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POSTERS

Table of Contents

POSTERS – RESEARCH

POSTERS – EDUCATION

Abstracts submitted to the virtual The Biochemistry Global Summit (25th IUBMB Congress, 46th FEBS Congress and 15th PABMB Congress) from 9th to 14th July 2022 and accepted by the Congress Organizing Committee are published in this Supplement of *FEBS Open Bio*. Late-breaking abstracts are not included in this supplement. The abstracts are available as two PDF files: Talks (Plenary Lectures, Symposia and FEBS Special Sessions) and Posters.

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* Each poster has been given a unique number beginning with the letter P; the next part relates to the session in which the poster will be presented (see p.68 for key).

P-01.1 Cancer and metastasis
P-01.2 Neurodegeneration and regeneration
P-01.3 Diabetes and obesity
P-01.4 Cardiovascular diseases
P-01.5 Ageing
P-02.1 Host–pathogen interactions
P-02.2 Looking for new antibiotics
P-02.3 Molecular evolution
P-02.4 Microbial metabolism
P-02.5 Human microbiome
P-03.1 Cell division and cell cycle regulation
P-03.2 Apoptosis
P-03.3 Molecular immunology
P-03.4 Cell signalling
P-04.1 Proteins
P-04.2 Lipids
P-04.3 Saccharides
P-04.4 DNA and RNA
P-04.5 Metabolism and metabolic regulation
P-05.1 Food and nutrition in biochemistry
P-05.2 Sensors and nanotechnology
P-05.3 Synthetic biology
P-06.1 Genomics
P-06.2 Proteomics
P-06.3 Systems biology
P-07.1 Atomic and molecular imaging
P-07.2 Cellular imaging
P-07.3 Quantitative analysis of bioimages
P-E-01 Undergraduate teaching/Learning
P-E-02 Postgraduate teaching/learning
P-E-03.01 Faculty development

POSTERS - RESEARCH

Cancer and metastasis

P-01.1-001
Abstract withdrawn

P-01.1-002
Genetic ablation of p53 at the onset of HCC triggers tumor initiation via the CD24-TGFB axis in a pro-carcinogenic microenvironment

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Liver cancer is the 6th most common cancer in the world and ranks 4th in terms of death rates. Fibrosis due to environmental inflammation and progressive fibrotic tissue transformation into cirrhosis is one of the most common mechanisms in liver cancer development and is seen in 90% of hepatocellular carcinoma (HCC). Many mutations and impaired molecular mechanisms are involved in this process, and it has been reported that p53 (20-40%), which is one of the most frequently mutated genes in HCC, has a driver role especially for tumor initiation. On the other hand, the microenvironment–mutation signal interactions that initiate the tumor have not been clearly elucidated in the literature. In our studies, we observed that Ki67, a proliferation marker, increased significantly in P53 knockout (p53-KO) human liver organoids, which we created with the CRISPR method from healthy liver organoid to examine these interactions. Similarly, we showed that p53-KO cells were more proliferative by CellTiter-Glo-3D viability analysis. Then, we performed flow analysis to determine the changes in the levels of candidate progenitor surface markers in p53-KO organoids and to examine the possible contribution of these changes to the cancer initiation process in an inflammatory environment. We showed that some stem cell surface marker-positive cells were significantly changed in p53-KO organoids and that these changes were in interaction with the TGFB pathway. In IHC staining, we observed that the P53-KO organoids showed more atypical cell morphology as well as higher glycogen stores. Consistently, it was observed with the Elisa method that the albumin level was higher in the P53-KO group. As a result, it has been shown that p53-KO cells are more proliferative in response to pro-carcinogenic inflammatory signals at the onset of HCC and that there may be a triggering mechanism involved in the formation of tumor-initiating progenitor hepatocyte cells via the P53-TGFB pathway.

P-01.1-003
Glycine/serine metabolism controls brain invasion by cancer cells

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The Tumor Microenvironment (TME) is a critical regulator of metastases, and its metabolic composition is of prime importance controlling cancer cell proliferation and motility. The brain TME is characterized by its unique nutrient biology, in particular amino acids (1). Maybe for these reasons, brain metastases (BM), one of the most complicated metastases threatening patient's survival, show a different metabolism as compared to primary tumors. Therefore, it is crucial to understand which metabolic vulnerability cancer cells develop during metastasizing the brain. The increased metabolic demand of metastasizing cells make them dependent on continuous uptake of certain metabolites when intracellular synthesis can't deal with their metabolic requirements. We aimed to separately study the metastatic potential of lung and breast cancer, known to preferentially form BM, to dissect the metabolic nature of molecules released in the brain TME, and to investigate the genetic and metabolic tools that the two cancer types develop in order to invade the brain. We developed an experimental procedure mimicking the brain TME by collecting the brain extracellular fluid from mice and using it to culture cancer cells. We found that serine and glycine, released in the brain TME, stimulate cancer cell chemo-kinesis and support brain invasion. Thus, serine hydroxymethyltransferase (SHMT), a key enzyme in one-carbon metabolism, fine-tune the cytosolic serine level, a parameter deemed critical in controlling metastasizing cancer cell energy production and redox homeostasis. A drop in serine/glycine levels, activates the cellular sensor, AMP kinase, which possibly leads metastasizing cells to switch from a more motile to a survival phenotype. *In vivo* studies are on-going in order to further investigate the metabolic pathways included in brain invasion by parental and metastatic breast cancer cells and to possibly halt BM formation. 1-Bouzidi A et al 2020. Cell Death & Disease 11:1012

P-01.1-004
Investigation of the radiobiology of HPV-positive and HPV-negative head and neck squamous cell carcinoma (HNSCC) utilizing 3D spheroid models

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Head and neck squamous cell carcinoma (HNSCC) is the sixth most-common cancer worldwide, and remains a significant public health concern in the UK, with approximately 12,500 cases diagnosed and 4,000 deaths confirmed annually. This research is particularly important to the local, North West region given the high incidence of HNSCC which is three times the national average, and where strategies leading to effective treatment are being sought. Radiotherapy, in the form of ionizing radiation, is predominantly used to treat HNSCC patients which acts by inducing particularly DNA double strand breaks (DSBs). Interestingly, work from our group and others have shown the increased radiosensitivity of HPV-positive HNSCC, which leads to enhanced patient response, is largely linked with defects in the signalling and the efficiency of DNA DSB repair. Therefore, *in vitro* 3D spheroid models of HNSCC have developed to further study the difference in cellular mechanism between HPV-positive and HPV-negative HNSCC in responding to radiotherapy, including both conventional x-ray irradiation but also targeted proton beam therapy. Furthermore, poly(ADP-ribose) polymerase (PARP) inhibitors (veliparib, niraparib, olaparib and talazoparib) have examined and demonstrated for enhancing the radiosensitivity particularly of radioresistant HPV-negative HNSCC spheroid models. This project is now being further developed in patient derived HNSCC organoids.

P-01.1-005
Modulatory effect of *Clerodendrum volubile* leaf extract on doxorubicin-induced toxicities: *In vivo* and *in silico* approach

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Doxorubicin (DOX) is a commonly used chemotherapeutic drug. However, its nontarget organ toxicities pose a serious problem. This study is to assess the protective role of *Clerodendrum volubile* leaf extract (CVE) against DOX-induced toxicities in rats. In addition, the inhibitory activities of three phytochemical compounds (rutin, gallic acid and rosmarinic acid) from CVE against Carbonyl reductase 1 (CBR1) were examined. Rats were randomly divided into five groups: (a) Control group rats were given 0.9% NaCl as vehicle, (b) DOX group: A single dose of DOX (25 mg/kg; i.p.) was administered and rats were sacrificed 4 days after DOX injection, while groups (c-e) CVE-treated DOX rat groups were given 125, 250 and 500 mg/kg body weight of extracts orally for 12 consecutive days; 8 days before, and 4 days after the DOX administration. Computational techniques were used to determine the inhibitory activities of the compounds against CBR1. DOX intoxication caused a significant increase ($P < 0.05$) in serum marker enzymes: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, creatine kinase activities. The levels of liver and heart tissues antioxidant parameters: glutathione peroxidase, superoxide dismutase, catalase, and glutathione were significantly ($P < 0.05$) decreased in DOX-intoxicated rats with concomitant elevation of malondialdehyde levels. Pretreatment with CVE reversed the above trends. From the structural analysis, rutin and rosmarinic acid exhibited the highest binding free energies against CBR1, and also exhibited structural stability when bound with CBR1. Our study indicates the protective effect of CVE when used in combination with doxorubicin thus improving its chemotherapeutic application via inhibition of CBR-mediated metabolism.

P-01.1-006
Investigation of the efficacy of human umbilical cord tissue-derived mesenchymal stem cells' paclitaxel loaded exosomes on cervical cancer cells

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Umbilical cord (UC) tissue contains a high concentration of stem cell sources and is widely used for mesenchymal stem cell (MSC) isolation. Because of their differentiation potential and intracellular richness, umbilical cord-derived mesenchymal stem cells have risen to prominence in clinical studies in recent years. Exosomes (Exo), on the other hand, are released by many cells and provide interaction between cells with the gene and protein molecules carried in their structures; they can also be used as biomarkers. The purpose of this study is to look into the effect of exosomes released from umbilical cord-derived mesenchymal stem cells in the treatment of cervical cancer by making them functional with paclitaxel (PAC). Based on tissue incubation with type 1 collagenase, MSCs were isolated from the umbilical cord. CD44, CD90, CD105, and CD34 were used to identify isolated MSC. Exosomes were isolated from the MSC starvation medium using an ultracentrifugation method every 48 hours. Exosomes were characterized using particle size and structure, as well as CD9, CD63, and CD81 markers. MSC-derived exosomes were electroporated with paclitaxel (UC-Exo-PAC) and then incubated with HeLa cervical cancer cells for 24 hours before cytotoxicity and apoptosis assays were performed. Transmission electron microscopy (TEM) has revealed the particle size and structure of exosomes as 114 nm. Paclitaxel loaded into exosomes shows efficacy in a shorter time and at lower concentrations. UC-Exo-PAC increases cell apoptosis and decreases cell migration in cervical cancer cells. If the exosomes of umbilical cords excreted during birth are isolated, they can be used as a drug delivery system, and these

exosomes facilitate drug entry into the cell, making drugs more effective at low doses. The findings of this study will help to increase the potential for using exosomes in the development of new drug delivery systems.

P-01.1-007
Mechanisms regulating cell death and survival behind biological responses to novel Ru(III)-based nanosystems in triple negative breast cancer

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According to WHO, breast cancer (BC) is the most common cause of cancer death in women. The triple negative breast cancer (TNBC) phenotype is the most aggressive and has to date limited treatment options. Therefore, the discovery of novel therapeutical approaches is crucial. Our research group is focusing on a newly synthetized Ru(III) complex – named AziRu – incorporated in different nucleolipid nanostructures to improve its stability and *in vivo* delivery, showing enhanced antiproliferative activity against estrogen responsive breast cancer model both *in vivo* and *in vitro* (previously published in: Ferraro et al. (2020) Cells 9(6), 1412). Among our nanosystems, HoThyRu/DOTAP was the most effective against a TNBC *in vitro* model represented by MDA-MB-231 cells, displaying an IC_{50} of 10 μM. Since HoThyRu/DOTAP demonstrated a significant antiproliferative activity against TNBC, we next investigated its capability to trigger multiples cell death pathways i.e., apoptosis and autophagy. Fluorescent microscopy showed early apoptosis and autophagy activation already after 12h of HoThyRu/DOTAP treatments. The evaluation of the main proteins engaged in the regulation of cell death pathways after 48 and 72h of treatment suggested our Ru nanosystem as capable to sustain these processes over time. Moreover, we focused on metastatic potential of TBNC demonstrating that HoThyRu/DOTAP can inhibit migration and invasion of MDA-MB-231 cells, as proved by different functional assays and by the modulation of principal EMT related proteins. Finally, we set up a xenograft model of MDA-MB-231 and analyzed the efficacy of HoThyRu/DOTAP *in vivo* by performing ex vivo analysis to validate the activation of specific cell death pathways through proteomic and transcriptomic analysis. The induction of different cell death pathways by multi-target Ru-based formulations could represent a valid and appealing chemotherapeutic option for breast cancer treatment.

P-01.1-008
Functional characterization of a novel mutation in PRKCA, the major driver of Chordoid gliomas

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Chordoid gliomas (ChG) are a rare low-grade brain tumour, believed to be derived from Tanycytes. An analysis previously identified a novel mutation present in all ChGs: *PRKCA* p.D463H (Rosenberg S. et al. 2018, Nat Commun 9(1):2371). This mutation involves a D463H amino acid substitution at the kinase domain of the protein kinase C alpha (PKCα) and represents the hallmark of ChG. PKCα is a serine/threonine kinase, is widely expressed, and carries out the regulation of various functions. The D463H mutation affects a critical residue of the kinase domain of PKCα, suggesting that such change may modify enzyme/substrate affinity and specificity. We have shown the mutated form of PKCα maintains activity by kinase assay, then to study the effect of the mutation on the specificity of the kinase, we designed a peptide array comprising of 384 unique peptides corresponding to known phosphorylation sites. To identify if there is a change in substrates between the wild type and mutant, the peptide array analysis will reveal candidate substrates of PKCαD463H. These candidates will be explored in a context that is more representative of ChGs, for this we are establishing the PKCαD463H mutation in immortalized astrocytes using a CRISPR/Cas9 approach, as well as primary Tanycyte culture. These models will allow us to study the effect of the mutation on cellular processes (proliferation, adherence, polarity, migration), involved in the tumorigenesis. We are also exploring the effect of the D463H mutation on the tertiary structure of the protein and therefore its stability, by producing constructs by mutagenesis. The aim of the project is to identify novel and biologically relevant PKCαD463H substrate candidates, which will demonstrate the involvement and the role of this mutated kinase in various cellular functions implicated in the development of ChGs. The functional characterization of this novel mutation may then provide new targeting opportunities in ChGs.

P-01.1-009
NK-cell mediated apoptosis of human peritoneal mesothelial cells in the ovarian cancer microenvironment

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Transcoelomic dissemination of tumor cells via the peritoneal fluid occurs at an early stage of high-grade serous ovarian carcinoma (HGSC) enabling extensive tumor spreading within the peritoneum. Different immune cells are present in the ascites as part of the tumor microenvironment. Although there are several concepts describing the transcoelomic metastasis formation, the exact mode of peritoneal invasion is largely unknown. We analysed the effect of tumor-associated lymphocytes (TAL) on promoting tumor cell invasion via apoptosis-induced disruption of the mesothelial barrier. In this context, we found that natural killer cells (NK-cells) seem to be an important effector population within the TALs. Upon their activation by CD3-stimulated T-cells NK-cells strongly degranulate in the presence of human peritoneal mesothelial cells (HPMC) *in vitro*. Subsequently, HPMC apoptosis is induced, pointing towards granzyme B/perforin signalling. We further observed that death-receptor mediated apoptosis via tumor necrosis factor related apoptosis-inducing ligand (TRAIL) also plays a role. By applying a TRAIL blocking antibody to the co-culture of TALs with HPMCs, the amount of apoptotic HPMCs was significantly reduced, but not completely lost. This indicates that more than one apoptosis pathway is active in our system, which seems to depend on the

activation state of the NK-cells: TRAIL blocking had a strong effect on contact-dependent activation of NK-cells by T-cells prior to the co-cultivation with HPMCs. This was not the case for T-cell secretome activated NK-cells. The TRAIL expression also differs between these NK-cell activation states with strongest expression when activated by direct contact with T-cells, which is in accordance with the apoptosis results. The effects were maintained in ascites-cultured cells – albeit to a lesser extent – meaning that these *in vitro* results could be important in transcoelomic dissemination of HGSC by clearing the mesothelial barrier.

P-01.1-010
Identifying new interactors of MT1-MMP involved in its trafficking using biotin ligases

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Trafficking is one of the main mechanisms for regulating membrane type 1 matrix metalloproteinase (MT1-MMP), a key enzyme involved in extracellular matrix degradation, but the details of this mechanism have not been fully described. We seek to elucidate some of the underlying mechanisms of endocytosis of MT1-MMP by performing proteomic analysis of potential interactors of this metalloproteinase. We are using three mutants with defects in endocytosis, ΔCP, $C^{574}A$, and LLY^{573}/AAA, previously published in: Uekita T *et al.* (2001) J Cell Biol 155, 1345-1356. and Anilkumar N *et al.* (2005) FASEB 19, 1326-1328. We are working with BioID2, a promiscuous biotin ligase, fused to MT1-MMP (WT and mutants) to label neighbouring proteins, followed by streptavidin pulldown and mass spectrometry. We have confirmed that our BioID2-tagged mutants have a lower internalization rate in a biotin internalization assay and that the biotin ligase successfully labels proteins by detecting them on Western blot using streptavidin-HRP. As a complimentary approach, we are employing the wild biotin ligase BirA, which biotinylates a specific peptide (Avi tag), allowing for very efficient precipitation of protein complexes with streptavidin beads. We have introduced the Avi tag into the cytoplasmic tail of MT1-MMP in a way that allows us to minimize the disruption of most interactions in that region. Selected candidates will then be confirmed as MT1-MMP interactors and the role of their interaction with the enzyme in its key functions will be assessed. To summarize, we plan on using biotin ligases to identify novel binding partners of MT1-MMP related to its endocytosis and looking into the functional implications of their interactions. The aim is to clarify the mechanisms behind the trafficking of MT1-MMP and the way this form of regulation impacts processes MT1-MMP participates in.

P-01.1-011
Novel seminoma biomarker from liquid biopsies

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Seminoma (SE) is a type of testicular tumour, routinely diagnosed after surgical orchidectomy using tissue analysis by immunohistochemistry of differentially expressed protein markers. Cell-free DNA (cfDNA) is a promising molecular biology platform for noninvasive SE screening and monitoring. We aimed to investigate cfDNA methylation of SE tissue biomarker *OCT3/4* in liquid biopsies and determine whether *OCT3/4* could be translated to a liquid biopsy approach. To that end, for cfDNA analysis, twenty-four SE patients and thirty-five healthy volunteers from the general population were recruited. For genomic DNA (gDNA) analysis, SE tissue from recruited patient was sampled and testicular tissue samples of twelve patients with non-malignant diagnoses were retrieved from paraffin tissue archive, as a control group. DNA methylation of *OCT3/4* was analysed by pyrosequencing in gDNA from SE tissue and cfDNA from blood and seminal plasma. In addition, OCT3/4 expression analysis was performed by immunohistochemistry followed by morphometric analysis. Associations between protein expression and DNA methylation were assessed, and predictive power of DNA methylation between SE and controls was determined using receiver operating characteristic (ROC) curve. *OCT3/4* gDNA methylation was in line with its protein expression in SE. In blood plasma, DNA methylation of *OCT3/4* was determined as a possible biomarker for SE patient monitoring, while in seminal plasma *OCT3/4* methylation was detected as possible screening biomarker. Further analysis revealed only CpG1 site in seminal plasma as potential screening biomarker with detected sensitivity of 100% and specificity of 55%, corresponding to an AUC of 0.71. However, statistically significant CpG1 site detected in blood showed modest diagnostic performance. In conclusion, for the first time, we disclosed that cfDNA methylation of SE tissue biomarker *OCT3/4* has great potential as epigenetic biomarkers from liquid biopsy in SE diagnostics.

P-01.1-012
Cancer cell-free DNA fragmentation profile

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Tissue biopsies, while being the gold standard in oncology, have issues which are becoming more and more apparent. Tissue biopsies are invasive, have difficulty detecting tumor heterogeneity, and certain tumors are simply unapproachable or too risky to perform biopsies on. With the rise of molecular biology approaches in medicine, liquid biopsies have promised to be reliable and minimally invasive surrogates. Cell-free DNA (cfDNA) is one of the most prominent molecules in liquid biopsies, detected in most body fluids, and reflects the characteristics of its tissue of origin. The integrity of cfDNA has been shown as cancer biomarker, with cfDNA of tumor origin being molecules of either longer or shorter size in relation to cfDNA from non-cancerous cells, depending on body liquid of origin. To investigate the use of size profiling in cancer detection, we have analyzed different body liquids of patients with testicular tumors (TT) and compared it to matched healthy controls. Patients were recruited from KBC Sestre milosrdnice and KBC Zagreb while healthy controls were recruited from the general population. Body liquids were processed to produce acellular plasma, from which cfDNA was extracted. Long and short LINE-1 fragments were quantified absolutely by qPCR and from their ratio the cfDNA integrity index (CFI) was calculated. To detect possible confounding factors, we have subdivided our patients by diagnosis, and controls by fertility score. We have detected an increase in both the total amount of long fragments and CFI in TT patients, while no difference was found in total cfDNA amounts. In addition, an increase in both was noted in men from the control group with a lower fertility score. We have confirmed the potential of CFI, as well as cfDNA size profiling for cancer diagnostics. CFI has shown itself as a reliable molecular biomarker of cancer, detecting TT of all types, with potential applications in reproductive science as well.

P-01.1-013
Role of intrinsically disordered regions of promyelocytic leukemia (PML) protein in the formation of PML-bodies

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The scientific data collected to date suggests, that membrane-less organelles (MLO) play an important role in the organization of intracellular space and signaling pathways. These dynamic structures are formed and disassembled as a result of reversible liquid–liquid phase separation (LLPS) that is highly controlled in biological systems. PML bodies — nuclear MLO that are involved in the regulation of transcription, stress response, differentiation, and the transition of cells to the senescent state. Formation of so-called nuclear Alternative Lengthening of Telomeres (ALT)-associated PML bodies (APBs) results in the alternative lengthening of telomeres in ALT-positive tumor cells, suggesting potential therapeutic application. The key factor in these MLO is promyelocytic leukemia (PML) protein. Due to alternative splicing, PML has 7 major isoforms that differ in their intrinsically disordered (ID) C-terminal domains. In this work, we analyzed the role of ID regions of different PML isoforms in the formation of PML bodies. It was shown that each of the full-length PML isoforms independently forms nuclear liquid-like droplets, however, only the PML-II and PML-V C-terminal domains are incorporated into endogenous PML bodies and form dynamic liquid-droplet compartments. Droplets formed by the C-terminus of PML-II and PML-V isoforms in the PML knockout cells possess dynamic characteristics of LLPS. It was also shown that disruption of PML SUMOylation, increases diffusion of the C-termini of PML-II and PML-V isoforms in endogenous PML knockout HeLa cells, but not in wild-type cells. Our data indicate the ability of the C-terminal domains of PML-II and PML-V isoforms to promote formation of dynamic liquid droplets independently of the ordered N-terminal PML region and PML SUMOylation. This indicates an essential role of LLPS driven by PML ID regions in the formation of PML bodies. This work was supported by a grant from the Russian Science Foundation RSCF 19-15-00107. *The authors marked with an asterisk equally contributed to the work.

P-01.1-014
Effects of vitamin C supplementation on epigenetic DNA modifications levels in patients with chronic lymphocytic leukemia

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Chronic lymphocytic leukemia (CLL) is the most common adult haematological malignancy. In leukemias, epigenetic mechanisms such as DNA methylation and active DNA demethylations, carried out by TET (Ten-Eleven Translocation) proteins play a crucial role. Ascorbic acid may induce generation of epigenetic DNA modifications, serving as a cofactor of TET enzymes during oxidation of 5-methylcytosine to 5-hydroxymethylcytosine (5-hmCyt) and subsequently to 5-formylcytosine (5-fCyt) and 5-

carboxycytosine (5-caCyt). TET proteins may also oxidize thymine to 5-hydroxymethyluracil (5-hmUra). The plasma vitamin C level is often lowered in CLL patients and may phenocopy loss-of-function mutations of TET. Supplementation of vitamin C in vitamin C-deficient patients may potentially restore TET proteins activity and slow disease progression. Therefore, we have orally supplemented 29 CLL patients with 500 mg of vitamin C per day for six months. At the beginning and end of supplementation, plasma vitamin C concentration was measured by UPLC-UV/Vis. Moreover, DNA from peripheral mononuclear blood cells was isolated and the level of epigenetic DNA modifications was analysed by 2D-UPLC-MS/MS. After 6 months of supplementation there was noticeable increase in vitamin C levels in plasma (on average 3.44 fold) accompanied, in most cases, by a decrease in 5-hmCyt levels (on average 0.76 fold) followed by increase in the levels of 5-caCyt (on average 2.57 fold). The levels of 5-fCyt and 5-hmUra were also decreased. An inverse correlation between vitamin C concentrations in plasma and 5-hmUra levels was observed after supplementation. Oral supplementation of vitamin C seems to restore its plasma concentration in CLL patients and may modulate profile of epigenetic modifications in DNA probably through stimulation of TETs activity. This work was supported by Polish National Science Centre grants no. 2017/27/B/NZ7/01487 and 2020/39/O/NZ5/00852.

P-01.1-015
Development of the hybrid antitumor bispecific protein DR5-B-iRGD

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The mutant TRAIL variant DR5-B has enhanced antitumor activity due to its selective interaction with the DR5 death receptor. DR5-B binds to DR5 receptor as efficiently as wild type TRAIL, but has no affinity for other TRAIL receptors (DR4, DcR1, DcR2, and OPG). This makes it possible to avoid the inhibitory effect of decoy receptors and to enhance the apoptotic signaling in tumor cells. Previously, a technique was developed for the expression and purification of the recombinant DR5-B protein in *E. coli*. In current work, we have developed a genetic construct encoding DR5-B fusion protein with an iRGD peptide (CRGDKGPDC) at the C-terminus. The iRGD peptide specifically binds to integrins αvβ3 and αvβ5, overexpressed on the surface of tumor cells, with subsequent proteolytic activation and acquisition of affinity for the NRP-1 receptor involved in angiogenesis. The DR5-B-iRGD fusion protein was expressed in a soluble form in the *E. coli* SHuffle B strain and purified by metal affinity and ion exchange chromatography. Affinity measurements by ELISA showed that the DR5-B-iRGD fusion protein binds to the DR5 receptor as efficiently as DR5-B, and has a similar affinity for the integrins αvβ3 and αvβ5, as free iRGD peptide. The cytotoxic activity of the DR5-B-iRGD has been confirmed on various 2D tumor cell lines and 3D models of tumor spheroids *in vitro*. It has been shown that DR5-B-iRGD penetrates the 3D multicellular tumor spheroids faster and more efficiently and exhibits higher cytotoxicity towards cancer cells compared to DR5-B. Bispecific targeting of various signaling pathways involved in tumor development with the DR5-B-iRGD fusion protein is promising for anticancer therapy. The research was funded by Russian Science Foundation grant No. 21-14-00224, https://rscf.ru/project/21-14-00224/.

P-01.1-016
Prestine C60 fullerene as a novel agent in sonodynamic treatment of cancer cells

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Sonodynamic therapy is the therapeutic strategy that involves the use of sonosensitizers, which become cytotoxic upon exposure to high-frequency ultrasound (US). Cavitation that occurs during US propagation through the liquid causes gas bubbles to implode with short bursts of light, known as sonoluminescence. This important feature of US promoted the idea of its use as an alternative energy source for photosensitiser excitation. Pristine C_{60} fullerene (C_{60}), an excellent photosensitiser, was explored for the sonodynamic treatment of cancer cells. For that purpose, we analysed sonoluminescence and C_{60} effects on human cervix carcinoma HeLa cells exposed to 1 MHz US treatment. HeLa cells were shown to take up pristine C_{60} from the media in a time-dependent manner. The maximum value of intracellular C_{60} level reached 802 ± 66 ng / 106 cells after 24 h of incubation. The US experimental set-up consisted of US generator and US transducer mounted directly into the water-bath. To assess the luminescent effect of US, a photomultiplier tube, connected to an oscilloscope, was used. The obtained peak-to-peak voltage (Vpp) values of the photomultiplier tube evidenced its dose-dependent significant positive correlation with the output power of the US generator (0–500 W). For investigation of the combined effect of C_{60} and US, HeLa cells were incubated in the absence or presence of 20 μM C_{60} for 24 h and exposed to 1 MHz US at the spatial average, temporal average intensity ISATA in 5.4 W/cm^2 for different exposure times (≤60 sec). Treatment of cells with C_{60} and US resulted in a 59 ± 5% decrease in cell viability at 48 hours, a 342 ± 29% increase in caspase 3/7 activity, and phosphatidyl translocation in 88 ± 4% of cells at 24 hours, demonstrating a significant proapoptotic cytotoxic effect of 1 MHz US and C60 on HeLa cells. The ability of C_{60} to induce apoptosis of cervix carcinoma cells after sonoexcitation represents a promising novel approach for cancer treatment.

P-01.1-017
Cytotoxic effect of the antipsychotic drug spiperone in colorectal cancer cells via endoplasmic reticulum stress induction, intracellular lipids dysregulation and Golgi apparatus damage

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Approximately 50% of colorectal cancer (CRC) patients still die from recurrence and metastatic disease, highlighting the need for novel therapeutic strategies. Drug repurposing is receiving increasing attention as a faster and cheaper strategy to offer more effective treatment options. Epidemiological and pre-clinical studies support the antitumoral activity of psychotropic drugs and, recently, we demonstrated the cytotoxicity of the typical antipsychotic spiperone in several cancer cell lines (*Varalda M. 2020 Front Oncol 10, 562196*). Currently, we are dissecting the mechanism of action of spiperone in both differentiated CRC cell lines and undifferentiated stem-like cells (CRC-SCs). Our data show that spiperone can effectively reduce cell viability in both differentiated cells and CRC-SCs and decrease the clonogenic potential of CRC-SCs at clinically relevant concentrations, with negligible toxicity in non-neoplastic cells. Also, analysis of intracellular calcium (Ca^{2+}) kinetics, upon spiperone treatment, revealed a massive phospholipase C (PLC)-dependent endoplasmic reticulum (ER) Ca^{2+} release, resulting in ER Ca^{2+} homeostasis disruption. RNA sequencing revealed unfolded protein response (UPR) activation, ER stress, and apoptosis induction along with IRE1-dependent decay of mRNA (RIDD) activation after 20 hours of treatment. Lipidomic analysis showed a significant alteration of lipid profile mainly of sphingolipids, including ceramide and dihydroceramide accumulation. Golgi apparatus and mitochondrial damage were also observed after 16 hours of spiperone treatment. Our data suggest that spiperone can represent an effective drug in the treatment of CRC and that ER stress induction, along with lipid metabolism alteration, represents effective druggable pathways in CRC. Further studies are needed to identify other potential targets of this drug, but our findings represent a promising starting point for the design of new therapeutic strategies for CRC.

P-01.1-018
Ketone bodies as a supporting strategy in cancer therapy? Findings on cervical cancer cell line HeLa with CRISPR/Cas9-mediated silencing of the key ketone body metabolism gene – OXCT1

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Cancer cells demand increased amounts of glucose for their growth, simultaneously maintaining glycolysis at a higher level than normal cells. This observation had led to the question of whether it is possible to eliminate glucose from patients with cancer by using an alternative energy source. One of the proposed solutions concerns a ketogenic diet due to the fact that the major ketone body – β-hydroxybutyrate – attenuates inflammatory response. The rate-limiting enzyme in ketone bodies catabolism is succinyl-CoA:3-ketoacid-coenzyme A transferase 1 (SCOT1), encoded by the *OXCT1* gene. The main aim of the research was to analyze the (i) proliferation rate, (ii) invasive and migratory potential, and (iii) sensitivity towards commonly applied cytostatic agents in cervical cancer treatment. The knocking-out procedure of *OXCT1* was performed by the CRISPR/Cas9 technique using two plasmids selected on blasticidin and puromycin and confirmed at the protein level by Western blotting as well as immunofluorescent staining of SCOT1. The proliferation rate analysis of HeLa OXCT1 KD cells was performed using the resazurin reduction assay. Both, the invasion and migration tests were conducted using the Transwell assay. The cytotoxicity of selected cytostatic agents such as doxorubicin and cisplatin in a given concentration range (5–500 nM and 50 nM–10 μM, accordingly) was determined by MTT assay. We observed that the *OXCT1* knockout increases both the proliferation rate and the invasive and migratory potential of HeLa OXCT1 KD cells. Moreover, higher resistance towards cytotoxic agents was noticed as well. Our findings suggest that the *OXCT1* gene is involved in the regulation of cervical cancer proliferation. Switching to alternative energy sources – ketone bodies – may be the trigger for invasiveness and resistance to chemotherapy. Acknowledgments: This work was supported by the Doctoral Research Grant funded by the University of Lodz.

P-01.1-019
Towards diagnostic and therapeutic avenues against SARS-CoV-2 by combining CryoEM and molecular simulations

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Molecular simulations have been instrumental in identifying the structure–function relationships of biomolecules in the atomic level as well as providing a means for structure-based drug discovery, thereby explaining and guiding experimental findings. The increase in computational power, the new physics and

machine-learning-based algorithms is significantly driving the boost in the field and gives access to addressing biomolecular phenomena of increasing length and timescales. In this talk I will discuss examples where using state-of-the-art integrative structural biology methods that inject Cryo-EM experimental data into the simulation, we can reveal accurate protein-functional dynamics of the SARS-CoV-2 spike protein in an atomistic level. In this way we can a) reveal virus vulnerabilities by identifying cryptic binding sites exposed during the S protein conformational transition related to the recognition to the host cell and b) provide with the molecular motion and energetics of protein-antibody complexes which enables to suggest mutations that increase the spike-antibody affinity. These predictions are validated in further CryoEM experiments.

P-01.1-020
Analysis of the immune response of breast cancers reveals greater significance of an adaptive response over an innate response

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Marker genes have allowed for the categorization of breast cancers into subtypes basal, HER2-positive, luminal A, and luminal B. Immunotherapeutic success require (i) understanding the immune response and (ii) identification of biomarkers. We compared innate and adaptive immune responses by investigating the impact of the expression of genes in the MHC I and MHC II pathways on the patient survival from breast cancer (BC) subtypes. Clinical and gene expression data of BC patients from The Cancer Genome Atlas were analysed. Expression values of MHC genes for each subtype were divided into tertiles, which were used to classify levels of expression as high, mid, or low. Hazard ratios (HRs) and death ratios (dRatios) comparing the probability of patients dying at low levels to high levels of each gene for each subtype were calculated. Eighty-nine gene-subtype combinations (sGenes) were identified as significantly associated with survival; 64 were MHC I genes, and 25 were MHC II genes. Among MHC 1 genes, 41 were involved in ubiquitination, 12 were proteasomal, and 11 serve other functions. Among MHC II genes, 10 were involved in protein transport, 6 were HLA-D and 9 serve other functions. None of the 6 HLA genes of the MHC I pathway were significantly associated with survival; in contrast, 6 HLA-D genes of the MHC II pathway were. Only 6 sGenes were curated as cancer driver genes by the Cancer Genome Consortium, as such potential biomarkers may exist among the other 83. MHC I genes impact survival via pathway regulation and preparation of protein fragments, whereas MHC II do so via the presentation of epitopes to CD4+ T-cells. Patients whose tumour cells acquire the capability of professional antigen presenting cells experience better outcomes. Upregulating the expression of HLA-D genes of the MHC II pathway may prove beneficial. Variation in the composition of sGenes for each BC subtype suggests enhanced prognosis if gene expression is analysed at the subtype level.* The authors marked with an asterisk equally contributed to the work.

P-01.1-021
The antiproliferative and proapoptotic effect of the HDAC inhibitor - LBH589 in glioma initiating cells

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Glioblastoma multiforme (GBM) is the most frequent and aggressive type of brain tumor, mostly due to its poor response to standard anticancer treatments. One of the reasons for the drug resistance may be the presence within the tumor mass of a small population of cells termed the glioma initiating cells (GICs). The development of novel therapeutic approaches targeting the population of GICs is needed to improve the survival of the GBM patients. Latest interest in the role of epigenetic regulation in carcinogenesis has prompted a widespread quest for epigenetic modifiers with possible utility as chemotherapeutics. Histone deacetylases (HDACs) inhibitors are now intensively investigated as potential cytostatic agents in many types of cancer including GBM. The LBH589 is a pan-HDACs inhibitor known to possess antiproliferative activity in a variety of malignancies. Two human GICs cell lines GH2 and 12o12 were treated with LBH589 in the concentrations ranging from 25–400nM for 48 h. Luminescence-based analysis was performed to determine HDAC-inhibitory potency of LBH589. The MTT and CellTiter-Glo tests were used to measure cell viability. Annexin V binding and caspase 3 expression were determined to detect apoptotic cell death. The Caspase 9-Glo assay and Western blot analysis was performed to determine caspases 9 activity and expression. Our findings showed that LBH589 is a potent inhibitor of HDACs. Treatment with LBH589 resulted in strong dose-dependent reduction in viability of both GH2 and 12o12 cells as shown by MTT assay and reduced ATP production. Increased Annexin V membrane binding together with hyperactivity and overexpression of caspase 3 and 9 indicated apoptotic death of GICs. Altogether, our results show that HDACs inhibitors such as LBH589 might be considered as potential cytostatic agents in GBM treatment. This study was supported by Polish National Agency for Academic Exchange (NAWA) within the frames of the Bekker program PPN/BEK/2020/1/00105 (to MK).

P-01.1-022
Syntenin-1-mediated small extracellular vesicles promotes lung cancer progression by increasing onco-miRNAs secretion

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Small extracellular vesicles (sEVs) play a pivotal role in tumor progression by mediating intercellular communication in the tumor microenvironment (TME). Syntenin-1 induces malignant tumor progression in various types of human cancers, including human lung cancer and regulates biogenesis of sEVs. However, the function of syntenin-1-regulated sEVs and miRNAs in sEVs remains to be elucidated. In the present study, we aimed to demonstrate the role of oncogenic Ras/syntenin-1 axis in the release of sEVs and elucidate the function of syntenin-1-mediated miRNAs in sEVs in lung cancer progression. The results revealed that oncogenic Ras promoted the release of sEVs by inducing syntenin-1 expression; disruption of syntenin-1 expression

impaired the release of sEVs as well as sEV-mediated cancer cell migration and angiogenesis. Moreover, we identified three miRNAs, namely miR-181a, miR-425-5p, and miR-494-3p, as onco-miRNAs loaded into syntenin-1-dependent sEVs. Remarkably, miR-494-3p was highly abundant in sEVs and its release was triggered by syntenin-1 expression and oncogenic Ras. Ectopic expression of the miR-494-3p mimic enhanced the migration and proliferation of lung cancer cells as well as tube formation in endothelial cells; however, the miR-494-3p inhibitor blocked sEV-mediated effects by targeting tyrosine-protein phosphatase nonreceptor type 12 (PTPN12), a tumor suppressor. sEVs promoted tumor growth and angiogenesis by downregulating PTPN12 expression; however, the miR-494-3p inhibitor significantly suppressed these effects in vivo, confirming that miR-494-3p acts as a major onco-miRNA loaded into lung cancer cell-derived sEVs. Eventually, the oncogenic Ras/syntenin-1 axis may induce cancer progression by increasing miR-494-3p loading into sEVs in lung cancer cells in the TME.

P-01.1-023
Peroxiporin-dependent oxidative stress triggers tumor induction in pancreatic cancer cells

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Aquaporins (AQPs) are transmembrane channels that facilitate water and small solutes transfer across membranes. Being involved in a wide range of physiological functions and diseases [1], AQPs have great potential for pharmacologic targeting and drug discovery. Recent studies showed that the altered expression of several AQPs is associated with various cancers, though the underlying molecular mechanism remains unclear. The overexpression of AQP3 and AQP5 was detected in pancreatic ductal adenocarcinoma (PDA) biopsies [2], being postulated their association with tumor progression [3]. In this work, we evaluated the contribution of AQP3 and AQP5 to the settings of tumorigenesis using a loss-of-function strategy. BxPC3 cells, a model of PDA that expresses high levels of AQP3 and AQP5, were silenced for AQP3 and/or AQP5 expression. AQP3 and AQP5-silenced cells were evaluated for their membrane permeability to water, glycerol and hydrogen peroxide, impact on cell migration, and effect on several markers of tumor (EGFR, ERK1/2, c-Fos, c-Jun,) and epithelial mesenchymal transition (EMT; E-cad and Vim). Our results confirmed the roles of AQP3 and AQP5 on cell membrane permeability to glycerol and hydrogen peroxide, corroborating their activity as aquaglyceroporin (AQP3) and peroxiporins (AQP3 and AQP5). Silenced cells showed slower recover of the wounded area (around 10%) evidencing the association between AQP3 and AQP5 and cell migration. Moreover, AQP3 and AQP5-silenced cells express lower levels of ERK1 (0.5- and 0.7-fold), ERK2 (0.4- and 0.8-fold) EGFR (0.6-fold for AQP3), c-Fos (0.3- and 0.5-fold) and c-Jun (0.1- and 0.7-fold), and E-cad (0.6-fold for AQP3) and Vim (0.2- and 0.6-fold) These results reveal a relation between the expression of AQP3 and AQP5 and the expression of key players of the MAPK signaling pathway and EMT, highlighting AQP3 and AQP5 importance in tumor biology. These findings may foster new strategies towards the development of antitumoral therapies. [1] Soveral G. et al. (2016) Aquaporins in health and disease: new molecular targets for drug discovery, CRC Press [2] Direito I et al. (2017) *J Surg Oncol* 115 (8):980-996 [3] Marios C et al. (2015) *BBA* 1848(10):2576-2583

P-01.1-024
Modulation of PP1/CAV1 interaction using bioportides as an anticancer strategy

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Prostate cancer (PCa) is a disquieting cause of men's death worldwide and there is an urgent need to develop new effective therapeutic strategies. Protein phosphatase 1 (PP1) recently emerged as a promising therapeutic target in cancer. In this context, the main goal of this work is to develop peptides to disrupt a key PP1 complex for PCa development, thus impairing PCa progression. The peptide designed to interrupt the interaction between PP1 and caveolin-1 (CAV1) was synthetised using microwave-assisted solid phase synthesis and coupled to penetratin to allow an efficient cell delivery. The efficacy of the synthetised peptide (and a scrambled homologue – peptide control) was evaluated *in vitro*, using androgen-dependent (LnCaP) and androgen-independent (PC-3) cell lines. The prostate cancer cells were incubated with different concentration of the peptides and cellular uptake, cells viability and the expression of several proteins were measured. We found that, after 48-h incubation, the peptides significantly decrease the LnCaP and PC-3 cells viability in a concentration-dependent manner. We also shed light on the expression of proteins involved in signaling pathways modulated by PP1/CAV1 interaction, including PP1α, p-PP1α, AKT, p-AKT, GSK3β and p-GSK3β. We observe a significant decrease in the phosphorylation of AKT, which suggest the disruption of the target interaction. These results highlight the potential of the synthetised peptide to negatively impact the PCa cells proliferation, by interfering with PP1/CAV1 complex. Further analyses are now required to confirm the disruption of the target interaction and to better elucidate the mechanisms of cells death.

P-01.1-025
Implications of aquaporin-3 and aquaporin-5 on pancreatic cancer cell biomechanics and cell-cell adhesion

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Aquaporins (AQPs), a family of transmembrane proteins, are responsible for the bidirectional transfer of water and small solutes across cell membranes in different human tissues. AQPs have important physiological functions in both exocrine and endocrine pancreas. AQP3 and AQP5 are known to be overexpressed in exocrine pancreatic cancer, playing key roles in cell migration, cell proliferation and invasion. Our study aimed to

evaluate changes in the biophysical, biomechanical and morphological properties of pancreatic cancer cells, as well as their changes upon silencing of AQP3 and/or AQP5. Our results show that silencing AQP3 and AQP5 had implications on cell migration, with the silenced cells showing slower recovery of the wounded area. We assessed membrane fluidity of the different cells using multi-photon scanning fluorescence microscopy to measure laurdan generalized polarization (GP). Silenced AQP5 and AQP3/5 cells (combined silencing of both AQPs) showed lower GP values, meaning that they have higher membrane fluidity. Using atomic force microscopy (AFM), we evaluated biomechanical and morphological properties. AQP5 and AQP3/5 silenced cells were shown to be softer than the control. Furthermore, these silenced cells were also shown to be smaller, with lower volume, higher height and with a higher surface roughness, when compared with the control cells. Through cell–cell adhesion measurements conducted by AFM, we also saw that the energy and force necessary to detach two cells were lower in AQP-silenced cells than for the control, showing that these AQPs have implications on cell–cell adhesion. These findings suggest that AQP3 and AQP5 contribute to cell migration and cell–cell adhesion. Water channels play a significant role in tumor development. Our findings provide new insight into possible strategies toward the development of antitumor therapies. *The authors marked with an asterisk equally contributed to the work.

P-01.1-026
The investigation of anticancer properties of some bispidine and phosphonate derivatives

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The search of therapeutical and (neo)adjuvant anticancer agents remains relevant in medicine. The polyamines (PAs) are widely investigated in connection with their involvement in regulation of cellular growth and proliferation. Their metabolism can be a potential target for the novel anticancer agents as PA catabolism is often reduced in tumors leading to their accumulation in actively proliferating cells. A set of 10 bispidine and 5 piperidine-phosphonate derivatives have been synthesized and evaluated for the biological activity. The IR and NMR spectra were used for the investigation of the final structures. The modification with ß-cyclodextrin complexes was performed to make the compounds water-soluble. Biological activity was studied at cell-free and cellular levels. The cell-free model contained the enzymes involved in PA metabolism and allowed evaluation of a direct influence of the tested compounds on these enzymes. The O-dianisidine/peroxidase-based method revealed the activatory effect of some bispidine derivatives on PA catabolism. The next step included the study on cancer cell lines, namely MCF7, PC3 and SKBR3. The MTT test showed some antiproliferative potential of bispidines and phosphonates. No correlation was observed between these methods. As some of the tested compounds had secondary amino-groups in their structure, the protonation constants were measured. It is known that natural PAs contain two and more secondary amino-groups and are positively charged at physiological pH. Our investigation revealed a correlation between protonation constants and cytotoxicity of the tested compounds. Taken together, these results suggest a possible mechanism of bispidine antiproliferative action mediated by targeting PA catabolism. We hypothesize that protonation facilitates penetration of the compounds into cancer cells and mediates their cytotoxicity. This study has been supported by the RUDN University Strategic Academic Leadership Program.

P-01.1-027
Sensitizing properties of neobavaisoflavone towards doxorubicin in human glioma cell lines

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Polyphenols are a broad group of plant-derived compounds, including coumarins, stilbenes, lignans, phenolic acids, and flavonoids. They exert many beneficial properties to health, such as anti-oxidant and anti-inflammatory effects. These compounds are also known for their anti-neoplastic properties, which is why they are in the spotlight of cancer research. Ineffective treatment is currently the gravest problem in oncology. This issue concerns high-grade gliomas, which are one of the deadliest neoplasms. There are promises about the ability of several polyphenols to modulate the activity of chemotherapeutics through the sensitization of tumor cells towards them. Therefore, the exploration of this effect might give a chance to develop more effective therapies. In our previous work, we showed that neobavaisoflavone, a *Psoralea corylifolia* L. isoflavone, sensitizes human glioblastoma U-87 MG cells towards doxorubicin, reducing their viability [previously published in: Maszczyk M et al. (2021) Molecules 26(15), 4516]. We also discovered that the isoflavone enhances apoptosis and induces changes in the cell cycle when combined with doxorubicin. The aim of this study was to evaluate the effect of neobavaisoflavone on the activity of doxorubicin in human anaplastic astrocytoma SW1783 cells. The WST-1 cell proliferation assay and image cytometry analyses, including the assessment of the cell cycle distribution and Annexin V assay, were performed. The results revealed that the combination of neobavaisoflavone and doxorubicin causes changes in the

subpopulation of apoptotic cells and the cell cycle. Our findings provide the basis for further research aimed at elucidating the molecular mechanism underlying the neobavaisoflavone sensitizing properties.

P-01.1-028
The effect of apoptosis inducer MIM1 on MDA-MB-231 human triple-negative breast cancer cells: from targeting Mcl-1 to inducing growth inhibition and apoptosis

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Lack of sufficient knowledge about the tumorigenesis process, the mechanism of drug resistance or, finally, the escape of cancer cells from apoptosis is the cause of the uneven and difficult fight against cancer. Breast cancer is the most commonly diagnosed cancer and the leading cause of cancer death among females. Triple-negative breast cancer (TNBC) is characterized by the poorest overall survival of all breast cancer subtypes. Lack of effective targeted therapies and the poor prognosis encourage intensive research to develop additional and better systemic treatment options for patients with TNBC. MIM-1(Mcl-1 Inhibitor Molecule 1) may selectively inhibit Mcl-1 protein and finally induce Mcl-1-dependent cancer cells death. It was stated that an elevated level of Mcl-1 protein in breast cancer is associated with rapid tumor progression, drug resistance, and poor prognosis to chemotherapeutic agents. Thus, the significant role of Mcl-1 makes the possibility of using its inhibitors in the treatment of breast cancer. The aim of this study was to assess the effect of Mcl-1 inhibitor MIM1 on MDA-MB-231 breast cancer cells viability and apoptosis. The obtained results from the *in vitro* experimental panel indicated that MIM1 significantly affects MDA-MB-231 cells viability – the highest cytotoxic effect (above 50%) was observed under conditions when breast cancer cells were exposed to apoptosis inducer at concentration 100 μM for 24 h. Moreover, following the image cytometry technique, MIM1 was found to induce mitochondrial membrane permeabilization – one of the key features of the mitochondrial-intrinsic apoptotic pathway, as well as DNA fragmentation in MDA-MB-231 cells. Our results provide the first evidence that the use of MIM1 may contribute to the loss of cell viability and apoptosis induction in MDA-MB-231 cells. In addition, our findings indicate that blocking of Mcl-1 protein could be considered as a target in triple-negative breast cancer treatment.

P-01.1-029
Doxycycline and minocycline induce apoptosis in amelanotic melanoma cells

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Malignant melanoma is a type of cancer derived from specialized melanin-producing cells called melanocytes. It is responsible for 65% of deaths related to skin cancer. High mortality and relatively low survival of patients with melanoma are observed, despite the common availability of various therapies. Doxycycline (Doxy) and minocycline (Mino) are widely used semisynthetic tetracycline antibiotics. In addition to antimicrobial action, they show also many non-antibiotic properties, including anti-cancer activity. The purpose of the study was to assess the anti-cancer potential of doxycycline and minocycline using C32 and A375 human amelanotic melanoma cell lines. The obtained results indicated that both doxycycline and minocycline inhibited cell proliferation and decreased cell viability of amelanotic melanoma cells. The effect was proportional to drug concentration and the time of the treatment. Considering the calculated values of the EC_{50} parameter, doxycycline appeared to be a more potent drug than minocycline. Nevertheless, both tested tetracyclines were able to induce apoptosis in tested cells. The apoptosis was confirmed by positive results of the annexin V assay. Moreover, Doxy and Mino decreased mitochondrial membrane potential and activated initiator and executioner caspases. It was found that A375 and C32 cells differed in their sensitivity to the treatment and the type of dominant initiator caspase. Additionally, the anti-melanoma effect of doxycycline and minocycline was investigated using confocal microscopy bioimaging. The analysis of cell morphology, the arrangement of the actin cytoskeleton, and the intracellular level of cytochrome c confirmed the effectiveness of the treatment with tetracyclines. In summary, the study showed the pleiotropic anti-cancer action of doxycycline and minocycline against amelanotic melanoma cells and will contribute to better understanding tetracyclines pharmacology and their mechanisms of anti-melanoma action.

P-01.1-030
The effect of cobalamin status on glioma cells

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Cobalamin (vitamin B12) deficiency results in an impairment of thymidine synthase function and thus DNA synthesis is interrupted in cobalamin-deficient cells. Vitamin B12 depletion is especially acute for rapidly dividing cells, such as cancer cells, however, different types of cancer may differ in sensitivity to cobalamin status. In the present study, we developed an *in vitro* model of cobalamin deficiency in a glioma cell line. The cells were treated with cobalamin antagonist to induce hypocobalaminemia and the state was detected by immunoenzymatic analysis of homocysteine level. Using an image cytometry, we demonstrated that cobalamin antagonist caused a significant inhibition of glioma cells proliferation via cell cycle arrest in the G2 phase. Moreover, as compared to the previous study, the cytostatic effect of the cobalamin antagonist was more pronounced in glioma cells than in normal human astrocytes. We also performed an *in silico* analysis to characterize the molecular interactions between the studied cobalamin antagonist and transcobalamin II, a ligand for CD320 protein, which is overexpressed in numerous human cancer types. We have demonstrated that the compound shares similar interactions with transcobalamin II as naturally occurring cobalamins and thus may act as a competitive inhibitor of this transporter protein. We believe that our findings may become the basis for further experiments concerning cobalamin transport blocking as a potential therapeutic strategy in oncology.

P-01.1-031
Expression pattern of circRNA and their putative function in primary and recurrent glioblastoma

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Glioblastoma (GBM) is one of the most lethal and the most aggressive malignant brain tumors. Recently, the world of non-coding RNAs, with special regard to circular RNAs (circRNAs), has become a field of intensive research. Their role has been extensively studied in various diseases, and a growing body of evidence shows that their disruption might play an important role in cancer development. CircRNAs can affect cellular processes associated with cancer at many levels. They can sponge miRNAs and proteins, regulate parental gene expression and, eventually, they can also be translated into proteins. The aim of our study was to identify circRNAs differentially expressed in GBM, that are potentially involved in GBM aggressiveness, invasiveness, and tumor recurrence. We performed RNA sequencing on GBM primary and recurrent patient-derived tissues as well as on blood samples from GBM patients. We conducted differential analysis, distinguishing dysregulated circRNAs among primary and recurrent GBM samples, and we also managed to establish circular RNAs expression pattern among different GBM molecular subtypes, namely classical, mesenchymal, proneural, and neural. Moreover, we discovered circulating circRNAs with biomarker potential in blood samples. Consequently, we performed functional experiments to determine potential interactions between circRNAs, microRNAs and RBPs. The accomplished global-scaled analysis allowed us to select circular candidates that might serve as putative diagnostic or therapeutic targets in glioblastoma which nevertheless need further investigation. *The authors marked with an asterisk equally contributed to the work.

P-01.1-032
Molecular mechanism of action and clinical relevance of circCLIP2 in glioblastoma

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Gliomas have always been one of the greatest challenges for medicine due to their location, significantly impeding the use of conventional methods of diagnosis and treatment. Nowadays, much effort has been made to explore the way in which malignancy develops and to identify key players and mechanisms. Circular RNAs (circRNAs), a type of regulatory RNAs, have been found to play an important role in a variety of biological processes, including cancer. In order to establish their role in gliomagenesis and GBM progression, we performed RNA sequencing of GBM tissue, which revealed the disruption of several circRNAs in tumor samples. Moreover, with these results, we provided circRNAs expression patterns of specific subtypes of GBM, as a step forward to personalized treatment of GBM patients. One of the differentially expressed circRNAs – circCLIP2 – emerges more often in the mesenchymal subtype of GBM, which is considered as the one with the poorest patient prognosis. Our results also indicated decreased migration potential of GBM cells upon circCLIP2 knockdown. Furthermore, we indicated the impact of circCLIP2 knockdown on EMT process and ECM rearrangement of GBM cells. *The authors marked with an asterisk equally contributed to the work.

P-01.1-033
Fanconi anemia-associated head and neck squamous cell carcinoma morphologic and metabolic mitochondrial characterization: search for new potential therapeutic targets

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Fanconi Anemia (FA) is a rare genetic disorder characterized, among other features, by an increased risk of malignancies. Indeed, FANC-genes mutations cause DNA repair systems defects, as well as genomic and chromosomal instability, and have been associated with cancer progression even in patients not affected by FA. The most common solid tumor in FA patients is head and neck squamous cell carcinoma (HNSCC), with a 500–800-fold increased risk than the healthy population, even without exposure to typical risk factors. Moreover, HNSCCs in FA patients arise at a significantly younger age and are more aggressive, with survival rates under two years. Other than the defective DNA repair systems, altered mitochondrial biogenesis and dynamics, as well as metabolic dysfunctions, have been associated with FANC-genes mutations and FA pathogenesis. Since literature is missing a mitochondria morphologic and metabolic characterization in FA-associated HNSCCs, we aim to provide data to fill this gap. We used two HNSCC cell lines, with and without the FANC-A gene mutation. We performed spectrophotometric, oximetric, luminometric assays, and western blots to evaluate oxidative phosphorylation, respiratory complexes expression and functionality, cellular energy state, mitochondrial biogenesis and dynamics, autophagy and mitophagy processes, oxidative stress damages, and antioxidant defences. Our results highlight how FANC-A-mutated HNSCCs have strong similarities with mitochondrial abnormalities reported in literature for FA. Specifically, unbalanced mitochondrial biogenesis and dynamics lead to dysfunctional oxidative phosphorylation and a subsequent metabolic shift towards anaerobic metabolism, linked to increased and uncompensated oxidative stress and a worse

prognosis for this type of tumor. In conclusion, we address FA-related mitochondrial dysfunctions as potential therapeutic targets to treat HNSCCs in FA and cancer patients with FANC-genes mutations.

P-01.1-034
Novel lncRNA/miRNA/mRNA axes significant in ovarian cancer metastasis

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Ovarian cancer (OC) is a leading cause of death among gynecological cancers in women around the world. Recently a model of non-coding RNAs and a competitive endogenous RNA (ceRNA) interaction in the lncRNA/miRNA/mRNA triplet have been suggested as a possible mechanism of epigenetic regulation of gene expression. The aim of this work is to identify new lncRNAs potentially involved in the regulation of tumor-associated proteins mediated by 10 tumor-suppressive miRNAs: miR-124-3p, -125b-5p, -127-5p, -129-5p, -137, -148a-3p, -193a-5p, -203a, -339-3p, and -375, involved in metastasis of OC. We analyzed transcriptome profiles of OC samples from TCGA database and revealed 354 DE lncRNAs and 546 DE mRNAs, 45.6 and 48.4% of them, respectively, were upregulated. According to Spearman's coefficients it was 102 lncRNA-miRNA and 131 mRNA-miRNA pairs with negative and 67 lncRNA-mRNA pairs with positive correlation. After excluding RNAs with a prevalence of less than 70% of the samples, 10 lncRNAs, 13 mRNAs, and 5 miRNAs were selected for experimental analysis. The qRT-PCR was performed on a set of 46 paired (tumor/normal) OC samples. We have detected 16 triplets, 15 of which included miR-203a-3p. After checking for the presence of miRNA binding sites in lncRNA and mRNA sequences, the number of potential direct bindings was reduced. As a result, we selected 7 triplets associated with OC metastasis: OIP5-AS1/miR-203a-3p/c-Met, ZEB1, ZEB2; MLK7-AS1/miR-203a-3p/c-Met, ZEB1, ZEB2; MALAT1/miR-148a/BCL2. Therefore, we found shifted interactomes, which can be associated with metastasis of OC. This work was supported by the Russian Science Foundation, grant no. 20-15-00368.

P-01.1-035
PD-L1 protein expression profiling in a cohort of HIV positive and negative cervical cancer from South Africa

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Cervical cancer is a major disease burden in women globally. Despite advances in management, mortality remains high particularly in developing countries. HIV is associated with worse outcomes in cervical cancer. PD-L1 plays a significant role in the pathology of several cancers. Furthermore, therapies directed towards this pathway have shown promising results. This study investigates the differences in PDL1 expression in both HIV-positive and -negative cervical carcinoma using immunohistochemical methods. The PDL1 data was correlated with clinicopathological parameters Eighty FFPE tissue blocks were used in our study The H&E slides were reviewed by a pathologist and the selected tissue blocks were cut and stained using immunohistochemical methods with the DAKO clone 22C3 antibody. The PDL1 expression was done using the Combined Positive Score. The Combined Positive Score was >1, in 51.3% of cases (20 of 40 HIV negative cases plus 21 of 40 HIV positive cases). The HIV status was not significantly correlated with the expression of PDL1. In addition, the distribution of PD-L1 expression was heterogenous in the 80% of samples with a homogenous distribution in 2.5%. Survival analysis showed that different expression levels did not significantly affect survival in this cohort. We successfully demonstrated PDL1 expression in our study cohort. The PDLI expression was similar in both our study cohorts, with no significant difference in the two groups and the expression of PDL1 was not prognostic in our study. Furthermore, the pattern of PD-L1 expression was heterogenous in most of our cases. *The authors marked with an asterisk equally contributed to the work.

P-01.1-036
Validation and optimization of the next-generation cancer therapeutics by targeting Protein Kinase D2

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Protein kinase D2 (PKD2) exhibits a central role in cancer and is involved in processes such as proliferation, invasion, migration, tumour growth, tumour cell viability and tumour angiogenesis. PKD2 is a member of the protein kinase D (PKD) family, which consists of three isoforms (PKD1, PKD2 and PKD3). PKD2 is known for its pro-oncogenic effects, while PKD1 acts as a suppressor in cancer-related environments. However, these three kinases exhibit a high sequence and structural similarity, which complicates the rational development of PKD2-specific compounds. As the ATP-site of kinase is perfectly conserved, preventing selectivity, isoform-specific inhibition of PKD2 can only be achieved via an allosteric mechanism. We performed a structure-based virtual screen on a presumed allosteric site of PKD2 (interdomain interactions with lower sequence conservation). 28 compounds of the top scoring molecules were purchased and biologically evaluated, yielding 1 compound, specifically inhibiting PKD2 with an IC50 below 25 μM. Further optimization of this compound was performed by testing 80 analogues, yielding three more PKD2 specific inhibitors. Another 23 analogues were tested and yielded again one PKD2 specific compound. All compounds were tested *in vitro* in our radioactivity kinase assay, an ADP-Glo assay and an aggregation assay & *in cellulo* in a pSer298 activation assay, a migration assay, a proliferation assay in cells. The radioactive kinase activity assay is considered to be the gold standard of protein kinase assays. This assay requires the P81 phosphocellulose paper to bind phosphorylated substrates, but P81 paper production has been discontinued. However, our lab identified and validated LSA-50 paper as an alternative. In conclusion, a novel class of PKD2 specific compounds were identified by evaluating the structures *in vitro* and *in cellulo*. While the compounds are specific for PKD2, the potency is at the micromolar range and further optimisation is ongoing.

P-01.1-037
Vincristine- and cisplatin-induced multidrug-resistant neuroblastoma cells retain sensitivity to mitochondria targeting agents

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Drug resistance remains a major obstacle in treating pediatric solid tumors including neuroblastomas (NBL). The treatment itself often drives the development of resistance, promoting selection of cells with more aggressive phenotype via upregulation of drug pumps, enhanced self-renewal or mitochondrial dynamics. To elucidate the mechanisms of induced resistance, we mimicked the repeating cycles of chemotherapy regime *in vitro*. CHLA-15 cells derived from NBL of a patient at diagnosis were repeatedly exposed to standard chemotherapeutics, vincristine (VIN) and cisplatin (CIS). Pulses of single-drug treatment at IC_{50} for 72 hours were followed by incubation in fresh media. By gradually increasing the drug concentration in pulses, we successfully generated chemoresistant cells, CHLA-15res (VIN or CIS), which exhibited at least 3-fold higher resistance (IC_{50}) to the selected drugs. CHLA-15res cells were even less sensitive than CHLA-20 NBL cells that were derived from the relapsed tumor of the same patient after combinational therapy. Importantly, CHLA-15res cells showed cross-resistance to other structurally and functionally unrelated chemotherapeutics and targeted drugs while retained sensitivity to agents targeting mitochondrial functions, doxycycline, phenformin and mdivi-1. Although we detected upregulation of the P-glycoprotein (Pgp) pump in both CHLA-15res cells, notably in CHLA-15resVIN, this could not explain the mechanism of acquired cross-resistance to non-Pgp substrates like CIS. Surprisingly, in sphere formation analysis, only CHLA-15resCIS cells showed increased self-renewal capacity. Thus, the differences in stemness potential also pointed to other mechanisms of resistance, e.g., those involving mitochondrial dynamics or autophagy. These data provide a valuable insight to therapy resistance development and show mitochondrial drugs as promising candidates to target multidrug-resistant NBL cells. Supported by the Czech Science Foundation (No. GJ20-00987Y).

P-01.1-038
Inhibiting mitochondrial translation triggers integrated stress response accompanied by downregulation of MYC oncoproteins and induction of cell death in neuroblastoma

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Mitochondria play a central role in metabolism, cell death control and regulation of stem cell identity in both healthy and cancer cells. Several antibiotics, including tetracyclines, are known to inhibit mitochondrial translation, a process vital for maintaining mitochondrial integrity and function. MYC-driven cancers rely on enhanced mitochondrial biogenesis, which might make them vulnerable to such inhibition. Here, we utilized doxycycline (DOXY) to block mitochondrial ribosomes in the highly lethal childhood tumor, neuroblastoma (NBL). DOXY effects were first examined in paired NBL cell lines derived from the patient at diagnosis (CHLA-15) and at relapse with therapy resistant disease (CHLA-20). While CHLA-20 cells show broad resistance to anticancer drugs, both cell lines were highly sensitive to DOXY effects. DOXY-mediated inhibition of mitochondrial translation led to heavily fragmented/aberrant mitochondrial morphology, loss of mitochondrial membrane potential, excessive ROS production, impaired cell proliferation and efficient induction of cell death. These cytotoxic effects were validated using other mitochondrial ribosome-targeting antibiotics. Moreover, DOXY-pretreated cells formed significantly fewer neurospheres, suggesting an effect on NBL stem-like cells. Immunoblots of lysates from DOXY-treated cells identified a dose- and time-dependent downregulation of OMA1 (a surrogate for mitochondrial stress) and activation of the integrated stress response pathway, including eIF2α phosphorylation and CHOP upregulation. These changes were found consistently across a panel of seven NBL cell lines and were associated with marked downregulation of N-MYC and c-MYC proteins, the major high-risk NBL oncogenic drivers. Together, we identify mitochondrial translation as a promising therapeutic target in NBL. Supported by the Czech Science Foundation (No. GJ20-00987Y) and the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868).

P-01.1-039
A link between the p53 pathway and cancer stemness-associated transcription factors in childhood sarcomas: MDM2 positively correlates with c-MYC and KLF4

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Sarcomas belong to the most common tumors in children. Therapy resistance, aggressive tumor regrowth and metastasis are observed in up to 30% of sarcoma patients and have been attributed to the acquisition of cancer stemness. Sarcomas frequently carry mutations in the p53 pathway, which has been recently recognized as a crucial regulator of differentiation and stemness. Here, we therefore aimed to determine if aberrations in the p53 pathway associate with the expression of key stemness proteins in sarcoma. An extensive expression correlation analysis of stemness-associated transcription factors (SOX2, OCT4, NANOG, KLF4, c-MYC) and members of the p53 family (p53, p63, p73) including their negative regulators (MDM2, MDMX) was performed in a panel of 32 patient-derived and 4 established osteosarcoma, rhabdomyosarcoma and Ewing's sarcoma cell lines. Our data show that highly dysregulated expression of p53 (indicative of *TP53* mutations or deletions) correlates with SOX2 and OCT4 upregulation in all sarcoma subtypes, and also with

NANOG upregulation in case of Ewing's sarcoma cells. Interestingly, a previously unrecognized positive correlation between the expression of MDM2 and transcription factors c-MYC and KLF4 was identified across all three groups of sarcomas. While it must be established by mechanistic studies, this correlation suggests a potential mechanism of MDM2-mediated p53 degradation protecting sarcoma cells from the activation of the p53 pathway, which is commonly induced under high levels of c-MYC or KLF4. Overall, we provide strong evidence interlinking the expression of p53, MDM2 and stemness-associated factors in childhood sarcomas, which might have important implications for targeting stemness in sarcomas. Supported by the Ministry of Health of the Czech Republic (No. NU20J-07-00004), the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/ 0000868) and the Brno Ph.D. Talent Scholarship funded by the Brno City Municipality.

P-01.1-040
Untangling multi-target activities of benzylated dihydrochalcones as potent inhibitors of cancer cell growth

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Dihydrochalcones constitute a family of natural flavonoids that are well known for their anti-inflammatory, anti-bacterial and also anti-cancer properties. We investigated synthetic and semi-synthetic derivatives of MF-15, a benzylated dihydrochalcone found in *Melodorum fruticosum*, in an *in silico* profiling approach and discovered that this structural class possesses a wide range of bioactivities that also translate to *in vitro* anti-proliferative activity on different cancer cell lines, previously published in Mayr F. et al. (2020) Int. J Mol Sci 21, 7102 and Kafka M. et al. (2020) Cancers 12, 2092. The most potent compound in the series, BndDHC51, was shown to inhibit 17β-hydroxysteroid dehydrogenase type 5 (17β-HSD5) (91% at 10 μM) as well as the androgen receptor (AR). BndDHC51 also displayed a concentration-dependent inhibition of liver cancer cell proliferation (IC50 = 0.07 μM for Hep3B, IC50 = 0.55 μM for HUH7) showing a more than 10-fold higher activity than the parent compound MF-15 (IC50 = 6.4 μM for Hep3B, IC50 = 8.6 μM for HUH7). HEK293T cells were inhibited by BndDHC51 only with an IC50 of 12.8 μM, suggesting selective inhibition of the compound on malign cell growth. *In silico* analyses were conducted to elucidate the binding modes of the BndDHC51 scaffold to 17β-HSD5 as well as the AR DNA binding site. Furthermore, the different effects on these targets and the different cell lines were mapped out to elucidate the relation between the individual target effects and the inhibition of cell proliferation in cancer cells. The scaffold's impressive polypharmacological profile with the dual anti-androgenic effect, in combination with its previously shown anti-inflammatory properties render it an extraordinary lead structure from the class of dihydrochalcones. V.T. is funded by the Austrian Science Fund (FWF) project T942. A.C.P. and A.G. received funding from RFI Objectif Végétal (Région Pays de la Loire, France).

P-01.1-041
Free fatty acid receptor-dependent effect of short chain fatty acids (SCFAs) and long chain fatty acids (LCFAs) in colorectal cancer – an *in vitro* study

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Recent studies suggest the involvement of free fatty acids (FFAs) and FFA receptors (FFARs) in the pathophysiology of colorectal cancer (CRC). An increase in FFAR4 (activated by long chain fatty acids, LCFAs) expression and decrease in FFAR2 (activated by short chain fatty acids, SCFAs) expression are observed in patients with CRC. The aim of the study was to evaluate the effect of natural SCFAs (acetate (0.1–400 mM), butyrate (0.1–50 mM)) and LCFAs (stearate, palmitate (both 0.1–200 μM)) (alone or in combination) on cell viability in human colonic epithelial (CCD-841 CoN) and adenocarcinoma (HT-29 and SW-480) cell lines. Moreover, the effect of selected FFAR agonists on cell migration was investigated. Cell viability and migration were determined after 48h incubation with tested compounds using MTT assay and migration test, respectively. qPCR was applied to identify the changes in FFAR2 and FFAR4 expression. Acetate and butyrate (50 mM and 2.5 mM, respectively) notably decreased HT-29 and SW-480 cell viability as compared to CCD-841 CoN ($P < 0.001$). Similar effect was observed for LCFAs (25 μM) and the combination of SCFAs with LCFAs ($P < 0.01$). SCFAs and its combination with LCFAs increased the expression of FFAR2 in cancer cells ($P < 0.05$) as compared to control. SCFAs and SCFAs with LCFAs significantly increased FFAR4 expression in CCD-841 CoN and SW-480 cell lines as compared to control ($P < 0.001$). Acetate, LCFAs along with their combination and combination of SCFAs with LCFAs significantly decreased cell migration in SW-480 line ($P < 0.05$). To sum up, our study confirmed anti-tumor properties of natural FFAR2 agonists, which notably decreased adenocarcinoma cell viability. Noteworthy, the anti-tumor effect of SCFAs was linked to increased expression of FFAR2; LCFAs enhanced this effect too. However, SCFAs and SCFAs with LCFAs also significantly increased FFAR4 expression in human colonic epithelial cells suggesting that FFAs may have dual effect on tumor development.

P-01.1-042
Long noncoding RNA profile in neuroblastoma and glioblastoma

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Long noncoding RNA (lncRNA) expression profile is correlated with the malignant transformation process and plays an important role in the classification, diagnosis and prognosis of

malignant pathologies. Although neuroblastoma occurs in children, while their nervous system develops, and glioblastoma occurs in adults, both have largely unexplained origins. A comparison of their lncRNA profiles might help explain why neuroblastoma responds quite well to the specific treatment while glioblastoma is still one of the most aggressive types of tumors. For this purpose, HTB-14 (U87), HTB-11 and normal human astrocyte cell lines were grown according to their specifications and RNA was extracted using TRIzol Reagent. Further, a lncProfiler qPCR array kit was used, which includes a negative control and 5 housekeeping genes that allow quantification of lncRNAs in any cellular compartment (RNU43 and rRNA 18S for nucleoli, snRNA U6B for nucleus, GAPDH and lamin A/C for cytoplasm). The obtained cDNA was amplified using SYBR Green qPCR MasterMix and specific primers on a Real Time PCR System. Gene expression was evaluated using $2^{-\Delta\Delta Ct}$ method. Both tumor cell lines showed a similar expression profile, among them worth mentioning: BC200 with an expresion ranging from a 50-fold increase in U87 to 150 in HTB-11, this lncRNA being associated with increased resistance to treatment. RNCR3 has a 10 fold increase in both tumor lines, activating the Akt/GSK3-β signaling pathway. One key difference noted was in the case of H19 which has increased values in glioblastoma but is underexpressed in neuroblastoma, this lncRNA being correlated with the malignancy grade. The differences of lncRNAs expression profiles noted between cell lines could reveal distinct pathways of oncogenesis, therefore an evaluation of these potential biomarkers in patients is necessary. *The authors marked with an asterisk equally contributed to the work.

P-01.1-043
Application of unique enzymatically-isolated apple pectin to enhance anticancer activity of irinotecan towards colon cancer cells

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Colorectal cancer (CRC) is the second cause of cancer death worldwide. Because of the multifactorial character of cancer, searching for an effective therapeutic substance is oriented towards the so-called multifunctional drugs which at the same time affect several biological targets. Anticancer activity of various pectins has been already recognized for several years. Commercially available pectins are usually prepared from citrus peels or apple pomace by hot acid extraction. Pectins extracted using this method are, in comparison with the native molecule, depleted in RG I and RG II regions that are considered to be crucial for anticancer potency of pectins. Pectin studied in the present work (PX) was enzymatically extracted from apple pomace using endo-xylanase. This procedure protects native molecules, especially its neutral sugars that are not damaged during isolation. The aim of the study was to evaluate the mechanism of anticancer activity of enzymatically-isolated apple pectin as well as the potential of co-operation of PX with irinotecan (SN-38). Anticancer activity of SN-38, PX, and their combinations was studied in two colon cancer cell lines HCT116 and HT29. It was demonstrated that PX might be a promising candidate for an adjunct to irinotecan therapy. PX reduced the viability of colorectal cancer cells, induced apoptosis and autophagy. Moreover, PX synergistically enhanced the cytotoxic and proapoptotic effect with irinotecan. The pectin interaction with intestinal epithelial cells can occur through several mechanisms, i.e., interplay with galectin-3 strongly expressed by tumor cells and inhibitory effect on toll-like receptors The study is a part of the current trend to search for new compounds of anticancer activity, which assumes the strategy of hitting more than one molecular target simultaneously.

P-01.1-044
Anticancer ruthenium(III) complexes interfere with breast cancer (BC) iron homeostasis

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Breast cancer (BC) represents one of the most common types of cancer, and continues to be the second leading cause of cancer-associated mortality in women worldwide. Altered iron metabolism is considered an important hallmark of cancer and, in particular, of breast cancer. This metal is also involved in the regulation of diverse cell death pathways (ferroptosis, autophagy and apoptosis), though the possible molecular mechanism is still unknown. Thus, Fe can actively participate in cell death pathways triggered by non-canonical metallodrugs such as ruthenium agents. In this context, we have focused on the development of an original strategy for the *in vivo* delivery of a multi-target Ru (III)complex (AziRu), based on biocompatible self-assembling nucleolipid nanoaggregates. Throughout *in vitro* and *in vivo* preclinical studies, our Ru(III)-based agents have demonstrated to be very effective in counteracting growth and proliferation of human BC cells, including the highly aggressive triple-negative subtype, by the activation of diverse cell death pathways, previously published in: Piccolo M et al. (2021) Cancers 13, 5164;Ferraro MG et al. (2020) Cells 9, 1412. Since Ru and Fe share several chemical-physical properties and Fe is actively involved in the metabolic rewiring of the cancer cell, here we report the effects of ruthenotherapy on cellular iron homeostasis in BC phenotypes. Iron deregulations were correlated with the Ru-dependent activation of cell death pathways through immunoblot analysis and fluorescent microscopy. Moreover, investigations on the effects of a combination therapy consisting of Ru-based nanosystems and Fe modulators (such as chelators or donors) on tumor intake/growth were performed. Interestingly, our results reveal that ruthenium treatment can induce imbalance of iron cancer, causing cellular responses that deserve further consideration. Therefore, modulation of iron metabolism can significantly change responsiveness of cancer cells to chemotherapeutics.

P-01.1-045
Cytotoxic effect of novel Zn(II) niflumato complex with neocuproine on endometrial cell lines

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Non-steroidal anti-inflammatory drugs (NSAIDs) belong to the most commonly used therapeutics worldwide. Since numerous studies indicate that inflammatory processes play an important role in early stages and development of various types of cancer, they are currently studied from a completely new point of view. Based on our previous research experience in synthesis of metal complexes with NSAIDs and studies of their biological properties, a novel zinc(II) niflumato complex with neocuproine was designed and its effect on two epithelial cell lines (control hTERT and endometriotic 12Z) was investigated (Smolko et al. (2021) J Mol Struct 1237, 130312). In addition to the traditional cell cytotoxicity assay, a xCELLingence real-time cell analysis was also used to evaluate the cytotoxic potential on both cell lines. Obtained results revealed that the cytotoxic effect was more pronounced in the case of the 12Z cell line afflicted with inflammation. While one of the proposed mechanisms of action of the studied complex is interaction with nuclear genomic DNA with subsequent effect on gene expression, the DNA binding properties of the complex were investigated by spectroscopic techniques. The fluorescence experiments performed on DNA samples isolated from the studied cell lines suggest stronger binding of the complex towards DNA of 12Z cell line via intercalation that might explain its higher cytotoxicity. To confirm that the complex is able to bind to genomic DNA *in vitro*, the cell nuclei from the unaffected cells as well as from cells treated with the complex were separated from lysed cell samples by ultracentrifugation and analysed by UV-VIS spectroscopy. While a decrease in DNA absorption maximum indicating intercalation of the complex to the nucleic acid was observed for both cell lines treated with the complex, a more significant change was observed in the 12Z sample. Obtained results indirectly confirm that the studied complex selectively effects DNA of 12Z cell line.

P-01.1-046
Investigation of novel anticancer metal complexes based on non-steroidal anti-inflammatory drugs

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Recently, non-steroidal anti-inflammatory drugs (NSAIDs) are studied for their potential role in cancer prevention and treatment. Alongside conventional drugs, such as acetylsalicylic acid, profens and coxibs, metal complexes based on NSAIDs are also receiving growing interest in this research area. Although numerous studies indicate enhanced antiproliferative activity of metal complexes in comparison to their parent drug, the mechanism of their action remains mostly unclear. Our recent studies focused on the investigation of structure–activity relationship of cobalt (II) complexes with fenamic acid derivatives (flufenamic and niflumic acid) have shown that even slight differences in structure can result in different effects on various cancer cell lines. More thorough investigation of their cytotoxic activity has revealed that while the niflumato complex induced autophagy and apoptosis in prostate cancer cell line (PC-3), a similar effect was not observed in the case of analogous flufenamato complex [Previously published in: Smolko L et al. (2020) J Inorg Biochem 210, 111160]. As a continuation of our research, a series of complexes of bioactive metals (copper, zinc and cobalt) with diflunisal were designed and studied on different cancer cell lines; cervical (HeLa), breast (MCF-7), prostate (PC-3) and colorectal (HT-29) to explore the role of metal central atom on their cytotoxic mechanism on cellular level. The results obtained not only confirm that metal complexes indeed show greater potential than original drugs, but also provide a valuable insight into their mechanism of action. Slovak grant agency (VEGA 1/0148/19) is acknowledged for financial support for this work.

P-01.1-047
A new cathepsin D targeting system based on liposome-bound pepstatin A

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Cathepsin D is an aspartic protease, overexpressed in many cancers, where it plays an important role in tumor development, progression and metastasis. While physiologically an intracellular protein, it is excreted to the extracellular matrix in pathological conditions, making it an appropriate target for drug delivery systems. Here we present the development and evaluation of a new delivery system for tumor targeting, based on liposomes, functionalized with pepstatin A – a natural peptide inhibitor of cathepsin D. We exploited its high affinity for cathepsin D and used it as a targeting moiety on the surface of liposomes. We then confirmed the ability of the newly developed system for *in vitro* inhibition of cathepsin D. In a further step, we applied the pepstatin A-conjugated liposomes to several cathepsin D-expressing breast cancer cell lines and showed their binding to the cell surface, thus verifying their usefulness as a targeted drug delivery system.

P-01.1-048
PNPT1 is an unfavourable prognostic biomarker in lymphatic cancer

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Lymphoma is the most common type of blood cancer. The origin of lymphoid cancer has been related to defects in mitochondrial physiology. We focus on five genes involved in mitochondrial RNA transport, mitochondrial fission and fusion, and inner and outer membrane translocases, respectively *PNPT1, DNM1L* and *OPA1*, *TIMM23* and *TOMM20*. We have developed *in silico*

studies with the objective of considering them as potential diagnostic and prognostic biomarkers, as well as therapeutic targets. We used *The Molecular Complexes Detection* (MCODE) add-on of Cytoscape 3.7.2, using scoring criteria ≥ 3 and nodes ≥ 3. Function enrichment analysis of the same model was performed using the *STRING* tool, considering $P < 0.05$ as a cut-off criterion. Genes related to mitochondrial function in lymphoid cancer were selected by the *Cytohubba* plugin of *Cytoscape 3.7.2*, considering MCC ≥ 6. Expression levels were validated, by the *Ualcan* program. Overall survival in lymphoid cancer cases was studied using the *Kaplan-Meier* tracer database. *Odds ratios* with 95% confidence intervals were calculated. Our results suggest that *PNPT1* represents an unfavourable prognostic biomarker for lymphoid cancer. On the other hand, *TIMM23* and *DNM1L* do not show statistically significant values for lymphatic cancer. Finally, *TOMM20* and *OPA1* are favorable prognostic markers for lymphoid cancer. Therefore, our study concludes that *PNPT1* is an unfavourable marker in lymphatic cancer and could be a candidate target for this type of tumor.

P-01.1-049
Clinical significance of endogenous DNA modifications in acute leukemias

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Acute leukemias (AL) are characterized by genomic instability, which may arise from endogenous modifications of DNA nucleobases. The most significant sources are processes of active DNA demethylation and deamination, as well as oxidation by reactive oxygen species. We analyzed the levels of 5-methylcytosine and their oxidation products, 2'-deoxyuridine, 5-(hydroxymethyl)-2'-deoxyuridine, and 8-oxo-2'-deoxyguanosine in the minimally-invasive material: the cellular DNA from peripheral blood cells and urine of patients with acute myeloid and lymphoblastic leukemias (AML, ALL) and myelodysplastic syndromes (MDS), using isotope-dilution two-dimensional ultra-performance liquid chromatography with tandem mass spectrometry, as well as profiles of expression of proteins involved in their generation and removal. We found that the most diagnostically useful for discriminating AML and ALL patients from the control group were 5-hydroxymethylcytosine, and 5-(hydroxymethyl)-2′-deoxyuridine. The highest prognostic value of the analyzed parameters in predicting the transformation of MDS into AML was observed for 5-carboxyl-2'-deoxycytidine and 5-(hydroxymethyl)-2′-deoxyuridine (previously published in: Skalska-Budala A. et al. (2022) Cells 11, 888; Rozalski R. (2021) et al Sci Rep 11, 21345). Moreover, we noted significant correlations of the analyzed parameters with the severity of the disease. The AL patients also presented with the symptoms of oxidative stress and aberrant deamination of the DNA. The observed relationships were also associated with specific patterns of proteins expression which suggest that the reduction or loss of TET protein activity and/or deficiency of essential cofactors of the catalyzed reaction (e.g. vitamin C) may be a factor predisposing to the development of acute leukemias. This work was supported by Polish National Science Centre grant no. 2015/19/B/NZ5/02208 *The authors marked with an asterisk equally contributed to the work.

P-01.1-050
Vitamin C concentration inside both healthy cells and cancerous cells from prostate tissue

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For a long time, many reports have indicated the important role of ascorbic acid in the prevention and treatment of cancer. The anti-cancer effect of vitamin C is related to its strong antioxidant properties. Our studies, for the first time, analyze vitamin C level before and during the supplementation of ascorbate, not only in plasma, but also inside target organ – prostate. The methodology of determination of vitamin C concentration in plasma by UPLC-UV is well known. We developed a quick and simple procedure to determine the intracellular concentration of ascorbic acid by UPLC-MS/MS. The material comes from patients with prostate cancer (blood and tissue). Except determination of vitamin C in tissue homogenates, the important thing is also to define the amount of thymine by UPLC-UV method which helps us to estimate the quantity of cells in homogenates. In consequence, we may define intracellular concentration of ascorbic acid per cell. Preliminary data showed no significant difference ($P > 0.05$) in vitamin C concentration inside the cell between healthy and cancerous tissue. Correlation between intracellular and plasma concentration of ascorbic acid was also not observed. This study was supported by the National Science Centre, Poland (grant No. 2017/27/B/NZ7/01487).

P-01.1-051
The DNA methylation of LGALS3 gene as a prostate cancer biomarker

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The unmet challenge for clinicians in prostate cancer (PCa) management represents its differentiation from benign prostate hyperplasia (BPH) due to the lack of specific diagnostic biomarkers.

Contemporary research is directed towards cfDNA from liquid biopsies as potential PCa biomarkers, especially DNA methylation since it plays an important role in prostate cancer development. In the present work, CpG methylation of the *LGALS3* gene in cfDNA isolated from blood and seminal plasma of PCa and BPH patients was investigated using pyrosequencing; as well as DNA methylation of *LGALS3* in tumor tissue, surrounding non-tumor tissue, and BPH tissue. Liquid biopsy samples were taken from patients with elevated PSA levels before prostate biopsy, who were subsequently divided into two groups (42 with PCa and 55 with BPH) according to the histopathology report. Statistically significant higher cfDNA methylation in seminal plasma of BPH patients was found compared to cancer ones. Still, in both groups, low methylation frequency was found; the median in BPH patients was 4 %, and in PCa patients, 3 %. In tumor tissue, there was a statistically significant DNA hypermethylation of *LGALS3* compared to surrounding non-tumor tissue and BPH tissue. However, cfDNA from blood plasma did not reflect that change. In conclusion, DNA hypermethylation of the *LGALS3* gene represents an event specific for prostate cancer compared to surrounding non-tumor tissue and BPH tissue, but it does not reflect itself on cfDNA in blood and seminal plasma as liquid biopsy samples. Therefore, cfDNA methylation of *LGALS3* in blood and seminal plasma did not meet the supposed potential for differentiation between PCa and BPH; but DNA methylation of *LGALS3* in tissue could be used as a biomarker for PCa.

P-01.1-053
Curcuminoid-porphyrin agents as novel antimetastatic agents

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Designing optimal (neo)adjuvant therapy is a crucial aspect of the treatment of oncological diseases. Standard methods of chemotherapy, radiotherapy, and immunotherapy represent effective strategies for cancer treatment. However, in some cases with high metastatic activity and high levels of circulating tumour cells, the efficacy of standard treatment methods is insufficient and results in treatment failure and reduced patient survival. It is well known that the majority of deaths of oncology patients, are not caused by the primary tumour but by metastasis. Classical neoadjuvant therapeutic regimens aim to shrink tumours using drugs that target cancer cells (mainly cytostatic drugs) and only indirectly affect metastasis-initiating cells. Nevertheless, some polyphenol compounds such as curcuminoids display strong potential for the repression of metastatic spread by various independent mechanisms. Combination of their structural motif with anticancer agents that display high activity against cancer cells could lead to development of new type agents for the shrinking primary tumour and repression metastatic activity. Inspired by this idea, we prepared a series of porphyrin derivatives substituted by curcuminoid functional groups. Their biological studies showed their application led to decreased mobility of cancer cells and the anticancer efficiency of porphyrin was significantly improved by curcuminoid substitution. *The research was funded by the Ministry of Health of the Czech Republic (grant no. NU21-08-00407). The authors also thank Operational Programme Research, Development and Education, within the project Center for Tumor Ecology—Research of the Cancer Microenvironment Supporting Cancer Growth and Spread (reg. no.CZ.02.1.01/0.0/0.0/16_019/0000785).* *The authors marked with an asterisk equally contributed to the work.

P-01.1-054
Xanthohumol C and its derivatives – *in vitro* anticancer preliminary study against human and mouse cells

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The common hop (*Humulus lupulus*) – the main raw material in the brewing industry) – is a source of many biologically active compounds such as hop bitter acids (humulones and lupulones) and flavonoids. The most important chalcone found in hops is xanthohumol (XN), which plays an important role as a natural substance with antioxidant, antitumor and antimicrobial properties. It is accompanied by over a dozen prenylated chalcones present in much smaller amounts (from 10 to 100 times less than XN), which include desmethylxanthohumol, xanthogalenol, 4'-*O*-methylxanthohumol, 3'-geranylchalconaringenin, 3',5'-diprenylchalconaringenin, 5'-prenylxanthohumol, flavokawain, xanthohumol B, C, D and E, α,β-dihydroxanthohumol and isodehydrocycloxanthohumol hydrate. All the compounds have a free hydroxyl group at C-2', and so they may isomerize to flavanones. Xanthohumol is not currently approved as a drug by the US Food and Drug Administration (FDA), but is available as a dietary supplement and ingredient in medical foods. Actually there is also a clinical trial underway to investigate the safety of using xanthohumol by healthy people. In order to support further research on therapeutic use of plant extracts, the aim of our project was to obtain biologically active compounds naturally found in hops, their chemical derivatization and evaluation of anticancer activity using the human and mouse cell lines. To better understand the influence of xanthohumol C and its derivatives on the aggressive kind of triple-negative breast cancer (TNBC) we used cells that do not express the estrogen receptor (ER), progesterone receptor (PgR) or the gene for human epidermal growth factor receptor 2 (HER-2). The non-TNBC cell lines and non-tumorigenic cells were used as a control. Monika Stompor-Gorący acknowledges support from the National Science Centre NCN Poland, grant number 2020/39/D/NZ9/02023).

P-01.1-055
EGFR mutations and lung cancer: the era of precision medicine in oncology

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Activating mutations of the epidermal growth factor receptor gene (*EGFR*) are present in about 10-15% of Caucasian patients with advanced non-small-cell lung cancer (NSCLC). The aim of our study was to determine the frequency and type of *EGFR* mutations in the investigated population and to evaluate characteristics of patients with detected mutations. The study included

375 patients with lung cancer in the period from January 2018 to January 2019. Patient data were extracted from the patient record database of the Institute for Pulmonary Diseases of Vojvodina. DNA was isolated from 351 histological/cytological specimens using the cobas® DNA Sample Preparation Kit. Liquid biopsy-based circulating tumor DNA (ctDNA), an alternative source for patients with inadequate biopsy, was isolated from 24 K2EDTA blood plasma samples using the cobas® cfDNA Sample Preparation Kit. Real-time PCR analysis was based on the cobas® EGFR Mutation Test V2. Results: There were 151 (40.3%) female and 224 (59.7%) male patients. The median age was 64 (range 36-86). The majority of patients were active and former smokers; 209 (55.7%) and 97 (25.9%), respectively. There were 69 (18.4%) non-smokers. The most common type of lung cancer was adenocarcinoma, diagnosed in 358 (95.5%) patients. *EGFR* mutations were detected in 42 patients (11.2%), whereas the wild-type gene was detected in 333 patients (88.8%). The types of *EGFR* mutations were as follows: deletions in exon 19 (47.6%), exon 21 L858R point mutation (26.2%), exon 21 L861Q (2.4%), exon 18 G719X (2.4%) and insertions in exon 20 (21.4%). The highest incidence of *EGFR* mutations was observed in females as well as in non-smokers. The obtained differences were statistically significant ($P < 0.001$). *EGFR* mutations are predictive biomarkers of response to tyrosine kinase inhibitors (TKIs). Tumor genomic profiling using clinical samples has become increasingly important in the era of precision medicine in oncology. *The authors marked with an asterisk equally contributed to the work.

P-01.1-056
3D spheroid culture system of HCT116 colon and A549 lung cancer cells in the study of the cellular response induced by antitumor unsymmetrical bisacridines in these cells

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3D spheroid culture is a promising tool in cancer research, especially in drug development and testing. Here, the effects of antitumor unsymmetrical bisacridines (UAs) on 3D spheroids of HCT116 colon and A549 lung cancer cells were studied. The influence of four UAs: C-2028, C-2041, C-2045 and C-2053 on the morphology, size and viability of A549 cells was tested and compared with the results obtained for HCT116 cells [Kulesza J. et al. (2021) Molecules 26, 6262]. Furthermore, the spherogenicity of HCT116 and A549 cells after UAs treatment was evaluated, together with the ability of the cells to return to proliferation following UAs exposure. We showed that UAs treatment of A549 spheroids resulted in a gradual reduction of their size in the case of three tested compounds (C-2028, C-2045, C-2053) and after 14 days the spheroids were almost 20% smaller than on day 0. C-2041 and etoposide, did not cause the reduction of spheres below baseline sizes. Cell viability evaluated using the 7-AAD dye revealed that both HCT116- and A549- cultures showed a significant amount of non-viable cells after treatment with UAs and this fraction was generally higher in 2D than in 3D. Moreover, 14 days after exposure to C-2041, C-2045 and etoposide, 100% of A549 cells were able to form spheroids, while C-2028 and C-2053 completely inhibited the spherogenicity of these cells. In HCT116 cells, some degree of spherogenic potential was visible after incubation with all UAs and irinotecan, but 100% of spheres were formed only after C-2041 treatment. The colony formation assay showed that 72h exposure of A549 and HCT116 cells to UAs (except C-2041) resulted in a complete inhibition of their proliferation. Obtained results showed the importance of testing potential antitumor drugs in both 2D and 3D environments and proved that UAs exhibit anticancer properties in both culture models. Studies supported by the National Science Center, Poland, Grants No. 2016/23/B/NZ7/03324 and 2019/33/B/NZ7/02534.

P-01.1-057
Gamma-glutamyl transferase as a novel biomarker in prostate cancer

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Prostate cancer (PCa) is the second most common cancer worldwide. Several characteristics intrinsic to this type of tumour have hampered the discovery of accurate diagnostic and prognostic biomarkers. Gamma-glutamyl transferase (GGT) plays a notable role in glutathione and xenobiotics metabolism. This enzyme has gained support over the last years, due to its potential use as a biomarker in PCa. We obtained two types of samples from patients diagnosed with or without PCa: (i) histological sections of needle biopsies and (ii) isolated exosomes by ultracentrifugation and filtration from serum and urine samples. In this study, we analysed the prostatic expression of GGT by immunolabelling and measured its enzymatic activity in the exosomal fraction. The results showed a higher tissue expression of GGT enzyme in patients with PCa in comparison with patients without PCa. This outcome correlated with the enzymatic activity in exosomes derived from patients diagnosed with PCa. This would support the possible application of GGT as a biomarker for this disease. Research Group Cancers of Epithelial Origin Funding: Instituto de Salud Carlos III (ISCIII) "P118/00526", co-funded by European Regional Development Fund (ERDF), "A way to make Europe".

P-01.1-058
MiRNA expression clustering in carotid paragangliomas

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Paraganlioma represents a rare neuroendocrine neoplasm developing from chromaffin or glomus cells. Carotid paragangliomas (CPGLs) arise in carotid artery bifurcation, being the most common head and neck paragangliomas. These tumors are

characterized by variable potential to metastasize and require studying of their molecular phenotype to define mechanisms of tumor initiation and progression. In the study, we first performed miRNA sequencing and clustering of 60 CPGLs using the equipment at EIMB RAS "Genome" center. The miRNA counts were obtained using miRge3; ConsensusClusterPlus was used for unsupervised clustering resulting in six clusters (mi1–mi6). Differential expression analysis and enrichment of KEGG pathways led to the following description of the clusters. Cluster mi1 was characterized by increased expression of miRNAs involved in the Hippo signaling pathway, as well as alanine, aspartate, and glutamate metabolism. Expression of miR-7-5p, which participates in several tumor-associated pathways, was decreased in mi2. Differentially expressed miRNAs in mi3 were involved in RNA degradation, apoptosis, and transcriptional dysregulation in tumors. Mi4 was marked by downregulated expression of hsa-miR-375-3p involved in downregulation of protein kinase B signaling and angiogenesis, as well as upregulation of endothelial cell apoptosis. Cluster mi5 was characterized by reduced expression of miR-324-3p, which has been described as a tumor suppressor inhibiting tumor growth and metastasis. Cluster mi6 showed a significant decrease in miR-27a-5p, acting like tumor suppressor and promoter of metastasis in several tumors. Supervised clustering was performed for differentially expressed miRNAs between tumors with or without a mutation in the known driver genes for PGL; it did not result in a clear division of samples into clusters depending on the presence of mutations. This work was financially supported by the Russian Science Foundation, grant no. 21-14-00353. *The authors marked with an asterisk equally contributed to the work.

P-01.1-059
The ion channel transmembrane BAX inhibitor motif-containing 4 promotes glioblastoma cell invasion

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Glioblastoma multiforme (GBM) is the most frequent malignant primary brain tumour. The development of efficient therapies is hampered by drug targeting restrictions and high tumour heterogeneity. This works aims at exploring the impact of the transmembrane BAX inhibitor motif-containing (TMBIM) family of ion channels on GBM. Gene expression and patient survival analysis with TCGA (The Cancer Genome Atlas), the CGGA (Chinese Glioma Genome Atlas), and Ivy GAP (Ivy GBM Atlas Project) datasets revealed an expression dysregulation of several TMBIM genes in gliomas. TMBIM1, 4 and 6 are particularly upregulated in GBM, and increased expression of TMBIM1 and 4 is positively correlated with glioma grade and is associated with a significant reduction of low-grade glioma patient survival rates. Human glioblastoma cell lines (U87 and U251) were used as models to explore the effects of TMBIM expression dysregulation on GBM. TMBIM4 knock-down (KD) using specific siRNAs induced a strong inhibition of 2D individual cell migration and invasion, without affecting 2D collective migration, cell viability, intracellular ROS or cell attachment. Additionally, TMBIM4 KD lead to a robust reduction of U87 3D *in vitro* cell invasion and to a significant reduction of *in vivo* tumour invasion measured in a mouse orthotopic GBM mouse model. Ongoing studies aim at evaluating the impact of TMBIM4 expression dysregulation on currently available glioma therapeutic approaches, and at dissecting the molecular mechanisms underlying the effects of TMBIM4 on glioma cell invasion. Data obtained so far support further exploration of TMBIM1, 4 and 6 as potential markers for glioma progression, or possibly as new therapeutic targets. This work was supported by the FCT [grant numbers UIDB04567/2020 and UIDP/04567/2020 to CBIOS and PhD grant UI/BD/151424/2021 to M.M.].

P-01.1-060
Investigation of the effect of STAT3 inhibition on apoptotic process associated with JAK/STAT signaling pathway in A-498 and ACHN renal carcinoma cells

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Renal cell carcinoma (RCC) is a type of kidney cancer and one of the 10 most common cancers in the world. Activation of STAT3 is associated with the expression of oncogenic genes and has been observed in many malignant states such as escape from apoptosis, proliferation, and angiogenesis. The aim of this project was to investigate the potential role of atiprimod, a STAT3 inhibitor, on PI3K/AKT signaling and apoptosis in STAT3-dependent manner in ACHN and A-498 renal carcinoma cells. As a result, it was observed that atiprimod suppressed cell viability for 48h. Firstly, the inhibitive effect of selected doses of atiprimod (2 and 5 μM) for 48 h was determined by examining the expression of STAT3 and STAT5. The colony formation ability of each cell line was suppressed by atiprimod and the expression of both Akt and mTOR was reduced. Moreover, atiprimod-mediated inhibition of STAT3 induced downregulation of angiogenesis and uncontrolled proliferation of both ACHN and A-498 renal cancer cells, and cells were triggered to undergo apoptosis by increasing MMP loss following atiprimod treatment for 48h. As a conclusion, negative regulation of STAT3/5 by atiprimod which induced apoptotic cell death in A-498 and ACHN cells was an important therapeutic target in suppressing the survival of renal cancer cells. The results to be obtained provided important clues about the response mechanisms of different renal cancer cells to the treatments to be applied depending on the STAT3 levels. *The authors marked with an asterisk equally contributed to the work.

P-01.1-061
Targeting fatty acid metabolism-associated EMT signaling by palbociclib in combination with celastrol in pancreatic cancer cells

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Pancreatic cancer (PC) is the 7th leading cause of cancer-related deaths worldwide. Dysregulation of cyclin D-CDK4/6–Rb pathway leading to loss of critical Rb checkpoint has been observed in PC. Palbociclib, an inhibitor of CDK4/6, acts by blocking the Rb phosphorylation. However, the response of palbociclib (PD) it creates in the metastatic process and inhibition of fatty acid

(FA) metabolism differs between PC. Therefore, celastrol, which is a protease inhibitor and a regulator of lipid metabolism, was used in this project to investigate the effect of combinational treatment with PD on suppression of EMT through inhibiting FA metabolism in MiaPaCa-2, Panc-1 and CFPAC-1 PC cells. The co-treatment of celastrol and PD reduced cell viability more than the alone treatment in each PC cell line. Celastrol+PD had more significant effect on reduction of the FA metabolism in CFPAC-1 cells than Panc-1 and MiaPaCa-2 cells. The various response to drug treatment was further investigated by evaluation of EMT signaling. Celastrol+PD led to an important decrease in cell migration of MiaPaCa-2, Panc-1 and CFPAC-1 cells compared to PD alone treatment. β-catenin, which could not be inhibited by only PD treatment, was significantly suppressed by the combination of celastrol in MiaPaCa-2 cells. Furthermore, celastrol increased the apoptotic effect of PD, especially in CFPAC-1 cells. As a conclusion, although various genotyping differences of MiaPaCa-2, Panc-1 and CFPAC-1 have been demonstrated, celastrol enhanced the effect of PD to downregulate FA metabolism-related EMT and increase apoptotic cell death. * The authors marked with an asterisk equally contributed to the work.

P-01.1-062
Activation of modified cytidine-based prodrugs by bacterial amidohydrolases: application to gene-directed enzyme prodrug therapy

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Gene-directed enzyme prodrug therapy is an emerging strategy for cancer treatment based on the delivery of a gene that encodes an enzyme which is nontoxic per se, but is able to convert a prodrug into a potent cytotoxin. While the development of enzyme-activated prodrugs is promising, there are several limitations, such as the lack of suitable enzyme variants and the limited choice of chemical bonds that could be activated. Therefore, the aim of this study was to determine the ability of bacterial amidohydrolases YqfB and RL_D8 to activate prodrugs that would affect the viability of eukaryotic cancer cells. First, several human cancer cell lines, which stably express the genes encoding the YqfB or the RL_D8 enzymes, were generated by retroviral transduction. In parallel, a number of cytidine derivatives were selected *in vitro* as possible substrates of the YqfB and RL_D8 enzymes. These potential prodrugs were also tested *in vivo* for their possible toxicity before the activation. Next, the transduced cells expressing the bacterial amidohydrolases were exposed to several concentrations of the new prodrugs (in the range of 1 to 100 μM) and their viability was assessed using the MTT assay. Finally, the data obtained was processed and statistical analysis was performed. The results show significant decrease in the viability of cell lines expressing either the YqfB or the RL_D8 amidohydrolase, compared to the control cell lines transduced with a vector without a gene insert. These results imply that the bacterial enzymes used in this study, together with the cellular cytidine deaminase, can convert the nontoxic prodrugs to a well-known chemotherapeutic drug 5-fluorouridine in eukaryotic cancer cell lines. In conclusion, our results suggest that bacterial YqfB and RL_D8 amidohydrolases, together with the modified cytidine-based prodrugs, may serve as future enzyme-prodrug systems for gene-directed enzyme prodrug therapy.

P-01.1-063
7-Azaindole derivatives as candidates for anticancer therapy: synthesis and evaluation of the antiproliferative effect

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This study aimed to evaluate the anticancer properties of newly synthesised agents inspired by clinical candidates targeting ATR. To bypass the resistance to conventional chemotherapy, attention is paid to the inhibition of alternative targets such as members of the DNA damage response pathway. Among them, ataxia telangiectasia and Rad3 related kinase (ATR), the major replication stress responder, is one of the most attractive targets. Based on the structure of several ATR inhibitors in clinical testing, 40 novel compounds were prepared and evaluated on a panel of nine cancer and one non-cancer cell line. Two 7-azaindole derivatives (compound 10 and 22, at the concentration of 10 μM) proved a significant decrease in cell proliferation with partial selectivity towards cancer cells. The growth percentage dropped to 30% and 54% compared to control untreated cells after 48 hours of treatment. Compound 22, which expressed the most pronounced cytotoxic effect, was selected for detailed broad oncology panel screening containing 104 kinases that could be potentially targeted. Unexpectedly, compound 22 proved the highest inhibitory activity towards Flt4, cKit, TrkA and Aurora-A kinases. The biological activity of compounds 10 and 22 was additionally analysed in detail on selected cancer cell lines. Taking together, we showed that 7-azaindole derivatives could represent novel pharmacophores bearing anticancer activity. This study was supported by the InoMed project (Reg. No. CZ.02.1.01/0.0/0.0/18_069/0010046) co-funded by the European Union.

P-01.1-064
SDHB R230H mutation associated with malignant jugulotympanic paraganglioma

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Jugulotympanic paraganglioma (JTPGL) is a rare neoplasm that arises from the extra-adrenal paraganglia located in the middle ear or temporal bone region. JTPGLs are slow-growing tumors with predominantly non-aggressive behavior. Metastatic JTPGLs are diagnosed in 2% of cases, however, all tumors have the potential to metastasis. Genetic markers for metastatic paraganglioma have been poorly investigated. We study a case of a man who was diagnosed with left JTPGL at the age of 18 and underwent surgical removal of the tumor. At the age of 32, the patient was found to have distant metastasis to the liver. Histological

examination of biopsy confirmed the liver metastasis from JTPGL. Immunohistochemical (IHC) analysis of metastasis biopsy showed the absence of S100 expression in tumor cells with pronounced diffuse cytoplasmic expression of synaptophysin and chromogranin. IHC analysis of SDHB subunit expression was performed using monoclonal primary antibody [21A11AE7]. To estimate the mutation status of the SDHB gene, whole-exome sequencing of the patient's primary tumor, metastases, and blood was carried out on an Illumina NextSeq 500 System. IHC analysis revealed weak diffuse staining of the SDHB subunit in the metastasis indicating the deficiency of the SDH complex. According to the literature, negative or weak diffuse SDHB expression can be associated with a germline mutation in any of the SDHx genes. In the studied patient, we found germline SDHB variant NM_003000: c.G689A, p.R230H (chr1:17349179, rs587782604) described as pathogenic/likely pathogenic in ClinVar. Thus, we detected the SDHB germline variant associated with the change in the expression of the SDHB subunit and, possibly, having a high impact on the SDH complex. These results confirmed the pathogenicity of this variant. We also showed that the tumor malignancy was associated with SDHB mutation in a studied case. The study was funded by grant MK-5956.2021.1.4.

P-01.1-065
Examination of fatty acid metabolism and induction of epithelial-mesenchymal transition pathway via modulation of miR-33a levels in Caki-1 and Caki-2 renal cell

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Renal cell carcinoma (RCC) is the most common kidney cancer type. It affects over 400,000 individuals worldwide according to Global Cancer Statistics, 2020. Since existing therapeutics are limited in suppressing the disease development, new therapeutic approaches are needed. Fatty acid metabolism is known to influence the viability of cancer cells, also the regulation of epithelial-mesenchymal transition (EMT) and cell death pathways. microRNA (miRNA) is a single stranded RNA, that plays critical roles in regulation of gene expression. miR-33a is the major regulator of fatty acid metabolism and was shown to involve in tumor formation and progression when it is overexpressed. In RCC cells, while miR-33a overexpression supresses the proliferation of cells, miR-33a inhibitor promote cell growth and proliferation. In this study, it was aimed to investigate the role of miR-33a expression on fatty acid metabolism and EMT in renal cell carcinoma cells. Alterations in the expression of key molecules involved in fatty acid metabolism and EMT due to modulation of miR-33a expression, was demonstrated by immunoblotting. The effect of miR-33a modulation on cell survival and colony formation capability were also investigated. miR-33a was overexpressed and suppressed in Caki-1 and Caki-2 RCC cells by treatment with miR-33a mimic and miR-33a inhibitor, respectively. Expression profile of important players of fatty acid metabolism such as fatty acid synthase (FASN) and acetyl CoA carboxylase (ACC) was modified by miR-33a mimic and inhibitor treatment. Expression levels of mesenchymal markers N-cadherin and Vimentin was also regulated by miR-33a mimic and inhibitor. Effect of miR-33a levels on cell survival and colony formation capability was also screened for Caki-1 and Caki-2 cells. In conclusion, modulation of miR-33a levels in Caki-1 and Caki-2 renal cell carcinoma cells affects the fatty acid metabolism and epithelial-mesenchymal transition in addition to colony forming ability. *The authors marked with an asterisk equally contributed to the work.

P-01.1-066
Targeting lysyl oxidases for breast cancer therapy: bioinformatic analysis and evaluation of 4-thiazolidinone derivatives as LOXL2 inhibitors

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Lysyl oxidase (LOX) and LOXL 1-4 are amine oxidases, which catalyze the cross-linking of elastin and collagen in the extracellular matrix, promoting cell migration and formation of metastases. The inhibition of these enzymes, and particularly of LOXL2, has been proposed as a therapeutic strategy to prevent breast cancer metastasis. However, the importance of inhibitors selectivity is unclear. A bioinformatic-based approach was performed to explore the role of each LOX on breast cancer and its subtypes. The TCGA database, with the GEPIA 2.0 and the TIMER 2.0 platforms was used to characterize the expression profile of LOXs and its impact on breast cancer patient survival and on tumor infiltrates. Our results suggest that the therapeutical inhibition of LOXL1 and LOXL2 but not of LOXL4 may be beneficial in breast cancer. For LOXL2, a higher expression in breast cancer tissues when compared with normal tissues was found. The overexpression of LOXL2 has a negative impact on disease-free survival of basal and HER2+ subtypes. A high expression of LOXL2 was associated with increased cancer associated fibroblasts and with lower infiltration of B and T cells in all breast cancer subtypes. These results allow us to speculate that specific therapeutic inhibition LOXL2 may have advantages in breast cancer treatment. Additionally, docking studies were conducted using a small library of 4-thiazolidones derivatives to find novel putative LOXL2 inhibitors. The inhibitory activity of five selected compounds was evaluated. One of the compounds inhibited LOXL2, with an IC50 value in the low micromolar range. To identify compounds with most remarkable activity and to explore their potential therapeutic interest and specificity, further studies will be carried out. Funding: FCT (UIDB/04567/2020, UIDP/04567/20209 and UIDB/05380/2020 to SF), and U. Lusófona/ILIND program Fazer+ (ILIND/F+/EI/01/2020). *The authors marked with an asterisk equally contributed to the work.

P-01.1-067
Pharmacophore-based virtual screening for β-tubulin colchicine-site inhibitors

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Tubulin inhibitors have potential as antimitotic leads, gathering widespread research interest for cancer therapy applications. The

polymerization of α/β-tubulin plays a key role in mitosis. Thus, in rapidly dividing cancer cells, it offers an enticing target for drug screening programmes. Notably, inhibitors of natural and synthetic origin act as destabilizing agents and exert their effect by interaction at the β-tubulin colchicine-binding domain. In order to search for new tubulin inhibitors, pharmacophore-based virtual screening was employed, an established *in silico* drug discovery approach. To cover a large part of the active space, several pharmacophore models for tubulin inhibitors were developed with two different types of modelling software, LigandScout and Discovery studio. Models were optimised against a dataset of active inhibitors from literature (n = 100) and a corresponding decoy set (n = 4,901). The best performing models were used to virtually screen vendor databases of natural products (n = 736) and synthetic compounds (n = 208,761). Among predicted hits, candidates were prioritised for *in vitro* screening based on consensus overlap, fit values, and drug-like properties. For hit validation, a set of 46 purchased compounds were tested in a cell-free fluorescence based tubulin polymerisation assay (Cytoskeleton, inc). Compounds were screened at 30 μM and inhibition was observed across varying scaffold types. Thiadiazole, sulfonate, and benzodioxole derivatives showed activity within range of the colchicine control (3 μM). As a next step, a dose-response study will be performed on the best candidates and IC50 values determined. Furthermore, potent inhibitors will be tested in cancer cell lines to assess activity. The combination of *in silico* screening and *in vitro* validation provides us with a powerful tool to identify novel tubulin inhibitors from synthetic and natural origin. Project funded by EUREGIO (IPN 119) 'HERBAL'.

P-01.1-068
Transcriptional factors and genes expression regulation in response to alternating oxygenation conditions, *in vitro*

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Hypoxia-inducible factor-1 (HIF-1) is a key regulator of tumor cell hypoxia of several genes related to oxygen homeostasis in response to hypoxic stress. Carbonic anhydrase 9 (CA9) has been found to be a stable marker of acute or chronic hypoxia. *N*-myc downstream-regulated gene 1 (NDRG1) is a tumor suppressor with the potential to suppress metastasis, invasion, and migration of cancer cells. It is regulated under stress conditions such as starvation or hypoxia. NDRG1 regulation is both induced and controlled by HIF-1α-dependent and -independent pathways under hypoxic conditions. We aimed to define the time-dependent pattern of CA9 and NDRG1 mRNA and protein expression in human glioblastoma cell lines in extreme hypoxia and after re-oxygenation as well as under normoxic conditions. Here, the regulation of the transcription factors HIF-1α, SP1, CEBPα, YB-1, and Smad7 was examined in a time-dependent manner. The human malignant glioma cell lines U87-MG, and GaMG were cultured for 1, 6, and 24 h under hypoxic (0.1% O_2) conditions with following re-oxygenation. The mRNA expression of NDRG1, CA9, HIF-1α SP1, CEBPα, YB-1, and Smad7 was measured via semi-quantitative RT-PCR analysis. Protein expression was analyzed using western blotting. Long-term (24 h), but not short-term hypoxia led to the induction of NDRG1 expression in human glioma cell lines while CA9 was induced upon short- and long-time hypoxic oxygenation conditions. CA9 and NDRG1 expression was found to correlate with the protein expression of HIF-1α, SP1, CEBPα, YB-1, and Smad7. These transcriptional factors, each separately or in combination, may possess the potential to become important regulative molecules that can be targetted during antitumor targetting.

P-01.1-069
Herbicides and structurally relevant Schiff bases as human protoporphyrinogen oxidase inhibitors

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Protoporphyrinogen oxidase (PPO, EC 1.3.3.4) catalyses the oxygen-dependent six-electron oxidation of protoporphyrinogen IX to protoporphyrin IX. Relevant work found that PPOs are overexpressed in some cancer types (e.g. colorectal cancer). In addition, targeting PPO could be a promising way to enhance photodynamic activity based on the application of 5-aminolevulinic acid. Possible human PPO inhibitors could be based on the structural motif of herbicides targeting plant PPO. However, prospective strategies for the preparation of anticancer agents could combine additional structural motifs, such as Schiff bases, for their molecular design. Schiff bases possess a wide range of biological activities, including the repression of oncological processes and signalling pathways. Schiff bases bearing 2-hydroxyaryl moieties can bind arginine groups and thereby possibly target Arg-98 or Arg-59, which are key amino acids that maintain PPO activity. Therefore, beside commercial herbicides, we study two novel series as potential inhibitors: 2-hydroxybenzaldehyde-based on anticancer pharmacophores Schiff bases and chromanone derivatives. Some tested inhibitors display potent activity against human PPO, but prepared compounds did not display any significant effect on enzyme activity. Nevertheless, obtained compounds could be used to better prediction PPO herbicidal toxicity and improve the design of synthetic inhibitors. The research was funded by the Ministry of Health of the Czech Republic (grant no. NU21-08-00407). The authors also thank Operational Programme Research, Development and Education, within the project Center for Tumor Ecology—Research of the Cancer Microenvironment Supporting Cancer Growth and Spread (reg. no.CZ.02.1.01/0.0/0.0/16_019/0000785).

P-01.1-070
Novel synthetic inhibitors of Il-6R based on structure motif of pentamethinium salts

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Interleukin-6 (IL-6) and its receptor play important roles in the tumor development (e.g. development of malignancies, promotes

tumor growth, inhibits apoptosis and induces angiogenesis). Blocking IL-6 or inhibiting its signaling could be a promising therapeutic strategy in the treating cancer. At the present time, targeting IL-6 receptor by monoclonal antibodies is an intensively studied therapeutic strategy. Nevertheless, its limits (e.g. high cost, invasive route of administration, and high rate of immunogenicity) decrease its clinical usability. Therefore, development of inhibitors with low molecular weight is highly requested for their superiority in oral absorption, low toxicity, and low antigenicity. Despite the immense importance of this task and the realized efforts, known compounds are only few. An interesting structure motif could be represented by pentamethinium salts. A docking binding study and microscale thermophoresis assay showed that tested compounds displayed high affinity for IL-6R. Nevertheless, *in vitro* their effect on the STAT3 signalling was smaller, than in this case of tociluzumab. This effect was probably caused by fast mitochondrial localization of both tested compounds. However, obtained data strongly implies that bis polymethinium scaffold represents a promising structure motif for the inhibition of IL-6R. The research was funded by the Ministry of Health of the Czech Republic (grant no. NU21-08-00407). The authors also thank Operational Programme Research, Development and Education, within the project Center for Tumor Ecology—Research of the Cancer Microenvironment Supporting Cancer Growth and Spread (reg. no.CZ.02.1.01/0.0/0.0/16_019/0000785).

P-01.1-071
Long non-coding RNAs EPB41L4A-AS1 and SPINT1-AS1 are upregulated in ovarian cancer

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Ovarian cancer (OC) is one of the most lethal gynecological cancers in the world because of its late detection when the disease has already spread and, in combination with the arising of treatment resistance and relapse, OC is associated with poor patient prognosis. Long non-coding RNAs (lncRNAs) are transcripts longer than 200 nucleotides that regulate gene expression at several levels, playing a role in the pathogenesis of various diseases, including OC, correlating with important features for the clinical setting. RNA-seq data of unmatched ovarian serous cystadenocarcinoma and normal ovary tissues was obtained from The Cancer Genome Atlas and Genotype-Tissue Expression, respectively, both included in the database GEPIA. LncRNAs were manually identified in the top significantly dysregulated gene list and selected according to their fold change. The endogenous expression levels of the selected transcripts were measured by RT-qPCR in A2780, PEO1 and SKOV3 cancer and IOSE80T normal ovarian cell lines. Our analyses from the RNA-seq data show 2 non-coding transcripts EPB41L4A-AS1 and SPINT1-AS1, that are previously unrelated to OC according to the literature. Both of them are significantly upregulated in the three studied OC cell lines in comparison to the normal one. Besides, SPINT1-AS1 is upregulated in ovarian carcinoma tissues compared to normal ovary. Our results point that the two lncRNAs may play an oncogene role within ovarian cancer cells. This fact opens the possibility of using them as novel biomarkers for OC, with diagnostic and, presumably, therapeutic value.

P-01.1-072
Cloning and purification of the HYB domain of the E3 ubiquitin-ligase HAKAI, a promising therapeutic target for cancer

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HAKAI is an E3 ubiquitin-ligase that mediates ubiquitination of E-cadherin leading to its degradation, which in turns causes down-regulation of cell-to-cell contacts and induces epithelial-mesenchymal transition (EMT). Hakai induces cell proliferation, invasion, and metastasis in cancer [1]. The HYB domain was first described in the E3 ubiquitin-ligase HAKAI, as part of a homodimer coordinated by zinc [2]. The HYB domain is considered an atypical phosphotyrosine binding domain, as most proteins harbour archetypical SH2 or PTB domains. This domain is required for the specific interaction of HAKAI with its main known substrate, E-cadherin in a phosphotyrosine-dependent manner. Therefore, HAKAI, and specifically its HYB domain, is considered a promising therapeutic target for novel anti-cancer strategies. In order to study the *in vitro* behaviour of the HYB domain, a high amount of the pure homodimer is needed. In this work, we show the cloning of the sequence encoding the HYB domain of HAKAI. The expression system included a vector harbouring the GST protein tag. This tag allowed us to purify the expressed HYB fragment with affinity chromatography columns. An even higher degree of purification was achieved with a subsequent molecular exclusion chromatography. In summary, these results will allow us to further explore the potential of the HYB domain as a therapeutic target in cancer by providing a model to test the effect of different molecules in the interaction with specific HAKAI substrates. [1] A. Rodríguez-Alonso, A. Casas-Pais, D. Roca-Lema, B. Graña, G. Romay, and A. Figueroa, "Regulation of Epithelial–Mesenchymal Plasticity by the E3 Ubiquitin-Ligases in Cancer," Cancers, vol. 12, no. 11, p. 3093, Oct. 2020, doi: 10.3390/cancers12113093. [2] M. Mukherjee et al., "Structure of a novel phosphotyrosine-binding domain in Hakai that targets E-cadherin," The EMBO Journal, vol. 31, no. 5, pp. 1308–1319, Mar. 2012, doi: 10.1038/emboj.2011.496. *The authors marked with an asterisk equally contributed to the work.

P-01.1-073
Hydrazones bearing heterocyclic moiety as specific agents for killing cancer cells

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Cancer is a leading cause of death worldwide. Its treatment is currently complicated and demanding with several unpleasant side effects. The increasing resistance of cancer towards drugs is also problematic; for these reasons searching for new anticancer compound is still required. One of the most promising types of cytostatic agents is based on heterocyclic hydrazones. The mechanism of their anticancer action has been assumed to be based on the chelation of metal ions, inhibition of the enzymes responsible for the biosynthesis of RNA and DNA as well as other

biological processes, intercalation to DNA, disruption of the cellular metabolic processes or formation of the redox systems producing reactive oxygen species. It is expected a combination of the above-mentioned mechanisms. We designed and synthesized series of novel hydrazones derived from benzimidazole and imidazopyridine hydrazines. Target hydrazones are substituted with 2-hydroxyaryl- or 2-N-heteroaryl moieties. Anticancer activity evaluation demonstrated that several derivatives display significant anticancer activity and great selectivity toward cancer or leukaemia cell lines. The research was also funded by the project "Center for Tumor Ecology - Research of the Cancer Microenvironment Supporting Cancer Growth and Spread" (reg. No. CZ.02.1.01/0.0/0.0/16_019/0000785) supported by the Operational Programme Research, Development and Education. The research was also funded by the Ministry of Health, Czech Republic, grant no. NU21-08-00407

P-01.1-074
Endothelin axis in gallbladder cancer invasiveness

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Gallbladder cancer (GBC) is the most common and fatal biliary tract carcinoma with a 5-year survival rate ranging from 4% to 60%, depending mainly on the disease stage detection. Unfortunately, less than 10% of patients have resectable tumors and almost 50% of them have metastasis. In the search for new prognostic biomarkers, it has been shown that Endothelin-1 (ET1) signaling through ET_AR and/or ET_BR receptors promotes cell invasion and its related with poor prognosis in several cancer models. The aim of this work is to study the role of ET1-axis in GBC cells invasiveness. In this study, protein and mRNA levels were measured by western blot and RT-qPCR, respectively. ET1 levels and activation were measured by ELISA and fluorimetric assays, respectively. Cell invasion was measured by matrigel-coated transwell assay. Interestingly, results showed that ET_AR and ET_BR expressed in GBC cell lines and its protein levels were higher in cells with a more aggressive origin, suggesting that ET1-axis could be a suitable marker to predict GBC poor prognosis. Moreover, GBC cells under treatment with a non-selective ET1 receptor antagonist, Macitentan, decreased protein levels of ZEB1, a well-known invasion-related marker. Finally, the use of Macitentan also decreased the invasive capacity of a GBC cell line, suggesting that ET1-axis could be a suitable therapeutic target in order to impair GBC invasiveness. Despite the low incidence worldwide, these results are promising for countries where this cancer predominates.

P-01.1-075
Screening of histone modifying gene expression in prostate tumors

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Histone modifications (HM) are one of the major molecular characteristics of prostate cancer (PCa). They occur at both individual loci and genome-wide level at early carcinogenesis and accumulate during further cancer progression. Due to their multiple roles in cellular processes, epigenetic regulators have emerged as modulators of treatment response and, therefore, potential prognostic and predictive biomarkers of PCa. In the present study, expression of HM regulatory genes was screened using RT^2 Profiler PCR Arrays (Qiagen) and Custom TaqMan Array plates (Thermo Fisher Scientific) and further validated by means of single-assay RT-qPCR. In total, 123 PCa, 43 non-cancerous (NPT) and 16 benign prostatic hyperplasia (BPH) tissues were included in the analysis. The initial screening step revealed deregulation of various HM genes. Differential expression of lysine methyltransferases (*KMT5A*, *KMT1E*) and demethylases (*KDM5B*, *KDM5D*) was common between PCa and BPH. Histone deacetylase *HDAC1* was highly expressed in PCa and at low levels in both NPT and BPH (all $P < 0.05$). Among other HM gene groups, *ASH1L*, *DZIP3* and some others were downregulated in PCa as compared to NPT, whereas *ESCO2* was upregulated in tumors (all $P < 0.05$). Down-regulation of *KDM4C*, *KDM7B*, *KMT1E* and *MYSM1* correlated with biochemical disease progression. Single-gene assay experiments confirmed associations between *KMT5A* and *KDM5D* expression and sample histology (all $P < 0.05$). Besides, *KDM5D* expression levels were associated with the presence of *TMPRSS2-ERG* fusion transcript (both $P < 0.05$). Various associations were also detected between gene expression and patients' age, prostate-specific antigen level and prostate mass. In summary, the present study confirmed differential expression of various HM regulators in PCa, with histone methylation-associated genes being among the most commonly deregulated ones. This is in agreement with other studies indicating their potential as prognostic biomarkers for PCa.

P-01.1-076
Polyamine oxidase induction in lymphocytes of the brain glioma patients after phytohemagglutinin stimulation

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The mechanisms of growth and progression of malignant brain gliomas stimulation by tumour-associated inflammation (TAI) are not clear enough. Peripheral blood mononuclear cells (PBMC) participate in TAI. Polyamines (PA) are strongly important regulators of such processes as cell growth and apoptosis. Diamine oxidase (DAO) and polyamine oxidase (PAO) produce cytostatics. We performed mitogenic stimulation of PBMC from glioma patients with phytohemagglutinin (PHA), explored the DAO and PAO activities and the red blood cells aggregation. PHA was diluted within a concentration gradient. Cells were cultured according to standard technique. To evaluate the degree of RBC aggregation, the cellular fraction of heparinized venous blood was used to determine the shift in the minimum of the surface plasmon resonance (SPR) curve in degrees, representing the grade of blood cells aggregation. The activities of DAO and PAO were assayed spectrophotometrically. Statistical processing of the obtained data was carried out in the package "Statistica-7"

using nonparametric methods. We found that the DAO and PAO activities were increased in cultural liquid after PHA stimulation of lymphocytes in all groups except the group of spinal hernias. The PHA concentration-dependent decrease in proliferative activity of PMBC was mainly associated with decrease of SPR indices. The dynamics of the mentioned characteristics differed in various groups of patients. *The authors marked with an asterisk equally contributed to the work.

P-01.1-077
Antiproliferative activity of fluorescent and photosensitive colchicine derivatives in 2D and 3D models of triple-negative breast cancer

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Triple-negative breast cancer is an aggressive type of cancer, the effective treatment of which is constitutively being developed. In this research, the biological activity of multifunctional conjugates of anticancer compound colchicine with fluorescent 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) and photosensitive halogenated BODIPY was studied in 2D and 3D culture of triple-negative breast cancer epithelial cell line UFH-001. In both cell models, the conjugation of colchicine with BODIPY moieties decreased its undesirably high cytotoxicity, for which it cannot yet be widely used in cancer treatment. While the half-maximal inhibitory concentrations (IC_{50}) of colchicine and its corresponding derivatives without BODIPY ranged in UFH-001 monolayer in low nM, the IC_{50} of colchicine-BODIPY conjugates were in the range of hundreds to thousands of nM. After photoactivation, however, the cytotoxicity increased by 1-2 orders of magnitude. A similar effect was observed in 3D spheroids of UFH-001. The conjugates of colchicine and BODIPY caused the disintegration of spheroids after 72 h at concentrations of dozens of μM. This antiproliferative activity, together with favorable phototoxicity and fluorescent properties make the presented colchicine-BODIPY conjugates a promising tool to combat triple-negative breast cancer. This work was supported from the grant of Specific university research grant No. A1_FPBT_2022_001 and A2_FPBT_2022_049.

P-01.1-078
Exposure to the pro-inflammatory prostaglandin E2 disrupts E-cadherin/Caveolin-1-mediated tumor suppression to favor Caveolin-1 enhanced migration, invasion and metastasis of melanoma cells

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Caveolin-1 (CAV1) suppresses the function of proteins that favor tumor development, yet also promotes migration and metastasis of cancer cells. E-cadherin promotes CAV1-dependent tumor suppression and decreases lung metastasis of B16F10 murine melanoma cells in immunocompetent C57Bl/6 mice. Inflammation is considered a hallmark trait that promotes tumor development and metastasis. In this context, cyclooxygenase-2 and its product prostaglandin-E2 (PGE2) are viewed as important participants, but whether they could modulate E-cadherin/CAV1-dependent tumor suppression is not known. Here, we exposed cells to pro-inflammatory PGE2 and evaluated whether this altered CAV1-dependent behavior of cancer cells *in vitro* and *in vivo*. We used murine B16F10 and human A375 melanoma cells, with low levels of endogenous CAV1 and E-cadherin and first evaluated how co-expression of E-cadherin modulated CAV1 function and then how this was affected by PGE2. CAV1 expression augmented migration, invasion and metastasis of melanoma cells and these effects were abolished by transient co-expression of E-cadherin. Importantly, however, exposure to PGE2 reverted the inhibitory effects observed *in vitro* and *in vivo* following E-cadherin expression and increased CAV1 phosphorylation on tyrosine-14. We also observed that E-cadherin/CAV1 mediated suppression of subcutaneous tumor formation and CAV1-dependent metastasis of melanoma cells was prevented by PGE2 incubation. In summary, pro-inflammatory PGE2 overrides tumor suppression by the CAV1/E-cadherin complex. Liberated CAV1 is then phosphorylated on tyrosine-14 and promotes migration/invasion/metastasis. Therefore, these observations reveal how a pro-inflammatory environment, caused here by PGE2 exposure, can convert a potent tumor suppressor complex into a promoter of malignant cell behavior. Acknowledgments: FONDAP 15130011 (AFGQ), Fondecyt 1210644 (AFGQ), 1200836 (LL) and 1211223 (LLG); ANID PhD student fellowships (AC, NDV).

P-01.1-079
Evaluation of immunostimulatory effect of combination of herbal extracts and chemotherapeutic drugs by quantitative alteration of interleukin-2

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Clinical trials have shown that biologically active compounds isolated from certain herbs are effective in treating many types of cancer without noticeable side effects improving patients' life quality. Recently, cytokine immunotherapy has been an important type of cancer treatment and continues to be a key contributor to the current clinical cancer research. It has been shown that plant extracts or derivatives can bring to the induction of endogenous anti-cancer factors such as p53, IL-25, TNF-α. This study aims to elucidate the mechanism of antiproliferative effect of certain Armenian herbs and chemotherapeutic compounds in HeLa human cervical cancer cells via the estimation of IL-2 quantity changes. The plant materials were harvested from Tavush region of Armenia. Nor-NOHA, L-NAME, and 5-FU were used as chemotherapeutic drugs. To determine potential cytotoxicity (MTT test), cells were treated with ethanol extracts of different herbs, 5-FU, nor-NOHA, and L-NAME in different combinations. IL-2 was determined using an ELISA assay kit. The cytotoxic effects of about ten different plants were studied. The extracts of herbs with higher antiproliferative activity were selected to further elucidate their immunostimulatory properties. Our study shows that only the *Inula helenium* L. and *Hypericum alpestre* herbal extracts and the synergic effect in combination with nor-NOHA have an immunostimulatory effect in the HeLa cells. The combination with the aforementioned compound increases the IL-2 quantity by 2–2.5 times compared with the HeLa control cells. The rest of the combinations didn't change the quantity of IL-2 and were the same as in the control samples. This study reveals one of the immunostimulatory mechanisms of the anti-cancer effect of different herbal extracts in combination with chemotherapeutic compounds. Herbal extracts can be widely used in anti-cancer therapy, moreover in some cases in combination with traditional chemotherapy.

P-01.1-080
Simultaneous expression dysregulation of redox and calcium signalling-related genes: impact on breast cancer patient survival

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Calcium and reactive oxygen species (ROS) homeostasis are interdependent. Expression dysregulation of genes involved on Ca^{2+} and ROS homeostasis can modulate cancer cell proliferation, migration, and survival. Many of the mechanisms involved remain unclear and an integrated view does not yet exist. This is the case for breast cancer (BRCA), the most frequent cause of cancer death in women. A data mining approach using the TCGA database was adopted. Genes with statistically significant differences between the expression in normal and tumor tissues ($P < 0.05$), and with higher impact in survival rate (hazard ratio (HR) < 0.8 or > 1.2), were included in this analysis. The combined analysis of the expression dysregulation of a selection of genes involved in Ca^{2+} (TRPM8, CALM2, CAMK2G, ATP2C2, PLCD1, ORAI1, STIM1 and TMBIM4) and redox (LOXL2, LOXL3, PRDX4, TXN, TXNRD1, GLRX2, GLRX3, SOD2 and NOX4) signaling and homeostasis, on BRCA patient survival was analyzed. HR values at 5 years post-diagnosis were determined for all four expression quartiles of each gene withing the population that either expresses a high (quartile 75) or low level (quartile 25) of a second gene. When an increasing or decreasing trend in HR was obtained the gene pair was selected for further analysis. Our results show thar the simultaneous expression dysregulation of a reduced number of gene pairs had an impact on patient survival. These pairs include: GLRX3/LOXL3, PRDX4/LOXL2, NOX4/PRDX4, TRPM8/CAM2G, ATP2C2/TRPM8, CAMK2G/STIM1, ATP2C2/GLRX2, PLCD1/TXNRD1 and NOX4/TMBIM4. This approach may contribute to the identification of more robust breast cancer biomarkers of prognosis. Validation of these findings using *in vitro* models of breast cancer cell phenotypes associated with progression, will contribute to the dissection of the intricate molecular mechanisms involved in the control of Ca^{2+} and ROS in the context of breast cancer. Funding: FCT (grants UIDB04567/2020 and UIDP/04567/2020 to CBIOS).

P-01.1-081
Role of PPM1D/WIP1 in regulation of basal and genotoxic stress-induced autophagy

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PPM1D/WIP1 is a Ser/Thr phosphatase activated upon genotoxic stress in a p53 dependent manner and involved in regulation of DNA damage response (DDR). Wip1 is amplified and over expressed in common human cancers and thus exerts oncogenic functions. Oncogenic Wip1 negatively regulates cellular responses to chemotherapy such as apoptosis and senescence. Here, we aimed to investigate the role of oncogenic Wip1 in induction of basal autophagy and chemotherapy-mediated autophagy in breast cancer cells. Etoposide was used to induce autophagy and chloroquin (CQ) for inhibition. GSK2830371 was used to inhibit Wip1. LC3I/II conversion P62 degradation, total and phosphorylated (Ulk1 p-Ser757) levels were by analysed WB. Wip1-Ulk1 interaction was demonstrated by co-IP analysis. LC3 puncta formation was analysed by and immunofluorescence staining. Apoptosis, cell cycle, and senescence were measured by AnnexinV/7AAD, BrdU/PI analysis and SAβ-gal staining, respectively. Colony formation assay was used to assess cells' ability to colonize. Utilizing Wip1 over-expressing MCF-7 cells, we showed that oncogenic Wip1 increased basal autophagy and mediated etoposide-induced autophagy as confirmed by accumulation of LC3-II, degradation of p62, and formation of LC3II puncta. Inhibition of Wip1 increased phosphorylation of Ulk1 from Ser757 both in basal and etoposide-induced autophagy. Further, endogenous Wip1 co-precipitated with Wip1 during

basal and etoposide-induced autophagy. Inhibition of autophagy or Wip1 enhanced apoptosis in response to etoposide but not senescence. In conclusion, our data suggest that oncogenic Wip1 may increase basal autophagy to support the survival of cancer cells. In addition Wip1-dependent autophagy in response to chemotherapy agents may cause resistance to chemotherapy. Thus targeting of Wip1-Ulk1 may lead enhancement of the chemotherapy response as a promising therapeutic strategy. This work is supported by TUBITAK Gr.N . 119S135.

P-01.1-082
Chromosomal aberrations and homologous recombination DNA repair activity in response to olaparib combined with inhibitors of ATR/CHK1 pathway in ovarian cancer cells sensitive and resistant to PARPi

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Treatment of ovarian cancer (OC) remains challenging despite recent advances in therapeutic approaches employing poly(ADP-ribose) polymerase inhibitors (PARPi). Olaparib, a first-in-class PARPi that induces DNA damage, has proven to be effective in the treatment of OC, however emerging resistance limits achieving a sustained response. Recent reports showed that combinations of olaparib with inhibitors of ATR/CHK1 pathway facilitate combating resistance to PARPi. We investigated genotoxic effects induced by olaparib, ATR inhibitor (ATRi, ceralasertib), and CHK1 inhibitor (CHK1i, MK-8776) alone or in combination in OC cell lines sensitive (PEO1) and resistant (PEO1-OR) to olaparib. We evaluated the expression level of γH2AX histone as a DNA double-strand (DSB) biomarker and RAD51 protein as an indicative of homologous recombination (HR) repair activity. The formation of γH2AX and RAD51 foci at damaged sites was assessed by immunofluorescence co-staining. Metaphase chromosomes were analyzed for structural abnormalities. Each drug alone or in combination increased γH2AX in PEO1 cell line, indicating augmented generation of DSB in olaparib-sensitive OC cells. Interestingly, RAD51 was overexpressed in PEO1-OR cells in response to olaparib, conferring resistance to PARPi. PEO1-OR cells were less susceptible to chromosomal aberrations caused by olaparib, ATRi, and CHK1i in comparison with a olaparib-sensitive cell line. The studies showed that combination therapy induced the formation of DSB and chromosomal aberrations in PARPi-sensitive cells. PEO1-OR cells resistant to olaparib exhibited significantly decreased susceptibility to genotoxic effects induced by tested compounds due to enhanced RAD51-mediated DNA repair. The combination of olaparib with either CHK1i or ATRi represents a promising perspective in overcoming PARPi resistance in OC. *This research was funded by the Polish National Science Centre (Project grant number: Sonata Bis 2019/34/E/NZ7/00056).*

P-01.1-083
Bromodomain inhibitor I-CBP112 breaks multidrug resistance conferred by overexpression of ABC transporters in cisplatin-resistant triple negative breast cancer

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Multidrug resistance (MDR) is a common phenomenon that develops in response to chemotherapy. This process desensitizes cancer cells to cytostatic drugs by increasing the expression of inter alia genes encoding ABC (ATP-binding cassette) transporters, which remove drugs outside the cell. In our recent study, I-CBP112, a bromodomain inhibitor, emerged as a potent silencer of *ABCC* gene transcription by enhancing LSD1 recruitment to chromatin in triple-negative breast cancer cells. In the follow up project, we aimed to check if I-CBP112 can be considered as anticancer agent in cisplatin-resistant cells derived from MDA-MB-231 cell line. Cisplatin-resistant cells responded similarly to basal MDA-MB-231 cell line with *ABCB1*, *ABCC1*, *ABCC2*, *ABCC3*, *ABCC5*, *ABCCG2* repression at the mRNA and protein level following I-CBP112 administration. As a consequence, higher autofluorescence of anthracyclines was observed inside cells pre-treated with bromodomain inhibitor. The increased drug accumulation led to increased drug toxicity, that was revealed after measuring resorufin fluorescence intensity and Caspase-3 activity. Bromodomain inhibitor arrested cell cycle progression before they entered the M phase. Importantly, I-CBP was found non-toxic to human blood-derived monocytes and lymphocytes, neither increased drug toxicity in the culture of these cells and hepatocytes, nor caused red blood cell hemolysis. Concluding, I-CBP112 may be considered as a promising component of anticancer therapy towards cancer featured by multidrug resistance due to its ability to repress expression of genes encoding ABC transporters. Our data from MDA-MB-231 cell model suggests the possible use of I-CBP112 in the combination with standard chemotherapy that is often applied to woman diagnosed with triple-negative breast cancer. Grant: LIDER/22/0122/L-10/18/NCBR/2019.

P-01.1-084
The importance of PFK-II isoenzymes (PFKFB3 and PFKFB4) in BRAFi-resistant and -sensitive melanoma cells

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Even though the treatment targeting mutated BRAF – the most common molecular disorder observed in melanoma patients – has been implemented for several years, the emerging resistance

to applied inhibitors is the main obstacle of its effectiveness. In this context, recent data showing that glycolysis inhibition can induce cell death in BRAF inhibitor (BRAFi) resistant cells provide a strong rationale to search for effective and specific treatment targeting glycolytic pathway in melanoma cells. Of note, it has been recently reported that glycolytic pathway in cancer cells can be targeted by inhibition of cancer specific isoenzymes (PFKFB3/PFKFB4) of phosphofructokinase II (PFK-II). What is more, numerous publications indicate a correlation between these isoenzymes and tumor aggressiveness, suggesting their importance in the process of carcinogenesis. Thus, the research hypothesis assumes that cancer specific isoenzymes of PFK-II (PFKFB3/4), may constitute the novel target for anti-melanoma therapy affecting both, growth and BRAFi resistance of melanoma cells. In the first step, the expression of PFKFB3/4 was examined in both wild-type and resistant melanoma cell lines, using RT-qPCR and western-blot to confirm their presence in BRAFi-resistant and -sensitive melanoma cells. Then, the cells were exposed to specific PFKFB3 and PFKFB4 inhibitors and the functional analysis, including proliferation, migration, survival, apoptosis, invasiveness and metabolic activity, was performed. The significant response of chosen cell lines confirmed the specificity of inhibitor applied in our study. Of note, we observed the dose dependent response for PFKFB3 inhibitor, whereas the cells exhibit slightly lower sensitivity to the PFKFB4 inhibitor. Our study suggests that currently available anti-melanoma therapeutic strategies may significantly benefit from agents targeting PFKFB3 and PFKFB4 activity.

P-01.1-085
Gadolinium doped TiO_2 nanoparticles for cancer therapy: experimental and numerical study of cytotoxicity and radiation dose enhancement

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Pancreatic cancer is the 7th leading cause of cancer-related deaths, with one of the lowest 5-year survival rates due to its poor early-stage prognosis. We investigate the potential of Gd-doped TiO_2 nanoparticles (Gd-TiO_2 NPs) for pancreatic tumor treatment with irradiation. The treatments with NPs and X-rays/blue light may promote the creation of reactive oxygen species, leading to the arrest of cell proliferation and cell death. Moreover, combined use of Gd-TiO_2 with X-rays can lead to dose enhancement effect (DEE). Theoretical simulation using Geant4 and cell viability assays using two pancreatic ductal adenocarcinoma cell lines (Panc-1 and MIA PaCa-2) were performed. Gd-TiO_2 NPs were synthesized by hydrothermal method and characterized with X-ray diffraction (XRD), Transmission electron microscopy (TEM), Energy Dispersive X-Ray Analyzer (EDX) and UV-VIS spectroscopy. XRD results showed the anatase phase of the TiO_2 crystals, TEM indicated NP size ranging from 5-8 nm, and EDX analysis validated the presence of Gd^{3+} ions incorporated in TiO_2 crystal lattice. UV-Vis spectra confirmed that doping TiO_2 with Gd results in a reduced bandgap width compared to pure TiO_2 crystals, allowing for broader excitation of NPs. Gd-TiO_2 exhibited good biocompatibility in the dark. Thus, prior to testing the X-ray/photo-induced cytotoxicity, we examined numerically the potential of the system to induce DEE on the irradiated monolayer. We simulated experimental set-up of one well in a 96-well plate, with a cell monolayer immersed in water filled with Gd-TiO_2 and irradiated with 50 keV X-rays, corresponding to the K-edge of Gd. Energy deposited in cells was compared in a system with and without NPs. The preliminary results showed potential for dose enhancement, presumably due to contribution from Auger and photo-electrons created in the interaction of X-rays with NPs, which imply the potential of Gd-TiO_2 NPs to be used in both photodynamic and radiotherapy of pancreatic cancer.

P-01.1-086
Identification of mesenchymal stem cell subpopulations from bone marrow adipose tissue of Fanconi anemia and acute myeloid leukemia patients

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Mesenchymal stem cells (MSCs) are important components of bone marrow homeostasis. Studies showed that bone marrow stromal cells, particularly bone marrow adipose tissue (BMAT) and its dysregulation is highly related to leukemia pathogenesis. We hypothesized that exposure to xenobiotics and chronic inflammatory environment may lead to dysregulation of BMAT and enhanced lipolysis. This fatty acid-rich bone marrow niche may support leukemia rather than the physiological regulators. MSCs are a heterogeneous population and sub-populations may be involved in pathophysiology of aplastic anemia, myelodysplastic syndrome, and myelofibrosis. In the present study MSCs and BMAT were isolated from bone marrow aspirates of Fanconi anemia (FA) and acute myeloid leukemia (AML) patients and healthy bone marrow transplant donors. FA is characterized by xenobiotic sensitivity and AML predisposition, and may contribute to identification of these population-based shifts. Besides typical MSCs cell surface markers (CD105, CD73, CD90), we tried to discriminate these sub-populations based on positivity of LEPR, CD140a, Nestin, CD271, FABP4. In addition, we performed time-course analysis by adipogenic differentiation markers and determined mRNA expression of PPAR-Gamma, PRDM16, UCP1, PLIN1, LPL1 and ATGL genes. In addition, metabolome analyses were performed from cell culture supernatants to determine lipid profile. BMAT is a hot topic subject for cancer and leukemia pathogenesis. This study aims to shed light on identifying these BMAT-MSCs and present the disease-specific alterations for leukemic niche concepts.

P-01.1-087
Effect of desethylamiodarone on mitochondrial mechanisms and cyclooxygenase-2 in MCF-7 and 4T1 breast cancer cell lines

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Breast cancer (BC) is the most frequent cancer type in women and the second primary cause of cancer-related death worldwide. The triple-negative (TN) form of the disease represents about 10–20% of all BC cases. Novel compounds significantly interfering with mitochondrial energy production may have therapeutic value in triple-negative breast cancer (TNBC). This criterion is clearly fulfilled by desethylamiodarone (DEA), which is a major metabolite of amiodarone, a widely used antiarrhythmic drug, since the DEA previously demonstrated anti-neoplastic, anti-metastasizing, and direct mitochondrial effects in B16F10 melanoma cells. Additionally, the more than fifty years of clinical experience with amiodarone should answer most of the safety concerns about DEA. Accordingly, in the present study, we investigated DEA's potential in TNBC by using a TN and a hormone receptor-positive (HR+) BC cell line. DEA reduced the viability, and invasive growth of the 4T1 cell line and led to a higher extent of the MCF-7 cell line. DEA increased calcium influx, lowered mitochondrial transmembrane potential, and induced mitochondrial fragmentation. On the other hand, DEA failed to significantly affect various parameters of the cellular energy metabolism as determined by a Seahorse live-cell respirometer. Cyclooxygenase 2 (COX-2), which was upregulated by DEA in the TNBC cell line only, accounted for most of 4T1's DEA resistance, which was counteracted by the selective COX-2 inhibitor celecoxib. All these data indicate that DEA may have potentiality in the therapy of TNBC.

P-01.1-088
The APC gene: DNA methylation and protein expression in prostate cancer

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Prostate cancer (PCa) represents a malignancy with high incidence and prevalence rates which are expected to rise further in the following years. In the search for new biomarkers, DNA methylation has been recognized as a key event in PCa development and progression. Therefore, in the present research, DNA methylation of the *APC* gene in liquid biopsies and tumor tissue of PCa patients, as well as its expression on protein level were investigated. Liquid biopsy samples (blood and ejaculate) and prostate tissue samples were taken from 42 patients with early-stage PCa and 55 with benign prostate hyperplasia (BPH). The degree and pattern of DNA methylation were investigated using pyrosequencing, while protein expression of *APC* was analyzed using immunohistochemistry. In the blood and seminal plasma of prostate cancer patients compared to BPH patients, there was no significant difference in cfDNA methylation of the *APC* gene. Moreover, literature data suggesting DNA hypermethylation in tumor tissue compared to surrounding healthy tissue or BPH tissue have not been confirmed. Analysis of *APC* protein expression showed that *APC* has higher expression in tumor epithelia than epithelia of surrounding healthy tissue or BPH tissue. In tumor stroma, *APC* had lower expression compared to the stroma of surrounding healthy tissue or BPH tissue. Based on these results, cfDNA methylation of APC does not have the potential as a biomarker for prostate cancer and its differentiation from BPH. Changes in *APC* expression on protein level have potential as prostate cancer biomarkers for immunohistochemistry purpose since their expression in tumor epithelium and stroma differs from surrounding healthy and BPH tissue.

P-01.1-089
Tissue transglutaminase as a potential therapeutic target in ovarian cancer tumor microenvironment

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Tissue transglutaminase (TG2) is a multifunctional enzyme that was found overexpressed in ovarian cancer and linked to metastasis and chemo- and radiotherapy resistance. TG2 function in cancer cells has been generally correlated to enhanced tumor progression. However, its functions in the host tissues were less investigated. We assessed the role of TG2 in the host by using a TG2KO syngeneic ovarian cancer mouse model. Decreased tumor burden and increased survival was observed in TG2KO mice upon i.p. injection of ID8 mouse ovarian cancer cells. Cell subsets in the peritoneum were immunophenotyped using FACS. Lack of TG2 allowed increased infiltration of $CD8^+$ T cells, while myeloid cells were found in decreased numbers in the peritoneal ascites. TG2 loss increased $CD8^+$ T cell activation and differentiation towards an effector phenotype by attenuating STAT3 phosphorylation. Cancer cells retrieved from the ascites of TG2KO mice showed an IFN-γ-responsive gene signature and were more

prone to apoptosis. In summary, these results demonstrate decreased tumor progression and increased T cell activation in the absence of TG2 in the host (previously published in: Sima LE et al. (2021) Journal for ImmunoTherapy of Cancer 9, e002682). In human OC tumor microarrays (TMAs) analyzed by image cytometry, an inverse correlation was seen between human stromal (but not tumor) TG2 expression and $CD8^+$ T cells presence. Besides expression in cancer cells, TG2 was also identified in a cancer associated fibroblast (CAF) subset and in cells surrounding the blood vessels, upon multiplex immunohistochemistry (mIHC) analysis. We currently aim to evaluate the impact of a TG2-directed therapy on the cross-talk between tumor microenvironment cells and on the tumor phenotype, as we propose TG2 as a new immunomodulatory target. This research was supported by funding from the US Department of Veterans Affairs, Robert H Lurie Comprehensive Cancer Center, and UEFISCDI (PN-III- P1-1.1-TE- 2019-0670).

P-01.1-090
Branched peptides for therapy of brain metastatic cancer

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Triple-negative breast cancer (TNBC) represents 10–20% of all breast cancer (BC) and is characterized by the absence of known targeted receptors (estrogen, progesterone, and human epidermal growth factor receptor-2). It presents an aggressive phenotype, a lower 5-year overall survival, a high number of relapses, and a high metastization rate, mainly into the brain. The use of chemotherapy remains the only therapeutic option, although its efficacy is poor. Recently, anticancer peptides (ACPs) have gathered hope as a potential cancer treatment due to their potency, low toxicity, and no drug resistance development. In our lab, we have studied a promising ACP, selective and active towards TNBC cell lines, and a blood–brain barrier (BBB) peptide shuttle (BBBps), which penetrates the brain. In a recent study, we conjugated both peptides, due to the inability of ACP to cross the BBB. The dual-active peptide synthesized demonstrated equal anticancer activity with the same low toxicity, and good translocation capacity. To further develop multipurpose peptides able to BBB translocation and improved TNBC elimination, we designed two new branched peptides bearing different domains. For drug testing purposes, 3D *in vitro* co-culture models are essential for being more representative and reliable than 2D models. Here, we addressed the need for innovative tumorigenic 3D co-cultures to study BM's drug treatments. We characterized and tested these newly designed peptides using 2D models, TNBC spheroids, and our 3D *in vitro* co-culture model showing increased efficacy to hinder BM while maintaining BBB integrity in comparison to our reference peptides. Our results demonstrated higher efficacy of these new branched peptides with manageable toxicities. The translocation capacity was also observed, which makes these new molecules potential candidates for the elimination of TNBC primary tumor in the breast and secondary tumor in the brain.

P-01.1-091
Characterization of new non-invasive biomarkers of prostate cancer by Raman spectroscopy

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We have used surface-enhanced Raman spectroscopy (SERS) as the basis for the development of a rapid, non-invasive and low-cost diagnostic system transferable to clinical use. Raman results suggest significant compositional changes in the urine of prostate cancer (PCa) patients. The parallel metabolomic study by GC/MS showed that 5-methylthioadenosine (5-MTA), sarcosine and adenosine values were altered in urine from PCa patients. The SERS spectra showed an enhanced sensitivity to nitrogenous bases and nucleosides, being insensitive to sarcosine variations. Thus, altered concentrations of adenosine and 5-MTA could present potential as PCa biomarkers, as it correlates with Raman response. The urine Raman spectra and chemometric analyses as PCA (Principal Component Analysis) and LDA (Linear Discriminant Analysis) allow to classify samples according to its pathological condition (healthy vs. PCa and other prostate conditions, including benign prostate hyperplasia and ASAP). The resulting trained model based on Raman-SERS analysis is a valuable tool for the early, non-invasive fast screening of patients. Funding: - Instituto de Salud Carlos III (ISCIII) "P118/00526" cofunded by European Regional Development Fund (ERDF), "A way to make Europe". - Universidad de Alcala (UAH) "CCG20/CCS-059". *The authors marked with an asterisk equally contributed to the work.

P-01.1-092
Cinnamic acid derivatives as chemosensitizers that modulate activity of anthracyclines antibiotic

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Doxorubicin (DOX) is classified by World Health Organization (WHO) as an essential medicine for cancer. However, its clinical application is limited due to resistance development and cardiotoxicity. Recently, inhibition of carbonyl reduction of anthracyclines (ANTs), catalyzed by enzymes from carbonyl reductase (CBR) and aldo-keto reductase (AKR) families, emerged as a potential way to simultaneously bypass cancer resistance and

alleviate cardiotoxicity of ANTs. In this context, we evaluated the potential application of synthetic cinnamic acid derivatives (CA). The tested compounds were found to chemosensitize A549 human lung cancer cell line towards DOX-induced viability and proliferation reduction. Co-treatment with DOX + CA significantly inhibited the migration of A549 cells. The addition of CA alleviated DOX-induced viability reduction in H9c2 rat cardiomyoblast cell line. Accordingly, CA reduced DOX-induced reactive oxygen species (ROS) generation and increased glutathione levels. The compounds were also found to limit inflammatory response in RAW 264.7 macrophage cell line. Inhibitory properties of the compounds toward CBR1and AKR1C3 were simulated by molecular modelling and confirmed *in vitro* in enzyme inhibition assay with recombinant proteins. The possible products of cytochrome P450-mediated metabolism of CA were also established to evaluate the potential impact of first pass effect. Our results suggest that CA are promising candidates for DOX adjuvant therapy that may simultaneously chemosensitize cancer cells and alleviate cardiotoxicity.

P-01.1-093
Deamination of adenine compounds in different organs of rats with DMBA-induced cancer

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Cancer treatment is a continuous struggle around the world and research into its treatment and prevention is constantly in progress. Any research aimed to clarify the development of cancer and the mechanisms of its treatment is a matter of current interest. The entire metabolism of the cell, including purine and pyrimidine nucleotides changes during cancer. It was interesting for us to study the activity changes of enzymes involved in purine metabolism in the neoplastic and healthy tissues of rats with cancer. We study the deamination alterations of AMP, ADP, ATP, as well as adenine and adenosine in the liver and breast tissue homogenates in rats with DMBA-induced breast cancer. Rats were treated with an intraperitoneal injection of *Hypericum alpestre* herb (2.4 mg/kg) for 8 weeks. Data show significant elevation of deamination level of the adenine compounds in the group with the breast cancer treated with *Hypericum alpestre* herb compared with rats with DMBA-induced breast cancer. In the liver homogenate of rats with breast cancer during the development of the disease was observed potent reduction of deamination levels, compared with the *H. alpestre*-treated group, where deamination levels significantly increase, becoming closer to the healthy control groups. The levels of deamination of adenine compounds after the treatment with *H. alpestre* herb become very closer to the values typical to the samples of healthy animals. Thus, in conclusion, the mentioned herb's anticancer activity can be expressed by elevation of the deamination levels of adenine compounds.

P-01.1-094
Modulation of microRNA in glioblastoma, after inhibition of key signaling components of EGFR/PI3K/Akt/mTOR pathway

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Glioblastoma (GBM) is the most frequent and aggressive primary malignant brain tumor and PI3K/Akt/mTOR signaling pathway is activated in almost 90% of cases. MicroRNAs (miRs) have been shown to have a key role in progression of GBM. Our aim was to target key signaling molecules of EGFR/PI3K/Akt/mTOR pathway and modulate of miR expression, which could be a potential therapeutic approach to suppress GBM development. Two cell lines: U-87 MG (ATCC HTB-14) and A172 (ATCC CRL-1620) were treated for 30 minutes with inhibitors: pictilisib 50nM (PI3K inhibitor) and erlotinib 500nM (EGFR inhibitor). Then ARN extraction, reverse-transcription to obtain cDNA, and real-time qPCR for miRNA profile evaluation were performed. The analyzed miRNA panel comprised miR-7, miR-21, miR-34a, miR-200c 3p and U6 as control. After the treatment of U87 cells with pictilisib and erlotinib, the results obtained showed an over-expression for miR7 in cells treated with pictilisib (1.6-fold regulation) and erlotinib (1.7-fold regulation), respectively miR200c3p in pictilisib-cells treated (2.15-fold regulation). After the treatment of A172 cells, the results showed an over-expression for: miR7 in cells treated with pictilisib (2,3-fold regulation) and erlotinib (2,6-fold regulation), miR21 in pictilisib-treated cells (1.95-fold regulation), miR34a in cells treated with pictilisib (1.62-fold regulation), and miR200c3p for pictilisib-treated cells (3.5-fold regulation). On the other hand, an under-expression of miR200c3p for erlotinib-treated cells (-2.45-fold regulation) was observed. In conclusion, targeting key signaling components: EGFR and PI3K, mir7 – known as a tumor suppressor down-regulated in GBM – showed overexpression, suggesting that targeting signaling components pathways can be seen as a potential therapeutic approach in GBM. Acknowledgement: Grants PNIII.P2-2.1-PED-2019-3141(382/2020), PN 19.29.01.04, COP A 1.2.3., grant ID: P_40_197/2016, 31PFE/2021. *The authors marked with an asterisk equally contributed to the work.

P-01.1-095
Efficacy of 6 active fractions of the *Macrovipera lebetina obtusa* snake venom in human dermal microvascular endothelial and fibrosarcoma cells

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The venom of *Macrovipera lebetina obtusa* (MLO) has remarkable properties that are hard to overlook. While, no specific toxin can be found in the venom, its 38 protein components achieve high efficiency by forming complexes with other non-enzymatic proteins. Moreover, we have previously demonstrated that the crude venom as well as one of its components – obtustatin – is capable of reducing sarcoma tumor by 50% and 30%,

respectively. Obtustatin which belongs to the family of short desintegrins, acts by inhibiting the angiogenic activity of α1β1 integrin. However, the reason for higher efficiency of the crude venom is not yet understood. Thus, here we propose an experimental study that will shed light on the activity of MLO venom components. We tested 38 fractions isolated from MLO venom *in vitro* in the human dermal microvascular endothelial (HDEC) and fibrosarcoma (HT-1080) cells and found 6 cytotoxic fractions which according to the N-terminal sequencing, mass spectrometry, and collision-induced fragmentation by nESI-MS/MS of mass fingerprinting belongs to the dimeric disintegrins VLO4 and VLO5, dimeric desintegrin Lebetase 3, short disintegrin obtustatin and serine proteinases.

P-01.1-096
Role of HMGB1 protein in the proteome of exosomes derived from ovarian cancer cells

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Ovarian cancer is one of the most lethal gynecological malignancies worldwide because it tends to be detected late, when metastasis has taken place. Early diagnosis, when the tumor is still localized in the ovaries, is a clear advantage, since this rate then increases up to 92% (1). Several studies investigated the association of high mobility group box (HMGB) proteins with cancer due to their importance and the variability functions inside and outside the cells. HMGB1 and HMGB2 proteins are the most abundant members among HMGB family and they contribute to a variety of hallmarks of cancer such as sustained proliferative signaling, cell death resistance, replicative immortality, instability in the genome and increased mutations rates, tumor-promoted inflammation, growth repressors insensibility, cellular energetics deregulation, immune destruction evasion, metastasis, and angiogenesis stimulation. Moreover, HMGB1 has been repeatedly proposed as a diagnostic and prognostic biomarker for human ovarian cancer (2). High concentrations of exosomes are found in various body fluids, including blood, urine, saliva, and seminal plasma. The proteins and RNAs enriched in exosomes reflect the specific physiological conditions and functions of their samples. These exosomes and their biomolecules are ideal biomarkers for liquid biopsy. In the present work, we analyze the influence of HMGB1 on the protein content of extracellular vesicles derived from ovarian cancer cells. To do that, exosomes obtained from SKOV3 and HMGB1 derivative knockout cells were used to analyze the differences in the proteomic composition by using Trapped Ion Mobility Spectrometry time-of-flight (timsTOF) technology. (1) Reid, BM et al. (2017). Cancer Biol. Med., 14(1), 9-32 (2) Cámara-Quílez M et al. (2020). Cancers, 12(9), 2435

P-01.1-097
Chicken chorioallantoic membrane as a relevant model for studying tumor biology

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The complexity of tumors requires appropriate models for analysis of their development, biological features as well as therapeutic response. 3D spheroid and chicken CAM tumor models are much simplified in comparison to rodent models, but adequately represent many features of tumors and overcome their limitations such as costs, long duration of the experiments and some ethical concerns. Here, we provide a comparison of a variety of cell lines and PDXs in 3D spheroids *in vitro* with chicken CAM *in vivo* tumor model. Murine glioblastoma multiforme cells GL-261, human glioblastoma multiforme lines U87 MG and U251 MG, human skin melanoma cells SKMEL-188, hamster melanoma cells BHM MA and BHM AB, and human uveal melanoma PDXs and patient-derived cells were used to generate spheroids and tumors engrafted on CAM. As applications of these models we show U87-MG glioblastoma tumor response on photodynamic therapy with AGuIX nanoparticles carrying porphyrin and uveal melanoma response to chemotherapy with quisinostat. Morphology images of tumors were compared. Growth rate was estimated both in 3D spheroids and tumors engrafted in CAM. The use of CAM model enables the generation of higher tumor volumes in shorter time compared to 3D spheroid model. The engraftment success ratio between cell lines was compared. Some of the cell lines showed increased embryos mortality, requiring more careful insight into optimization of the model maintenance. In conclusion, chicken CAM is promising model for cancer biology research but, for some of the cell lines, it is more challenging to develop the tumor. This is not correlated to ability of spheroid formation. Acknowledgements: This research was supported by CMUJ nr K/ZDS/007190 and NCN UMO-2020/37/B/NZ4/01313

P-01.1-098
Analysis of mutations in lung adenocarcinoma using the next generation sequencing

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Non-small cell lung cancer (NSCLC) accounts for about 80 % of all lung cancer cases and belongs to the leading causes of cancer

deaths. Adenocarcinoma is the most common histological subtype. The treatment is based on surgery, radiotherapy, chemotherapy and targeted therapy. An integral part of a decision making on the use of targeted therapy is the identification of a causal mutation or a predictor of treatment efficacy. The growing availability of the next generation sequencing (NGS) methods provides a platform for this purpose. The aim of the study was to analyse the spectrum of mutations and chromosomal rearrangements present in tumour samples of 265 patients treated for lung adenocarcinoma at University Hospital in Pilsen from 2017–2020. Such an analysis has implications for the choice of targeted therapy. All clinical stages of the disease were included in the study group, ranging from Ia1 to IVb stage. RNA was isolated from the tumour tissue and the analysis was performed using the Archer FusionPlex Comprehensive Thyroid and Lung (CTL) panel targeting 36 genes commonly mutated in solid cancers. Oncogenic alterations have been revealed in 155 out of 265 samples (58.5%). The most prevalent mutations were those in KRAS (76 cases), EGFR (32 cases), TP53 (9 cases), BRAF (4 cases) and ERBB2 (4 cases) oncogenes. EML4-ALK fusion was detected in 10 samples tested. The NGS approach is a promising tool for the wider application of targeted therapy in the sense of personalized medicine. The work was supported by the grant of Ministry of Health of the Czech Republic - Conceptual Development of Research Organization (University Hospital in Pilsen, 00669806) and by the Charles University Research Fund (Progres Q39 and SVV 260539).

P-01.1-099
Dynamic influence of paclitaxel on inflammasome components and pyroptosis in breast cancer cells

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Progressive inflammation sustained by inflammasome activity and inflammatory programmed cell death – pyroptosis – influences the pathogenesis and development of breast cancer, especially of triple-negative breast cancer. Therapies based on chemotherapeutic drugs (paclitaxel, 5-fluorouracil, cisplatin, docetaxel, etc.) were used for the treatment of a wide spectrum of cancers. In the context of breast cancer, paclitaxel (PTX)-based treatment is responsible for the dysregulation of NLRP3 inflammasome complex, pro-inflammatory cytokines release and pyroptosis cellular death initiation by caspase-1 activation. The aim of this study was to investigate the effects of different concentrations of PTX on inflammasome assembly and pyroptosis mechanisms in MCF7 and MDA/MB 231 breast cancer cell lines versus MCF12A normal breast cell line used as reference. To accomplish this study, PTX-treated cells' viability was quantitatively and qualitatively determined. The caspase-1 activity mediated by the inflammasome complex was determined from culture media. The gene and protein expression of key components of inflammasome complex and pyroptosis pathways (caspase-1, IL-1β, IL-18, NLRP3, ASC, etc) were determined using real-time PCR and immunofluorescence (IF) techniques. PTX treatment considerably decreased tumor cell viability. The highest levels of casapase-1 were observed in MDA/MB 231 triple-negative breast cancer cells exposed to PTX. Real-Time PCR and IF revealed the abnormal expression of caspase-1, IL-1β, IL-18, NLRP3, ASC, etc in breast cancer cells exposed to PTX, compared to control. PTX proved to have a strong cytotoxic effect on breast cancer cells, stimulating the assembly of the inflammasome complex and activating the caspase-1-induced pyroptotic cell death.

P-01.1-100
PKA basal activity is required for protein trafficking from perinuclear recycling endosomes involving Rab-coupling protein

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The endosomal recycling pathway is crucial in the function of signaling receptors and nutritional receptors and plays an important role in a variety of physiological and pathological conditions, including cancer progression. This pathway includes peripheral endosomes and the perinuclear recycling compartment (ERC). Our laboratory has described that recycling of epidermal growth factor receptor (EGFR) from ERC requires basal protein kinase A (PKA) activity. The Rab-coupling protein (RCP), an effector of the small GTPase Rab11, is a substrate of PKA that has been involved in recycling of EGFR during migration/invasion of tumoral cells. Here we study the role of RCP in PKA-mediated trafficking out of ERC. We used HeLa cells to compare the effect of phospho-inert and phospho-mimicking mutants of RCP on receptor recycling from ERC, evaluating confocal microscopy images of fluorescent markers in live cells and cell surface binding. Results: While PKA phospho-inert RCP mutants inhibit recycling of EGFR from ERC, increasing its colocalization with transferrin in ERC, the phosphomimetic RCP mutants have opposite effects, increasing recycling from this compartment. Our results suggest that RCP role in recycling from ERC is regulated by PKA activity and might be a potential therapeutic target against tumoral cell migration/invasion. Acknowledgements: FONDECYT#1181907, National Agency for Research and Development (ANID)/Scholarship Program/DOCTORADO BECAS CHILE/2021-21110001, CONICYT Basal Project grants AFB-170004 and AFB-170005, Basal FB210008.

P-01.1-101
Expression profiling reveals dysregulated microRNAs involved in the progression of melanocytes to primary and metastatic melanoma

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Malignant melanoma is a highly aggressive type of skin cancer, deadly in advanced stages. Few treatment options are available, and its global incidence is increasing fast. MicroRNA molecules (miRNAs) are small, single-stranded, non-coding RNAs that target the 3′-untranslated regions to inhibit gene expression, being part of epitranscriptomic regulatory mechanisms. Knowledge of cancer miRNA-mediated regulation has significantly improved

over the years, with networks of miRNAs emerging as capable of controlling key signalling pathways responsible for tumour cells' growth and propagation, however research is ongoing. In this context, the purpose of our study was to investigate the melanoma-associated dysregulated expression of miRNAs involved in the progression of melanocytes to primary and metastatic cancer. Expression profiling was performed on melanocytes and melanoma cells originated from primary and metastatic samples (lymph node and brain), representing stages of tumour progression. ARN was isolated from each cell line, reverse-transcribed to cDNA, and the expression of 84 miRNA molecules was evaluated using qPCR array. The results highlighted the up-regulation of some miRNAs (e.g. miR-210, miR-140-5p, miR-96-5p), while others, such as miR-338-5p and miR-193b-3p, were down-regulated over the course of cancer development from melanocytes to primary cells and further to metastatic melanoma. Moreover, several miRNAs were found to be increased during early tumour progression but decreased in metastatic phase, including miR-122-5p, miR-29b-3p and miR-139-5p. Bioinformatic analysis revealed potential targets of the miRNAs of interest, among them IGF2, MAP2K6 and HIF3a, important genes involved in several signalling pathways. Understanding the essential roles of miRNAs in each phase of melanoma progression and metastasis development will contribute to the achievement of future miRNA-targeted therapy, as well as better prevention, diagnosis and clinical management.

P-01.1-102
The influence of inhibitors targeting epigenetic factors in cervical cancer *in vitro* model

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Cervical cancer is one of the leading causes of cancer deaths among females worldwide, high-risk Human Papillomavirus (hr-HPV) infection being the main cause. Epigenetic alterations, such as changes in viral and host genome DNA methylation patterns, as well as histone modifications are involved in cervical carcinogenesis. Inhibitors targeting DNA modifying enzymes, particularly histone modifying ones, may represent a potential cancer treatment. The aim of this study was to investigate the impact of epigenetic inhibitors on the expression pattern of 13 chromatin remodeling enzymes in *in vitro* model. The study was performed on CaSki cell line (HPV16 positive), treated for 24 and 48 h with two inhibitors in different concentrations: vorinostat (1 μM and 5 μM) and procaine (0.1 mM and 0.5 mM). Cell cultures were kept in the incubator at 37°C in an air atmosphere containing 5% CO_2. Total RNA isolated from the samples was used to generate cDNA. The expression levels of the gene panel (KDM1B, KDM2B, KDM3C, KDM4C, KDM5A, KDM5C, KDM6A, EZH2, EHM2, DNMT1, DNMT3B, TET1, HDAC1) were quantified in treated and non-treated cells in Real-Time PCR using β-actin as a housekeeping gene. Statistical analysis was performed using GraphPad Prism 5.0. The results showed that both concentration of vorinostat inhibits the KDMs, EZH2 and EHMT2 expression levels at 48 h. On the other hand, HDAC1 gene expression level significant decreases after 24 h with 1 μM vorinostat treatment compared with untreated cells ($P = 0.0018$). Like vorinostat, procaine had a similar influence on the studied genes. Moreover, DNMT1 expression level decreases significantly after 24h under treatment with 0.1 mM procaine ($P = 0.0034$), while DNMT3B expression levels did not change. Epigenetic drugs in cervical cancers can restore the balance of epigenetic factors. Thus, modulation of investigated genes expression could be a new therapeutic approach in cervical cancer therapy. Acknowledgement TE39/2020. *The authors marked with an asterisk equally contributed to the work.

P-01.1-103
HIF-2α regulates the invasiveness capacity of glioblastoma stem-like cells through A2BAR under hypoxic conditions

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Glioblastoma (GBM) is the most common and deadly malignant brain tumor, with a median survival rate between 15 and 17 months. GBM contains a cellular subpopulation known as glioblastoma stem-like cells (GSCs) that persist in hypoxic niches and can infiltrate healthy brain tissue: they are considered responsible for recurrence. Hypoxia stabilizes HIF-2α and increases A_{2B}AR expression, making it a central player in the cellular adaptation of cancer cells in the hypoxic microenvironment. HIF-2α and A_{2B}AR can modulate the aggressiveness of different cancer models. However, the role of these two proteins in the GSC invasiveness phenotype under hypoxic conditions is still unknown. This study aims to understand the role of HIF-2α and A_{2B}AR in modulating the migratory/invasive capacity of GSCs under hypoxia. A_{2B}AR and HIF-2α expression was evaluated in GBM tissue in the TCGA database. GSCs derived from U87MG (GSCs-U87MG) and primary culture (GSCs-PC) were cultured under normoxia (21% O_2) and hypoxia (0.5% O_2). MRS1754 was used as an A_{2B}AR antagonist and siRNA to decrease HIF-2α expression. mRNA levels of TWIST1, SNAIL, MMP9, HIF-2α, and A_{2B}AR were evaluated by PCR. The migratory and invasive capacity of GSC-U87MG and GSCs-PC was evaluated using transwells and transwell-matrigel, respectively. A_{2B}AR expression was associated with GBM tissue and necrotic areas. The migratory and invasive capacity of GSCs increased under hypoxic conditions. A_{2B}AR blockage decreased the invasiveness capacity of GSCs, downregulating MMP9, SNAIL and TWIST1, and similar results were obtained in HIF-2α knockdown (KD). Additionally, KD of HIF-2α reduced A_{2B}AR expression. These results suggest that HIF-2α acts through A_{2B}AR signaling to regulate the invasiveness phenotype of GSCs under hypoxic conditions.

P-01.1-104
New tissue transglutaminase inhibitors for preventing ovarian cancer dissemination

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Tissue transglutaminase (TG2) is a multifunctional protein that regulates cell adhesion to the extracellular matrix by forming a complex with fibronectin (FN) and integrins. In ovarian cancer (OC), this interaction enables intraperitoneal disease dissemination and metastasis, which renders TG2 as a promising therapeutic target. We previously proposed MT-4 as an improved small molecule inhibitor (SMI) for TG2-FN protein–protein interaction, based on dedicated molecular and cellular assays (previously published in: Sima LE et al. (2019) Mol Cancer Ther 18, 1057-1068). We are currently continuing our drug development efforts by characterizing new MT-4 analogues, namely: #2997, #2998, #3002, #3010, and #3011. Three SMIs showed promising effects and target specificity. Inhibitor #2997 decreased SKOV3 OC cell migration in a wound-healing assay. Compound #3002 inhibits SKOV3 cell adhesion and downstream pFAK signaling, as well as G1-S cell cycling. Interestingly, #3011 totally prevented spheroid formation, identically to *Tgm2* gene KO. This compound is extremely promising for intraperitoneal treatment of OC patients for prevention of cell aggregation. Moreover, we analyzed the adaptive signaling in attached OC cells in the presence of SMIs using SILAC-based phosphoproteomics. IPA analysis showed that cells attached in the presence of MT-4 had upregulated Z-scores associated to the sirtuin signaling pathway. Sirtuins represent druggable co-targets that could enhance our SMIs efficacy in OC treatment. Secondly, we adapted a mesothelial clearance assay to a microfluidic chip for real-time imaging. An inhibitory action of MT-4 on mesothelial cells displacement by SKOV3 spheroids was observed, similarly to TG2KO. This data underlines the progress of our TG2 target drug development initiative for OC treatment. This research was supported by US Department of Veterans Affairs (I01 BX000792–06), Robert H Lurie Comprehensive Cancer Center and UEFISCDI (PN-III-P2-2.1-PED-2019-1543).

P-01.1-105
New therapeutic approach in breast anti-cancer treatment using a combination of *Rumex obtusifolius* extract and L-arginine pathway inhibitors

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The increasing threat of cancer leads to a search for new and alternative sources to battle the disease. Natural products have always been a very important source of active compounds, many of which have also shown anticancer activity. Anticancer action of herbal extracts in combination with synthetic analogs of amino acids targeting the arginase and NOS family can be a possible promising anticancer approach against mammary carcinogenesis. The goal of our study was to explore the anticancer potential of *R. obtusifolius* (RO), 2.4 mg/kg/day, i.p.) combined with inhibitors of the L-arginine pathway (nor-NOHA 3 mg/kg/day, i.p. and L-NAME 30 mg/kg/day, i.p.), administered for 8 weeks (after tumor development, every 4th day) against 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary carcinogenesis in rats. Mammary gland tumors were induced by a single dose of 25 mg of DMBA diluted in soy oil (1 mL) given injected subcutaneously, 2nd and 3rd pair of breasts. To determine the anti-cancer properties, we assess arginase activity, nitrite ions, MDA, IL-2, and polyamine changes in the blood. We have shown *R. obtusifolius* herbal extracts combined with inhibitors of arginase (nor-NOHA) and NO-synthase (L-NAME) can influence the regulation of cancer cell metabolism, which has been reflected in changes in tumors' size and animals' mortality. Combined treatment of *R. obtusifolius* extracts and chemicals significantly decrease tumor multiplicity and incidence. We have also found synergistic interactions between tested plant extracts and synthetic compounds against induced mammary tumors. Histological changes discontinue in the treated groups (there is a 2 from 8 rats' good prognosis in RO+nor-NOHA+DMBA group). The long-term goal of our research is to find effective anti-cancer plant extract/chemotherapeutic agent combinations, explore the molecular mechanism of their anti-cancer action, and prepare to propose these combinations for patenting as a test anti-cancer preparation.

P-01.1-106
Galectin-8 induces epithelial–mesenchymal transition in a model of fully polarized epithelial cells

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Epithelial cells that have apical/basolateral polarized phenotype can undergo changes towards a more mesenchymal migratory

phenotype during processes of organogenesis, tissue repair, and cancer development. Factors able to induce this epithelial–mesenchymal transition (EMT) are therefore of great interest. Galectin-8 (Gal-8) is a carbohydrate-binding protein that is overexpressed in several tumors and upon secretion by a nonconventional mechanism can elicit a variety of cellular responses interacting with cell surface glycoproteins. Tumor cells usually co-exist with fully differentiated cells within the epithelia where they are growing. Our previous studies show that Gal-8 promotes EMT in sub-confluent Madin-Darby Canine Kidney (MDCK) epithelial cells by trans-activating the epidermal growth factor receptor (EGFR). However, whether fully differentiated epithelial cells might undergo similar Gal-8-induced changes remain unknown. MDCK cells were grown in matrigel to form three-dimensional (3D) cysts with their apical surfaces facing a central lumen and their basal surfaces contacting the extracellular matrix and exposed to added recombinant Gal-8. Apical and basolateral markers and cyst structure were analyzed by confocal immunofluorescent microscopy. Gal-8 induced loss of apical/basolateral polarity and cyst structure, reverted by EGFR inhibitors. Gal-8 can induce EGFR-mediated EMT in fully polarized epithelial cells and therefore might potentially contribute to transform normal epithelial cells adjacent to tumor cells overexpressing Gal-8. Acknowledgements: FONDECYT 1181907 and 1211829, ANID/BASAL grants FB210008 and ACE210009.

P-01.1-107
Spin-labeled Michael acceptors to study covalent inhibition of thioredoxin reductase

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Maintaining the redox balance is important for living organisms. During cellular metabolism, including the metabolism of xenobiotics, reactive oxygen/nitrogen species are formed. Their formation is controlled by antioxidant systems, mainly glutathione- or thioredoxin-dependent systems. The ability to modulate signaling pathways with exogenous substances/compounds that affect the thioredoxin-dependent system creates a real opportunity to expand the use of existing drugs, and also contributes to the search for new pharmacotherapeutic methods for cancer treatment to activate apoptosis pathways in tumor cells. Human thioredoxin reductase I has a high potential as a target for new types of anticancer drugs. The project is aimed at the search and study of new natural compounds with high selectivity and inhibitory activity against thioredoxin reductase I. In our work, we obtained 1-aryl-3-arylidenepyrrolidine-2,5-diones in highly productive direct diazo transfer reactions and subsequent involvement of the resulting diazo compounds in Rh II catalyzed O–H, S–H, and N– H insertion reactions delivered 4-substituted 1-aryl-3-arylidenepyrrolidine-2,5-diones of certain regiochemistry and geometric configuration [1]. These products are intended to be studied as Michael acceptors capable of inhibiting thioredoxin reductase, a promising cancer target. The resulting Michael acceptors were used to introduce a spin label in order to study the process of covalent binding of the enzyme to the inhibitor. The results obtained allow us to conclude that there is a relationship between the molecular structure and selectivity of inhibitors. 1. Chupakhin E. et al. (E)-3-Arylidene-4-diazopyrrolidine-2, 5-diones: Preparation and Use in RhII-Catalyzed X–H Insertion Reactions towards Novel, Medicinally Important Michael Acceptors //Synthesis. – 2021. – T. 53. – №. 07. – C. 1292-1300. Acknowledgments: The reported study was funded by I. Kant Baltic Federal University, project number 882

P-01.1-108
ANP0903, a novel nanoformulated darunavir analog, kills hepatic cancer cells by inhibiting the proteasome system

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Liver cancer is one of the most common causes of cancer death worldwide. In recent years, significant progress has been made in the development of systemic therapies, but there is still a need for new drugs and technologies that can increase survival and quality of life for patients. Several preclinical and clinical reports suggest that HIV-1 protease inhibitors, in addition to antiretroviral properties, possess pleiotropic pharmacological actions, including antitumor effects [Maksimovic-Ivanic D et al. (2016) Int J Cancer 140, 17131726]. The possible use of some derivatives of hydroxyethylamine, the core of the HIV-1 protease inhibitor Darunavir, as molecules with proapoptotic activity on tumor cells, has already been demonstrated, [Facchinetti V et al. (2015) Med Chem Res 24,533542] [Rinaldi R. et al. (2021) Cells, vol. 10, no. 11], therefore a novel Darunavir analog, indicated as ANP0903, has been synthesized. This molecule showed enhanced and dose-dependent cytotoxicity towards the HepG2 liver cancer cell line compared to the non-pathological IHH liver cell line. Moreover, a nanoformulation using PEGylated liposomes was developed to enhance intracellular uptake. Several assays were performed to elucidate the molecular mechanisms explaining the cytotoxic effect of ANP0903 and of its liposomal form. The results showed a cytotoxic effect against tumor cells mainly through the inhibition of the proteasome, which triggers the processes of autophagy and apoptosis, resulting in cell death. The proposed liposomal formulation represents a promising approach to deliver a novel therapeutic agent to cancer cells and enhance its activity.

P-01.1-109
Impact of the transcription factor ONECUT2 on the cellular plasticity of breast tumors

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Intratumoral heterogeneity complicates patient treatment and can occur through the transition to different cellular phenotypes. This process is associated with the acquisition of independence from an oncogenic driver, such as the estrogen receptor (ER) in breast cancer, resulting in tumor progression, therapeutic failure and metastatic spread. Here we analyze the role of ONECUT2 (OC2) as a negative regulator of the ER axis in luminal subtypes of breast cancer. Gene expression profiling of OC2-engineered luminal cell lines has allowed us to generate a OC2 activity signature that reveals high negative correlation with ER activation pathways. We find that OC2 is a negative regulator of ER

expression and a repressor of its transcriptional program. Finally, we show that OC2 is required for cell growth and survival and that it can be targeted with a small-molecule inhibitor providing a novel therapeutic strategy for patients with OC2 active tumors.

P-01.1-110
Identification of new key players of cell competition in a colon cancer model

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Cell competition is a mechanism through which weaker cells are recognized by their fitter neighbors and eliminated through apoptosis. Detection and elimination of these cells is important to maintain healthy tissues and prevent diseases. In a cell competition assay between two colon cancer cell lines, we observed LoVo and LS174T engage in cell competition, with LoVo behaving as loser, being eliminated by apoptosis, arresting cell cycle at G0/G1 phase and migrating less; while LS174 behaves as winner, proliferating and migrating more, as previously reported. To identify new players of cell competition we performed RNA-seq of winner and loser cells. Using a stringent analysis and confirmed by RT-PCR, we identified eight genes upregulated in winners and three genes downregulation in losers. Functional validation confirmed three genes upregulated in winners, AQP3, MYT1 and NRIP1, are required for the elimination of losers. We observe at mRNA or protein levels, upregulation of AQP3, VIMENTIN, EGFR, HER2 and mTOR in winners, and downregulation of EGFR and mTOR in losers, suggesting cell competition promotes epithelial–mesenchymal transition (EMT) in winners, and that this is due to AQP3. Conditioned media experiments suggest that improved survival of winner is based on the presence of a survival factor in culture media. RT-PCR analysis of candidate genes show that AQP3, MYT1 and NRIP1, increase expression when in the presence of the media from the co-culture, indicating that secreted factors present in the media are enough to upregulate gene expression. Mass spectrometry analysis of the cell culture media identified VGF (neurosecretory protein VGF precursor) as a candidate factor leading to upregulation of the candidate genes, and consequent cell competition. Although further validation is need, VGF has been linked to EGFR and EMT, adding to its potential role in cell competition triggering. Our data improves knowledge on the role of cell competition in cancer development

P-01.1-111
High antioxidant and cytotoxic activity of *Rumex obtusifolius* seed extract and metabolomic characterization of its bioactive phytochemicals

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In recent years, increasing attention is paid to plants as a promising source of new anticancer compounds. The inhibition of cancer cell growth by bioactive phytochemicals is frequently associated with redox homeostasis disruption. The proliferation is regulated by reactive oxygen species (ROS) and antioxidant properties of phytochemicals can cause scavenging ROS, thereby blocking cell growth. We aimed to evaluate the antioxidant and anticancer properties of *R. obtusifolius* L. (seed) (RO) ethanol extract in relation to metabolomic characterization of its bioactive components. Antioxidant potential of RO extract was evaluated using chemical (ABTS, DPPH), electrochemical (CV), and cellular (CAA) tests. MTT assay was used to assess cytotoxic effect of the plant extract against HT29 and MCF-7 cell lines. Based on obtained data, it can be concluded, that RO extract exhibits high antioxidant activity in all used assays. The plant extract also exhibited significant cytotoxic activity against both tested cell lines even at 0.25 mg DW/ml concentration. HPLC equipped with photodiode array detector and high-resolution mass spectrometer (HPLC-DAD-HR-MS) enabled identification of more than 240 compounds in the extract. Antioxidant profiling using HPLC followed by post-column derivatization with ABTS allowed to identify several compounds displaying high antioxidant activity. These compounds are catechin, epicatechin monogallate, several procyanidins, etc. Our study identified compounds responsible for the antioxidant activity of *R. obtusifolius* extract as well as demonstrated its ability to inhibit cancer cell growth. However, more deep analysis is needed to confirm its anticancer potential. In further, the possible anticancer therapeutic targets and mechanisms of action of identified phytochemicals will be elucidated using advanced simulation tools of computational biology. The anticancer potential of the extract in the rat mammary carcinogenesis *in vivo* model also will be studied.

P-01.1-112
ATM/ATR-CHK1/CHK2-p53 signaling axis primes breast cancer cell for survival upon genotoxic stress caused by the alkylating agent cisplatin

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The action of numerous anticancer drugs is accompanied by the formation of DNA damage, which stimulates signaling pathway in which ATM/ATR kinases act as damage detectors, and their substrates CHK1/CHK2 lead to activation of the p53 protein. The latter protein increases transcription of p21 which inhibits cell proliferation and, thereby, suppresses genes transcriptionally controlled by the rate of mitotic divisions. This group includes a significant number of genes whose products are involved in DNA repair. However, our experiments on p53 wild-type breast cancer cell line MCF7 show an increase in expression of *PARP1*, *BRCA1* and *RAD51* in response to cisplatin. Therefore, we aimed to identify all genes whose products participate in DNA repair, and which are overexpressed by cisplatin-induced activation of the ATM/ATR-CHK1/CHK2-p53-p21 pathway, as well as to determine the involvement of p53 protein in regulating cellular responses to genotoxic stress. Using next-generation RNA sequencing we compared the transcriptome of p53-deficient and proficient MCF7 cells treated with cisplatin. The results showed increased expression of 262 genes involved in DNA repair after treatment with cisplatin. A decrease in the expression of 298 DNA repair genes was observed in cisplatin treated and p53 transiently silenced cells. We further provided evidence that drug-induced upregulation of *PARP1*, *BRCA1* and *RAD51* expression depends on p53 levels and DNA damage response. Silencing of

p53 and pharmacological inhibition of ATM/ATR resulted in decreased protein and mRNA levels of the analyzed genes. Although previous reports documented p53 occurrence only at the promoters of two DNA repair genes such as *DDB2* and *TP53R2* upon genotoxic stress, our study shows that this protein is also recruited to promoter of i.a. *BRCA1*. In conclusion, DNA damage caused by cisplatin activates p53-dependent transcription of DNA repair genes. Grants: IDUB-60/2021

P-01.1-113
Integrated proteogenomics of leiomyosarcomas for metastatic risk prediction

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Non-uterine high-grade leiomyosarcomas (LMS) are rare but frequently lethal neoplasms. LMS often exhibit aggressive behavior, recur locally, and some metastasize to other organs. Thus, better understanding tumor biology is needed to predict individual risk, to tailor treatment, and to improve clinical outcomes. In this study, we used integrated deep proteogenomics at DNA, mRNA, and proteome levels to explore differentially expressed genes and proteins that play a role in driving aggressiveness and metastatic propensity of LMS. We selected 40 LMS cases, including 20 low-risk cases (disease-free survival (DFS) of more than 5 years) and 20 high-risk cases (DFS less than 3 years) that were all histologically high-grade and not distinguishable. DNA and mRNA were analyzed using next generation sequencing. Deep unbiased proteomes and phosphoproteomes were analyzed using LC-mass spectrometry. Differential expression analyses and gene pathway enrichment analyses were performed. Using integrated proteogenomics (quantifying >8,000 proteins and >18,000 transcripts) and comparing high-risk cases to low-risk cases, we discovered 90 differentially expressed genes, 64 up-regulated and 26 down-regulated. In upstream regulator analyses, inflammation-associated genes are associated with high-risk LMS, such as TNF, IL1, IL6, and IFN gamma. In canonical pathway analyses, inflammatory pathways such as tumor microenvironment pathway, IL17 signaling, TREM1 signaling, and HIF1 alpha signaling are also found up-regulated in high-risk LMS tumors. Our study identified differentially expressed genes and proteins and affected signaling pathways that may drive aggressiveness and metastatic propensity of non-uterine high-grade LMS. We have constructed a multiomic outcome risk prediction model for LMS that can help guide adjuvant therapy decisions for LMS patients after surgery.

P-01.1-114
Cross-talk between lipid and metal homeostasis regulates fitness of tumour cells in leukemia

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Although it has been shown that lipid metabolism plays role in tumour development, the molecular mechanism by which it influences cancer progression has been partially understood. Here, using the example of a frequent type of leukemia, the B-cell chronic lymphocytic leukemia, we studied the effect of lipid-transporting proteins on tumour cell fitness and the underlying mechanism of their action. Apolipoprotein E (ApoE) is an abundant serum protein regulating lipid homeostasis and cholesterol transport. Tumour B cells express several ApoE receptors on their surface, suggesting that ApoE could influence their physiology. We observed that recombinant microvesicle-associated ApoE is able to inhibit proliferation of leukemic B cells at physiological concentrations. Transcriptomic analysis identified a signature of metal poisoning upon ApoE treatment, implicating copper ions in the observed toxicity. This led us to discover that B cells, especially of leukemic origin, are intrinsically sensitive to cuproptosis, a type of cell death induced by the disbalance in copper homeostasis. A combination of ApoE and copper gives an optimal inhibitory profile to block proliferation of leukemic B cells. Interestingly, leukemic B cells are more sensitive to copper intoxication than normal B cells and, to counteract this, they express proteins protecting them from cuproptosis. To evaluate whether ApoE is expressed in the tumour niche in patients, guided by single cell transcriptomics data, we are investigating on the nature of ApoE-producing cellular subsets from patients' bone marrow to get a full picture of interactions which may regulate pathogenic B cell proliferation. In conclusion, we illustrate a new inhibitory circuit potentially targetable in B cell leukemia. Our findings suggest that further investigation of lipid-metal crosstalk in B lymphocytes and other cell types may lead to a better comprehension of patho-physiological processes and the development of new therapeutic tools.

P-01.1-115
Nanobodies as tools to restore the protases activity in tumoral context through the sequestration of the protease inhibitor SERPINB3

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Primary liver tumors developed from cancer stem cells are commonly originated from chronic diseases and chronic inflammatory events, such as alcohol abuse or viral infections leading to liver cirrhosis. The ongoing inflammatory process involved in cirrhosis generates diffuse tissue necrosis and regeneration, with remarkable fibrosis response and progenitor cell activation. Recent studies have documented that the protease inhibitor SERPINB3, also known as squamous cell carcinoma antigen-1 (SCCA-1), is overexpressed in the most aggressive forms of both hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA). SERPINB3 has been widely recognized as an anti-apoptotic and pro-proliferative factor involved in the carcinogenesis

process. The aim of the present study was to further investigate the role of this protease inhibitor exploiting new tools for its molecular tracking and for the development of possible inhibiting agents. Nanobodies (Nbs), the smallest antibody fragments known so far, were developed as tools for inhibiting the antiprotease activity of SERPINB3 and for tracking its expression in biopsies of patients with HCC and CCA. A nanobody library was generated by alpaca immunization with recombinant SERPINB3 and screened by means of Yeast Surface Display technology. Fluorescence Activated Cell Sorting was applied to select Nbs binders with high affinity towards SERPINB3. Selected Nbs were further characterized by flow cytometry, determining their affinity for the target antigen and mapping the recognized epitopes. The best candidates were finally expressed in soluble form for in vitro inhibitory assays meant to design their application to tumoral tissues environment to restore the apoptotic cascade and potentially reach tumoral mass reduction.

P-01.1-116
The epithelial-mesenchymal transition of A431 cells after treatment with ATP, ADP, AMP and adenosine

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Epithelial–mesenchymal transition (EMT) is a crucial process related to metastasis of cancer cells. Although some factors that influence the cellular rearrangement are already known, the exact mechanisms that induce and regulate this process are still not fully understood. The extracellular environment has been shown to play a key role in induction of this transformation. Among others, ATP is indicated as important EMT inducer. The adenosine triphosphate, besides its energy storage function, is also known signalling molecule. Numerous data proved this nucleotide may be actively release by cells in order to induce target signalling pathways via P2X and P2Y receptors. However, besides ATP, its degradation products also play important functions in extracellular environment. Therefore, it is indicated that ATP and products of its extracellular degradation, may play an important role in the induction of the signalling pathways related to the cancer progression and metastasis. Here, the influence of extracellular ATP, ADP, AMP and adenosine on the epithelial–mesenchymal transition in A431 cells will be presented. The EMT status in the presence and absence of tested nucleoside and nucleotides were validated by the level of markers of epithelial and mesenchymal states like E-cadherin, slug, snail, beta-catenin. Besides, invasion properties of cells in both, 2D and 3D models after treatment with tested compounds will be presented. Our data show that not only ATP but also its degradation products may affect cell EMT parameters of A431 cells in the concentration-dependant manner. Acknowledgment: This project was financially supported by the grant 2017/26/D/ST5/01046 from National Science Centre in Poland.

P-01.1-117
Drug delivery with pH-sensitive star-like dextran-graft polyacrylamide copolymer

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The development of precision cancer medicine relies on novel formulation strategies for targeted drug delivery to increase the therapeutic outcome. Polymer nanoparticles represent one of the innovative non-invasive approaches for drug delivery applications in cancer therapy. Among the currently most employed materials several biocompatible synthetic polymers, namely dextran-graft-polyacrylamide (D-g-PAA) copolymers, are being developed. In this study, the star-like D-g-PAA copolymer in anionic form (D-g-PAAan) was developed for the pH-triggered targeted drug delivery of the common chemotherapeutic drugs – doxorubicin (Dox) and cisplatin (Cis). The initial D-g-PAA copolymer was synthesized by the radical graft polymerization method, and then was alkaline-hydrolyzed to get this polymer in anionic form for further use for drug encapsulation. The monodisperse size distribution of both Dox- and Cis-loaded polymeric nanoparticles was estimated at 80 nm. The acidification of the buffer from pH 7.4 to 5.0 promoted release of Dox and Cis on 26.0 and 32.5% at 48 h, respectively. D-g-PAAan nanoparticles increased toxic potential of the drugs against human and mouse lung carcinoma cells (A549 and LLC), but not against normal human lung cells (HEL299). Thus, half-maximal inhibitory concentration (IC50) of Dox and Cis was decreased on 2–3 times at 48 h upon delivery with D-g-PAAan nanoparticles to LLC and A549 cells. The drug-loaded D-g-PAAan-nanoparticles promoted further oxidative stress and apoptosis induction in LLC cells by increasing intracellular reactive oxygen species generation and activation of caspase 3/7. The data obtained indicate that the strategy of chemotherapeutic drugs encapsulation within the branched D-g-PAAan nanoparticle allows not only to realize pH-triggered drug release but also to potentiate its cytotoxic, prooxidant and proapoptotic effects against lung carcinoma cells *in vitro*.

P-01.1-118
Small extracellular vesicles from hypoxic glioblastoma stem-like cells promote a highly chemoresistant phenotype in glioblastoma

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Glioblastoma (GB) has the worst prognosis of all brain tumors. GB contains highly chemoresistant glioblastoma stem-like cells (GSC) that produce large amounts of extracellular adenosine (Ado), especially under hypoxic conditions. Ado activates signaling pathways that promote a chemoresistant phenotype. Extracellular Ado is produced by CD73 and PAP and causes chemoresistance through an MRP1 and MRP3 dependent mechanism via A_3AR and A_2BAR receptors. Small extracellular vesicles (sEV) mediate intercellular

tumor communication, regulate chemoresistance, and promote malignancy in GB. We investigated whether hypoxic sEV-GSC express Ado axis markers, as well as MRPs, and promote a highly chemoresistant stem phenotype in GB receptor cells. We incubated GSC (from the U87 human GB cell line) under hypoxic and normoxic conditions, and isolated sEV from conditioned media by differential ultracentrifugation and measured mRNA and protein levels by RT-PCR and western blot. Size distribution and sEV concentration were analyzed using NanoSight, while its morphology was observed by transmission electron microscopy. Finally, hypoxic sEV-GSC were incubated for 48 h with U87-GB cells. We isolated samples highly enriched in sEV with a typical cup shape morphology. We detected transcripts for CD73, A_3AR, MRP1, and MRP3 in hypoxic sEV-GSC and detected CD73 overexpression in these vesicles. We also observed a morphological change towards a more chemoresistant GSC phenotype in U87 cells treated with hypoxic sEV-GSC. In conclusion, we observed Ado axis markers and MRP expression in hypoxic sEV-GSC and found these vesicles promote a highly chemoresistant phenotype in GB cells. We hypothesized that this phenotype is caused by the induction of these proteins in receptor cells via hypoxic sEV-GSC.

P-01.1-119
The importance of the MCPIP1 protein in the progression of hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the third most common cause of cancer mortality worldwide, with a higher incidence among men than women. There are several reasons for the development of HCC, with nonalcoholic fatty liver disease (NAFLD) currently the most common etiology of chronic liver disease in the United States. NAFLD progression is multifactorial, but inflammation is considered a key component of disease progression. One of the negative regulators of inflammatory response is MCPIP1 protein, which, as a result of RNase activity, regulates the level of many transcripts, including the proinflammatory cytokines Il -1β and IL-6. Furthermore, the MCPIP1 protein inhibits the biogenesis of several miRNAs. Recent studies show that the MCPIP1 protein plays an important protective role in cancer progression. MCPIP1 decreases during the progression of breast cancer and clear cell renal cell carcinoma. Furthermore, our recent research found that MCPIP1 protein level is reduced during NAFLD development. We are currently conducting research on the role of the MCPIP1 protein in the progression of HCC, using a unique mouse model with a specific knockout of the Zc3h12a gene in the liver. To induce HCC, we administered diethylnitrosamine (DEN) to 2-week-old mice. Our research showed that 40 weeks after DEN administration, mice deficient in the MCPIP1 protein in the liver develop larger and more numerous tumors. In addition, in these mice we observed a change in liver cell morphology and the presence of multiple collagen fibrils compared to the control. Our quantitative studies showed that in mice deficient in the MCPIP1 protein, there was increased infiltration of immune cells invasive to the liver, especially macrophages. It should also be added that the level of mesenchymal markers is increased. Our results so far indicate a significant protective role for the MCPIP1 protein in the progression of HCC. Funding: 2017/26/E/NZ5/00691, 2021/41/N/NZ4/04187 and MNS 2/2021.

P-01.1-120
Antibody fragment-drug conjugates as a novel strategy for targeted therapy of ganglioside GD2-positive tumors

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GD2-positive tumors account for approximately 10% of all cancers, and at the same time, many tumors overexpressing the marker, namely glioma, small cell lung cancer, neuroblastoma, and melanoma, are characterized by low therapy efficiency. Practically no research has been carried out on antibody–drug conjugates (ADCs) targeting GD2, although other ADCs have shown success in eliminating various tumors both in the clinical and preclinical setting. Many studies are underway to optimize the efficacy of ADCs, including those addressing their antigen-binding parts. Changing the antibody format within ADCs from full-length mAbs to antibody fragments can improve drug efficacy, specifically by enhancing drug penetration into solid tumors. Still, no data currently exists on antibody fragment-drug conjugates (FDCs) directed to GD2-expressing tumors. In our study, GD2-targeted FDCs were created based on scFv fragments or minibodies conjugated to the commonly used monomethyl auristatin E (MMAE) or F (MMAF) by thiol-maleimide chemistry. MMAE and MMAF are an example of structurally similar molecules that manifest key differences in intracellular processing and often contrasting efficacy. Comparison of the binding of the original GD2-specific molecules and the FDCs showed that site-directed conjugation did not alter the ability of the molecules to interact with ganglioside GD2. Analysis of the cytotoxic effects of the FDCs in a panel of GD2-positive and GD2-negative neuroblastoma and glioma cell lines showed that both FDC formats induce cell death in GD2-positive lines but not GD2-negative lines in the selected concentration range. Importantly, direct correlation was observed between the level of GD2 expression on the cells and cytotoxic effects, with a more pronounced activity for the minibody-based FDC. The antitumor activity of the FDCs was analyzed in the GD2-positive B78-D14 syngeneic mouse cancer model. The research was supported by the Russian Science Foundation grant 21-74-30016.

P-01.1-121
Cannabidiolic acid targets and inhibits translation initiation factor 2A to remodel the proteome in glioblastoma

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Cannabinoids have recently been recognized as possible therapeutic tools for tumor therapy, especially for CNS tumors[1], however their mechanism is poorly understood. Therefore, we focused on

defining the mechanism of action of cannabidiolic acid (CBDA) in a glioblastoma cell line (U87MG). Performing Drug Affinity Responsive Target Stability (DARTS) assay, we identified eukaryotic initiation factor 2A (EIF2A) as a CBDA interactor. This result was confirmed by western blot and CETSA (Cellular Thermal Shift Assay). A proteolysis proteomic analysis was used to characterize the protein region involved in the interaction with CBDA; obtained results indicated that the EIF2A C-terminal portion 460-480 plays a critical role in this molecular recognition. Hence, as EIF2A is involved in protein translation[2], pSILAC (pulsed stable isotope labeling by amino acids in cell culture) analysis was performed to assess the impact of CBDA treatment on protein expression. This nascent proteome investigation in 2D and 3D cell model showed that CBDA had comparable effects to those seen in U87MG cells with siRNA-based EIF2A silencing, both in terms of overall protein expression reduction and biological effects, suggesting that CBDA specifically inhibits EIF2A. In a 2D cell culture, GSEA analysis of the proteome data indicated the activation of the EGF/EGFR signaling (p-value-4.6 E^{-9}) and hallmark epithelial-mesenchymal transition (p-value-2.42 E^{-9}) while 3D culture shows the hallmark mTORC1 signaling (p-value-6.9 E^{-6}). The CBDA inhibition decreases MOB2 expression and increases patient survival, as shown in the TCGA Glioblastoma multiforme (GBM) cohort. These findings strongly suggest CBDA as an efficient EIF2A inhibitor in glioblastoma, indicating that this molecule may be a good lead for developing novel medications for use in the co-treatment of patients with central nervous system cancers. [1]Cristino L et al. (2019) Nat Rev Neurol 16,9–29; [2]Komar AA et al. (2020) Int J Mol Sci 21,2054

P-01.1-122
Obtaining cell models with translocations involving the KMT2A gene

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Acute leukemia is an oncogenic disease 10% of which are associated with *KMT2A* translocation with negative prognosis. The translocation disrupts the connection of the chromatin binding part with the effector part of KMT2A (lysine histone methyltransferase) protein. Genes with different functions are fused to *KMT2A* gene as a result of translocations, however only 3 of more than 100 known partner genes are responsible for almost 90% of the cases. To understand the mechanisms of *KMT2A*-induced leukemias, we created cell models with different translocations. Since translocations form in only some of the small percentage of transfected cells, we are looking for an approach that allows selection of cells with the rearrangement most efficiently. We developed and compared several approaches forobtaining cell lines with chromosomal rearrangements. We created 2 double-strand breaks in rearranging genes, stimulated homology-directed repair, labeled cells with translocations and sorted them. Then, the genes of selective markers flanked by LoxP are removed. We started from transfection cells with 4 plasmids encoded Cas9, 2 guide RNAs and a selective cassette between the homology arms, which made HDR possible and labeled such cells. However, this approach had low efficiency. Then we integrated both gRNA genes and additional red fluorescent protein in a Cas9-encoded plasmid wit the aim of selecting transfected cells. After detection of the translocation event, we removed the selective cassette by Cre-recombinase. However, this approach takes up to several months due to transient expression of selective markers. To accelerate the selection of cells with translocation, we applied a gene trap strategy. The selective cassette contains a splicing acceptor and expresses only after integration into the target intron of *KMT2A* gene. This method is suitable to create a panel of cell lines with different *KMT2A* translocations. This work was supported by the Russian Science Foundation under Grant No 19-75-10056

P-01.1-123
Interactions of antitumor unsymmetrical bisacridines with ABC transporters: MDR1, MRP1 and MRP2

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Up-regulation of ATP binding cassette (ABC) proteins leads to increased drug release, which reduces chemotherapeutic effect and is one of the reasons for multidrug resistance (MDR). Unsymmetrical bisacridines (UAs) are novel, patented anticancer drugs, undergoing extensive preclinical studies. UAs interacted with DNA G-quadruplexes, inhibited the c-Myc protein in some cancer cells and subsequently induced apoptosis. The aim of the presented studies was to evaluate whether UA compounds are the substrates for ABC proteins, MDR1, MRP1 and MRP2, and if they can influence the expression of selected pumps in LS174T and HCT116 colon and H460 lung cancer cells. The cytotoxicity of the tested derivatives: C-2028, C-2041, C-2045 and C-2053 revealed that their IC_{80} values varied between 0.03 and 0.36 μM. Only in the case of LS174T colon cells treated with C-2045 the IC_{80} was much higher (1.3 μM). These colon cells possess the UGT1A10 enzyme active in the glucuronidation of C-2045 (RT-PCR and HPLC analysis), what likely decreases the effectiveness of C-2045 in these cells. An ATPase assay using the vesicles with MDR1, MRP1 and MRP2 transporters showed that native UAs were substrates for the MDR1 pump, but not for MRP1 and MRP2. Furthermore, RT-PCR analysis revealed variable influence of bisacridines on the expression of the studied pumps. In LS174T cells, C-2045 compound increased the expression of MDR1, MRP1 and MRP2, while other derivatives demonstrated weak impact on these transporters. Contrary, in HCT116 and H460 cells UAs generally caused slight decrease or no effect on the expression of the studied ABC pumps. Interestingly, in H460 cells, C-2041 significantly downregulated the expression of MDR1, while C-2045 upregulated. Summing up, we demonstrated the influence of UAs on the activity and expression level of selected ABC pumps what can be beneficial in future therapy. These studies were supported by the National Science Centre, Poland, No. 2019/33/B/NZ7/02534.

P-01.1-124
Integrin-bearing melanoma ectosomes promote migration of recipient vascular endothelial cells

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The transfer of integrins by tumor-derived ectosomes might enhance proangiogenic phenotype of recipient endothelial cells.

This study evaluated αvβ3 and αvβ5 integrin-bearing ectosomes as a proangiogenic factor. Ectosomes were isolated from WM793 and WM1205Lu melanoma culture conditioned media by low-vacuum filtration dialysis followed by centrifugation at 18,000 x *g*. Melanoma cells and ectosomes were analysed for αvβ3 and αvβ5 integrin expression by western blot. Changes in total and surface integrin expression in HUVECs were assessed by western blot and flow cytometry after 18 h of incubation with WM793 and WM1205Lu ectosomes. Migratory properties of ectosome-treated HUVECs were analyzed in wound healing assay in the presence of anti-integrin antibodies or RGD peptides (Echistatin and Cilengitide). Higher expression of αvβ3 integrin in WM1205Lu cells and ectosomes vs WM793 cells and ectosomes was observed, whereas αvβ5 integrin expression showed no significant differences. Total and surface expression of αvβ3 integrin was increased in HUVECs after incubation with WM1205Lu ectosomes but not with WM793 ectosomes. An increase in total αvβ5 integrin expression was observed only after incubation with WM1205Lu ectosomes, while αvβ5 surface expression remained unchanged. In a wound healing assay, incubation of HUVECs with WM793 or WM1205Lu ectosomes caused 2,1- and 2,8-fold increases of the wound closure rate, respectively. When RDG peptides or anti-integrin antibodies were used for incubation, the effect of ectosomes on HUVEC migration was significantly reduced It suggest that αvβ3 and αvβ5 integrins transferred by ectosomes act synergistically with those expressed by HUVECs. Summing up, melanoma cells are able to enhance migratory phenotype of HUVECs by transferring αvβ3 and αvβ5 integrins via ectosomes. Functional effect exerted by ectosomal integrins on endothelial cells points to their relevance in development of integrin-targeted antiangiogenic therapies for melanoma.

P-01.1-125
Investigation of CD24, CD44, VEGFa and MGMT expression levels in serum samples of glioblastoma patients

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Glioblastoma multiforme (GBM) is the most aggressive primary brain tumor. Despite surgical resection and subsequent chemotherapy and radiotherapy, GBM with a poor prognosis results in approximately 12–15 months of survival. CD24, which is among the GBM stem cell markers, has been associated with many types of cancer, including solid tumors and GBM. CD44 protein expression, which correlated with the molecular subtype of GBM, was found to be positively associated with glioblastoma radiation resistance. Vascular endothelial growth factor (VEGF) promotes tumorigenesis and angiogenesis of human GBM stem cell-like cells whereas excessive expression of VEGF is associated with poor prognosis in glioma. O6-methylguanine-DNA methyltransferase (MGMT) gene promoter methylation, which causes a decrease in MGMT protein expression, plays a role in predicting the positive response to temazolomide. Therefore, determination of MGMT gene expression levels is crucial for the use of alkylating agents as a therapy option. In our study, CD24, CD44, VEGFa and MGMT protein interactions, which are thought to be related to poor prognosis in GBM, were investigated using ELISA method in serum samples obtained from peripheral blood of 10 healthy individuals and 70 GBM patients. The data were analyzed statistically, together with information about patients' survival rate, response to treatment, recurrence and prognosis of the disease. The level of CD24 protein in patient serum samples was found to be statistically significantly higher than healthy serum samples ($P = 0.039$). MGMT protein level was found to be significantly correlated with CD24 and CD44 protein levels ($P = 0.000$). In conclusion, CD24, CD44 and MGMT protein associations can be suggested as GBM prognostic and predictive biomarkers.

P-01.1-126
Recombinant immunotoxin targeting CD64 is cytotoxic to acute myeloid leukemia cells *in vitro*

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Among hematologic malignancies, acute myeloid leukaemia (AML) remains an unmet medical need as it is primarily treated with chemotherapy, which is associated with serious side effects. Therefore, antibody-based targeted therapy is preferred as it can target and specifically eliminate malignant cells. An immunotoxin (IT) is a chimeric molecule consisting of targeting molecule and a toxic component that specifically kills the target cells. H22 (scFv)–ETA' is an immunotoxin consisting of a humanised single chain fragment (scFv) antibody and a truncated version of Pseudomonas exotoxin A (ETA'). H22(scFv)–ETA' was recombinantly expressed in *E. coli* Bl21 (DE3) and channelled into the periplasmic space and purified by metal ion affinity chromatography and size exclusion chromatography. The cytotoxic efficacy of H22(scFv)–ETA' was evaluated by annexin V bioassay and binding assays using flow cytometry. We also constructed a diagnostic fusion protein version of H22(scFv)–ETA' in which the toxic component ETA' was removed and replaced with the protein SNAP–tap to generate H22(scFv)–SNAP. SNAP–tag enables efficient tumour targeting and diagnosis of molecular biomarkers of cancer. We have shown that H22(scFv)–ETA' is cytotoxic to AML cancer cells expressing CD64. CD64 is highly expressed on monocytic blast cells in patients with AML and not in normal haematopoietic stem cells, making it a suitable target antigen. H22(scFv)–ETA' showed significant toxicity *in vitro* against CD64-positive cell lines HL-60 and U937. Binding studies showed specific binding to both HL-60 and U937 cell lines. We also demonstrated specific binding of H22(scFv)–SNAP to HL-60 and U937. The development of a successful scale-up production of H22(scFv)–ETA' and H22(scFv)–SNAP is crucial for the production of large quantities to enable further preclinical/clinical studies. The current phase of our study is focused on optimising the productivity of H22(scFv)–ETA' and its large-scale production.

P-01.1-127
Galectin-8 induces epithelial–mesenchymal transition in a model of fully polarized epithelial cells

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Epithelial cells that have apical/basolateral polarized phenotype can undergo changes towards a more mesenchymal migratory phenotype during processes of organogenesis, tissue repair and cancer development. Factors able to induce this epithelial-mesenchymal transition (EMT) are therefore of great interest. Galectin-8 (Gal-8) is a carbohydrate binding protein that is overexpressed in several tumors and upon secretion by a nonconventional mechanism can elicit a variety of cellular responses interacting with cell surface glycoproteins. Tumor cells usually co-exist with fully differentiated cells within the epithelia where they are growing. Our previous studies show that Gal-8 promotes EMT in sub-confluent Madin-Darby Canine Kidney (MDCK) epithelial cells by trans-activating the epidermal growth factor receptor (EGFR). However, whether fully differentiated epithelial cells might undergo similar Gal-8-induced changes remain unknown. MDCK cells were grown in matrigel to form three-dimensional (3D) cysts with their apical surfaces facing a central lumen and their basal surfaces contacting the extra cellular matrix and exposed to added recombinant Gal-8. Apical and basolateral markers and cyst structure were analyzed by confocal immunofluorescent microscopy. Gal-8 induced loss of apical/basolateral polarity and cyst structure, reverted by EGFR inhibitors. In conclusion, Gal-8 can induce EGFR-mediated EMT in fully polarized epithelial cells and therefore might potentially contribute to transform normal epithelial cells adjacent to tumor cells overexpressing Gal-8. Acknowledgements: FONDECYT 1181907 and 1211829, ANID/BASAL grants FB210008 and ACE210009, BASAL FB 210008.

P-01.1-128
Silencing of ABCC6 transporter alters the expression level of proteins involved in adhesion and migration of hepatocarcinoma cells

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ABCC6 is a member of the superfamily of ATP-binding cassette (ABC) transporters whose mutations cause Pseudoxanthoma Elasticum, an autosomal recessive metabolic disease characterized by progressive ectopic mineralization processes. As extracellular ATP supplier, ABCC6 might contributes to the modulation of the purinergic pathway thus playing a potential role in cancer cell biology [Bisaccia F et al. (2021) Int J Mol Sci 22, 1-12]. Silencing or inhibition of ABCC6 activity caused a rearrangement of actin filaments in the cytoskeleton, reduced migration rate in hepatocarcinoma cells (HepG2) and decreased the expression of CD73 (ecto-5′-nucleotidase) suggesting that the transporter could be a key regulator in the modulation of purinergic signaling by the reduction of extracellular ATP [Martinelli F et al. (2018) Front Mol Biosci 5, Ostuni A et al. (2020) Cells 9, 6]. RNA-Seq analysis of Abcc6 silenced cells allowed to highlight some of the molecular mechanisms underlying the observed effects. Transcriptome analysis, validated by Real-Time PCR and western-blotting, showed a dysregulation of gene encoding for proteins that play a key role in cell adhesion and migration confirming that ABCC6 could be a potential therapeutic target for anti-metastatic treatment.

P-01.1-129
Targeting peroxiporin AQP3 impairs hydrogen peroxide diffusion and cell proliferation in melanoma

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Peroxiporins are transmembrane proteins from the aquaporin (AQP) water channels family that facilitate H_2O_2 across biological membranes. H_2O_2 is a reactive oxygen species (ROS) with dual intracellular action: at low concentrations, it acts as a signaling molecule with an important role in redox signaling, inducing cell growth, development, and signal transduction pathways, and at high concentrations, it reacts with various cellular targets triggering oxidative stress and leading to cell damage or even cell death. Oxidative stress is a common factor in cancer biology and studies showed that peroxiporins have altered expression in several types of cancer, such as melanoma. In melanoma, melanocytes present uncontrolled proliferative characteristics and the peroxiporin AQP3 is found overexpressed. In this study, MNT-1 cells' membrane permeability to water, glycerol and H_2O_2 was evaluated by epifluorescence microscopy. Taking advantage of the well-known inhibitory effect of the gold compound Auphen on AQP3, here, we assess the inhibitory effect of a new series of gold compounds resultant from Auphen modifications aiming to improve its potency and selectivity ($C^{CO}N$, $C^{NH}N$ and $C^{CH2}N$). The inhibitory effect of gold compounds was investigated in cell proliferation and adhesion by the colorimetric MTT assay. From this series of compounds, $C^{NH}N$ and $C^{CH2}N$ have shown to inhibit AQP3 peroxiporin activity (H_2O_2 transport) in 88% and 75%, respectively. Interestingly, only $C^{CH2}N$ interfered with the AQP3 glycerol permeability. However, all the three inhibitors were effective in decreasing MNT-1 proliferation, although $C^{CO}N$ and $C^{CH2}N$ induced higher reduction of proliferation (19% and 38%, respectively). Overall, our data show that the impairment of glycerol and H_2O_2 permeability via AQP3 can affect melanoma progression, unveiling AQP3 a promising drug target for cancer therapies.

P-01.1-130
Gold compounds inhibitory effect on aquaporin-3 impairs melanoma cell migration

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Aquaporins (AQPs) are transmembrane proteins that facilitate the diffusion of water and glycerol across cell membranes. Aquaglyceroporin AQP3 was found to be overexpressed in human tumors, including melanoma, being involved in cell proliferation and migration. Thus, AQP3 has emerged as a novel therapeutic target for cancer, prompting the discover of AQP modulators with anticancer properties. The gold compound Auphen was reported as a potent AQP3 inhibitor, affecting cell proliferation in AQP3-expressing cells. Here, we tested a gold compound series resulting from Auphen modifications as an attempt to boost potency and selectivity towards AQP3 and investigate their impact on melanoma cell migration. The inhibitory effect of gold compounds on aquaglyceroporin glycerol permeability was evaluated in yeast cells transformed with a plasmid encoding individually human aquaglyceroporin paralogs (AQP3, AQP7, AQP9 and AQP10) through stopped-flow fluorescence. The effect of the compounds on melanoma cell viability and cell migration was assessed by MTT assay and wound closure assay, respectively. Permeability assays showed that RBA29 and CCON were the most potent AQP3 inhibitors, although weaker than Auphen, followed by CCH2N and CNHN, with STAM013 exhibiting a weaker effect. Moreover, RBA29, CCON and CCH2N significantly inhibited AQP10 glycerol transport, similar to Auphen, showing weaker effect on AQP7 and AQP9. The effect of gold compounds on melanoma cell migration, where AQP3 is highly expressed, revealed RBA29 and CCON as the strongest inhibitors (impairment of 74.3% and 73.6%, respectively), followed by CNHN (52.4%). STAM013 and CCH2N almost did not affect cell migration suggesting that gold compounds impact on cell migration might be due to their ability to inhibit AQP3. Therefore, our data revealed RBA29 and CCON as novel potent AQP3 inhibitors that strongly impair melanoma cell migration, unveiling their potential as anticancer drugs in tumors with high expression of AQP3.

P-01.1-131
Regulating asparagine-linked protein glycosylation is a new target in breast cancer research

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Asparagine-linked (*N*-linked) protein glycosylation is a dynamic process regulated by intra- and/or extra-cellular *milieu*. It is fundamental to normal somatic cells and cells undergoing oncogenic transformation. Our laboratory has demonstrated when the process is upregulated the cell proliferation is enhanced and reduced in their absence. Thus, "Glycome – the hidden code in biology" plays a central role. Breast cancer is a global health problem affecting nearly 2.1 million women each year. The disease is multi-factorial, exists in four molecular subtypes, has no geographical boundaries, affects women from low-, medium-, and high-income countries, women with diverse ethnic backgrounds and faith. The current death rate is approximately 500,000 women per year and is expected to surpass 1.04 million in 2040 if no major changes in prevention or treatment are forthcoming. The generalized treatments cannot cure the disease. Since all breast cancer cells and their microvasculature express *N*-linked glycoproteins with diverse structures, i.e., the *N*-glycans, and play a critical role in angiogenesis and tumor progression, we have tested a small molecule and a natural product, tunicamycin, that blocks the catalytic activity of *N*-acetylglusosaminyl 1-phosphate transferase (GPT), a specific step in protein *N*-glycosylation pathway in the endoplasmic reticulum (ER). The outcome has been overwhelmingly exciting. Tunicamycin inhibits quantitatively *in vitro* and *in vivo* angiogenesis, and inhibits the breast tumor progression of multiple subtypes in pre-clinical mouse models with no toxicity. Mechanistic details support ER stress-induced cell cycle arrest followed by unfolded protein response (*upr*) signaling mediated apoptotic cell death. The process is multi-genic and requires Glycome. It interferes with the Wnt signaling as well. Thus, the conclusion is tunicamycin is going to be a stand-alone glycotherapy treating breast cancer of all subtypes.

P-01.1-132
Cell migration and wound healing assays revisited: proposal of an improved approach

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Cell migration is a central process in the development and maintenance of multicellular organisms. However, it can be dysregulated in many pathological conditions, such as cancer, where it plays a critical role in the development of distant metastasis. Wound healing assays are commonly used to quantify cell migration under different physiological and therapeutic conditions. The assay consists in creating a physical wound in a confluent cell monolayer, using a pipette tip, followed by monitorization of cell motility with live cell imaging. In this work, we used a highly aggressive, invasive and poorly differentiated triple-negative breast cancer (TNCB) cell line (MDA-MB-231). The dysregulation of Wnt/β-catening signaling pathway [1] is highly associated with TNBC migration and, consequently, cancer malignancy. iCRT-3 and niclosamide are two known inhibitors of this pathway, thus, were used as standards. 24 h after seeding, wound was performed, and non-adherent cells were removed. Compounds were diluted in serum-free media and added in concentrations ranging from 10 to 100 μM. Cells were observed under the microscope to set wound area (μm^2) at 0 h and for the following 48h. The area of the wound was measured using an adapted script [2], allowing a quick and accurate data acquisition, and the % of wound closure was calculated. The measurement of the healing speed, calculated from the slope in a plot of area of the wound against time, allows the quantitative comparison of wound healing across different concentrations and compounds. The results confirm that treatment with both inhibitors slows the

healing process. iCRT-3 requires higher concentrations (100 μM), while niclosamide halts the healing process at concentrations as low as 10 μM. This platform can be easily adapted for other compounds and cell lines. [1] Ryu W et al (2020) Experimental and Molecular Medicine 52, 832-842 [2] Suarez-Arnedo A et al (2020) Plos One 15, e0232565

P-01.1-133
Prospection of markers associated with proteolytic processing in biological samples of patients with melanoma

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Melanoma is aggressive skin cancer and a lethal melanocytic neoplasm with an increasing annual number of cases. Since the protein composition (proteome) of tumoral cells may be regarded as a description of the cellular status, the evaluation of protein expression patterns in biopsy samples may lead to the discovery of cancer-related markers and new drug targets. In the context of tumoral development, proteolytic processing plays an important role in the dispersion of tumor cells to distant sites (metastasis) as well as in mediating irreversible signaling events. The main goal of this project is to probe the Biobank of the São Paulo of Cancer Institute (ICESP) aiming at the prospection of molecular markers derived from proteolytic processing events. In this work, we have applied an N-terminomics approach to investigate proteolytic processing events in plasma samples of patients with early/localized or systemic melanoma. Plasma samples from melanoma patients were subjected to gelatin-zymography and significant activity was found in samples from systemic disease, indicating active gelatinases in such samples. Albumin and IgG-depleted plasma samples were submitted to Terminal Amine Isotopic Labeling of Substrates (TAILS) and the corresponding prime-side peptides were analyzed by LC-MS/MS and database searching. We identified > 100 unique peptide signatures, corresponding to proteolytically processed substrates in each disease state (early/localized or systemic melanoma): the majority of them were derived from ordinary plasma proteins, such as fibrinogen, complement proteins, apolipoproteins among others. Prime and non-prime subsite specificity mapping of the peptide set suggested active serine protease enzymes. In conclusion, data obtained in this work provided a systems-wide profile of proteolytic signaling in melanoma, with important translational potential for disease prognosis. Funding: Fundação de Amparo à Pesquisa do Estado de São Paulo–(FAPESP) grant#2019/072828

P-01.1-134
Adapted suspension tumor cells rebuild metabolic pathways for survival

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Metastatic spread of cancer cells is the main cause of cancer-related death. As cancer cells adapt themselves in a suspended state in the blood stream before penetration and regrowth at distal tissues, understanding their survival strategy in an anchorage-independent condition is important to develop appropriate therapeutics. We have previously generated adapted suspension cells (ASCs) from parental adherent cancer cells to study the characteristics of circulating tumor cells. In this study, we explored metabolic rewiring in MDA-MB-468 ASCs to adapt to suspension growth conditions through extracellular flux analyses and various metabolic assays. We also determined the relationship between AKT activation and metabolic rewiring in ASCs using the AKT inhibitor, MK2206. ASCs reprogramed metabolism to enhance glycolysis and basal oxygen consumption rate. RNA-sequencing analysis revealed the upregulation in the genes related to glycolysis, tricarboxylic acid cycle, and oxidative phosphorylation. The changes in the metabolic program led to a remarkable dependency of ASCs on carbohydrates as an energy source for proliferation as compared to parental adherent cells (ADs). AKT activation was observed in ASCs and those generated from pancreatic and other breast cancer cells, and AKT activation inhibition in ASCs decreased glycolysis and oxygen consumption. AKT activation is an important strategy for obtaining energy through the enhancement of glycolysis in ASCs. The regulation of AKT activity and/or glycolysis may provide a strong therapeutic strategy to prevent the metastatic spread of cancer cells.

P-01.1-135
Inhibition of PD-L1 expression and restoration of T cell proliferation in tumor-coculture induced by NDRG2 expression in breast cancer cells

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The aim of the present study was to evaluate the effect of NDRG2 expression in regulating PD-L1 or PD-L2 on malignant breast cancer cells. Overexpression and knockdown of the NDRG2 gene in human and mouse cancer cells were applied and quantitative real-time PCR and Western blot analysis were performed. T cell proliferation and TCGA analysis were conducted to validate negative correlation of the PD-L1 expression with the NDRG2 expression. We found that NDRG2 overexpression inhibits PD-L1 expression in human breast cancer cells through NF-κB signaling. NDRG2 overexpression in 4T1 mouse breast cancer cells followed by PD-L1 downregulation could block the suppressive activity of cancer cells on T cell proliferation and knockdown of NDRG2 expression enhanced the expression of PD-L1, leading to the inhibition of T cell proliferation by tumor cell coculture. Finally, we confirmed from TCGA data that PD-L1 expression in basal and triple-negative breast cancer patients was negatively correlated with the expression of NDRG2. Intriguingly, linear regression analysis using TNBC cell lines showed that the PD-L1 level was negatively associated with the NDRG2 expression level. Our findings demonstrate that NDRG2 expression is instrumental in suppressing PD-L1 expression and restoring PD-L1-inhibited T cell proliferation activity in TNBC cells.

P-01.1-136
Hypoxia-inducible factors in breast cancers and their association with clinicopathological characteristics

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Hypoxia is a common feature associated with many types of cancer. The activity of the hypoxia-inducible factors (HIFs), the critical element of response and adaptation to hypoxia, enhances cancer hallmarks such as suppression of the immune response, altered metabolism, angiogenesis, invasion, metastasis, and more. The HIF-1α and HIF-2α isoforms show similar regulation characteristics, although they are active in different types of hypoxia and can show different or even opposite effects. Breast cancers present several unique ways of non-canonical hypoxia-inducible factors activity induction, not limited to the hypoxia itself. The hypoxia-induced factors' level has been recognized to correlate with a few breast cancer characteristics. Earlier studies did not observe the correlation between HIF-1α expression and clinicopathological characteristics such as molecular type, receptors status, tumor size, or age, however correlation with lymph node status, and higher expression level of HIF-1α in samples with higher age and bigger tumor size has been reported. The HIF-2α level was reported to correlate with HER2 status. The study aimed to determine the level of both HIFs' isoforms in clinical samples of breast cancer and corresponding normal tissue and investigate their association with various clinicopathological characteristics such as molecular type, grade, TNM staging, tumor size, and receptor status. Another investigated correlation includes features such as age, BMI, prior hormone therapy, and contraception. Including an extensive selection of clinicopathological characteristics enables a better understanding of the role of the hypoxia-inducible factors in breast cancers.

P-01.1-137
HIRA loss transforms FH-deficient cells

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Fumarate hydratase (FH) is a mitochondrial enzyme that catalyses the reversible hydration of fumarate to malate in the TCA cycle[1]. Biallelic inactivation of FH leads to hereditary leiomyomatosis and renal cell carcinoma (HLRCC), a cancer syndrome characterised by the presence of benign tumours of the skin and uterus, and a highly aggressive form of renal cancer[1]. Although HLRCC tumours metastasise early in the disease course, FH-deficient mice develop premalignant cysts in the kidneys, rather than overt carcinomas[2]. Interestingly, these cysts are positive for the tumour suppressor and senescence marker p21, arguing that senescence could hinder full-blown transformation in Fh1-deficient cells[3]. Consistent with this hypothesis, HLRCC patients harbour the epigenetic suppression of Cdkn2a, another key player of senescence[4]. However, how Fh1-deficient cells overcome these tumour suppressive events during transformation or if additional oncogenic events are required is still unknown. Here, we perform a genome-wide CRISPR/Cas9 enrichment screening to identify genes that, when ablated, enhance the proliferation of Fh1-deficient cells. We found that, when depleted, the Histone Cell Cycle Regulator (HIRA) enhances proliferation and invasion of Fh1-deficient cells in vitro and promotes transformation *in vivo*. Mechanistically, we determine that Hira loss enables the activation of MYC and its target genes, specifically in Fh1-deficient cells. The discovery and study of these oncogenic events will be instrumental for understanding mechanisms of tumorigenesis in HLRCC and the development of targeted treatments. 1. Tomlinson, I. P. M. et al. Nature Genetics 30, 406–410 (2002). 2. Pollard, P. J. et al. Cancer Cell 11, 311–319 (2007). 3. Zheng, L. et al. Nature Communications 6, (2015). 4. Cancer Genome Atlas Research Network. New England Journal of Medicine 374, 135–145 (2016).

P-01.1-138
Impact of BMI-1 and RING1 silencing/ inhibition on the autophagy process in endometrial cancer cells

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Autophagy is an intracellular degradation mechanism where cytoplasmic components (e.g. damaged or outdated organelles, single proteins or aggregates) are degraded by the lysosome. Autophagy dysregulation is associated with etiopathology of several diseases including cancer. However, the role of autophagy in cancer is ambiguous. It has been shown that autophagy inhibits cancer development, especially in the early stages of initiation. On the other hand, in late stages of tumor development autophagy is thought to be a mechanism supporting tumor progression and metastasis. Recent studies suggest that epigenetic changes can play an important role in the regulation of autophagy genes in tumors. BMI-1 and RING1 proteins are components of Polycomb repressive complex 1 (PRC1) that is commonly involved in inhibition of gene expression by ubiquitinylation of histone H2A. There is some evidence that BMI-1 and RING1 downregulation in cancer cells can induce autophagy. The aim of the study was to determine how downregulation of BMI-1 and RING1 via siRNA or BMI-1/RING1 inhibitors PTC-209 and PRT4165 affects autophagy in endometrial cancer cells HEC-1A and Ishikawa. PTC-209 is a novel small-molecule selective inhibitor of BMI1 expression that has been reported to interfere with post-transcriptional regulation of BMI-1 and down-regulate BMI-1 production. PRT4165 inhibits histone H2A E3 ubiquitin ligase activity of RING1A and its more active paralogue, RNF2. Moreover, the effect of PRC1 inhibition was tested in different glucose concentration. We have found that silencing and inhibition of PRC1 proteins can differently affect the autophagy mechanism in endometrial cancer cells which might be depending on molecular context. Moreover, the results showed significant

differences depending on the glucose concentration. It might suggest that PRC1 proteins play and important role in regulation of autophagy.

P-01.1-139
Photodynamic therapy with octahedral molybdenum clusters

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Molybdenum clusters with $\{Mo_6I_8\}^{4+}$ core produce singlet oxygen upon irradiation with a wide range of sources including blue light and X-rays. Their biocompatibility and low toxicity allow the application of Mo clusters in the photodynamic therapy (PDT) and X-ray induced photodynamic therapy of cancer cells. The testing of photosensitive properties of different modifications of molybdenum clusters was the main topic of the project. Molybdenum cluster $Na_2[Mo_6I_8(C_{17}H_{28}N_2O_4)_6]$ (1) have a high phototoxicity after 460 nm irradiation with IC_{50} 37 ± 4 nM in presence of serum on HeLa cells and surprisingly with no detected dark toxicity (> 50 μM). The cluster is localized on cell membrane with low interaction with proteins in the medium (IC_{50} 25 ± 5 nM HeLa, without serum). Nanoparticles made of cluster $Na_2[Mo_6I_8(N_3)_6]$ + Bis-dPEG®11-DBCO (1:4) (2) are phototoxic (IC_{50} 17.4 μg/mL, TRAMP C2 cells), with no detected dark toxicity (> 1.48 mg/mL). More importantly, radiotoxicity of (2) was found after exposure to 1 mg/mL nanoparticles detected as a significant decrease of proliferation after X-ray irradiation. Nanoparticles were localized in the lysosomes after 2 hours of incubation. In conclusion, molybdenum-based compounds have a very promising perspective in cancer therapy due the water stability, low toxicity, high phototoxicity, and radiotoxicity. This work was supported by grant no. 21-16084J given by the Czech Science Foundation.

P-01.1-140
On the quest for new aquaporin modulators for therapeutic applications

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Aquaporins (AQPs) are membrane water channels that facilitate the passive transport of water, glycerol and other small solutes across cell membranes in response to osmotic gradients. These proteins are essential for water homeostasis and energy metabolism and appear aberrantly expressed in a wide range of clinical disorders including cancer. For that reason, AQPs have emerged as promising pharmacological targets. However, most reported modulators are non-selective and toxic, which make them difficult to be used in clinical trials. The urgent need to discover efficient and selective modulators for therapeutical use, led us to develop an innovative structure-based *in silico* computational workflow to identify AQPs inhibitors. In this approach, the combination of ensemble Molecular Docking, coupled to Molecular Dynamics and Molecular Mechanics Poisson-Boltzmann Surface Area (MM/PBSA) calculations, allows the trustful discrimination between active/inactive compounds with inhibitory effect on AQPs function. From a proprietary compound database from Sigma-Aldrich, we were able to identify one polyphenol derivative (RoT) as a promising AQP inhibitor with proven drug-like properties. For the experimental validation of this compound, we investigated its inhibitory effect on AQP1 and AQP3 by measuring water (Pf) and glycerol (Pgly) permeability of human red blood cells that endogenously express these AQPs, using stopped-flow spectroscopy. Our results showed that RoT strongly inhibits glycerol permeability via AQP3 (IC50 = 6.02 ± 0.13 μM) and significantly reduces water permeability via AQP1 (IC50 = 26.28 ± 0.02 μM). Considering that AQP1 and AQP3 are both overexpressed in cancer tissues, these results warrant further studies to evaluate RoT anti-cancer properties in cellular cancer models.

P-01.1-141
Role of let-7d and miR-18a expression in biology, radio- and chemosensitivity of head and neck cancers

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Head and neck cancers (HNSCC) are mostly squamous cell carcinomas, difficult in treatment due to resistance to chemo- and irradiation therapy resulting in a low percentage of 5-year survival of the patients. Development of molecular markers that would enable personalization of treatment could help overcome this problem. The *aim of the study was the* evaluation of the effect of let-7d and miR-18a on biology and radio- and chemosensitivity of HNSCC. SCC-25 and SCC-040 cell lines with overexpression of let-7d, miR-18a and both miRNAs together were analyzed regarding their influence on proliferation, migration and response to ionizing radiation and chemotherapeutics. The changes in gene expression were determined by microarray analysis and potential miRNAs' targets were identified. Obtained results were verified using the TCGA data. The increased proliferation ratio for lines with expression of let-7d or miR-18a and decreased one for lines with expression of both miRNAs was indicated. No changes in cell migration were observed. Increased sensitivity to irradiation was demonstrated only for SCC-040-7d and 7d+18a, and for SCC-040-7d and SCC-25-7d+18a to 5-FU and doxorubicin. let-7d and miR-18a affect the expression of genes involved in metabolic processes, regulation of biological and cellular processes or response to stimuli. Regarding the TCGA data, let-7d directly regulates SLC16A14, CBL, CPEB4, ITGB8, DSC3, HAS2 and HBEGF, and miR-18a: TRIM2 and PSAT1. Patients with high expression of both miRNAs have shorter disease-free survival time and more advanced types of cancers. In *conclusion,* let-7d and miR-18a cause the acquisition of a specific phenotype by the cell and regulate its response to irradiation and chemotherapeutics. Potentially, let-7d and miR-18a can be used as diagnostic, prognostic and predictive markers in clinical practice.

P-01.1-142
miR-18a as a potential biomarker in treatment personalization of invasive cutaneous melanoma

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Invasive cutaneous melanoma is an aggressive form of cancer undetected until the advanced stage, when its mortality is high. For this reason, it is necessary to identify the specific biomarker which allows the detection of melanoma at an early stage. In many studies, the potential of miRNAs as a promising biomarker was noticed. The biological role of miR-18a-5p/3p and their utility as biomarkers were examined. Expression levels of miR-18a-5p/3p were analyzed depending on the clinicopathological parameters based on the TCGA data. Potential target genes for miR-18a-5p/-3p were verified using the StarBase v3.0 database. Next, proliferation, cell cycle, response to irradiation was checked using the MEVO cell line with overexpression of miR-18a-5p/3p. The changes in gene expression were checked using RNAseq and qRT-PCR methods. No significant differences between clinicopathological parameters and levels of miR-18a-5p/3p were indicated. Longer patients' DFS and OS were associated with low miR-18a-5p expression. Negative correlation for over 330 genes in patients with melanoma, which are associated with cell adhesion, migration, apoptosis, negative regulation of transcription or alternative splicing was noticed and BTG2 as direct target for miR-18a was indicated. In the case of cell line, higher expression of miR-18a-5p/3p was displayed more often in cells in S and G2/M phase. They are more sensitive to irradiation. The obtained results show that miR-18a could be a potential prognostic biomarker, which is independent of any clinical parameters. miR-18a targets are involved in many important processes and has potential role in response to the treatment.

P-01.1-143
Role of survivin on radioresistance and metabolism in the context of head and neck squamous cell carcinoma

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Recent studies point to survivin as a radioresistance marker. We aimed to determine at which radiotherapy cycle survivin is a key factor to promote radioresistance and if it is related to the metabolic status. Survivin and metabolic markers mRNA expression was analyzed in normal, adjacent and tumoral mucosa from a well-characterized human cohort (33 patients with HNSCC). FaDu cells cultured with 3.5, 6 or 10 mM of glucose were treated with doses of 3 Gy radiation until obtain an accumulative dose of 15 Gy. Cells were collected at 3, 9 and 15 Gy and qPCR of pro-tumoral and key metabolic genes was evaluated. AZD5582 was used as survivin inhibitor and apoptosis at 48 h was determined with the cell death detection ELISA plus kit. We show that survivin is increased in tumoral tissue and is correlated with adjacent and normal tissue levels. We also found positive associations between survivin and metabolic markers gene expression in tumoral tissue and with glucose levels. In FaDu cells, we observed increased levels of survivin expression along with radiation, especially in low concentration of glucose and without effect of hypoxia. mRNA expression of metabolic markers that were correlated with survivin in tumoral tissue follows the same pattern as survivin in the cellular model. As happened with survivin, pro-tumoral and radioresistance markers are dependent of the radiotherapy stage and glucose levels. Survivin inhibition together with radiation and high glucose levels promote a higher sensitivity to apoptosis. These findings point to survivin as a molecular link between metabolism and radioresistance.

Neurodegeneration and regeneration

P-01.2-001
Anxiolytic, antidepressant and antioxidant effects of cotinine and 6-hydroxy-L-nicotine in an Aβ-induced rat model of Alzheimer disease

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Alzheimer's disease (AD) is a progressive, unremitting neurodegenerative disease and the most common cause of dementia. Cerebral β-amyloid (Aβ) deposition, accumulation of neurofibrillary tangles of protein tau and neuronal loss are key hallmarks of AD. Besides progressive cognitive decline, neuropsychiatric symptoms, such as anxiety, depression, and apathy, were also linked to these pathological changes. Nicotine was reported to reduce anxiety and improve memory, learning and attention, but the therapeutic use in AD was limited by its cardiovascular and addictive side-effects. Here, we aim to evaluate the anxiolytic, antidepressant, and antioxidant potential of two structural related nicotine derivatives, namely cotinine (COT) and 6-hydroxy-L-nicotine (6HLN) in a rat model of AD induced by intracerebroventricular (i.c.v.) infusion of Aβ25-35 peptide. For this, COT and 6HLN were chronically and intraperitoneally administered to rats infused i.c.v. with Aβ25-35 peptide and their behavior was monitored in specific tasks. Anxiety-like behavior was assessed using elevated plus maze test (EPM) and open field test (OFT) while depressive behavior was evaluated using forced swimming test (FST). The oxidative stress biomarkers were monitored from homogenized amygdala samples. The results revealed positive effects of COT and 6HLN on behavioral changes induced by Aβ25-35 peptide in rats. Thus, both compounds intensified the locomotor activity and significantly reduced the anxiety-like behavior in EPM and OFT tasks and abolished the depressive behavior in FST task. Also, the treatment reduced the oxidative stress in the amygdala of Aβ25-35-treated rats by increasing the specific activities of antioxidant enzymes and lowering the level of protein and lipid oxidation products. Taken together, our data suggest that COT and 6HLN could represent viable therapeutic agents for ameliorating AD condition. *The authors marked with an asterisk equally contributed to the work.

P-01.2-002
Selection of an Anticalin against the transferrin receptor 1 with potential for brain drug delivery

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While the blood–brain barrier (BBB) acts as a physical barrier that prevents free entry of circulating substances to the central nervous system (CNS), including those intended for therapeutic applications, so-called molecular Trojan horses offer a promising approach for the non-invasive delivery of therapeutics to the brain. This concept relies on the application of natural or genetically engineered proteins that bind to receptors involved in the receptor-mediated transcytosis (RMT) at the BBB, in particular the transferrin receptor (TfR). The fusion of therapeutic proteins with such binding domains has been demonstrated to enable transcytosis into the brain parenchyma. Subsequent to the preparation of a recombinant homodimeric TfR ectodomain and its functional characterization, we selected a cognate Anticalin candidate via phage display and bacterial cell surface display from a random library based on the human lipocalin 2. After affinity maturation, the engineered lipocalin binds TfR with an affinity (KD) of 3.8 nM and, notably, in a non-competitive manner with the natural ligand, Transferrin·Fe^{3+}. Analysis of an array of overlapping 16-mer peptides immobilized on a membrane, thus covering the entire amino acid sequence of the murine TfR ectodomain (698 residues), revealed a single linear epitope that is recognized by the Anticalin, which maps to a surface region remote from the Transferrin-binding site in the three-dimensional structure of TfR. Due to its dynamic binding characteristics, as evidenced by real-time surface plasmon resonance (SPR) measurements, this Anticalin (T4B11LN6) shows promise as a vehicle to mediate the brain delivery of biopharmaceuticals. *The authors marked with an asterisk equally contributed to the work.

P-01.2-003
Butyrylcholinesterase with pralidoxime analogs scavenges nerve agents

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Nerve agents' (NA) neurotoxicity is primarily induced by phosphorylation of acetylcholinesterase (AChE), the key enzyme in the neurotransmitter acetylcholine (ACh) pathway. Its akin enzyme, butyrylcholinesterase (BChE), an enzyme without known essential physiological function, can serve as a protector of AChE from inhibition by NAs by binding them in a stoichiometric ratio and thus lower their concentration in the body. Current therapy, consisting of compounds containing oxime group (i.e. pralidoxime) as reactivators of phosphorylated AChE, has limited reactivation potency for phosphorylated BChE. Therefore, a search for a BChE reactivator that would create an enzyme-oxime catalytic scavenging system is still ongoing. With that purpose, in this study, several pyridinium oximes were selected, tested, and identified as efficient reactivators for cyclosarin-inhibited BChE with up to 300-fold higher overall kinetic reactivation rate than their analog pralidoxime. Also, tested oximes did not affect the viability of SH-SY5Y neuroblastoma cells upon 4- as well as 24-hours treatment. Furthermore, the cytotoxicity profiles showed that, at oxime-assisted catalytic BChE degradation of cyclosarin, in post-treatment approach, 50 % of cells were still viable, while in pre-treatment almost all of the cells were protected from cytotoxic cyclosarin effects. The most promising analogs were additionally evaluated in *ex vivo* conditions together with exogenous BChE. Obtained results demonstrated up to 80% of restored phosphorylated cholinesterase activity within a short time. Taken all together our findings offer a platform for the further antidote and BChE based scavenger's development for exposure to organophosphates.

P-01.2-004
GM1 oligosaccharide as a novel neuroprotective agent for amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by accumulation of ubiquitylated proteinaceous inclusions and progressive degeneration of upper and lower motor neurons (MNs). Although its etiopathogenesis is still poorly understood, pathogenic proteins mislocalisation, misfolding and aggregation (i.e. SOD1 and TDP-43), glutamate toxicity, oxidative stress, impaired axonal transport, mitochondrial dysfunction, and RNA processing failure have been described to play a crucial role. Additionally, a major change in glycosphingolipids metabolism and, in particular, a depletion of GM1 ganglioside has been reported in spinal cord and in neuromuscular junctions of SOD1 ASL mice. GM1 has been considered as a master regulator of the nervous system and accumulating evidence is pointing out its role in preventing neurodegeneration through its bioactive portion: the GM1 oligosaccharide (OligoGM1). Exploiting proteomic and biochemical approaches, we demonstrated that OligoGM1 directly triggers TrkA-MAPK pathway activation inducing neuronal differentiation and protection by modulating mitochondria biogenesis and function, calcium flux and redox balance in neuronal cells (Chiricozzi, Glycoconj J 2021, 38(1), pp. 101–117). Based on these outcomes, we decided to test the OligoGM1 neuroprotective potential in MNs from SOD1G93A rat embryos, an ALS *in vitro* model. By biochemical analyses, we observed that the pre-treatment with OligoGM1, as well as GM1, significantly increases neuronal survival and preserves neurite networks both in WT and in SOD1G93A MNs injured with glutamate. Additionally, the mislocalisation of TDP-43 from nucleus to cytoplasm due to glutamate exposure was significantly ameliorated and the disrupted mitochondrial network has been recovered by OligoGM1/GM1 administration. Although the precise mechanism should be investigated, these data indicate for the first time OligoGM1 as a neuroprotective molecule in ALS context.

P-01.2-005
Mice harboring the FXN I151F point mutation present decreased frataxin levels, a Friedreich Ataxia-like phenotype and mitochondrial alterations

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Friedreich Ataxia (FA) is caused by mutations in the frataxin (FXN) gene. The most prevalent mutation is a GAA expansion in the first intron of the gene which causes decreased frataxin expression. Some patients present the GAA expansion in one allele and a missense mutation in the other allele. One of these mutations, FXNI154F, was reported in cellular models to result in decreased content of mature frataxin and increased presence of an insoluble intermediate proteoform. By introducing this mutation in the murine FXN gene (I151F, equivalent to human I154F), we have now analyzed the consequences of this pathological point mutation *in vivo*. $FXN^{1151F/wt}$ heterozygous mice were obtained from the Jackson Laboratory, where CRISPR/Cas9 was used to introduce the desired mutation. Homozygous $FXN^{1151F/1151F}$ mice were obtained by crossing HET mice. Functional and biochemical analyses were performed to characterize the consequences of the mutation on frataxin and on mice. We have observed that FXNI151F homozygous mice present low frataxin levels in all tissues, with no evidence of insoluble proteoforms. Moreover, they display neurological deficits resembling those observed in FA patients. Biochemical analysis of heart, brain and cerebellum have revealed decreased content of components from OXPHOS complexes I and II, decreased aconitase activity, and alterations in antioxidant defense. These mitochondrial alterations are more marked in the nervous system than in heart, precede the appearance of neurological symptoms, and are similar to those observed in other FA models. We conclude that the primary pathological mechanism of the I151F mutation is frataxin deficiency, as in patients carrying GAA expansions. Therefore, patients carrying the I154F mutation would benefit from frataxin replacement therapies. Furthermore, our results also show that the FXN^{1151F} mouse is an excellent tool for analyzing tissue-specific consequences of frataxin deficiency and for testing new therapies.

P-01.2-006
Determination of the domain structure of DISC1 in mammalian cells identifies a region crucial for its aggregation in schizophrenia

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While the pathology of schizophrenia remains unclear, disrupted protein homeostasis has recently been suggested as its biological basis. Several proteins have now been shown to form insoluble aggregates in this condition, including Disrupted in Schizophrenia 1 (DISC1), a multi-functional scaffolding protein important for neurodevelopment. However, delineation of DISC1 domain structure has only recently been attempted using high-throughput analysis in *E. coli*, which identified four distinct domains: D, I, S and C. This data was used to confirm and refine the structure of DISC1 in mammalian cells, as well as to identify the region of DISC1 responsible for its aggregation. To do so, DISC1 domain borders were refined by combining previously published bioinformatics data with the recent empirical data. Constructs encoding variants of three domains were cloned, with borders modified based on theoretical predictions. These were transfected into HEK293 cells and tested for stability via a proteasome inhibition assay. Modified versions of the D and C domains showed improved stability over their empirical counterparts, while the I region may not represent a stable folded domain by itself. The D, S and C domains were further shown to be functional in isolation via interactions with known binding partners. To investigate which structural region might be responsible for its aggregation, DISC1 fragments were expressed in SH-SY5Y cells, with localization patterns viewed by fluorescent microscopy. Single domains showed diffuse cytoplasmic localization, while the combination of domains D and I showed clear aggregation. We therefore hypothesized that the unstructured region between domains D and I is responsible for DISC1 aggregation propensity, which was verified using further truncation constructs. Together, our data will aid in generation of high-quality animal models to study the mechanism and behavioral consequences of DISC1 aggregation, and its relevance for schizophrenia.

P-01.2-007
Protection of AD neurodegeneration in the presence of IFNγ-stimulated microglia depend on miR-124-3p level regulation

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Alzheimer's disease (AD) is a devastating disorder affecting over 50 million people worldwide. Although microglia, neuroinflammation and miRNA are key factors in AD, their interplay is not clear. We found increased miR-124 in APP Swedish SH-SY5Y cells (SWE) and in PSEN1 iPSC-derived neurons [1], along with microglia miR-124 co-localization in AD brain samples [2]. To explore miR-124 cell-to-cell crosstalk in neuroinflammatory conditions, we co-cultured SWE cells with IFNγ-stimulated CHME3 microglia cells. miR-124 regulation in SWE cells was done with its inhibitor/mimic, cell viability by flow cytometry, gene/miRNA expression by RT-qPCR, and proteomics by mass spectrometry. Exosomes were isolated by ultracentrifugation and characterized by western blot. miR-124 was found elevated in SWE cells/secretome, and in IFNγ-microglia after co-culture. miR-124 inhibition reduced SWE viability and induced stress-gene expression, while intensified inflammatory markers in IFNγ-microglia. miR-124 mimic sustained SWE cell viability and relieved IFNγ-microglia activation. Proteomics identified 113 microglial responsive proteins, most involved in neurodevelopment and synaptic function (21), inflammation (17), vesicular trafficking (15), and mitochondria dynamics (10). The mimic inhibited 72 proteins (e.g., MAP2K6), and raised 21 (e.g., EFEMP1). Since the processing of miRNAs depends on Dicer, we silenced it in microglia to investigate whether miR-124 was generated by microglia or resulted from passage from SWE cell exosomes. Microglia still revealed increased expression of miR-124, validating paracrine signaling of miR-124 from the SWE cells. In sum, neuronal miR-124 levels reshape microglia plasticity and support neuronal survival in an inflammatory milieu. These findings validate miR-124 regulation in AD and support the development of miRNA-based strategies in AD therapeutics. [1] Garcia et al. (2021) Cells, 10:2424. [2] Brites D (2020) Glia, 68:1631-42. Supported by FCT.

P-01.2-008
Prenatal hippocampal neurotoxicity of di(2-ethylhexyl) phthalate involves changes in the PTEN/Akt/mTOR signaling pathway

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Plastic products are a big part of everyday life for the people around the world. Phthalates that enhance elasticity and sustainability of plastics, easily migrate to the environment. There are many studies which prove phthalate toxic effects on the endocrine system, but target brain structure, as molecular mechanisms of neurotoxic effect aren't yet investigated. To assess prenatal toxicity test rats were exposed to di(2-ethylhexyl) phthalate (DEHP dosage 500 mg/kg/day). Cognitive functions and anxiety level were evaluated at 10-weeks of age. Tests have shown elevated anxiety levels and space memory disorder, induced by DEHP prenatal exposure. To determine the precise molecular mechanisms underlying this toxicity, N-methyl-D-aspartate (NMDA) receptor subunit composition, phosphatase and tensin homolog/protein kinase/target of rapamicyn (PTEN/Akt/mTOR) pathway phosphorylation, as well as brain derived neurotrophic factor (BDNF) levels were assessed in relevant subcellular fractions of the hippocampus of control and DEHP exposure. Male rat's hippocampus analysis revealed that DEHP prenatal exposure induces changes of NMDA glutamate receptor composition to N-methyl-D-aspartate receptor (NR2B) subunit phenotype and activation of NMDA-associated nitric oxide (nNOS) synthase, increase of extracellular signal regulated kinase (Erk) phosphorylation level decrease of AKT and PTEN phosphorylation, also enhancement of mTOR content and its phosphorylation level. Caspase-3 activity was also increased in DEHP-treated hippocampus that additionally proves targeted neurotoxicity. BDNF level in DEHP-exposed hippocampus was also highly increased, which could be regarded as a compensatory mechanism against hippocampal neurotoxicity. Therefore, it could be suggested that the hippocampus is one of the direct targets of DEHP prenatal toxicity and he mechanism of these actions involves changes in NMDA-dependent PTEN/Akt/mTOR signaling pathway that triggers compensative elevation of BDNF. *The authors marked with an asterisk equally contributed to the work.

P-01.2-009
Droplet-digital PCR protocol for precise, quantitative and allele-specific determination of endogenous transcripts associated with polyQ diseases

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Polyglutamine (polyQ) diseases are caused by the expansion of CAG trinucleotide tracts within specific genes. Patients usually harbor two alleles that differ in the length of CAG tracts: normal (NORM, < 30 CAG) and mutant (MUT, > 40 CAG). Because CAG tracts are quite common in the human transcriptome, it is challenging to design allele-specific methods and therapeutic strategies that selectively engage CAG expansion in the MUT transcripts. To circumvent this problem, we first identified SNPs that are linked to the NORM and MUT alleles of ataxin-3 (*ATXN3*) and huntingtin (*HTT*) genes. We then designed the SNP-based quantitative droplet-digital PCR (ddPCR) protocol and used it to precisely determine the levels of endogenous NORM and MUT transcripts in cellular and mouse models of spinocerebellar ataxia type 3 (SCA3) and Huntington disease (HD). We analyzed the ratio between NORM and MUT alleles of *ATXN3* and *HTT* mRNAs in human fibroblasts, iPSCs, neural progenitors and neurons derived from SCA3 and HD patients as well as in the mouse brain tissue. Additionally, the ddPCR assay allowed us to estimate the absolute number of *ATXN3* and *HTT* transcripts per cell. Our quantitative protocol is designed to explore differences in NORM and MUT allele expression and may be useful in studies aimed at understanding allele-specific regulation of polyQ mRNA transport, localization and turnover. Additionally, this method can be implemented to reliably monitor silencing efficiency in allele-selective therapeutic approaches for polyQ diseases.

P-01.2-010
Sweroside supports memory function by increasing mRNA expression of BDNF, CREB, NPY and decreasing AChE activity and brain oxidative stress in the scopolamine-induced zebrafish model (*Danio rerio*)

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Alzheimer's disease (AD) is a neurodegenerative and multifactorial disorder which involves various mechanisms of disease onset and progression. AD is associated with memory disorders and cognitive decline. Neurovascular dysfunction, cholinergic changes, inflammatory processes, oxidative stress and mitochondrial dysfunction are critical factors in the pathogenesis and development of the disease. Decreased autophagy and the ability to regulate the production of brain-derived neurotrophic factor (BDNF), cAMP response binding protein (CREB) and neuropeptide Y (NPY) are also reported as emerging disease factors. It was recently discovered that several plant iridoids have significant neuroprotective effects, being able to slow down the process of neurodegeneration. Many studies show that some iridoids have antioxidant potential and can promote neuronal growth factors. We focused on the natural iridoid namely Sweroside (Swe) which was previously characterised as having beneficial effects on the SNC, without adverse effects. Swe (1, 3 and 5 μg/L) was administered chronically (16 days) to zebrafish by immersion. The dementia model was established by treating the zebrafish with scopolamine (100 μM) 30 min before the behavioral tests. Anxiety was measured using the novel tank diving test (NTT), while memory was assessed using the Y maze test and the novel object recognition (NOR) test. The impact of Swe on the cholinergic and oxidative state of the animal model was evaluated immediately after the behavioral tests. We also evaluated the gene expression of the BDNF, NPY and CREB and we quantified the level of CREB protein by the immunoblotting technique. Our results show that Swe can improve the cognitive dysfunction of amnesic fish by increasing the gene expression of BDNF, NPY and CREB and by inhibiting AChE. AChE inhibition was also correlated with improved memory parameters, as suggested from the behavioral approaches (NTT, Y maze and NOR). *The authors marked with an asterisk equally contributed to the work.

P-01.2-011
Translocation of pyridinium oximes through blood–brain barrier

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Oximes are investigated as antidotes against toxic organophosphates (OP), which interrupt neurotransmission by inhibiting acetylcholinesterase (AChE) in the synapses of central and peripheral nervous system. To be fully efficient in their action as antidotes, oximes have to cross the blood–brain barrier (BBB) and reactivate OP-inhibited AChE in the brain. The three antidotes, so far approved for medical poisoning treatment, do not cross the BBB efficiently, thus prolonging the poisoning symptoms. To improve their efficacy, new potential antidotes are being synthetized using diverse scaffolds and modifying the known structures of the oximes. In this study, we evaluated the capacity of newly synthesised pyridinium oximes to cross a BBB model, based on *in vitro* cell monolayer of HBEC-5i cells. Initially, cytotoxicity screening determined how oximes act on HBEC-5i cells. According to the results, the concentrations of oximes for the BBB translocation assay were selected, to avoid paracellular leakage of BBB model and overestimation of oximes passing through the cell barrier. Most oximes were translocated in a percentage up to 50 %, which is significant result, compared to currently used antidotes. Furthermore, it was shown that there was no significant difference in oxime translocation after 4 or 24 h. In addition, membrane integrity was high throughout the experiment (above 85%) and the results represent translocation through the intact BBB cell monolayer model. Furthermore, the potential to cross BBB also opens up a new perspective for this compounds to be a base for development of BBB-active drugs for treatment of many other diseases or conditions such as Alzheimer's disease or cancer. Acknowledgement: This work was supported by the Croatian Science Foundation under the project UIP-2017-05-7260 and Portuguese Funding Agency, Fundação para a Ciência e a Tecnologia, FCT IP (grants: PD/BD/128281/2017 and PTDC/BIA-BQM/5027/2020).

P-01.2-012
SC-Nanophytosomes formulation to support a mitochondria-targeted approach: physical-chemical characterization and therapeutic potential for neurodegenerative diseases

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Mitochondrial dysfunction is a common pathological hallmark of many degenerative diseases, including Parkinson's disease (PD). Thus, developing therapeutic strategies to modulate mitochondrial function is a great challenge. With this main goal, we built a multi-functional formulation, assembling *Codium tomentosum* Stackhouse polar lipids and *Sambucus nigra* L. elderberry anthocyanin-enriched extract (EAE-extract). The polar lipids were chosen to improve the functional organization of the lipid bilayer of the mitochondrial membranes since they are rich in anionic phospholipids with a high relative abundance of omega-3 polyunsaturated fatty acids, the precursor of mature cardiolipin. EAE-extract, dominated by four cyanidin-glycosides, is selected by the ability of elderberry anthocyanins to overcome the complex I-related mitochondrial dysfunction [1]. SC-Nanophytosomes engineered with 600 μM algae phospholipids and 0.5 mg/L of EAE-extract are nanosized vesicles (diameter = 108.74 ± 24.74 nm) with a negative surface charge (Zeta potential = -46.93 ± 6.63 mV) and versatile shape. SH-SY5Y cells assays showed that SC-Nanophytosomes have the competence to improve the activity of the mitochondrial respiratory chain complexes and preserve the mitochondrial membrane potential in the presence of rotenone, a complex I inhibitor. Using green-fluorescent labelled SC-Nanophytosomes, it was shown that they have the competence to target mitochondria using a caveolae-mediated endocytosis process to overcome the plasma membrane cell barrier. This SC-Nanophytosome formulation is being tested by oral administration in rotenone-induced PD-like pathology in a rat model. The preliminary results showed positive outcomes on the disabling motor symptoms as well as on several biochemical pathological hallmarks exhibited by brain cells of this PD animal model. Overall, data indicate that SC-Nanophytosomes have the potential to support new mitochondria-targeted therapy for neurodegenerative diseases.

P-01.2-013
Astrocytic S100B protein acts as dual-function chaperone inhibiting aggregation and preventing the formation of zinc-induced toxic Aβ oligomers

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Amyloid beta (Aβ) aggregation and imbalance of metal ions are major hallmarks of Alzheimer's disease (AD), with Zn, Cu and Fe accumulating in plaques. Among these, Zn binding to Aβ promotes its oligomerization into highly toxic polymorphic species that strongly affect cell viability (previously published in: Lee MC et al. (2018) Sci. Rep. 8, 4772). S100B is an abundant brain protein, which is upregulated in AD, and that accumulates nearby plaques upon its secretion by astrocytes. Recently, we uncovered that S100B is able to suppress Aβ42 aggregation, thus acting as protective chaperone (previously published in: Cristovão JS et al. (2018) Sci Adv. 4(6), 1702). Additionally, its ability to bind Ca, Cu and Zn ions confers it an important role in the regulation of trace metal homeostasis in the brain (previously

published in: Hagmeyer S et al. (2017) Front Mol Neurosci 10, 456). Here, we combine biophysical and kinetic approaches to explore the effect of S100B Zn-binding properties in the context of Aβ42 aggregation. From detailed kinetic analysis of ThT monitored Aβ42 aggregation, we show that equimolar ratios of S100B prevents highly toxic off-pathway oligomers that are formed when monomeric Aβ42 self-assembles in the presence of Zn. In this scenario, S100B action redirects Aβ42 aggregation pathway into the formation of mature inert fibrils, as inferred from TEM imaging, which are however less toxic than the Zn-oligomers. In agreement with an action as biological metal ion scavenger, we observed that the effect of S100B over zinc-mediated Aβ oligomerization is similar to that of EDTA. Altogether, these data shows that S100B potentially exerts a dual protective function in the AD brain, acting both as a suppressor of aggregation as well as an inhibitor of Zn-induced toxic oligomer, by means of Zn scavenging. FCT/MCES (Portugal) is acknowledged for funding (UID/MULTI/04046/2013) and for PhD grants 2021.06393.BD (to AJF) and SFRH/BD/101171/2014 (to JSC). *The authors marked with an asterisk equally contributed to the work.

P-01.2-014
A humanized yeast model system to assess the risk of environment contaminants to Parkinson's disease progression

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Parkinson's disease (PD) is the most common neurodegenerative movement disorder, mainly affecting the older population. Neuropathologically, PD is characterized by the loss of dopaminergic neurons and the occurrence of toxic cytoplasmic inclusions of insoluble aggregates of α-synuclein (α-syn). Although 10% of PD cases arise from specific gene mutations, this disorder is considered multifactorial, with environmental toxicants playing an important role in the etiology. For this reason, we aim to take advantage of the well-established "humanized PD" yeast model to screen compounds that could pose a risk for the development and progression of PD. Indeed, yeast cells expressing recombinant human α-syn have been used as a widespread system in screens to identify genetic or chemical compounds that prevent α-syn-induced toxicity, resulting in significant insights into the pathogenesis of PD. Applying the reverse principle, we exposed yeast cells expressing α-syn to different toxic compounds and assessed their effect on PD hallmarks, including α-syn localization and aggregation. We found α-syn localization shifted from the cytoplasmatic membrane to the cytoplasm in response to a number of commonly used pesticides, aggregating into vesicles analogous to Lewy bodies. We categorized the compounds according to their ability to induce toxicity and α-syn aggregation and performed several complementary functional analyses, including assessment of mitochondrial dysfunction/ROS production and lipid accumulation. Our results generated new information on the effects of environmental toxicants on PD development and will thus contribute to future risk assessment of chemicals.

P-01.2-015
In vitro study of microglial activation by LPS and radiation and its effects on neighboring cells

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Microglial cells are involved in the brain's physiology, representing its first line of immune defence. While immune activation of microglial cells is crucial to protect the nervous system and maintain their physiology, chronic neuroinflammation is involved in several neurodegenerative disorders. The aim of our work was to study microglial activation and its effects on surrounding nervous cells. Microglial human cell lines HMC3 and SH-SY5Y were used as biological model. Microglial cells were exposed to LPS or ionizing radiation, thought to induce microglial activation *in vivo*. The activation was evaluated by cytokine measurement. Microglial activation effects on surrounding tissue were explored by medium transfer or co-cultivation with SH-SY5Y cells, used here as a model of neuronal cells. We evaluated cellular viability, ROS production, apoptosis induction and cell cycle alterations up to 6 days of growing in media received from activated HMC-3 or in presence of these cells. Microglial cells released increased level of IL-6 starting short time following LPS treatment (3 h) and after a longer time following irradiation (5 days). Increased mRNA expression of several pro-inflammatory cytokines was also found. Co-cultivation of SH-SY5Y cells with resting HMC-3 lead to increased cellular viability, while incubation with activated HMC-3 cells lead to reduce viability. These changes were correlated with increased ROS levels of neuronal cells exposed to activated microglia. We conclude that HMC-3 cells present a different activation kinetic dependent on activation factor, possible due to different underlying mechanisms. Microglial cells have a dual role on SH-SY5Y cells, depending on their activation status, with a detrimental effect in case of microglial activation. Acknowledgements: This work was supported by Romania Ministry of Education Grant nr. 46PD/2020 and Norway-Romania Grants: RO-NO-2019-0510, CTR 41/2020.

P-01.2-016
A novel extracellular chaperone prevents tau aggregation and proteotoxicity

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The microtubule-associated protein tau is implicated in the formation of oligomers and fibrillar aggregates, that evade proteostasis control and spread from cell to cell. Tau pathology is accompanied by sustained neuroinflammation and, while the release of alarmin mediators aggravates disease at late stages,

early inflammatory responses encompass protective functions. This is the case of the Ca^{2+}-binding S100B protein, an astrocytic protein augmented in Alzheimer's Disease and which we previously implicated as a proteostasis regulator, acting over Aβ42 aggregation (Cristóvao et al. 2018 Science Advances 4, eaaq1702). Here we report novel findings describing the holdase activity of S100B resulting in suppression of tau aggregation and seeding (Moreira et al. 2021 Nature Communications, 2, 6292). NMR structural analysis showed that S100B interacts with tau in a Ca^{2+}-dependent manner, notably with the aggregation prone repeat segments at the microtubule binding regions. This interaction, also evidenced by SAXS, involves contacts of tau with a cleft formed at the interface of the S100B dimer, which is exposed upon Ca^{2+}-binding. Aggregation kinetics and mechanistic analysis revealed that S100B inhibits tau aggregation through effects on secondary nucleation, as corroborated by seeding assays and direct observation of S100B binding to tau oligomers and fibrils in TEM immunogold labelling experiments. In agreement with a role as an extracellular chaperone and its accumulation near tau positive inclusions, we show that S100B blocks proteopathic seeding by tau oligomers as investigated using the tau biosensor cell model. Latest unpublished findings reveal that S100B inhibits the formation of tau liquid droplets and recovers tau from liquid-liquid phase separated states. Altogether, our findings establish tau as a client of the S100B chaperone, whose action suggests a novel extracellular system capable to handle proteotoxicity from early stages of neurodegeneration.

P-01.2-017
Molecular consequences of cobalamin deficiency in normal human astrocytes

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Hypocobalaminemia causes a number of neurological features including neuropathy, cognitive and psychiatric disturbances. It is also a risk factor for the top two common neurodegenerative diseases: Parkinson's disease and Alzheimer's disease. Growing evidence indicates that disturbances of astrocyte homeostasis might play an important role in the neurodegenerative process. Previously, we developed an *in vitro* model of cobalamin deficiency in normal human astrocytes by culturing the cells with cobalamin antagonist and we showed that cobalamin deficiency causes a non-apoptotic activation of caspases, suggesting astrogliosis or astrosenescence [previously published in: Rzepka Z et al. (2019) Cells 8(12), 1505]. The aim of the current study was to examine the expression of cytoskeleton proteins as well as senescence-associated proteins in cobalamin-deficient astrocytes. Western blotting and immunofluorescence with confocal laser scanning microscopy were applied. We revealed changes in the expression of a glial fibrillary acidic protein, vimentin, p16 INK4A, and p21 Waf1/Cip1 as well as in cellular morphological features, including cytoskeleton organization. The presented results may contribute to a better understanding of the molecular mechanism underlying the neurodegeneration process and nervous system disorders associated with cobalamin deficiency.

P-01.2-018
Analysis of capability of carbon-containing nanoparticles from combusted organics to mitigate cadmium- and mercury-induced excitotoxicity in presynaptic brain nerve terminals

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Cadmium and mercury are hazardous neurotoxicants. Carbon-containing nanoparticles (CNPs) are both the main component of air pollution and promising in nanotechnology. Here, the methodological approach for monitoring capability of CNPs to mitigate cadmium- and mercury-induced neurotoxicity was developed using presynaptic rat cortex nerve terminals. $CdCl_2$ and $HgCl_2$ caused a concentration-dependent increase in the extracellular L-[^{14}C]glutamate level in nerve terminals. Combined effects of Cd^{2+} or Hg^{2+} and CNPs were analysed and verified using classical reducing/chelating agents. CNPs were able to mitigate in an acute manner excitotoxic Hg^{2+}-induced increase in the extracellular L-[^{14}C]glutamate level in nerve terminals by 37%, thereby being a provisional Hg^{2+} scavenger. CNPs and Hg^{2+} acted as a complex in nerve terminals that was confirmed with fluorimetric data and Hg^{2+}-induced changes in spectroscopic features of CNPs. Whereas, CNPs did not cause analogical in intensity effect on Cd^{2+}-induced increase in the extracellular synaptosomal level of L-[^{14}C]glutamate. Beside the environmental/biotechnological implementation of data, the developed approach can be applicable for monitoring capability of different particles/compounds to mitigate Hg^{2+}-mediated threat. Funding: This work was supported by the National Research Foundation of Ukraine, Project #2021.01/0061.

P-01.2-019
Peptidomimetics based on a fragment of Blm10 protein as allosteric modulators of proteasome activity

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Human 20S proteasome provides cells with a cleaning system, removing mutated, misfolded, and oxidatively damaged proteins [1]. During aging, the effectiveness of the proteasome system gradually decreases, which leads to the accumulation of abnormal proteins and their aggregation. Impairment of proteasome function contributes to the development of several neurodegenerative diseases, such as Alzheimer's, diseases [2]. Therefore, the activation of the proteasome can be a promising therapeutic strategy to delay the onset of age-related disorders [3]. This approach is growing in popularity, but so far little is known about the mechanism of 20S stimulation by small molecules, hence the lack of good leading structures for the design of activators. Blm-pep is a 14-mer peptide, designed based on the sequence of the C-terminal end of an ATP- and ubiquitin-independent Blm10 activator of the 20S proteasome. It stimulates hydrolysis of peptides and some partially unstructured proteins. So far, we obtained dozens of analogs of Blm-pep. The crystal structure of one of them in the complex with h20S revealed its ability to bind into the two adjacent pockets, between the α2/ α3 and α3/α4 subunits of the 20S core particle. Binding a modulator in

them probably induces a signal transmission in the form of conformational changes, which induce opening the entrance to the catalytic chamber and propagate to the active sites, affecting the effectiveness of their working. Aiming to increase the stability of the open form of the proteasome, we designed modulators able to bind in both pockets simultaneously. Some of these compounds activate the proteasome more than the original activators and show increased proteolytic stability in human plasma. This study was financially supported by the NCN-funded grant 2019/35/O/NZ7/00227 References: [1] Groll M. et al. (1997) Nature, 386, 463–471 [2] Ciechanover A. et. al. (2015) Exp. Mol. Med. 47, e147 [3] Chondrogianni, N. et al. (2014) Biol. Med 71, 303–320

P-01.2-020
Radioprotective DNA-binding damage suppressor protein (Dsup) affects the functioning of neural system in *D. melanogaster*

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The unique tardigrade protein Dsup promotes stress resistance of different organisms by DNA protection from reactive oxygen spices (ROS). Dsup is a putatively intrinsically disordered protein (IDP) with few sequence similarities to non-tardigrade proteins except another DNA-binding HMGN protein (IDP). An overexpression of HMGN protein interferes astrocyte differentiation, additionally neurodegenerative trends have been observed after overexpression of the other DNA-binding protein TDP43 (Nagao M et al. (2014) Stem Cells 32, 2983–2997; Hazelett DJ et al. (2012) G3 2, 789–802). We hypothesized, that an unspecific binding mode of Dsup to DNA may affect the gene expression and functioning of neural system in model organisms. On that goal, we obtained for the first time *Dsup*-expressing lines of complex animal *D. melanogaster* and an increased resistance of these lines to ionizing radiation (γ-radiation) and stress associated with ROS has been confirmed. Transcriptome profiling of *Dsup*-expressing *D. melanogaster* lines revealed a broad down-regulation of gene expression and we assume that Dsup may act as a transcriptional repressor. Along with a significant number of altered biological processes associated with DNA compaction and transcription, many enriched categories attribute to neural system were observed (synapse organization, axon guidance, neuropeptide signaling pathway, G-protein coupled receptor signaling pathway, mushroom body development). Moreover, with negative gravitaxis assay, climbing deficit in *D. melanogaster* was determined. In conclusion, we demonstrate that DNA-binding Dsup protein can impact the neural system functioning in *D. melanogaster* and these data may be important for studies of IDPs in neural disorders. *The authors marked with an asterisk equally contributed to the work.

P-01.2-021
Long-term mitochondrial stress induces early steps of Tau aggregation

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Mitochondria are nowadays recognized as very important signaling organelles. Moreover, defective mitochondria are increasingly associated with the development of neurodegenerative diseases, such as Alzheimer's disease, in which Tau protein aggregates are observed in patients' brains. In the present study, we report that under long-term mitochondrial stress Tau undergoes enhanced dimerization, which is the first step of protein aggregation. In order to analyze the early stages of Tau aggregation, we took advantage of the bimolecular fluorescence complementation assay using cells transfection with plasmids that encoded Tau fused with N-terminal or C-terminal part of fluorescent Venus protein. Long-term mitochondrial stress was induced by prolonged rotenone treatment of HEK293T or SH-SY5Y cells and by NDUFA11 knockout in HEK293T cells. We observed a significant increase of reactive oxygen species (ROS) levels in both HEK293T and SH-SY5Y cells under applied mitochondrial stress conditions. Oxidative stress and Tau dimerization in HEK293T were significantly reduced by cells treatment with N-acetyl-L-cysteine, a well-known ROS scavenger. This observation indicated that early steps of Tau protein aggregation may be triggered by oxidative imbalance under long-term mitochondrial stress. Interestingly, we did not observe the increased formation of insoluble Tau aggregates, isolated with the use of differential ultracentrifugation from cells under mitochondrial stress conditions. We analyzed the interactome of endogenous Tau in SH-SY5Y cells in control conditions and under mitochondrial stress conditions using a co-immunoprecipitation approach followed by mass spectrometry analysis. We revealed increased interaction of Tau and a heat shock protein - HSP90B1, which acts as a cytoplasmic chaperonin. We consider this enhanced interaction as a protective response, counteracting Tau protein aggregation on a bigger scale under long-term mitochondrial stress.

P-01.2-022
Matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) as blood biomarkers of Alzheimer's disease

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Although matrix metalloproteinase-9 (MMP-9), tissue inhibitor of metalloproteinase-1 (TIMP-1) and CD147, play an important role in amyloid- and neurofibrillary tangles processing in the pathogenesis of Alzheimer's disease (AD), their contribution as blood biomarkers in patients with AD is still not clear. The aim of this study was to analyze levels of blood biomarkers, such as MMP-9, TIMP-1, CD147, and MMP-9/TIMP-1 ratio in patients with AD in comparison to subjects with normal cognition, and to evaluate their diagnostic values. The study included 36 patients with a diagnosis of AD according to NIA-AA (2011) criteria, who were confirmed to have low Aβ42 or Aβ42/40 ratio, and high total tau (t-tau) and phosphorylated-tau (p-tau) in cerebrospinal fluid, and 40 healthy subjects with normal cognition. Serum levels of MMP-9, TIMP-1, and CD147, were determined by enzyme-linked immunosorbent assay (ELISA), and the MMP-9/TIMP-1 ratio was calculated. Serum MMP-9 and TIMP-1 levels in patients with AD were significantly higher than in controls ($P < 0.001$ and $P < 0.001$, respectively), while there were no differences in CD147 levels and MMP-9/TIMP-1 ratio between AD patients and healthy subjects. The receiver operating characteristic (ROC) curve analysis showed that the cut-off value at

137.00 ng/mL for MMP-9, and at 260.41 ng/mL for TIMP-1 contributed to the separation between AD and controls, with a sensitivity of 80.6% and 86.1%, and a specificity of 70.0% and 77.5% (AUC = 0.798 and AUC = 0.876, respectively). These results indicate that MMP-9 and TIMP-1 provided a better compromise between specificity and sensitivity in distinguishing AD from healthy subjects than the MMP-9/TIMP-1 ratio and CD147. *The authors marked with an asterisk equally contributed to the work.

P-01.2-023
Thermodynamics of oligomeric alpha-synuclein-membrane interactions

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Parkinson's (PD) disease is an increasingly prevalent and currently incurable neurodegenerative disorder linked to the accumulation of proteinaceous pathogenic inclusions in the nerve system. Misfolded alpha-synuclein oligomerisation and interactions with lipid bilayers are key steps in PD-associated neurotoxicity. However, despite the intensive effort put to understand aberrant protein oligomerisation and the downstream events, mechanistic details of oligomer-induced cellular toxicity are still missing. To address these questions, a systematic thermodynamic characterisation of toxic oligomeric alpha-synuclein-membrane binding was carried out. Synthetic liposome-alpha-synuclein oligomer systems were investigated directly in solution by the means of microfluidic techniques combined with widefield fluorescence microscopy. The study revealed that toxic oligomeric alpha-synuclein binds to the membrane surface with nanomolar affinity and precludes functional monomeric alpha-synuclein from membrane binding and functioning.

P-01.2-024
Potential neuroprotective effect of bezafibrate against mitochondrial dysfunction caused by 3-nitropropionic acid in rat striatum

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Huntington's Disease (HD) is a neurodegenerative disorder caused by a CAG nucleotide base repeat expansion in the huntingtin gene, leading to the accumulation of a mutant protein huntingtin (mHTT). mHTT has been shown to be neurotoxic, resulting in neuronal death mainly in striatum. Treatment for HD is limited and based on management of symptoms. Therefore, in the present study Wistar rats were injected with 3-nitropropionic acid (3-NP), to better elucidate mechanisms of neurodegeneration observed in HD. Striatum was used to evaluated parameters of mitochondrial function and the interaction of mitochondria with endoplasmic reticulum (ER) 7, 14, 21, and 28 days after 3-NP administration. The effects of a pre-treatment with the protective compound bezafibrate were also determined. Our results showed that 3-NP induced disturbances in redox homeostasis, by reducing antioxidant defenses, as observed by a decreased in glutathione concentrations (21 and 28 days), the activities of the enzymes glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase, and glutathione S-transferase, and by increasing lipid peroxidation (28 days). Moreover, an impairment of mitochondrial respiration was observed, with reduction of respiratory chain complexes activities, as well as a reduction of state 3, state 4, and non-coupled in 7 and 28 days. Furthermore, 3-NP impaired mitochondrial dynamics and ER-mitochondria crosstalk by increasing protein contact, as observed by increased protein levels of mitofusin-1, voltage-dependent anion-selective channel 1 and chaperone glucose-regulated protein 75. Besides, 3-NP induced a loss of the protein synaptophysin in 28 days. Finally, orally administration of bezafibrate prevented some of the biochemical alterations induced by 3-NP. In conclusion, mitochondrial dysfunction has an important role in the pathophysiology of striatum abnormalities observed in HD and bezafibrate may be considered as an adjuvant therapy.

P-01.2-025
Administration of *Plinia trunciflora* extract has neuroprotective effects in Wistar rats submitted to neonatal hypoxia-ischemia

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Neonatal hypoxia-ischemia (HI) results from the decrease in oxygen and glucose supply to the brain leading to severe neurological consequences. Medicinal plants have been studied as a therapeutic strategy to treat HI injury. *Plinia trunciflora* is a plant that has antioxidant and anti-inflammatory properties. Present study evaluated the neuroprotective action of *Plinia trunciflora* extract (PTE) in rats submitted to experimental HI through behavioral testing, histological and biochemical analysis. HI was produced in Wistar rats at postnatal day 7 (PND7). Pups received intraperitoneal injections of PTE (10 mg/kg) 1, 24, 48 and 72 hours after HI procedure; another group had PTE offered to dams, orally (800 mg/L), during pregnancy and lactation. At PND10, parameters of redox homeostasis and inflammation were assessed; from PND45 animals underwent behavioral testing. Thereafter, rats were euthanized for histological analysis. The HI

insult caused an increase in the lipoperoxidation levels and glutathione peroxidase activity, along with a decrease in concentration of reduced glutathione in hippocampus, cerebral cortex and striatum; additionally, HI provoked an increase of interleukin-1β in hippocampus. PTE given post-HI was able to prevent most of the pro-oxidant effects and neuroinflammatory response caused by the injury. HI injury caused anxiety-like behavior in Plus Maze and spatial learning deficits in the Morris Water Maze. In addition, HI insult caused neural tissue loss, mainly in the hippocampus. Both PTE treatments showed anxiolytic action in the Plus Maze task, however only given post-HI was able to reverse spatial memory impairments and neural tissue damage. Taken together, our results provide evidence for a neuroprotective potential of PTE administration after the neonatal HI event, however no neuroprotection was observed in the offspring of dams receiving PTE during pregnancy and lactation.

P-01.2-026
Melatonin improves oxidative stress, motor disabilities and neurodevelopmental delay caused by intracerebroventricular administration of L-2-hydroxyglutaric acid to neonatal rats

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L-2-hydroxyglutaric acid (L-2-HG) concentrations are highly elevated in brain and biological fluids of patients affected by L-2-hydroxyglutaric aciduria (L-2-HGA), a neurometabolic and neurodegenerative disease characterized by psychomotor delay and cerebellar ataxia, whose pathogenesis is still poorly understood. Since there is no treatment available for this disorder and previous *in vitro* results showed that L-2-HG provokes oxidative stress in brain of young rats, the present study evaluated the short- and long-term effects of an intracerebroventricular injection of L-2-HG to neonatal rats, as well as the neuroprotective role of intraperitoneal melatonin administration. We investigated whether L-2-HG could disrupt redox homeostasis, as well as neurodevelopment and motor activity. L-2-HG increased reactive oxygen species generation, provoked lipid peroxidation, decreased glutathione concentrations and increased superoxide dismutase and glutathione peroxidase activities in cerebellum. L-2-HG-injected rats also showed a poor performance in neonatal neurodevelopmental reflex tests such as righting, gait, cliff avoidance, negative geotaxis and hindlimb suspension, reflecting a marked delay in neurodevelopment. Finally, animals injected with L-2-HG showed deficits in gross and fine motor tasks in adulthood, as determined by the rota-rod and ladder rung walking tests. Noteworthy, melatonin a hormone associated with sleep-wake cycles, which also presents antioxidant properties, was able to prevent most L-2-HG-induced pro-oxidant effects, as well as to improve neurodevelopmental parameters and motor activity in L-2-HG treated rats. Our results may indicate that oxidative stress is involved in the pathophysiology of motor damage and neurodevelopmental delays characteristic of patients affected by L-2-HGA. Furthermore, the use of antioxidant scavengers of reactive oxygen species such as melatonin may represent a potential adjuvant therapy in the treatment of L2HGA.

P-01.2-027
MIN-102 enhance the oxidative profile of FRDA mouse model

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Friedreich's ataxia (FRDA) is a neurodegenerative disorder mainly characterized by neuromuscular and neurological manifestations, but also frequently cardiomyopathy and diabetes. It is caused by mutations in the FXN gene. There is currently no treatment for this disease, so in this project we have analyzed MIN-102 as a potential treatment for FRDA. Three different groups of mice were used for this study: C57BL/6J (as control), YG8sR and YG8sR treated with MIN-102 (for 7 months). The organs analyzed of each group were: heart, cerebellum and pancreas, according to the main disease symptoms. Using the Reverse Phase Protein Array (RPPA), we have seen in the three organs analyzed that MIN-102 is capable of restoring normal levels of proteins related to the antioxidant response, as well as pyruvate oxidation and the Krebs cycle. In relation to mitochondrial function, using western blot (WB) and RPPA analysis, we observed that MIN-102 seems to recover levels of relevant proteins in the pancreas. We also measured proteins important in apoptosis using WB and a Proteome Profiler array kit, and found that YG8sR doesn't have an increase in apoptosis compared to C57BL/6J, and MIN-102 doesn't affect this process either. However, we measured the levels of the GPX4 protein by WB, which is associated with ferroptosis, and we observed differences between C57BL/6J and YG8sR, and also that MIN-102 restores the levels of this protein in pancreas. Additionally, we are analyzing a RNAseq of heart and cerebellum samples to confirm our findings. MIN-102 appears to enhance the antioxidant response of the FRDA mouse model, as well as pyruvate oxidation and the Krebs cycle. Furthermore, this molecule improves mitochondrial function and reduces ferroptosis in the pancreas. RNAseq will be used to confirm our findings.

P-01.2-028
Comparative study of the affinities of natural aminosterols for lipid membranes and their relation with protection from misfolded protein oligomers

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Most of the neurodegenerative diseases, including Parkinson's and Alzheimer's, are associated with the self-assembly of specific peptides or proteins from their native soluble states into misfolded fibrillar aggregates known as amyloid fibrils, that are deposited in the extracellular space of the brain or within neurons. It is increasingly clear that small misfolded oligomers formed during the aggregation process or released by mature fibrils represent the main pathogenic species in these diseases, and play a pivotal role in neurotoxicity through their ability to interact and disrupt neuronal membranes. Natural compounds initially isolated from the gastrointestinal tract of the dogfish shark *Squalus acanthias*, named aminosterols, have been shown to be promising small molecules for the treatment of neurodegenerative diseases, since they protect neuronal cells from the action of toxic oligomers through the interaction and insertion within cell membranes. Recently, the aminosterol trodusquemine was reported to interact and incorporate within the superficial hydrophilic portion, and first hydrophobic part underneath, of lipid membranes, leading to the modulation of their physicochemical properties by reducing the net charge, increasing the mechanical resistance to indentation and remodelling the spatial distribution of cholesterol and GM1 lipids. These modulations contribute to increase the resistance of cell membranes to the toxic action of misfolded oligomers. Here we report, using fluorescence binding assays, fluorescence anisotropy, circular dichroism, dynamic light scattering, Ca^{2+} influx assays and ζ potential measurements, a comparative study between three different natural aminosterols. The results show how slight variations in the chemical structure of aminosterols lead to different binding affinities for large unilamellar vesicles that mimic the lipid composition of neuronal membranes and different protective abilities towards misfolded oligomers.

P-01.2-029
The fight against socially significant neurodegenerative diseases based on the paradigms of preventive and translational medicine

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Numerous attempts to develop a preclinical diagnosis of neurodegenerative diseases (NDD), Parkinson's disease (PD) and Alzheimer disease, by searching for peripheral biomarkers as changes in biological fluids were not successful. The drawback of this approach is that the search for markers is in patients at the clinical stage without guarantee that they are characteristic for the preclinical stage. Indeed, all markers detected so far are nonspecific. We have upgraded this approach by considering as preclinical markers only those changes in body fluids that are characteristic of both patients and animal models of the preclinical stage of NDD. It has been shown that no more than 25% of the markers found in the blood and tears of PD patients are characteristic of MPTP-treated mice. In addition, we have developed a fundamentally new for neurology approach to the early diagnosis of PD – a provocative test that has long been successfully used for the early diagnosis of chronic diseases of internal organs. It has been shown that the administration of α-methyl-p-tyrosine, a reversible inhibitor of dopamine synthesis (a provocative agent), to MPTP-treated mice in a model of the preclinical stage of PD induces a reversible decrease in the level of dopamine in the striatum to a threshold (30%), at which short-term motor disorders were observed. The development of preclinical diagnosis of NDD will make it possible to use preventive neuroprotective therapy by slowing down neurodegeneration and thereby prolonging the preclinical (asymptomatic) stage, the period of normal physical and social activity of patients. Thus, we proposed a new approach to the development of preclinical diagnosis and preventive therapy of NDD based on the paradigms of preventive and translational medicine. This project was funded by Ministry of Science and Higher Education of the RF (grant agreement № 075-15-2020-795 of 29.09.2020)

P-01.2-030
Role of γ-enolase in neuronal development: cell differentiation into neuronal-specific subtype

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Neurodegenerative diseases result in dysregulation of neurotrophic factors, molecules that are specific to different types of neurons and aid in neuron function, especially in proliferation, differentiation, and growth. Our lab previously showed neurotrophic activity exhibited by the C-terminal part of the γ-enolase, which is regulated by the cysteine peptidase cathepsin X. However, the exact role and importance of γ-enolase and its regulation with cathepsin X during differentiation into a specific neuronal subtype remain unclear. We investigated the role of γ-enolase and cathepsin X in the process of differentiation of the SH-SY5Y cell line into an individual neuronal phenotype by setting up an *in vitro* cell differentiation model. Proliferation was partially inhibited in cells treated with retinoic acid (RA), and brain-derived neurotrophic factor (BDNF), at the expense of stimulated differentiation. We further demonstrated that expression of the active form of γ-enolase is increased when cells are stimulated with RA alone or in combination with BDNF, while the expression and activity of cathepsin X, a regulator of γ-enolase neurotrophic activity, was also elevated in differentiated cells. Moreover, the inhibition of cathepsin X showed neurite outgrowth in differentiated cells, indicating its role in proliferation. We demonstrated that γ-Eno peptide, which mimics the C-

terminal part of the enzyme, increases neurite outgrowth of undifferentiated cells as well as promotes cell differentiation of RA alone or in combination with BDNF differentiated cells, indicating an important role of γ-enolase in the development of cholinergic neurons. So far, our results suggest that γ-enolase is important in the process of neuronal growth and differentiation, indicating similarity to cathepsin X inhibition. Therefore, exploring their function in specific differentiation processes is crucial in the design of new therapies to stimulate the regeneration of damaged neurons. *The authors marked with an asterisk equally contributed to the work.

P-01.2-031
Biological assessment of selected herbicides and pesticides in use as inhibitors of human cholinesterases and on HepG2 and SH-SY5Y cell lines

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The toxicity of organophosphorus compounds (OPs) remains a major public health concern due to their widespread use as pesticides and herbicides. Their common mechanism of action involves the inhibition of enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), crucial for neurotransmission. Both chronic and acute poisoning by OPs can leave long-lasting health effects even when patients are treated with standard medical therapy. We investigated *in silico* and *in vitro* interactions and kinetics of inhibition for human cholinesterases with selected organophosphate pesticides and herbicides. Overall, the pesticides ethoprophos and fenamiphos and herbicides anilophos and piperophos were the most potent inhibitors of both cholinesterases, with a slight preference to bind BChE known as a natural endogeneous bioscavenger of various xenobiotics. Molecular modelling enabled an evaluation of interactions important for specificity and selectivity of inhibition. The tested compounds did not affect the viability of the cell lines upon 4, 24 and 48 hours of treatment and were not hepatotoxic or neurotoxic at concentrations in which they displayed inhibition activity. Based on overall results, the selected compounds may be the original structures for the design and synthesis of a new class of pesticides and herbicides safe for use, or even drugs for treatment of various neurodegenerative diseases. Acknowledgments: This work was supported by the Croatian Science Foundation (IP-2018-01-7683 and UIP-2017-05-7260).

P-01.2-032
Characterization of protein aggregation promoted by PKA-RIb mutation in a rare neurodegenerative disease

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Dysregulation of cAMP signaling contributes to the etiology of several brain degenerative diseases. A missense mutation in protein kinase A (PKA) RIb regulatory subunit, the least studied isoform, was found in patients diagnosed with a rare neurodegenerative disease. The gap in knowledge regarding this gene and the devastating outcomes seen individuals with motor deficits are two critically important problems that we are currently researching. Our recent structural model led us to hypothesize that an amino acid substitution L50R may result in breaking the dimer formation. Biochemical studies as well as cell-based high-resolution image analysis suggest that this RIb missense mutation not only prevents RIb homodimerization but also eliminates the binding site that is created by dimer formation for A kinase anchoring proteins (AKAPs) binding. Consequently, PKA holoenzyme localization is affected as evidenced by accumulation of RIb into neuronal inclusions in human brain patients. A quantitative multiplex proteomics revealed that the phospho-signaling cascade is disrupted by the L50R mutation in postmortem brains. This study emphasizes the importance of precisely controlled PKA isoform subcellular localization and demonstrates how a mutation in PKA regulatory subunit drives aberrant cAMP signaling and neurodegeneration. This study provides insights into the molecular and cellular mechanisms of other neurodegenerative diseases where PKA function is dysregulated.

P-01.2-033
Molecular changes in Alzheimer's disease resulting from Western diet-induced brain insulin resistance

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Western diet (WD) is a type of nourishment based on ultra-processed foods, rich in simple sugars and saturated fats. Long-term consumption of WD may lead to disruption of insulin signaling and the development of insulin resistance. It is supposed that insulin resistance is one of the probable risk factors for Alzheimer's disease (AD). The aim of this study was to verify the hypothesis that WD can trigger the initiation and propagation of major neuropathological features of AD, such as amyloid-β (Aβ) plaques and neurofibrillary tangles. To this aim, male C57BL/6J wild-type mice were fed WD diet or standard diet (SD; CTR) from 3 months of age and analyzed at 4-, 8-, 12-, and 16-months of age. The effects of WD on the levels of the insulin signaling pathway components: p-IRS-1(Ser616), p-Akt(473), p-GSK-3β (Ser9) and on neuropathological AD biomarkers: p-Tau(Thr231) and APP/Aβ levels were analyzed in the entorhinal cortex and hippocampus. Entorhinal cortex proved to be more sensitive to WD-dependent insulin impairments than the hippocampus: immunoblotting analysis in the entorhinal cortex revealed an increase in p-IRS-1(Ser616) levels, indicating the development of brain insulin resistance under the WD. Moreover, a change in the localization of p-Tau(Thr231) in cellular compartments from fibers to nerve cell bodies indicated a progressive tauopathy. In addition, we also observed an age-dependent decrease in APP protein levels correlating with the appearance of Aβ peptides under the WD. Obtained results suggest that the WD diet, by inducing abnormalities in the brain insulin signaling, promotes the development of AD, and may be considered as a significant, modifiable AD trigger. Funding: Polish National Science Centre grant UMO-2014/15/D/NZ4/04361. *The authors marked with an asterisk equally contributed to the work.

P-01.2-034
Mitochondrial dysfunction and redox alterations in cellular models and the I151F mouse model of Friedreich Ataxia: role of Sirt3

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Friedreich Ataxia (FA) is a rare and orphan neurodegenerative disease, mainly caused by the presence in homozygosity of a GAA-triplet expansion in the first intron of the frataxin gene (FXN). Such mutation results in low levels (2 to 25% of controls) of frataxin, a mitochondrial protein. However, some patients present a point mutation in one FXN allele and expanded GAAs in the other. In FA, one of the most affected cells are dorsal root ganglia (DRG) neurons, where mitochondrial impairment has been described. The lack of FXN favor an oxidative stress situation disrupting the respiratory electron transport chain and OXPHOS activities. As a consequence, this redox alteration may lead to a decrease in NAD^+/NADH which may compromise Sirt3 activity. In this context, the main objective is to study the impact of mitochondrial metabolism and oxidative stress on Sirt3 activity and mitochondrial protein acetylation using i) primary cultures of DRGs neurons from neonatal rats in which FXN level has been reduced by lentivirus transduction and ii) DRGs obtained from a new mouse model of FA based on a I151F point mutation in FXN gene (equivalent to mutation I154F observed in humans). Our results show that FXN-deficient DRG neurons present decreased mitochondrial membrane potential both basal and at the maximal respiration rate, and reduced ATP levels. In addition, an alteration is also observed in the OXPHOS system, in glutathione levels and in antioxidant proteins such as Sod2. Due to this redox alteration, the NAD^+/NADH ratio is decreased, reducing sirtuin activity. Increased acetylation of mitochondrial Sirt3 targets may initiate a deleterious circle of mitochondrial metabolism impairment, finally compromising cell viability. In conclusion, these results provide new data on mitochondrial oxidative stress and a better understanding of the role of Sirt3 on mitochondrial protein acetylation and its effects on FXN deficient DRG neurons.

P-01.2-035
Composition comprising hydroxyl modified fullerene substances decrease plaque load in 5XFAD mouse model of Alzheimer's disease

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The potential of fullerenes and fullerene's water-soluble derivatives to bind to amyloid-β has been well documented *in vitro* and *in silico*. However, the anti-amyloid action of fullerenols in *in vivo* treatments has not been fully examined. In the present study we assessed the effects of the hydroxylated fullerene-water complex (3HFWC) on Alzheimer's disease (AD) neuropathological hallmarks in 5XFAD mice, a well-recognized AD animal model. The 3-week-old 5XFAD mice were exposed to 3HFWC water solution *ad libitum* for 3 months. The 3HFWC treatment started in the presymptomatic phase of pathology and analyses were focused on the effects on amyloid-β (Aβ) accumulation, plaque formation, gliosis, and synaptic plasticity in cortical and hippocampal tissue. The 3HFWC treatment significantly decreased the amyloid-β plaque load in specific parts of cerebral cortex, followed by the unchanged levels of Aβ. None of these changes were detected in the hippocampus. At the same time, 3HFWC treatment did not exacerbate the activation of glial cells, nor altered the expression levels of synaptic proteins. The obtained results point to the potential of 3HFWC, when applied in the presymptomatic phase of AD, to interfere with Aβ accumulation and amyloid plaque formation without exacerbating the other AD-related pathological processes.

P-01.2-036
Comparative analysis of B cell receptor repertoires revealed delay in regulatory B lymphocyte maturation in multiple sclerosis patients

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Regulatory functions of B lymphocytes play an important role in the development and suppression of the immune response. Previously it was shown that deficiency in B regulatory cells (Bregs) or decrease of its anti-inflammatory activity may lead to a number of immunological disturbances, in particular autoimmune diseases. The exact mechanism of Bregs functioning and development is only partially resolved, namely almost nothing is known about structure of its B cell receptors (BCRs). Here, we analyzed BCR repertoire of transitional Bregs subpopulation with CD19+ CD24highCD38high phenotype using high-throughput sequencing of B cells from patients with multiple sclerosis. Our data suggest that transitional B cells from patients with highly active multiple sclerosis carry IgG heavy chain with less amount of hypermutations in comparison with healthy donors. This study was supported by Russian Science Foundation grant # 17-74-30019

P-01.2-037
Dissecting the involvement of acid sphingomyelinase inhibition in GBA-dependent Parkinson disease

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Homozygous mutations in GBA gene, encoding for the lysosomal enzyme β-glucocerebrosidase (GCase), are responsible for Gaucher disease (GD). Interestingly, heterozygous mutations in this gene represent one of the major genetic risk factors for the

development of Parkinson's disease (PD). Recent evidence suggests that variants in other lysosomal genes could contribute to increase the penetrance of GBA mutation in the establishment of the pathological phenotype or could have a protective effect. Recent data reported in literature obtained in different GD models link the deficiency in the lysosomal enzyme acid sphingomyelinase (ASM) with an improvement of the pathological phenotype. Starting from this evidence, we decided to verify whether the ASM inhibition could have a protective role in GBA-PD using IPSC-derived dopaminergic neurons. The IPSCs were generated from human fibroblasts of healthy subjects, differentiated into dopaminergic neurons for 30 days and then treated with Conduritol-B-Epoxide (CBE), a pharmacological inhibitor of GCase. After 60 days in culture, amitriptyline, a functional inhibitor of ASM, was administered to the neurons for 3, 5 and 7 days. CBE-treated neurons showed a strong reduction in GCase activity and the consequent accumulation of glucosylceramide, that diminished after 5 days administration of amitriptyline. We have also observed a significant neurodegeneration in CBE-treated neurons which was partially reduced after 3 days treatment with amitriptyline. These results suggest that the inhibition of ASM could represent a strategy to partially ameliorate the neurodegenerative phenotype in GCase-related pathologies, including PD.

P-01.2-038
Regular exercise training improves the learning abilities of females and induces sex-specific changes in the brain and muscle tissue of healthy and hyperlipidemic mice

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Obesity and physical inactivity exacerbate the development of neurodegenerative diseases, whereas exercise is a promising therapeutic tool to improve cognitive functions. For therapeutic use, exercise should be personalized, and we need to better understand the role of skeletal muscle as an endocrine organ and muscle-brain communication. Therefore, our aim was to study the sex-dependent effects of long-term, medium-intensity treadmill running on the muscle-brain axis in healthy wild-type and hyperlipidemic, high-fat diet-fed (HFD) ApoB100-overexpressing transgenic mice. The Barnes maze test showed that exercise training significantly improved the learning abilities of WT and ApoB100 females, whereas, in males, no difference could be detected. To reveal the underlying mechanisms biochemical and immunohistochemical approaches were applied. The microglia marker IBA1 coverage showed a decreasing trend upon exercise in females, whereas it was elevated due to HFD and was further increased by exercise in the cortex and hypothalamus of ApoB100 males, which may indicate inflammation. The qPCR analysis revealed that metabolism-related genes modulating neural functions, such as leptin, the leptin receptor, and the lactate receptor changed sex-dependently in the brain in response to HFD or exercise. The weight of m. quadriceps increased only in males upon exercise and HFD, the latter indicating a possible fat accumulation in the muscle tissue. Moreover, the gene expression of myokine Il-6 and other factors regulating muscle work, integrity, and metabolism showed remarkable sex differences, revealing that this training was stressful for males but suitable for females. The altered regulations of metabolic pathways in the brain, and the more favorable skeletal muscle response in females can contribute to their better learning abilities. Our work contributes to making personalized exercise a therapeutic option in neurological diseases. This work was funded by NKFIH FK138390.

P-01.2-039
Effect of gamma-aminobutyric acid on membrane electrical properties: *in vitro* studies of rat cerebral cortex synaptosomes and model lipid bilayers

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Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system. GABA dysfunction causes neurological disorders such as Alzheimer's and Huntington's disease, autism spectrum disorders, fragile X syndrome, anxiety, depression, hypertension, cardiovascular diseases and chronic heart failure. Neurotransmitter-induced alteration of synaptosomal and model membranes is studied in view of clarifying its role in development of neurological disorders. The investigation of fundamental principles, governing cell signalling and transmission of nerve impulses, involves detailed characterization of biomembrane electrical properties. Lipid bilayers represent a basic simple model of biological membranes for reproducible measurements of membrane physicochemical characteristics with good control of the experimental parameters. In the present study, we address the effect of GABA on the electrical properties of lipid bilayers by electrochemical impedance spectroscopy. Pinched-off nerve endings synaptosomes have been providing for decades a powerful tool in neurobiology. Surface characteristics of rat cerebral cortex synaptosomes upon GABA addition (10^{-12}-10^{-3} mol L^{-1}) are studied *in vitro* by microelectrophoresis. The decreased zeta potential of synaptosomes measured immediately after isolation is due to reduced surface electrical charge in the presence of GABA. Na^+,K^+-ATPase activity is inhibited after incubation of synaptic membranes with GABA in contrast to acetylcholinesterase and Mg^{2+}-ATPase activities. GABA interaction with model membranes provides a valuable tool for exploring the membrane electrical and electrokinetic phenomena and unravelling molecular mechanisms of neurotransmitter action on membranes. Funding by Bulgarian National Science Fund (BNSF), Ministry of Education and Science of the Republic of Bulgaria, grant № KP-06-N58/6/2021, is acknowledged. *The authors marked with an asterisk equally contributed to the work.

P-01.2-040
Serotonin in the different brain structures of DAT-/- rats

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DAT-KO rats have knock-out of DAT (dopamine transporter) gene. This model was developed to research diseases caused by the accumulation of extracellular dopamine with a simultaneous decrease in its intracellular content. An influence of knock-out DAT gene to content of dopamine is well-known, but the influence of this knock-out on other biogenic amines, particularly serotonin is not well-studied. The aim of this research was to analyze the level of serotonin and its metabolite in the different structures of 1-month old DAT-/- rats. The study was performed by HPLC-ED method with using external standard, levels of serotonin (5-HT) and 5-hydroxyindoleacetic acid acid (HIAA) were expressed as ng/mg protein in the sample. The second aim was devoted expression level of MAO-A RNA analyzing. MAO-A is an enzyme which deaminizes amines such as dopamine and serotonin. There were found 30% decreasing of 5-HT level with a simultaneous increasing HIAA level by 3.8 times (0.8 ± 0.2 vs 3.2 ± 0.5, $P = 0.002$) in striatum of DAT-/- rats. In the hippocampus was shown a 1.8-fold decrease of HIAA. The most pronounced changes were observed in the cerebellum: DAT-/- rat's level of 5-HT decreased by 12 times in comparison with DAT+/+ rat's level (0.07 ± 0.01 vs 0.92 ± 0.03, $P = 0.007$) with unchanged HIAA amount. In the medulla oblongata the amount of 5-HT was lower than the level of detection, while in the control it is 0.6 ± 0.3. The MAO-A RNA level of DAT-KO rats in comparison WT rats was reduced: by 2.5 times in the striatum ($P = 0.003$) and by the 12 times in the medulla oblongata ($P = 0.0031$); in the cortex the MAO-A RNA level was higher in DAT-KO rats by 2.1 times ($P = 0.004$). Since serotonin enhances neurogenesis, we suggest that the pronounced decrease of the content of 5-HT which isn't related with increased MAO-A activity indicates a decrease in the intensity of neurogenesis in the brain of DAT -/- rats. The reported study was funded by RSF, project number 22-25-00124.

P-01.2-041
Cholesterol oxime olesoxime (TRO19622) as a potential reactivator of nerve agent-inhibited cholinesterases

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Olesoxime (cholest-4-en-3-one) is a cholesterol derivative originally developed in 2007 as an experimental drug for treatment of a neurodegenerative disorder amyotrophic lateral sclerosis (ALS). Olesoxime exhibited broad neuroprotective effects in a number of cell and animal models, prompting further preclinical studies for other neurodegenerative diseases and phase II/III clinical trials for treatment of ALS and spinal muscular atrophy (SMA). Although olesoxime failed to achieve relevant therapeutic effects in clinical studies, its ability to cross the blood–brain barrier, excellent safety and tolerability properties and presence of an oxime group reveal it as a potential antidote and centrally active reactivator for nerve agent-inhibited cholinesterases. Nerve agents (NA) are organophosphate compounds which irreversibly inhibit enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), serine esterases which hydrolyze neurotransmitter acetylcholine. Disruption of their activity leads to uncontrolled signal transduction in synapses, cholinergic crisis and potentially life-threatening symptoms. In this work, we evaluated the potency of olesoxime to reactivate AChE and BChE inhibited by NAs sarin, cyclosarin, tabun and VX and determined the binding affinity for the enzymes in terms of IC_{50} value. Molecular modelling enabled evaluation of oxime-enzyme interactions important for specificity and selectivity of both inhibition and reactivation. Olesoxime showed reactivation potential for cyclosarin-inhibited BChE, with the overall reactivation rate constant (k_r) of 476 ± 12 M^{-1} min^{-1} comparable to that of standard oxime HI-6 (780 ± 30 M^{-1} min^{-1}). We also tested the ability of olesoxime to assist a clinically used standard oxime 2-PAM and the promising results highlight the undiscovered capability of olesoxime as a novel NA poisoning antidote.

P-01.2-042
Every-other-day feeding affects the number of parvalbumin-expressing neurons and BDNF/Trk signaling in the cortex of 5xFAD mice

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Neuroprotective effects of food restriction were demonstrated in several animal models of stroke, traumatic brain injury, and neurodegenerative diseases. Since Alzheimer's disease (AD) is characterized by a long silent prodromal phase, the present study aimed to determine the effects of every-other-day (EOD) feeding on cortical responsiveness to PV interneurons and BDNF/TrkB signaling using 5xFAD mice, a well-characterized mouse model of AD. Female 5xFAD transgenic (Tg) mice and their non-transgenic littermates were exposed to ad libitum (AL) or EOD feeding regimen, beginning at 2 months of age. Neurons expressing PV were detected in the retrosplenial dysgranular cortex (RSGc), retrosplenial granular cortex (RSD), parietal cortex (PtA), and somatosensory cortex (S) of 6-month-old animals by immunohistochemistry. Analysis of the BDNF/Trk signaling was examined by western blot. TgAL mice showed a significantly reduced number of PV-positive cells in the RSGc, PtA, and S, while no changes were detected in the RSD. Interestingly, four months of EOD feeding reverted the number of PV-positive cells to control values in all three regions examined. BDNF was decreased in the TgAL mice, which was additionally decreased in TgEOD mice, while no significant difference in pro-BDNF was identified. Analysis of TrkB and pTrkB revealed a significant increase of TrkB in the TgEOD group, whereas a significant decrease in pTrkB was detected only in the TgAL group. The present study indicates that every-other-day feeding can ameliorate PV neuronal loss, and have an important role in further understanding the neural basis of AD-like-associated cognitive impairments in 5xFAD mouse model of AD.

P-01.2-043
Structural analysis and molecular dynamic processes observed in Aβ1-40 amyloid under interaction with nanoparticles by NMR spectroscopy and molecular dynamic simulations

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The formation of neurotoxic amyloid Aβ peptide oligomers is accepted as the Alzheimer's disease progression model which is responsible for the formation of insoluble deposits in the brain. It was shown that both copper and zinc ions are deposited in amyloid plaques [1]. In the presented study we perform structural analysis and explore molecular dynamic processes observed in both, free and bound states, of the Aβ1-40 amyloid in solution at the presence of copper nanoparticles of selected sizes (NP). Our previous work resulted in the expression of 13C,15N-labeled Aβ1-40 suitable for multidimensional NMR spectroscopy. Now, we explore the influence of copper NP on monomeric and profibril forms, using Aβ1-40 peptide as a model of the Aβ amyloid. The recently installed cryogenic probehead, make it possible to obtain high-quality experimental data even for NMR samples containing paramagnetic Cu(II) NP. Previously, based on 15N relaxation data, the mechanism formation of polydisperse Aβ protofibrils was described as a two-stage process for both Aβ1-40 and Aβ1-42, peptides [2]. In our study, the 15N relaxation experiments R1rho and Chemical Exchange Saturation Transfer (CEST) [3] were applied to the analysis interactions Aβ1-40 with NP. The PFG NMR spectroscopy is applied to distinguish between free and bound forms of Aβ amyloid. Finally, multiscale molecular dynamic calculations provide information about conformers and molecular interactions with NP at the structure and atomic levels. *Acknowledgment* This research was supported by a research grant 2021/41/B/ST4/03807 from National Science Centre (Poland). *References:* 1. James S.A., et al. (2017) ACS Chem. Neurosci., 8, 629-637 2. Fawzi N.L., et al. (2011) Nature, 480, 268-272 3. Vallurupalli, P. et al. (2013) Angew. Chemie. Int. Ed., 52, 4156-4159.

P-01.2-044
Fluorescence-activated cell sorting of dopaminergic neurons from the substantia nigra of mice as a new approach to study their functioning in norm and pathology

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The study of the molecular mechanisms of the functioning of dopaminergic neurons (DNs) of the nigrostriatal system of the brain is among highest priorities in neuroscience, since they play a key role in the regulation of motor behavior, and their death leads to the development of Parkinson's disease (PD). The aim of this work was to develop a method for selection a pure population of DNs from the substantia nigra (SN) and to assess the possibility of studying these neurons in norm and pathology. To this end, three objectives were solved. First, we found the optimal conditions for selecting DNs from SN of intact mice using two fluorescent markers, DRAQ5 (nuclear marker) and GBR-BP (original non-commercial stain). The latter is internalized into DAs via the dopamine transporter. Optimal conditions included using GBR-BP at a concentration of 50 nM and staining of DN in vibratome sections of SN prior to cell dissociation. When solving the second issue, it was proved that the sorted neurons are dopaminergic. Indeed, these cells were shown to express the genes for the synthesis of dopamine and dopamine transporter. The most convincing evidence is the demonstration that tyrosine hydroxylase was detected immunohistochemically in 97% of sorted neurons. When solving the third issue, we obtained evidence that in DNs sorted from SN cell suspension, it is possible to quantify changes in at least the expression of genes for proteins involved in dopamine metabolism and neurotransmission in animals in pathology, in this work, when modeling PD. Thus, when modeling the early clinical stage of PD in MPTP-treated mice, unidirectional and similar changes in the number of DNs and the expression of genes for tyrosine hydroxylase and dopamine transporter were found in sorted DNs and in DNs in the whole SN. This project was funded by Ministry of Science and Higher Education of the RF (grant agreement № 075-15-2020-795 of 29.09.2020).

P-01.2-045
Regulatory factors of neurite generation of human stem cell-derived neural progenitor cells

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Establishment of pluripotent stem cell technology opened new perspectives for studying hard-to-obtain human tissues, such as the neural tissue. Neural progenitor cells (NPCs) represent a cell population that play pivotal role in neural development and regeneration. In the present study, we focused on the initial step of neural regeneration, the formation of neurites in NPCs. We aimed at identifying the molecular and cellular factors that confine and modulate this process. Neurite outgrowth of GFP-expressing NPCs was studied under normal and injury-related conditions using a high-content screening system. We found that extracellular matrix components strongly influenced the rate of neurite formation but inhibitors of the non-muscle myosin II and the upstream regulatory kinase, ROCK1 were able to override the inhibitory effect of a restrictive environment. We also investigated the modulatory effect of microglial cells on neurite formation of NPCs. We observed that NPCs' differentiation and proliferation was distinctively influenced by microglia depending their activation state, i.e., naïve, stimulated with pro- or anti-inflammatory agents. Our results help better understanding of human neural development and regeneration at the progenitor level, thus providing opportunities for development of novel regenerative therapeutic interventions. This work has been supported by the National Research, Development and Innovation Office [the Hungarian Scientific Research Fund (OTKA)

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P-01.2-046
Identification of novel dopamine D2 receptor ligands – a combined *in silico* / *in vitro* approach

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Diseases of the central nervous system (CNS) are an alarming, global problem showing an increasing prevalence. The dopamine receptor D_2 (D_2R) has been shown to be involved in CNS diseases and is a major player in neurodegenerative diseases (NDDs) such as Parkinson's Disease (PD). While different D_2R-targeting drugs have been approved by the FDA, they all suffer from major drawbacks due to decreasing therapeutic efficacy and adverse effects during therapy. Combined *in silico* / *in vitro* approaches have been shown to be efficient strategies to find novel active scaffolds for established targets. In this study, in silico pharmacophore models were generated utilizing both ligand- and structure-based (LB and SB, respectively) approaches. SB pharmacophore models were based on the cryo-EM structure of the D_2R in complex with its agonist bromocriptine which was only published in 2021. Thus, this structure allows for novel insights into receptor-ligand interactions. Moreover, both LB and SB models were generated using the two modelling software packages LigandScout and DiscoveryStudio, thus, exploiting their different pharmacophore algorithms. Subsequently, different compound databases were screened for potential D_2R ligands. Selected virtual hits were investigated *in vitro*, quantifying their binding affinity towards the D_2R utilizing a cell-based homogenous time-resolved fluorescence (HTRF) assay. With this workflow we successfully identified six novel D_2R ligands exerting micro- to nanomolar (most active compound K_I = 4.1 nM) binding affinities. Thus, the developed pharmacophore models showed prospective true positive hit rates in between 4.5 and 12 %. The developed workflow and identified ligands could aid in developing novel drug candidates for D_2R-associated pathologies. Additionally, the developed workflow would contribute to the discovery of novel pharmacological tools.

P-01.2-047
Altered gene expression profile in ALS glial cells is associated with a modified chromatin structure

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Amyotrophic lateral sclerosis (ALS) is a lethal neurodegenerative disease where a selective and progressive loss of motoneurons occurs. Non-cell-autonomous mechanisms by astrocytes expressing ALS-linked mutant proteins (i.e. in SOD1, TDP43 and C9orf72) have been shown to mediate motoneuron death. Transcriptomic analyses show that a significant number of genes are up- and down-regulated in mouse astrocytes expressing mutant SOD1 (mutSOD1). Here, we show that epigenetic mechanisms, including global changes in chromatin accessibility (ATAC-seq) and in the distribution of the histone H3K9me3 repressive mark (ChIP-seq) accompany the altered transcriptomic profile of mut-SOD1 astrocytes. Immunofluorescence staining and quantitative confocal imaging indicate that ALS astrocytes display a significant reduction in the number of H3K9me3-immunoreactive nuclear foci relative to control astrocytes. Western blot assays confirm that the nuclear levels of this repressive epigenetic mark are significantly decreased in ALS astrocytes. We also find that a set of non-glial genes that are transcriptionally enhanced in mut-SOD1 astrocytes display reduced H3K9me3 enrichment at their promoter sequences. Together, these data support a model where a loss of H3K9me3-dependent epigenetic control at these non-glial genes contributes to their miss-regulation during ALS onset and/or progression. This work is supported by grants from FONDECYT 1211026 and 1181645.

P-01.2-048
Patterning of AQP4 molecular distribution through CNS development

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Aquaporin 4 (AQP4) is the most abundant water channel within the central nervous system (CNS). It is expressed in ependymal cells and glia limitans, including the foot processes of pericapillary astrocytes, where this protein plays an important role in the homeostasis of cerebrospinal fluid (CSF). However, most studies on its expression have always been carried out in adult animals, and its appearance and location pattern remain unknown throughout postnatal development. In the present work, we evaluated the localization and abundance of this channel by studying the expression of AQP4 mRNA by *in situ* hybridization (ISH) and qPCR. Jointly, we analyzed the protein expression by immunofluorescence and western blot assays against AQP4. These studies were carried out in the brain of wild-type (WT) C57BL/6 mice of 1, 3, 7, 11, 60 days and 18 months of age. The results obtained showed a significant change in the location of AQP4, which appears first in the corpus callosum and cerebellum at early ages and moves to the cortical areas in animals older than 11 days in a vascular-governed pattern. This observation might point out a role for AQP4 in fibrous astrocytes during myelination at the first postnatal weeks never previously described, in contrast to its widely studied function in the foot processes of protoplasmic astrocytes shaping the blood–brain barrier (BBB).

P-01.2-049
Oligomerization acts as gatekeeper for TDP-43 nucleocytoplasmic shuttling and modulates its aggregation patterns

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The invariably fatal neurodegenerative diseases amyotrophic lateral sclerosis and frontotemporal lobar degeneration (FTLD)

share a common neuropathological feature: ubiquitin-decorated aggregates comprising the RNA-binding protein TDP-43. Under physiological conditions, TDP-43 is predominantly nuclear and contained in droplets formed via liquid–liquid phase separation (LLPS). Previous studies have shown that RNA binding and self-oligomerization via the N-terminal domain (NTD) are required for TDP-43 to perform its essential functions in nuclear RNA processing. In disease, most commonly TDP-43 is depleted from the nucleus and instead found in cytoplasmic aggregates. Additionally, intranuclear TDP-43 inclusions hallmark two FTLD subtypes. To date, the mechanisms governing TDP-43 dysregulation and aggregation in distinct patterns are incompletely understood. Here, we studied the role of oligomerization and RNA binding in TDP-43 physiology and transition to pathology. Our data reveal that both TDP-43 oligomerization and RNA binding act as gatekeepers for nucleocytoplasmic shuttling and modulate its LLPS properties in human neural networks and HEK293 cells. Furthermore, we show for the first time that also in the cytoplasm TDP-43 exists as NTD-driven oligomer. Mimicking failure of the ubiquitin-proteasome system observed in TDP-43 aggregate-bearing cells in patients, we employed proteasome inhibition paradigms and found that TDP-43 aggregates via LLPS in the nucleus, but in an aggresome-dependent manner in the cytoplasm. The favored aggregation pathway depended on both TDP-43 state (monomeric versus oligomeric, RNA-bound versus RNA-unbound) and the subcellular environment. Taken together, RNA binding and oligomerization allow TDP-43 to maintain its localization in physiology and their disruption drives distinct aggregation pathways in the nucleus and cytoplasm, indicating independent molecular origins for the different inclusion types observed in TDP-43 proteinopathies. *The authors marked with an asterisk equally contributed to the work.

P-01.2-050
AQP4 deletion leads to ependymal abnormalities underlying congenital hydrocephalus pathology

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Aquaporin 4 (AQP4) is the most widely expressed water channel in the central nervous system (CNS). Its presence in ependymal cells, glia limitans, and pericapillary astrocyte foot processes is essential to achieve proper solute clearance and fluid homeostasis within the CNS. In the neural developmental context, the expression of AQP4 is reported to start postnatally and experiences a sharp increase during the first two postnatal weeks. Intriguingly, at the same developmental period, a small percentage of the offspring of AQP4 knock-out ($AQP4^{-/-}$) mice develop congenital hydrocephalus, produced by Sylvian aqueduct stenosis which leads to the premature death of the animal. Here, transcriptomic microarray analysis of the periaqueductal tissue at P11 was performed, showing relevant changes in gene subsets related to ependymal cell function in both, the hydrocephalic and non-hydrocephalic $AQP4^{-/-}$ mice compared to wild-type littermates. Independent qPCR experiments measuring expression levels of genes associated with ciliary function and development were conducted to validate the *in silico* analysis. To further explore these results, the ependymal surface lining the Sylvian aqueduct was examined by scanning electron microscopy (SEM) showing an impaired formation of the structural ciliary organization and planar polarity. In conclusion, our study shows for the first time a functional role of AQP4 contributing to the normal development of the aqueductal ependymal barrier.

P-01.2-051
Neurite outgrowth and guidance (NOG) motif derived peptides are biomimetic and can restore neuritogenesis in a CRASH syndrome cell model

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Homo- and heterophilic binding mediated by the immunoglobulin (Ig)-like repeats of cell adhesion molecules play a pivotal role in cell–cell and cell–extracellular matrix interactions. By performing protein sequence alignment, we found that specific Ig-like repeats from the extracellular domains of neuronal cell adhesion molecules share a highly conserved neurite outgrowth and guidance (NOG) motif, and presented evidence shows it is crucial in mediating such interactions during neural development and repair. Molecular dynamics simulations (MD) and docking were used to infer the role of the NOG motif in neuronal CAMs structure. We also found NOG-derived peptides could promote neuritogenesis in cell cultures at levels comparable with treatment with the whole proteins they were derived from (Scapin et al, 2021, CSBJ, 19:5622-5636). Among such proteins, the neuronal membrane receptor L1CAM is crucial in neuronal differentiation, and several studies suggest that many of its functional interactions are mediated by Ig2-Ig2 binding. X-linked mutations in the human L1CAM gene are summarized as L1 diseases, including the CRASH neurodevelopmental syndrome, which abrogates neuritogenesis in cell models. MD simulations provided a molecular rationale for CRASH phenotypes resulting from mutations I179S and R184Q in the NOG region of L1CAM. Docking simulations and SPR assays showed peptides could bind L1CAM Ig2 site even in the presence of mutations while L1CAM could not. *In vitro* assays on neuronal derived cells expressing mutant L1CAM confirmed *in silico* results: while treatment with recombinant L1CAM ectodomain proved ineffective, NOG peptides could rescue neuritogenesis in a cellular model of the CRASH syndrome (Gasparotto et al, 2022, Biomedicines, 10(1):102). Presented evidence describes the role of the NOG motif in neuronal CAMs structure and function and opens the route to the use of NOG-derived peptides as biotechnological and therapeutic tools. *The authors marked with an asterisk equally contributed to the work.

P-01.2-052
Neuroprotective strategies for neonatal hypoxia-ischemia

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Neonatal hypoxia-ischemia (HI) is a brain injury caused by oxygen deprivation to the brain due to birth asphyxia or reduced cerebral blood perfusion. HI remains one of the leading causes of

neonatal mortality and morbidity worldwide, and it often leads to lifelong limiting sequels such as cerebral palsy, seizures, or mental retardation. HI physiopathology involves oxidative stress, inflammation, and apoptosis, among other mechanisms. Therapeutic hypothermia is the current standard of care for HI, but it does not provide complete neuroprotection and can only be applied to a subset of newborns that meet strict inclusion criteria. Given the unpredictable nature of the obstetric complications that contribute to neonatal HI, prophylactic treatments that prevent, rather than rescue, HI brain injury are emerging as a therapeutic alternative. Natural compounds or nutraceuticals that have antioxidant, anti-inflammatory, or antiapoptotic properties are suitable candidates for this purpose. A growing number of preclinical *in vivo* studies shows that nutraceuticals can prevent and reduce HI-induced brain damage and cognitive impairments, including omega-3 fatty acids, melatonin, and several phenolic compounds such as stilbenoids or flavonoids. Our lab is interested in evaluating the neuroprotective activity of a plant-derived phenolic compound. We use the Rice-Vannucci method to induce HI to mouse pups at postnatal day 7, by ligation of the left common artery followed by hypoxia. Our results indicate that pretreatment with the phenolic compound can significantly reduce the amount of tissue loss and the severity of the HI-induced brain lesion in a dose-dependent manner. Future experiments will examine whether this compound can also preserve myelinisation and regulate astrocyte and microglia activation. If results are positive, we will supplement maternal diet during pregnancy with the phenolic compound as a dietary neuroprotective strategy for neonatal HI.

P-01.2-053
The vitamin K-dependent survival factor Gas6 is expressed by brain glial cells and is upregulated in human multiple sclerosis brain lesions

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Gas6 is a vitamin K-dependent protein that is involved in regulation of cell survival and the inflammatory/immune response in various tissues through acting via TAM receptors. In the central nervous system (CNS), Gas6 acts on glial cells to promote myelination and suppress proinflammatory signalling. However, little is known about the cellular sources of Gas6 in the CNS, nor the regulation of its expression in the human brain undergoing pathology. The aims of this study were to: (i) study the expression of Gas6 in the mouse brain; (ii) determine Gas6 expression status in post-mortem brains from patients with the demyelinating disease multiple sclerosis (MS). Animal procedures and Imperial College Brain Tissue Bank analyses were carried out with approval from relevant national and local ethics committees. Whole brain tissues were obtained from mice at different ages (embryonic – adult). *In vitro* primary cultures were set up of microglia and astrocytes from neonatal mouse brains. Samples from lesion and matched non-lesion areas were isolated from human MS brains (secondary progressive MS). Gas6 and TAM receptor expression at mRNA and protein levels were determined by RT-qPCR, western blot and ELISA. Gas6 and TAM expression was detected in whole mouse brain, showing a steady increase throughout postnatal development. Gas6 was also expressed by cultured astrocytes and microglia, with higher expression in the latter. In human MS brains, both Gas6 gene and protein expression were found to be higher in lesion tissue as compared to non-lesion. Gas6 expression is inherent to the brain, with glia as distinct cellular sources of the protein. Gas6 also appears to be upregulated in human MS lesions. These results provide an insight into the role of Gas6 as a regulator of inflammation, immunity and tissue repair in the CNS. Further work should elucidate a potential role for exploiting Gas6/TAM signalling as a therapeutic target for these diseases. *The authors marked with an asterisk equally contributed to the work.

P-01.2-054
The role of MEF2-HDAC transcriptional complexes in neuronal protection in stroke

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Ischemic stroke is a debilitating event that can lead to severe disability or death. While ischemic stroke has well documented negative effects on central nervous system (CNS) grey matter, it can even more severely damage white matter. In the eye, retinal ganglion cells (RGCs) are CNS neurons that transmit visual information from the retina via the optic nerve to the brain. Strokes of the optic nerve cause permanent blindness due to RGC axon damage and retrograde cell death. New data suggests that inhibition of myocyte enhancer factor 2A (MEF2A) transcription factor activity promotes RGC survival following injury. In this study, we tested if class IIa HDAC (histone associated class IIa histone deacetylases) mediated repression of MEF2A impacts neurosurvival and neuroprotection, providing new insight into the mechanisms underlying the pathophysiology of ischemic injury. In order to investigate the role of MEF2A, MEF2D and HDACs in neuroprotection, we used primary cultures of rat E18 hippocampal neurons grown in the starvation media. Surviving neurons spontaneously exhibit neurite outgrowth, and measurement of the longest neurite represents an assay for axon growth as a model for axon regeneration. To test whether HDAC-mediated repression promotes axon extension and neuronal survival, MEF2A expression was depleted with siRNA and mutant MEF2A, MEF2D and HDAC4/5 proteins were used. Surprisingly, MEF2A silencing didn't cause significant neurite extension, even after KCl stimulation. Some interesting changes we found for constitutively active and desumoylated MEF2D mutants indicating a possible role of this protein in neuronal survival. Moreover, increased HDAC4/5 nuclear localization, inhibited promoted neurite outgrowth and survival in primary neurons. These results suggest that enhancement of HDAC activity might be protective in neurons following stroke. Supported by National Science Center in Poland (Narodowe Centrum Nauki), grant number: 2020/39/D/NZ4/01250

P-01.2-055
Insights into the protective role of natural aminosterols in TDP-43-associated proteinopathies

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A number of neurodegenerative conditions, such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD), are associated with a common histopathology within neurons of the central nervous system, consisting in the deposition of cytosolic inclusions of TAR DNA-binding protein 43 (TDP-43). In the last years, natural aminosterols such as squalamine and trodusquemine showed significant protective effects against the pathological aggregation and toxicity of alpha-synuclein and amyloid-beta peptide. Our study with natural aminosterols was aimed to probe their effects on inhibiting the cytotoxicity of TDP-43 toward motor neurons in neurodegenerative diseases. The protective effect of natural aminosterols against the deleterious deposition of TDP-43 in neuronal cytoplasmic inclusions was evaluated by exploiting the high resolution power of Stimulated Emission Depletion (STED) microscopy in murine NSC-34 cells, in which we have expressed human full-length TDP-43. We also assessed the effects of aminosterols using a well-established transgenic *C. elegans* model of ALS, in which TDP-43 forms inclusions over time in the large muscle cells, thus leading to age-dependent paralysis. We show that naturally occurring aminosterols prevent the mislocalisation of overexpressed human wild-type full-length TDP-43 in our cell model. In particular, they decrease the cytosolic TDP-43 accumulation with a re-localization of the protein in the nucleus, and significantly rescue TDP-43-associated cytotoxicity. When administered to TDP-1 worms, aminosterols significantly decrease the formation of muscle inclusions and fully recover worm motility. This study provides important evidence that natural aminosterols could be rationally optimized in drug discovery programs to target TDP-43 toxicity in ALS and other devastating neurodegenerative diseases. The study was supported by Fondazione Cassa di Risparmio di Firenze (TROTHERALS to C.C.).

P-01.2-056
The crosstalk between periphery and CNS in relation to Parkinson disease

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Degeneration of the dopaminergic neurons are the characteristic feature of the Parkinson disease, affecting around 7–10 million people worldwide [Dogra N et al. (2021) Cellular and Molecular Neurobiology, pp.1-18], among them 90% cases are sporadic [Michel P.P et al (2016) Neuron, 90(4), pp.675-691]. Despite the different proposed mechanism, the involvement of gut-brain axis has attracted much attention, in which infiltration of peripheral cytokines like IL-6 and TNFα are supposed to a risk factor of PD [Dogra N et al. (2021) Cellular and Molecular Neurobiology, pp.1-18, Chen H et al (2008) American journal of epidemiology, 167(1), pp.90-95.]. To understand the effect of cytokine and dopamine auto-oxidation due to α-synuclein aggregation, we treated the SHSY5Y cells with TNFα in the presence and the absence of dopamine. Significant cell death was observed in TNFα with dopamine treatment compared to TNFα alone. Future experiments will be to understand the interaction between different cell lines such as IHH, HepG2, HMC-III microglia, and SH-SY5Y in PD.

P-01.2-057
Intracellular thermometry reveals amyloid-β 42 aggregation leads to cellular thermogenesis

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The aggregation of amyloid-β 42 (Aβ42) is a hallmark of Alzheimer's disease, and the highest risk factor of this neurodegenerative disease is age. It is still not known what the biochemical changes are inside a cell which will eventually lead to Aβ42 aggregation. Thermogenesis has been associated with cellular stress, the latter of which may promote aggregation. Using super-resolution microscopy, we visualise the nanoscale fibrillar structures of exogenously added Aβ42 formed in the cytoplasm of live HEK293T cells. We then perform intracellular thermometry measurements using fluorescent polymeric thermometers (FPTs) to show that Aβ42 aggregation leads to an increase in the average cell temperature. This rise in temperature is mitigated upon treatment with an aggregation inhibitor of Aβ42. To investigate the intertwined pathways of Aβ42 and mitochondrial dysfunction, we additionally perform mitochondrial metabolism assays. We show that the main contribution to the thermogenesis effect observed in cells undergoing Aβ42 aggregation is due to the exothermic elongation process and is less dependent of mitochondrial damage that can otherwise lead to thermogenesis. To conclude, we present a diagnostic assay which could be used to screen small-molecule inhibitors to neurodegeneration-related amyloid proteins in physiologically relevant settings.

Diabetes and obesity

P-01.3-001
Finding a receptor isoform selective and thermally stable insulin analogue for a better life comfort of diabetic patients

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Insulin is a hormone that has a key role in glucose metabolism and energy homeostasis. Insulin elicits its functions through binding to the insulin receptor (IR), which exists in two isoforms, IR-A and IR-B. Both isoforms have different tissue distribution. The longer

IR-B is predominant in adult hepatocytes (more than 90%), skeletal muscle and subcutaneous fat (both about 70% IR-B), while the shorter IR-A is almost exclusively expressed in the brain, lymphatic tissues, or embryo. We are aiming to design insulin analogues, which could increase the comfort of diabetic patients. First, such an insulin analogue should bind preferentially to insulin receptor isoform B and therefore enable a more physiological control of internal glucose metabolism. Second, thermally more stable insulin could eliminate a constant need for refrigeration and could give patients more freedom and comfort. We systematically designed dozens of insulin analogues based on the known structures of the insulin–receptor complexes. We assessed binding specificities of all analogues through a competitive binding assay with radioactively labelled insulin in cell cultures which only express IR-A or IR-B. We have chosen several analogues with increased selectivity for IR-B to assess their thermal stability. We measured the increase of fluorescence of Thioflavin T, which is emitted upon binding to insulin insoluble fibrils. One insulin analogue with an extended and amidated C-terminus of B-chain showed more than 3 times higher selectivity for IR-B and more than 4 times higher thermal stability than human insulin. We have also shown that this analogue is as efficient as human insulin in lowering blood glucose in mice (Páníková *et al*., J. Med. Chem. 64, 14848, 2021 and PCT/CZ2021/050123). Inspired by these findings we are continuing our search for more stable and receptor-specific insulin analogues. We are also searching for an explanation of the increased thermal stability of the insulin analogues.

P-01.3-002
Could some areas of brain be impaired in diabetes mellitus and protected by nicotinamide?

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Diabetes mellitus (DM) induces central nervous system abnormalities including changes in protein functions, but the molecular mechanisms are still unclear. The aim of study was to elucidate whether expression of key proteins in diabetic brain areas (cerebral cortex, hippocampus and cerebellum) is impaired and whether nicotinamide (NAm) treatment can correct this. Studies used a model of type 2 DM induced by high-fat diet combined with a low-dose STZ injection in male Wistar rats treated for 2 weeks with or without NAm (100 mg/kg, b.w.,i.p.). The levels of vascular endothelial growth factor (VEGF), sirtuins 2 (SIRT2), ionized calcium binding adaptor molecule 1 (Iba-1), zonula occludens-one (ZO-1), phospho-tau were assessed by immunoblotting followed by densitometric analysis. NAm affected neither blood glucose level nor body weight in diabetic rats. DM led to VEGF level elevation in cortex and hippocampus, which was normalized by NAm. Levels of tight junction protein ZO-1 were down-regulated only in the cortex and were partially increased by NAm. So, NAm can positively affect blood–brain barrier functions that are dependent on expression of VEGF and ZO-1 proteins. Levels of SIRT2, NAD-dependent protein deacetylase were increased in diabetic cerebral cortex and normalized by NAm. Phospho-tau level, the major microtubule associated protein of a mature neuron, was increased in cortex and cerebellum and wasn't changed in hippocampus in DM. NAm partially decreased it in cerebellum, normalized in cortex, didn't affect in hippocampus. Iba-1, microglia/macrophage-specific protein, was increased only in hippocampus in DM and normalized by NAm. Thus, NAm is not excluded that through modulating NAD-dependent processes leads to improving angiogenic factors expression, interaction of tight junctions' proteins, activation of microglia and regulating the assembly and stability of microtubules in the axons of neurons in investigated brain areas in DM.

P-01.3-003
High-throughput N-glycoprofiling of human complement C3 in recent onset type 1 diabetes mellitus

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The complement system plays a key role in immune responses, and has a significant role in the pathogenesis of many diseases with immune basis. Its most abundant and central component, glycoprotein C3, contributes to the development of type 1 diabetes (T1D) by enhancing the organ-specific autoimmune inflammatory processes. Moreover, it is known that changes in glycosylation can modulate inflammatory responses and direct role of glycans in the pathophysiology of diabetes is also confirmed. Despite the growing recognition of the involvement of complement and N-glycans in the pathological process leading to diabetes, N-glycosylation of C3 remains poorly investigated, as there has been no appropriate analytical method for large scale studies of the C3 N-glycome. Therefore, we developed a novel high-throughput and cost-effective glycoproteomic workflow for a site specific N-glycosylation LC-MS analysis of human C3 to reveal the possible role of C3 glycosylation in T1D development. The method includes C3 enrichment from human plasma in a 96-well format using Concanavalin A lectin affinity matrix, Glu-C digestion of enriched glycoproteins, hydrophilic interaction chromatography-based solid-phase extraction (HILIC-SPE) glycopeptide purification, and nano-LC-MS analysis. The method was successfully applied to healthy subjects (N = 14) sampled at 3 time points and showed unprecedentedly that C3 N-glycome is stable in healthy individuals during homeostasis. To evaluate its diagnostic potential, we studied plasma samples from 61 children and adolescents (1-16 years) newly diagnosed with T1D as well as their 84 (4-22 years) unaffected siblings. Our study revealed significant differences in C3 N-glycan profiles between the two groups, associating the T1D with an increase in the proportion of glycan structures with more mannose units and confirming the relevance of newly developed method for further research of C3 N-glycosylation's role in diabetes and other diseases.

P-01.3-004
Are inflammatory markers necessary to differentiate retinopathy subgroups?

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This study was aimed to investigate the role of the Orexin-A, IL-34, and Follistatin-like protein 1 (FLP-1) in different types of

retinopathy patients. Sixty-five patients with different retinopathy groups Non- Diabetic Retinopathy Group (n = 25), Non-Proliferative Diabetic Retinopathy Group (n = 20), Proliferative Diabetic Retinopathy Group (n = 20) were included. For comparison, 38 healthy controls (n = 22) were also included in the study. Also, in all subjects, surgical operational status and the relationship between biomarkers and routine biochemistry tests were examined. The biomarker levels were estimated using a quantitative enzyme-linked immunosorbent assay. Serum IL-34 levels (2.58-63.12 (4.26)) pg/mL were significantly higher in the Proliferative Diabetic Retinopathy Group compared to other groups ($P = 0.009$). In addition, serum Orexin-A levels (48.7 ± 16.32) pg/mL were significantly higher in the Non-Proliferative Diabetic Retinopathy Group compared to other groups ($P = 0.025$). Also, there is a positive correlation between serum IL-34 levels and FLP-1 levels in the proliferative retinopathy group ($r = 0.545$, $P = 0.013$) Serum IL-34 levels are significantly altered between retinopathy groups. Further investigations are needed for the role of biomarkers to differentiate retinopathy groups.

P-01.3-005
Exploring the proinflammatory effect of emerging chlorinated contaminants in adipocytes

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Obesity has become pandemic owing to the increased prevalence of a sedentary lifestyle and a low cost of energy-dense food. It is associated with the increased risk of diabetes mellitus (DM) and cardiovascular diseases (CVD). Chlorinated paraffins (CPs) are the emerging persistent organic pollutants (POPs) produced on an industrial scale and commonly found in fatty food. While the effects of recently banned short-chain CPs on human health are well known, the knowledge about medium-chain CPs is still lacking, even though their physicochemical properties and their bioaccumulation potential are similar. Recent research has uncovered a synergistic effect of POPs and obesity in the promotion of DM and CVD. However, the mechanism remains unknown. Here, we aimed at developing a reliable methodology to study a hypothetic stimulating effect of CPs on the inflammatory signalling of adipocytes *in vitro*. The effects of different doses of CPs were investigated in 3T3-L1 adipocytes by measuring parameters of redox homeostasis, bioenergetics, and inflammatory signalling by fluorescent probes, qRT-PCR, and ELISA. In parallel, a method based on gas chromatography coupled with high-resolution mass spectrometry was developed for the analysis of CPs' accumulation in adipocytes and culture media. We have successfully quantified the relationship between different concentrations of CPs and their accumulation in adipocytes. Our results show that CPs are accumulating in the adipocytes with a tendency to be washed out when a medium without CPs is added. Also, even though the Oil Red O staining shows a higher accumulation of fat droplets when CPs are added, the viability of these cells is not affected. In conclusion, although CPs were generally nontoxic to investigated cells, we found a significant non-linear effect of their exposure on adipocyte signalling pathways, which may be translated into increased risk of chronic diseases in obesity including DM and CVD.

P-01.3-006
BAT mitochondria: the therapeutic target in the fight against obesity

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Obesity is a global health problem affecting 650 million people worldwide and an important predisposing factor for cardiometabolic diseases. In this multi-organ framework, adipose tissue is critical for energy homeostasis. Our recent work demonstrates that modulation of brown adipose tissue (BAT) activation or white adipose tissue (WAT) browning protects against obesity. BAT is a potential therapeutic target for obesity because of its capacity to burn fat during thermogenesis, thus increasing the energy expenditure. Although it is mainly mediated by uncoupling protein 1 (UCP1), recent studies suggest that UCP1-independent thermogenesis may also contribute to this function. In addition, BAT mediates beneficial effects through the secretion of adipokines. We have found that BAT mitochondrial activity can be modulated by a protein localized in the mitochondrial inner membrane. The deletion of this protein in BAT enhances both thermogenic capacity and energy expenditure under high fat diet, protecting against obesity development. Furthermore, mice lacking this mitochondrial protein maintained BAT temperature after cold exposure, correlating with more cellularity and smaller lipid droplets in brown adipocytes. Surprisingly, these animals did not have a higher content of UCP1, suggesting an alternative mechanism responsible for a higher leak respiration. In fact, transplantation of BAT lacking this protein into WT mice was sufficient to reduce obesity and increase BAT activity. The recipients of BAT from KO animals showed an induced browning in the subcutaneous WAT. Taken together, our data indicate that deletion of this mitochondrial protein in BAT might be beneficial in obesity through two mechanisms: increasing BAT thermogenesis and inducing WAT browning through an endocrine mechanism. The tools to inhibit this target *in vivo* have been developed in humans, and we can therefore now evaluate its potential use as a therapeutic target.

P-01.3-007
Fibroblast growth factor 21 and CL 316,243 synergistically ameliorate metabolic abnormalities in dietary obese mouse

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Obesity is accompanied by impaired insulin tolerance, elevated plasma glucose and lipids, ectopic lipid accumulation in tissues, etc. Fibroblast Growth Factor 21 (FGF21) is a potential

therapeutic agent shown to lower body weight, improve insulin sensitivity and lower blood glucose. It was also reported to have an antilipolytic effect in adipocyte cultures. These effects are more pronounced in obese than in lean mice. CL 316,243 (CL) is a beta-3 adrenergic agonist that induces lipolysis and acutely lowers blood glucose in lean mice. This glucose-lowering effect is blunted in obese mice. So, a combination of FGF21 and CL could be a beneficial treatment of obesity-associated impairment of glucose and lipid metabolism. We tested the effects of such combination on chow-fed and dietary obese C57BL/6J mice. In both lean and obese mice, 35 μg CL acutely induced lipolysis leading to a rise of free fatty acids (FFA) in plasma. FFA are known to induce insulin secretion. As expected, the insulin surge led to an acute drop of blood glucose in lean, but not in obese (insulin-resistant) mice. The 4-8 day pretreatment with 47.25 μg FGF21 (2x daily) increased insulin sensitivity and lowered basal insulin levels in obese mice. Injection of CL to FGF21-treated obese mice resulted in smaller rise of insulin but significant decrease of blood glucose. Thus, FGF21 sensitized the obese mice to glucose-lowering effect of CL. In 3T3-L1 adipocyte cell culture, 40 μg FGF21 lowered both basal and CL-stimulated lipolysis. In our model of dietary obese mice, we demonstrate that 24 h after CL injection the FGF21 pretreated mice exhibited lower FFA levels and were protected from hepatic triglyceride accumulation. In conclusion, we demonstrate beneficial effects of combined FGF21 and CL treatment in obese mice. FGF21 sensitizes the obese mice to insulin and restores glucose-lowering effect of CL. Also, FGF21 modifies the lipolytic effect of CL preventing ectopic lipid accumulation in liver.

P-01.3-008
Characterization of the oligomeric forms of human amylin

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A broad range of human diseases, including neurodegeneration and type II diabetes, are associated with misfolding and aggregation of a specific protein or proteins [1]. The rapidly aging population, sedentary lifestyle, and dietary changes mean that more and more people suffer from both neurological and cognitive dysfunctions characterized by progressive neurodegeneration and dementia, as well as from metabolic diseases. In 2015 over 415 million people were affected by type 2 diabetes (T2DM). By 2040, this number is expected to rise to 642 million [2]. Recent studies have increasingly focused on hIAPP (human islet amyloid polypeptide) oligomers found in the pancreas of T2DM patients. They are considered more toxic than mature fibrils [3]. These oligomers could be removed from the cells and their toxicity would be reduced if the intracellular proteolytic systems were functioning properly [1]. In order to understand the mechanism of the influence of the emerging cytotoxic oligomers on the proteolytic systems, it is necessary to carefully study and characterize their forms. We synthesized and purified human amylin. Various incubation conditions were tested in order to select the optimal ones for capturing the soluble oligomers. We monitored the progress of oligomerization using a number of techniques, including SDS-PAGE electrophoresis, fluorometric assays with thioflavin T and also immunodetection with A11 antibody, specific for oligomeric forms. Additionally, we managed to isolate and purify individual oligomers. Results of these experiments will be presented. This study was financially supported by an NCN-funded grant 2019/33/B/NZ7/00112. References: [1] Ciechanover A et al. (2003), Neuron, 40, 427-446 [2] International Diabetes Federation (2015), IDF Diabetes Atlas, 7th Ed. Brussels, Belgium, International Diabetes Federation [3] Engel MF et al. (2008), Proc. Natl. Acad. Sci. U.S.A.,105, 6033-6038

P-01.3-009
Intestinal region- and layer-dependent TNFα induction and TNF receptor expression may contribute to myenteric neuroprotection of duodenum in type 1 diabetic rats

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Tumor necrosis factor alpha (TNFα) has an essential role in neuroinflammatory modulation, however, depending on its different receptors (TNFR1 and TNFR2), TNFα has dual role in cell degeneration or survival. Therefore, our aims were to reveal the effects of chronic hyperglycaemia and insulin treatment on TNFα and TNFRs expression in myenteric ganglia and other intestinal layers of different gut segments. TNFα and TNFRs expression was evaluated by fluorescent and immunogold labelling in myenteric ganglia of duodenum, ileum and colon. Tissue levels of TNFα and TNFRs were measured by enzyme-linked immunosorbent assays in muscle/myenteric plexus-containing (MUSCLE-MP) and mucosa/submucosa/submucous plexus-containing (MUC-SUBMUC-SP) homogenates. In controls, increasing density of TNFα-labelling gold particles was revealed in myenteric ganglia from proximal to distal segments and elevated TNFα levels were seen in MUSCLE-MP than in MUC-SUBMUC-SP homogenates[1]. The density of TNFR1 gold labels was the lowest, while TNFR2 density was the highest in the duodenal ganglia. In diabetics, the TNFα density were significantly increased in the duodenal ganglia, while insulin did not prevent it. TNFα concentration was also increased in MUSCLE-MP homogenates of diabetic duodenum, while decreased in MUC-SUBMUC-SP samples of diabetic ileum and colon[1]. The TNFR2 density was only significantly altered in the diabetic duodenum with decrease in the ganglia, while no significant changes in TNFR1 density was observed. In diabetic MUSCLE-MP homogenates, both TNFR levels robustly decreased in the duodenum, which markedly influenced the TNFR2/TNFR1 proportion both in the ganglia and their muscular environment. These findings support that type 1 diabetes has region-specific and intestinal layer-dependent effects on TNFα and TNFR expression and assume that myenteric neuroprotection may be mediated via TNFR2-TNFα-pathway in the duodenum. [1]Previously published in: Bódi N et al. (2021) Cells 10(9), 2410.

P-01.3-010
The renoprotective effects of taurine against diabetic nephropathy via P38 MAPK and TGF-β/smad2/3 signaling pathways

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This study aimed to investigate the possible renoprotective effects of taurine via p38 MAPK and TGF-β/smad2/3 signaling pathways in a diabetic nephropathy model in rats. 29 Wistar albino rats were divided into 4 groups: control, taurine (1% with drinking water), diabetes (45 mg/kg Streptozotosin), diabetes+taurine. After 12 weeks, kidney and serum were collected for biochemical and histological studies. The histological analysis showed that there were significant changes in tubule dilatation and loss of tubule brush border in the kidney of the diabetes group. These changes were significantly decreased with taurine. However, there were no significant changes in serum creatinine and blood urine nitrogen levels among groups. Further, taurine significantly reduced the protein expression of NADPH oxidase 4, known as a major enzymatic source for oxidative stress in the kidney. Also, there was a significant decrease in reactive oxygen species and malondialdehyde levels by taurine whereas the decreased superoxide dismutase activity level in the diabetes group was significantly increased with taurine. On the other hand, taurine resulted in significant decreases in both mRNA and protein expression of fibronectin, which is a major extracellular matrix protein related to the fibrosis process, These findings were parallel to Masson Trichrome staining. The matrix metalloproteinases (MMP)-2 and MMP-9 mRNA expression levels and their activities increased significantly in diabetes compared to the control, and these increases were significantly reached to higher levels with taurine. Also, the decreased mRNA expression of extracellular matrix metalloproteinase inducer (EMMPRIN) increased with taurine in the diabetes+taurine group. Moreover, it is found that taurine suppressed the p38 MAPK and TGF-β/smad2/3 signaling pathways. All these findings indicate that taurine may be an effective practical strategy to prevent renal diabetic injury.

P-01.3-011
Bone marrow transplant reduces hyperglycemia and the cell number in the stromal vascular fraction of visceral adipose tissue in spontaneous lean type 2 diabetic Goto-Kakizaki rats

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Hyperglycemia can influence the activation of inflammatory pathways in the bone marrow (BM) and this was previously referred to as 'metabolic memory'. Tissue leukocyte recruitment from the BM stem cell differentiation involve the visceral adipose tissue (vAT) and stromal vascular fraction (SVF) and considered critical for chronic inflammation of type 2 diabetes (T2D). BM transplantation (BMT) was performed from Wistar (WT) to Goto-Kakizaki (GK) rats, a model of spontaneous lean T2D. Male rats (28 days-old) were submitted to a myeloablative regimen (2-days of 20 mg/kg busulfan; 1-day of 150 mg/kg cyclophosphamide) and the BMT was performed in WT (WT-WT, n = 4) and GK (WT-GK, n = 5) rats. We investigated the fasting blood glucose at day 30, 60, and 90 post-BMT and the SVF cell number, enzymatically extracted from vAT. GK rats present with diminished body weight (11%) and tibial length (9%), elevated glycemia (69%) and glucose intolerance. We observe increased blood counts for leukocytes (59%) and lymphocytes (63%) in GK rats (28-days-old). In addition to the differences found in blood leukocytes, SVF cells normalized by vAT weight showed surprisingly higher cell number (9 times) as compared to WT. At day 100 post-BMT, the glycemia was 12% lower in WT-WT and WT-GK, compared to the respective control group. Fasting glycemia was 14% lower in WT-WT and 22% in WT-GK at day 90 post-BMT as compared to their first 30 days. BMT also decreased SVF cell number in WT-GK showing no difference as compared to WT. BMT in GK recipients with WT donors attenuated progressive glycemia associated with reduced macrophages. Taken together, the results of our studies suggest BMT can influence glycaemic control. BM stem cells reconstitution could achieve AT modulation and systemic metabolism. *The authors marked with an asterisk equally contributed to the work.

P-01.3-012
Palmitate increases von Willebrand factor secretion without furin convertase upregulation

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Obesity is the main risk factor associated with insulin resistance and type 2 diabetes, accompanied by thrombosis and vascular

endothelium dysfunction. An increase in serum levels of saturated fatty acids, in particular palmitate, as a result of high fat dietary intake, is an important cause of proinflammatory response and cardiovascular complications. In addition, obesity is characterized by increased plasma von Willebrand factor (VWF). In patients with type 2 diabetes or insulin resistance, higher levels of secreted VWF are associated with an elevated risk of cardiovascular disease. VWF is a protein involved in hemostasis and is a marker of endothelial dysfunction. It is stored in the endothelial cells in specialized granules – Weible-Palade bodies – and is secreted after stimulus activation or vessel injury. We observed that an *in vitro* incubation of human umbilical vein endothelial cells (HUVECs) with palmitate results in increased basal and stimulated exocytosis of VWF. Moreover, palmitate increases VWF gene expression and protein maturation. VWF proprotein is cleaved to the mature form by furin convertase. Therefore, we hypothesized that the effect of palmitate on exocytosis and cellular content of VWF is possibly related to furin activity. Surprisingly, we have found a substantial decrease in the furin gene expression and the protein level in cells incubated with palmitate. Thus, the mechanism behind palmitic acid induced changes in VWF synthesis and secretion remains to be elucidated. This work was supported by a grant number 2016/23/B/NZ3/03116 from the National Science Center, Poland, and by statutory funds from the Nencki Institute of Experimental Biology.

P-01.3-013
Modelling diabetes in epithelial model membranes for (poly)phenol-lipid interaction studies

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Biomimetic models are valuable platforms to improve our knowledge on molecule–molecule interactions governing membrane-driven processes in (patho)physiological conditions including membrane permeability, transport, and fusion. However, current membrane models are oversimplistic and do not include the membrane's lipid remodelling in response to extracellular stimuli. In this study, we developed a cholesterol- and glycosphingolipid-rich model with complex lipid composition (PC/PE/Chol/GSL) designed to mimic epithelial membranes. The epithelial membrane model was tailored to mimic hyperglycemia conditions by the incorporation of synthesized glycated dimyristoyl-phosphatidylethanolamine (DMPE-glyc). Structural characterization of synthetic DMPE-glyc Amadori derivative was confirmed by mass spectrometry (MS) and quantification by nuclear magnetic resonance (NMR) confirmed cross-linking modification of 8% (total DMPE). The thermotropic behaviour of epithelial model was investigated by classical spectroscopic approaches namely by steady-state fluorescence using a fluorescent probe (diphenylhexatriene, DPH) and by dynamic light scattering (DLS) without the incorporation of exogenous probe. The transition temperature values (Tm) obtained by both approaches show good agreement highlighting the complementary character of spectroscopic techniques. Our results also show that hyperglycemia impacts the thermotropic behaviour of epithelial membrane models as well as membrane fluidity. In overview, the epithelial membrane models developed to mimic normo- and hyperglycemic scenarios will be used to investigate (poly)phenol-lipid and drug-membrane interactions essential in pharmaceutics, structural biochemistry and medicinal chemistry. Acknowledgements: AR acknowledges FCT (DL 57/2016/CP1346/CT0006) and financial support from project AgriFood XXI (NORTE-01-0145-FEDER-000041) and national funds (UIDB/50006/2020).

P-01.3-014
Upregulation of nitric oxide quantity by L-norvaline and L-arginine in high-fat diet and streptozotocin-treated male rats

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Endothelium produces various vasodilation agents, one of which is nitric oxide (NO). Hyperglycemia and hypercholesterolemia are associated with increased oxidative stress, which reduces NO bioavailability. In turn, it disrupts L-arginine (LA) transport by inactivation of NO synthase and by activation of arginase. L-norvaline (LN) is an optional arginase inhibitor which can increase NO production and ensure normal vascular endothelial activity. The bioavailability of NO in the cardiovascular system can also be increased by LA. We hypothesized that inhibition of arginase may have protective properties in the cardiovascular system, as elevating NO levels, may uphold against oxidative stress and hypercholesterolemia. We aim to investigate the antihyperglycemic properties of arginase inhibitor LN, both separate and combined with L-arginine in streptozotocin (STZ)-induced hyperglycemic rats. Rats were fed a high-fat diet for three weeks concomitant with the two-time injection of 30 mg/kg STZ to induce stable hyperglycemia and hypercholesterolemia. Treatment with LN and LA reduced fasting blood glucose levels in comparison with untreated STZ-administered rats. High concentrations of total cholesterol, low-density lipoprotein, and malondialdehyde in the blood were significantly decreased in both LN and LN-LA-treated rats compared with untreated HFD/STZ rats. Administration of LN and LA to STZ-induced hyperglycemic rats reversed the progression of pancreatic and kidney pathology based on the histological picture. The HFD/STZ group showed the accumulation of necrotic nuclei. In STZ_LN and STZ_LN_LA groups, histological analysis of the myocardium showed the structural repair of cardiac muscle fibers and nuclei. Thus, we suggest that both L-norvaline and L-arginine are anti-hyperglycemic agents and can be used in both pre-diabetic and anti-diabetic conditions.

P-01.3-015
Using inflammatory-redox combined indexes for evaluating diabetes mellitus patients – a preliminary study

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The objectives of this pilot study were the evaluation of parameters comprising both redox impairment and inflammatory burden

of type 2 diabetes mellitus (T2DM) patients, taking into consideration their smoking status, as such group-specific biomarkers are scarcely reported in literature. Our study included 68 subjects from the "N. Paulescu" National Institute of Diabetes, Nutrition and Metabolic Diseases in Bucharest, Romania. They were divided into two groups: a T2DM study group (n = 52, with 3 subgroups: non-smokers, smokers and ex-smokers) and a control group (n = 16). Routine biochemical parameters were assessed, along serum protein (AOPPs, AGEs) and lipid (Amplex Red-based method) oxidative damage, as well as inflammatory status (CRP, IL-6, IL-10). Cytokine ratios and oxidative-inflammatory status indices were evaluated (IH1, IH2). Glycemia was positively correlated with the inflammatory parameters (CRP/IL-10) and inversely with anti-inflammatory ones (IL-10). We observed significant differences in terms of serum redox and inflammatory status (notably: CRP/HDL, CRP/IL-6, IL-10/IL-6, IH1-comprising CRP and HDL levels and HDL lipid peroxidation) between T2DM patients compared to control and, moreover, between the subgroups based on smoking status (CRP, CRP/HDL, IH1). Of the assessed parameters, some may possess prognostic value for diabetics, especially when comparing subgroups with a different smoking history and could prove useful in clinical practice for assessing disease progress and therapeutic efficacy. Although the number of patients was small, IL-10/IL-6 presents itself as a relevant biomarker in evaluating the inflammatory status of T2DM patients and possibly in discriminating between different subgroups of diabetics; CRP/IL-6 was significantly higher in T2DM patients with a history of smoking versus non-T2DM subjects; IH1 was significantly higher in T2DM patients with a history of smoking versus non-smokers (T2DM and control).

P-01.3-016
Diabetic encephalopathy – beneficial effects of metformin treatment in a type 2 diabetes rat model

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Diabetic encephalopathy is a chronic complication of diabetes mellitus (DM) that affects the central nervous system and which is often associated with oxidative stress and neuronal injury. Tumor necrosis factor-α (TNF-α) is a pro-inflammatory cytokine that is implicated in neurotoxicity and proliferation of glial cells. Metformin is widely used as first-line therapy for type 2 diabetes due to its glucose-lowering effects. We evaluated the effect of metformin on diabetes-induced frontal cortex oxidative damage. There were four experimental groups (6 animals per group). A rat model of type 2 DM was induced by nicotinamide injection (110 mg/kg, i.p.), followed by streptozotocin injection (45 mg/kg, i.p.). After the model was developed, metformin was administered orally in drinking water (1 mg/ml; 100 mg/kg body weight per day) for two weeks. The dose of metformin used in this experiment corresponded to the human equivalent dose of 16 mg/kg, which is considered a therapeutic dose in humans. As expected, metformin down-regulated fasting blood glucose levels ($P < 0.001$) and serum fructosamine ($P < 0.001$) in treated animals. Additionally, metformin-treated animals had lower serum TNF-α when compared to untreated animals ($P < 0.001$). It also reduced concentrations of the thiobarbituric acid reactive substances (TBARS; $P < 0.001$) and advanced oxidation protein products (AOPP; $P = 0.001$) in the frontal cortex of treated animals. Furthermore, there was a positive correlation between fasting blood glucose level and concentrations of TBARS ($r = 0.786$; $P < 0.001$) and AOPP ($r = 0.731$; $P < 0.001$). Metformin exerts beneficial effects on diabetes-induced oxidative damage in the frontal cortex of rats.

P-01.3-017
HSPB1 influences certain symptoms of metabolic syndrome in a sex-dependent manner in transgenic mice

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Metabolic syndrome is a complex disease that causes several health problems. However, heat-shock proteins (HSPs) are able to maintain protein homeostasis during cellular stress, and they are involved in several processes related to cardiovascular diseases or diabetes. Therefore, our aim was to study the potential beneficial roles of HSPB1 in a mouse model of metabolic syndrome. As a model of the disease we used high-fat diet-fed APOB-100-overexpressing mice which were crossed with HSPB1-overexpressing animals to study the effects of this HSP. As expected, the body weight and serum LDL cholesterol levels were significantly higher of the APOB-100 animals compared to wild-types in both sexes. Interestingly, HSPB1 overexpression induced a further increase in weight gain and LDL concentration in female APOB-100 animals. In contrast, in APOB-100 males HSPB1 overexpression resulted in a lower level of LDL-cholesterol, while it did not influence body weight. On the other hand, the weight of the liver was increased significantly only in the male animals in response to obesity, suggesting the development of fatty liver disease. This was confirmed by hematoxilin-eosin staining of the liver sections; both the number and size of lipid droplets were significantly elevated in male APOB-100 animals. This is in accordance with our gene expression studies as well. The mRNA level of Lpl, which was found to be correlated with the severity of liver damage in obese patients, showed significantly higher level in male APOB-100 animals compared to females, although it was increased by obesity in both sexes. Moreover, in female APOB-100 animals HSPB1 overexpression restored the Lpl level, and decreased the expression of certain cytokines as well. These results show that HSPB1 may be involved in the regulation of obesity-related processes, however in a sex-dependent manner. The work was funded by NKFIH FK138390.

P-01.3-018
The inhibition of Galectin-1 in high glucose-stimulated ARPE-19 cells by OTX008 through TGF-β/EMT pathway

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Diabetic retinopathy (DR) is a very common neurovascular disease affecting the retina integrity and functions. An important cause of DR progression is the development of retinal fibrovascular tissue, which impairs the function of retina. Galectin-1 is a glycan-binding protein found to be upregulated in human proliferative DR progression, especially by dysfunction of Müller glial cells. In these cells, galectin-1 co-localized with VEGF-A and HIF-1α in hypoxic conditions. However, galectin-1 role in DR-induced fibrosis needs to be investigated, as well as the possibility to alleviate DR pathogenesis with galectin-1 inhibitors. In our experimental setting, we exposed ARPE-19 cells to normal (5 mM) or high glucose (35 mM) for 3 days. Then, the selective galectin-1 inhibitor OTX0008 (2.5 – 5 – 10 μM) was added for 6 days. We determined cell viability, ROS levels, protein and mRNA expression by ELISA, immunocytochemistry, Western Blotting and Real Time-PCR. Our results showed a significant up-regulation of galectin-1 expression in cells exposed to high glucose, with a reduction of cell viability and high levels of epithelial–mesenchymal transition (EMT) markers. The inhibition of galectin-1 by OTX008 5 μM and 10 μM improved cell viability and reduced the EMT process. This was exerted by a down-regulation of TGF-β pathway and a parallel reduction of NF-kB and ROS levels. Therefore, the OTX008-induced inhibition of galectin-1 could be considered a possible innovative target to improve retinal pigment epithelial cells homeostasis, preserving them from fibrosis and EMT during DR progression. Acknowledgements: This research was funded by a grant of the Ministry of Research, Innovation and Digitization, CNCS/CCCDI – UEFISCDI, project number PN-III-P4-ID-PCE-2020-1772, within PNCDI III.

P-01.3-019
Galectin-1 inhibition by OTX008 in high glucose-stimulated H9c2 cardiomyocytes as a new tool to regulate cardiovascular hypertrophy

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Galectin-1 is a β-galactoside-binding lectin emerged as a regulator of cardiac inflammation, hypertrophy and neovascularization. Its expression has been found to be upregulated in the cardiomyocytes from patients suffering of several cardiovascular disorders, such as acute myocardial infarction, heart failure and Chagas cardiomyopathy. However, galectin-1 role in diabetes-induced heart dysfunction has never been explored. Therefore, we aimed to investigate galectin-1 expression in rat H9c2 cardiomyocytes cultured in high glucose conditions, and to assess the effects of galectin-1 inhibition by OTX008 in diabetes-induced cardiac hypertrophy. In our experimental design, H9c2 cells were exposed to normal (5.5 mM) or high glucose (33 mM) for 2 days. Then, OTX0008 (0.1 – 0.35 – 0.75 μM), a selective galectin-1 inhibitor, was added for 6 days. Cell viability, ROS levels, protein and mRNA expression were assessed by ELISA, immunocytochemistry, western blotting and real time-PCR. Our data showed a significant increase of gene and protein galectin-1 expression in cardiomyocytes exposed to high glucose, with an increase of cell mortality and high levels of cardiac hypertrophy markers. OTX008-induced inhibition of galectin-1 reduced cell death and cardiac hypertrophy. These effects were associated to a down-regulation of TGF-β pathway, NF-kB and ROS content. Therefore, by selectively inhibiting galectin-1 through OTX008, diabetes-induced cardiac hypertrophy could be alleviated. This could be further investigated as a novel pathway to prevent and manage diabetes-induced heart injuries. Acknowledgements: This research was funded by a grant of the Ministry of Research, Innovation and Digitization, CNCS/CCCDI – UEFISCDI, project number PN-III-P4-ID-PCE-2020-1772, within PNCDI III.

P-01.3-020
High-throughput virtual and experimental screening of new free fatty acid receptor 1 modulators

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Free Fatty Acid Receptor 1 (FFAR1) is a G-coupled receptor protein (GPCR) expressed in pancreatic β-cells which enhances glucose stimulated insulin secretion (GSIS) upon ligand binding, therefore the discovery of new agonists or positive allosteric modulators (PAMs) might provide new therapeutic strategies for type 2 diabetes mellitus (T2DM). We present here results obtained using a combination of virtual and experimental high-throughput screening (vHTS/HTS) performed on a 1364 set of tested, pre-approved FDA compounds with known pharmacokinetic and toxicological profiles, given the reduced time necessary for approving such molecular entities. In order to run the vHTS, we first derived from the X-ray structure of FFAR1, crystalized with both binding sites occupied (pdb code 5TZY), four models corresponding to the presence or absence of ligand at these sites. Models were further subjected to regular and accelerated molecular dynamics simulations (MD, aMD) and structures along the trajectories were used to generate ensembles on which docking procedures of the compound set were performed. Ranking scores were pre-optimized on a validation set consisting of known modulators of FFAR1 alongside a set of generated decoys. In parallel, each molecule from the library was *in vitro* tested for agonistic and PAM action in a HTS campaign using intracellular calcium mobilization assay on CHO cells transiently transfected with humanFFAR1. The results of HTS were compared to the predictions based on vHTS in order to assess their accuracy. Hence combined vHTS/HTS is shown to provide novel

compounds for improved T2DM therapeutical treatment strategies. Acknowledgement: This work was supported by a grant of the Romanian Ministry of Education and Research, CNCS - UEFISCDI, project number PN-III-P2-2.1-PED-2019-5179 *The authors marked with an asterisk equally contributed to the work.

P-01.3-021
The prognostic value of complete blood count and biochemical markers and indexes in COVID-19 progression in patients with Diabetes mellitus

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COVID-19 requires a complex assessment of disease progression. We aimed to assess the prognostic value of biochemical and complete blood count (CBC) parameters and their indexes in the progression of COVID-19 in patients with Diabetes mellitus (DM). Data from 784 patients hospitalized at the University Clinical Center Nis were assessed. Patients were divided into the control and DM groups. Disease progression was defined as a lethal outcome or intensive care treatment requirement. Patients' laboratory data upon admission was observed. There were 163 subjects (20.8%) with DM and 621 (79.2%) controls. In 52.8% of DM patients and 45.6% of controls, progression was observed. The ANOVA test showed significance between progression and IL-6 ($P < 0.05$), neutrophil-lymphocyte ratio (NLR) ($P < 0.05$), and CRP-IL-6 ratio ($P = 0.02$) in both groups. In controls, CRP ($P = 0.012$) and LDH ($P = 0.000$) were also highlighted. ROC analysis showed the best results for IL-6 (AUC = 0.77 and 0.64). There was a significant positive correlation of CRP, NLR, and CRP-IL-6 ratio ($P = 0.05$) with progression, while IL-6 correlated more significantly ($P = 0.01$) in DM patients. In controls, all correlations were of high significance ($P = 0.01$). Commonly assessed CBC and biochemistry parameters showed a more significant link to progression in controls than in DM patients, whereas the most highlighted marker was IL-6.

P-01.3-022
MicroRNA detection in liquid-biopsy samples of women diagnosed with gestational diabetes mellitus

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Gestational diabetes mellitus (GDM) is a chronic hyperglycemia with onset during pregnancy and is associated with increased morbidity and mortality for both the fetus and the mother. Screening for GDM is usually performed at 24–28 weeks of gestation; however, molecular changes in body fluids can be detected even earlier. Epigenetic alterations, such as miRNA deregulation, are among the most promising liquid-biopsy biomarkers for GDM. In the present study, 10 miRNAs (miR-17, miR-29a, miR-195 and others) were quantified by means of reverse-transcription real-time PCR in blood samples. In total, 76 women were included in the study: 64 GDM cases, 7 healthy pregnant (noGDM), and 5 healthy non-pregnant women (HW). Samples (129 in total) were taken at 24–28 weeks of gestation (n = 59), 6-12 weeks after delivery (n = 28), and from umbilical artery (UA) and vein (UV) during delivery (n = 21 pairs). MiR-29a, miR-195 and some others showed differences between GDM and noGDM or HW groups and were associated with various clinical parameters. MiR-16a, miR-17 and miR-29a during pregnancy correlated with body mass index, while miR-222 with women's age (all $P < 0.05$). MiR-17 and miR-195 were increased in blood after delivery as compared to the pregnancy period (both $P < 0.05$). Furthermore, miR-17, miR-29a and miR-499a levels in the GDM after delivery were higher than in HW (all $P < 0.05$). Although most miRNAs did not show significant differences in paired UA or UV blood samples, levels of miR-195 in UA of GDM were lower as compared to noGDM, while miR-29a correlated with high- and low-density lipoproteins. Quantities of miR-17 and miR-499a in UV blood were also associated with plasma glucose levels (all $P < 0.05$). In conclusion, we identified associations of several miRNAs with GDM and respective clinical parameters, suggesting these miRNAs as putative biomarkers of the disease. Larger validation studies are required to confirm the clinical applicability of these miRNAs.

P-01.3-023
Characterization of the T lymphocyte profile in lean type 2 diabetic Goto-Kakizaki rats with different ages

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Goto-Kakizaki (GK) rats develop a well-defined resistance to insulin (RI) and type 2 diabetes mellitus (DM2) without presenting obesity. The characterization of the immune response in non-obese diabetic condition is not yet elucidated. Therefore, the aim of this study is to characterize the process of T lymphocyte differentiation during the development of DM2 in Goto-Kakizaki rats. GK and Wistar rats with 21, 60, and 120 days after birth were evaluated. Lymphocytes were isolated from the mesenteric lymph nodes. Expression of GLUT1 in the membrane, and T helper lymphocyte profile were evaluated by flow cytometry; expression of genes involved with lymphocyte polarization was determined by real-time PCR. We observed in the glucose and insulin tolerance test, an increase in the area under the curve, showing possible insulin resistance in animals with 21, 60, and 120 days. Regarding GLUT1 analysis, there is a higher expression in animals from the GK group at all ages in non-stimulated conditions compared to the Wistar control group. The percentage

of Th1 and Th17 cells was increased in GK rats with 120 days. Expression of GATA-3 in the GK group was lower at all ages when compared to Wistar. In relation to Foxp3 (Treg cell transcription factor), we observed a lower expression in the rats with 21 days from the GK group compared to the Wistar. T-bet expression was higher in 120 days GK rats. In relation to TNF-α, we observed an increase in GK at 60 and 120 days and for IFN-gamma, we observed greater expression for GK at all ages. In conclusion, we can state that GK animals with 21 days have a reduction in Th2 anti-inflammatory response markers (Gata-3 and IL-10) and Treg (Foxp3 and IL-10), indicating that reduction of immunosuppressor mechanisms early in the animal's life may favor pro-inflammatory responses in later stages of life. This is evidenced, in the expression of Th1 profile cytokines, which is increased only in animals with 60 and 120 days. *The authors marked with an asterisk equally contributed to the work.

Cardiovascular diseases

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Abstract withdrawn

P-01.4-002

Rational diagnostics of thrombophilia in risk groups during immunization by AstraZeneca anti SARS-CoV-2 vaccine

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Thrombotic complications are known for COVID-19. Also some sources report a risk of thrombosis after the immunization by vector-based vaccines. These facts make it important to evaluate the main parameters of the blood coagulation system in persons who were vaccinated by AstraZeneca anti SARS-CoV-2 vaccine. Blood samples of patients from 25 to 68 years old, women and men were collected in Ternopil region in August 2021 after 7 days after the 1st (n = 27) and the 2nd (n = 8) shots of vaccine. Soluble Fibrin (SF) and D-dimer were quantified using immunodiagnostics developed in Palladin Institute of Biochemistry. The concentration of fibrinogen was determined by the modified spectrophotometric method using a thrombin-like enzyme. The level of protein C (PC) was measured using chromogenic substrate S2366. Statistical data analysis was performed using the Wilcoxon-Mann-Whitney test. We did not find a statistically significant increasing of concentration of fibrinogen or D-dimer. However, we did find several markers of the predisposition to thrombophilia that are accompanied by chronic diseases, such as hypertonia, diabetes, obesity, etc, can lead to intravascular blood clotting. First of all, 63% of persons had SF (more than 7 mkg/ml) after the 1st shot of the vaccine. Also, they possess decreasing (level less than 80%) of the important anticoagulant protein – PC in 33% and 43% of persons after the 1st and the 2nd shots respectively. Our studies did not find the thrombotic events or predispositions to thrombosis caused by vaccination by AstraZeneca vaccine. However, 1/3 of studied vaccinated persons exhibited the risk of thrombophilia. These markers must be evaluated with the aim to avoid any unwanted issues during the vaccinations in the case of risk group patients.

P-01.4-003

A derivative of hydroxytyrosol has anti-inflammatory properties *in vitro* in the context of atherosclerosis

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The expression of cell adhesion molecules by the endothelium and the attachment of monocytes to endothelium are processes that play a major role in the early atherogenic process. Hydroxytyrosol, a compound naturally present in virgin olive oil, exerts anti-cancer, anti-angiogenic and anti-inflammatory properties, among other benefits, *in vitro* and *in vivo*. Hexyl hydroxytyrosyl eter (HT C6), a synthetic derivative of hydroxytyrosol, showed anti-angiogenic properties both *in vitro* and *in vivo* that improved those of the hydroxytyrosol itself. In this line, we studied whether HT C6 has improved anti-inflammatory effects as well. In this study, we studied the expression of important adhesion molecules, such as ICAM-1 and VCAM-1, expressed by the activated endothelium, in endothelial cells treated with different concentrations of HT C6 in the presence of a pro-inflammatory stimulus. We also explored the effect of this HT derivative in the expression of pro-inflammatory cytokines by the endothelium, like CCL2 . Interestingly, HT C6 reduced the expression and relative amount of these cytokines and adhesion molecules in endothelial cells. We will also provide data about the modulation of the expression of CCR2 (the receptor of CCL2) in monocytes by HT C6, and how this adhesion is altered in a co-culture of endothelial cells and monocytes. The results that we have obtained so far support the anti-inflammatory role of this compound and make it a promising candidate for the treatment of inflammations and cardiovascular disease.

P-01.4-004

Evidence of sialylation pathways alteration in peripheral blood of Brugada syndrome patients

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Brugada syndrome (BrS) is an inherited arrhythmogenic disorder with an increased risk of sudden cardiac death. Recent evidence

suggests that BrS should no longer be considered as a pure autosomal dominant disorder but an oligogenic or polygenic condition. Mutations in genes associated with BrS are found in about one-third of patients and mainly (20-25%) disrupt the cardiac sodium channel NaV1.5, which is considered the main cause of the disease. However, post-translational modifications such as sialylation can also impact voltage-gated channel's activity, but their role in BrS remains unknown. We conducted a retrospective study on a population composed by two groups: high risk BrS patients (n = 32) and healthy controls that tested negative to ajmaline drug challenge (n = 31). BrS patients underwent a combined endo-epicardial mapping procedure using a three-dimensional (3D) mapping system. Gene expression was evaluated by qPCR and protein sialylation by flow cytometry and western blot with specific lectins. Significant alterations in gene expression of enzymes involved in the biosynthesis, activation, transfer, degradation, and recycling of sialic acid and total protein sialylation were detected in PBMCs from BrS patients. These changes correlated with the phenotypic expression of the disease, such as the size of the arrhythmogenic BrS substrate and the duration of epicardial electrical abnormalities. Dysregulation of the sialylation machinery provides definitive evidence that BrS also affects extracardiac tissues and suggests that this may be an underlying cause of the disease. Detection of these changes at the systemic level and their correlation with the clinical phenotype suggest the existence of a biomarker signature for BrS that could be uncovered in a comprehensive multiomic study.

P-01.4-005
Curcumin improves hypolipidemic activity of rosuvastatin and prevents rosuvastatin-induced liver damage in hyperlipidemic model of Wistar rats

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The role of the lipid profile in the progression of cardiovascular diseases is well known to induce atherosclerosis and cardiovascular risk, and therefore, various animal hyperlipidemic models, including atherogenic diets, were developed. These models allow for screening of different compounds efficient in the prevention and treatment of cardiovascular diseases. So far, the hypolipidemic activity of the combined use of curcumin and rosuvastatin has not been investigated. The present study aimed to investigate the lipid-lowering efficiency of combination of curcumin and rosuvastatin in the rat model of atherogenic diet-induced hyperlipidaemia. In addition, the ability of curcumin to prevent rosuvastatin-induced liver damage was assessed. Sixty Wistar rats were divided into five study groups: Standard control received standard rodent diet; Atherogenic control received atherogenic rodent diet, i.e. western-type diet with added cholesterol and chlorate; Curcumin-atherogenic group received atherogenic diet and curcumin (200 mg/kg body weight) by oral gavage; Rosuvastatin-atherogenic group received atherogenic diet and rosuvastatin (10 mg/kg body weight) by oral gavage; Rosuvastatin-curcumin-atherogenic group received atherogenic diet and a combination of curcumin and rosuvastatin in mentioned doses and route. Hyperlipidemia was induced by feeding rats with an atherogenic diet for 14 days, and feeding further continued for the next 14 days in combination with the treatment. The serum lipid profile, atherogenic index, LDL/HDL cholesterol ratio, markers of hepatocytes injury, as well as the histopathology of liver and thickness of abdominal aorta were determined. Combination of curcumin and rosuvastatin resulted in a more effective decrease in plasma lipid levels, lower atherogenic index and LDL/HDL cholesterol ratio values. Curcumin seems to improve hypolipidemic activity of rosuvastatin and prevents rosuvastatin-induced liver damage in hyperlipidemic model of Wistar rats.

P-01.4-006
Effects of asperglaucide on endothelium function in 2K1C hypertensive rats

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Asperglaucide (ASP) is an amide isolated from *P. aurantiacum* having anti-inflammatory and antioxidant properties. This study aimed to investigate the effects of ASP on endothelial nitric oxide synthase (eNOS) expression, vascular fluidity and vascular endothelial function in the two-kidney one-clip (2K-1C) model of renovascular arterial hypertension. Forty male Wistar rats, aged 2 months were randomized to one of the following groups: control (C), sham-operated (SO), ASP treated, hypertensive (H) and H + ASP treated. Hypertension was induced by surgery and mean arterial pressure (MAP) was monitored by tail-cuff method during 4 weeks of ASP treatment (0.5 mg/kg/day administered intraperitoneally). At the end of experimental period, blood samples and thoracic aorta were obtained. Vascular dilator and constrictor responses were measured in organ baths while red blood cell deformability was determined by rotational ektacytometry. Protein and gene expression of eNOS were evaluated in thoracic aorta by immunocytochemical and quantitative PCR analysis, respectively. Asperglaucide treatment significantly decreased MAP in hypertensive rats and caused significant improvement in endothelium dependent vascular dilator and constrictor responses. Red blood cell deformability was significantly increased in hypertensive rats treated with ASP as compared to hypertensive rats alone. Administration of ASP lead to a significant increase in both eNOS protein and gene expression in both normotensive and hypertensive rats. Asperglaucide was found to significantly lower blood pressure in hypertensive rats 1 week after treatment by increasing endothelium-mediated relaxation response. Asperglaucide treatment resulted in improvement in phenylephrine-mediated contractile responses, which were impaired in the hypertension group. Asperglaucide also increased blood flow and eNOS gene expression in hypertensive rats. Acknowledgements: This study was supported by a grant from TUBİTAK #219S713.

P-01.4-007
Temporal relationship between systemic endothelial dysfunction and alterations in erythrocyte function in a murine model of chronic heart failure

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Endothelial dysfunction (ED) and red blood cell distribution width (RDW) are both prognostic factors in heart failure (HF), but the relationship between them is not clear. In this study, we used a unique mouse model of chronic HF to characterise the relationship between the development of peripheral ED and the occurrence of structural nanomechanical and biochemical changes in red blood cells (RBCs). Systemic ED was detected *in vivo* in 8-month-old Tgαq*44 mice, as evidenced by impaired acetylcholine-induced vasodilation in the aorta and increased endothelial permeability in the brachiocephalic artery. ED in the aorta was associated with impaired nitric oxide (NO) production in the aorta and diminished systemic NO bioavailability. ED in the aorta was also characterised by increased superoxide and eicosanoid production. In 4- to 6-month-old Tgαq*44 mice, RBC size and membrane composition displayed alterations that did not result in significant changes in their nanomechanical and functional properties. However, 8-month-old Tgαq*44 mice presented greatly accentuated structural and size changes and increased RBC stiffness. In 12-month-old Tgαq*44 mice, the erythropathy was featured by severely altered RBC shape and elasticity, increased RDW, impaired RBC deformability, and increased oxidative stress (GSH/GSSH ratio). Moreover, RBCs taken from 12-month-old Tgαq*44 mice, but not from 12-month-old FVB mice, co-incubated with aortic rings from FVB mice, induced impaired endothelium-dependent vasodilation and this effect was partially reversed by an arginase inhibitor. In the Tgαq*44 murine model of HF, systemic endothelial dysfunction accelerates erythropathy and, conversely, erythropathy may contribute to endothelial dysfunction. These results suggest that erythropathy may be regarded as a marker and a mediator of systemic endothelial dysfunction in HF. In particular, targeting RBC arginase may represent a novel treatment strategy for systemic endothelial dysfunction in HF.

P-01.4-008
The role of cardiac fibroblasts and macrophages in mouse model of experimental autoimmune myocarditis

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Myocarditis is a common cause of dilated cardiomyopathy associated with pathological tissue remodeling, ventricle stiffening and heart failure. In experimental autoimmune myocarditis (EAM) model, susceptible mice immunized with alpha myosin heavy chain and complete Freund's adjuvant develop acute myocarditis (at d21) followed by cardiac fibrogenesis, cardiomyopathy and systolic dysfunction (at d40). The aim was to define the role of cardiac fibroblasts and inflammatory macrophages in EAM. EGFP+ fibroblasts were sorted from control and inflamed hearts obtained from reporter Col1a1-EGFP mice. Analysis of total RNA sequencing showed that cardiac fibroblasts in myocarditis upregulate expression of genes involved in immune processes (mainly chemokine production), extracellular matrix reorganization and response to stress. Cardiac macrophages were obtained from LysM-Cre x R26R-YFP and LysM-Cre x R26R-YFP x Tgfbr2-fl/fl (lacking TGF-beta receptor 2 on macrophages) hearts at d21 of EAM and analyzed by RNA sequencing. In the absence of Tgfbr2, differential expression analysis of sorted cardiac macrophages in myocarditis showed attenuated production of cytokines (Ifnb1, Il23a, Il10, Il12b, Cxcl1, Tnf) and their receptors (Cxcr1, Ccr4). Transgenic αSMA-TK mice were used to study effects of ganciclovir-inducible ablation of myofibroblasts in EAM. Treatment with ganciclovir during post-inflammatory phase (d21-40) improved stroke volume, ejection fraction, cardiac output and reduced cardiac hypertrophy at d40, but the extent of cardiac fibrosis was comparable with PBS-treated mice. In conclusion, during acute inflammatory phase cardiac fibroblasts contribute to proinflammatory and profibrotic responses, while TGF-beta signaling on cardiac macrophages regulate production of inflammatory cytokines. During post-inflammatory phase, activated myofibroblasts mediate development of post-inflammatory cardiomyopathy and heart failure. Co-funded by the NCN, Poland 2020/37/N/NZ5/02079

P-01.4-009
Myocardial expression of heat shock protein HSP90 under essential hypertension, insulin-dependent diabetes mellitus and their combination

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Molecular chaperones are involved in protecting the heart from oxidative stress and accumulation of toxic misfolded proteins by regulating the balance of protein synthesis/degradation. HSP90 is one of the main cardioprotective chaperones that supports the quality control of improperly folded proteins, preventing the aggregation of damaged proteins in cardiomyocytes (CMCs), protects actin and myosin from thermal denaturation under stress, and also participates in programmed cell death in various diseases of the cardiovascular system, including those caused by complications of diabetes mellitus (DM). The expression of HSP 90 in CMCs under hypertension and/or DM was the main objective of our study. The experiments included 4 groups of male rats (5 animals in each group): Wistar-Kyoto rats (control 38-week-old); 57-week-old SHR (spontaneously hypertensive rats); Wistar-Kyoto rats with insulin-dependent DM (38-week-old WKY + 30-days DM); SHR rats with insulin-dependent DM (38-week-old SHR + 30-days DM). Insulin-dependent DM was modeled by a single intraperitoneal injection of Streptozotocin (65 mg/kg). The animals with the glucose blood levels ≥ 18 mmol/L were selected for the further experiments. The duration of DM was 30 days from the verification of hyperglycemia. The expression

of HSP90 in the left ventricular CMCs was assessed immunohistochemically using primary rabbit anti-HSP90 polyclonal antibodies (Sigma-Aldrich). According to the results obtained, the expression level of HSP90 in CMCs was significantly higher compared with the controls in all the experimental groups. At the same time, the expression of HSP90 in 57-week-old SHR rats and in SHR rats with insulin-dependent DM were at the same level. CMCs HSP90 content was the highest in the group of Wistar-Kyoto rats with insulin-dependent DM. It can be concluded that myocardial alteration caused by insulin-dependent DM or/and hypertension leads to the activation of HSP90 production in CMCs. *The authors marked with an asterisk equally contributed to the work.

P-01.4-010
Relationship between BECN1-induced cardiomyocyte autophagy and morphological changes of the myocardium in acute diphtheria intoxication

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Diphtheria is now rarely reported due to widespread immunization. However, sporadic cases of severe complications are high and the cause of death is mainly associated with toxin-mediated myocarditis. The exotoxin of *Corynebacterium diphtheria* inhibits protein synthesis and causes cell death in the heart. Autophagy is considered to be either a part of cardioprotective adaptation or a type of regulated cell death in myocardial injury. In case of diffuse diphtheria toxic injury, the role of Beclin 1 (BECN1) associated cardiomyocyte autophagy is still not clear. Experiments were carried out on 16 adult male Chinchilla rabbits with body mass 3–3.5 kg. Animals were divided into 4 groups: 1 control group (intact rabbits) and 3 experimental groups (rabbits with acute diphtheria intoxication 1, 3 and 5 days after the onset of the intoxication correspondently). Diphtheria intoxication was modeled by bolus intravenous injection of native diphtheria toxin (0.3 DLM/1 kg). The content of BECN1 in cardiomyocytes was evaluated using the immunohistochemical assay of the left ventricular myocardium. Morphological and morphometric examination of histological sections of the myocardium was also performed. Immunohistochemical study revealed a significant decrease in the content of BECN1 on days 1, 3 and 5 with a descending trend (0.12 ± 0.04, 0.04 ± 0.04 and 0.0 ± 0.00 vol.% respectively vs 2.77 ± 0.38 vol.%, $P < 0.05$). This descending tendency had a strong negative correlation with the increasing destruction changes (-0.75) and nuclear-cytoplasmic ratio (-0.96) in the myocardium and also a strong positive correlation with a decreasing quantity of morphologically safe myofibrils (+1.00) at all the experimental terms. Microscopy demonstrated acute myocardial inflammation (extensive necrotic changes, tissue edema, diffuse leukocyte infiltration). It is concluded that autophagy was reduced due to increasing destructive changes in the diphtheria-induced myocarditis.

P-01.4-011
Screening for small molecules that enhance the enzyme activity of human paraoxonase 1 in serum

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Human paraoxonase 1 (PON1) is a serum enzyme associated to high-density lipoprotein (HDL) and can hydrolyze a wide range of substrates, including organophosphorus compounds, lactones and aromatic carboxylic acid esters. PON1 metabolizes oxidized lipids associated with lipoproteins and therefore is involved in the antioxidant and anti-atherogenic functions of HDL. Reduced PON1 activity has been associated with increased risk for cardiovascular disease. Here, we screened a library of marketed drugs (~1000 compounds) to identify small molecules that can increase HDL-associated PON1 activity in serum. For this purpose, we used a kinetic absorbance assay in a 96-well plate set up using human HDL as source of PON1, paraoxon as substrate to measure the paraoxonase activity of PON1, as well as phenyl acetate as another substrate to measure the arylesterase activity of PON1, in the absence or presence of compounds. The screening revealed seven compounds that increase PON1-paraoxonase activity, while four of them also increase PON1-arylesterase activity. Analysis of the effect of these compounds on the enzyme activity of cardiovascular risk-associated PON1 polymorphisms (192Q/R, 55L/M) and mutations (M127R) narrowed the potential enzyme activators to three. Further mechanistic investigations of the effect of compounds on PON1 activity are underway. Overall, our results suggest that existing drugs can enhance HDL-associated PON1 enzyme activity in serum and pave the way to novel therapeutic approaches for atherosclerosis and cardiovascular disease. Acknowledgement: project "New therapies aiming to improve the atheroprotective and immunomodulatory properties of high density lipoprotein (HDL) for the treatment of autoimmune and cardiovascular diseases" (T2EDK-02361), co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call Research – Create – Innovate.

P-01.4-012
Assessment of *in vitro* cytotoxic, Nos2 and anti-inflammatory effects of DMSO- and water-soluble extracts of Greek biodiversity plants, in LPS- and IFN-γ-stimulated RAW264.7 macrophages

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In the last few decades, much effort has been devoted to the development of effective plant extracts as anti-inflammatory agents. The aim of this study is to investigate cytotoxicity, Nos2 (INOS) inhibitory, anti-inflammatory and antioxidant activities of Greek dittany, thyme and sage extracts in RAW 264.7 macrophages. Thus, the antioxidant content of DMSO and water-soluble extracts was evaluated using the DPPH assay. The anti-inflammatory capacity of extracts was evaluated in LPS+IFN-γ-induced RAW 264.7 macrophages, while cell viability and toxicity were assessed by the MTT assay. To identify mRNA expression of Nos2, IL-6 and Ptgs2 (COX-2), real-time RT-PCR

analyses were performed. The DPPH assay revealed strong scavenging activities of DMSO extracts. The estimated AEAC and TEAC (Ascorbic acid/Trolox EAC) values varied from 0.81 ± 0.09 to 0.24 ± 0.05 and 0.55 ± 0.09 to 0.16 ± 0.02, respectively. Aqueous extracts showed low but dose-dependent increase of activity. Sage extracts exhibited the highest total antioxidant capacity both in DMSO and aqueous solutions. According to MTT assays, all extracts exerted no significant cytotoxicity in RAW 264.7 cells up to the highest treated concentration. Results from RT-qPCR analyses indicated that all DMSO extracts significantly decreased Nos2 expression in a concentration-dependent manner compared to the LPS+IFN-γ-treated RAW 264.7 cells. In agreement, the majority of DMSO extracts from all plants significantly reduced IL-6 levels, while sage and dittany extracts, specifically inhibited Ptgs2 production ($P < 0.01$ and $P < 0.05$) at higher concentrations (250 and 125 μg/mL, respectively). Aqueous extracts did not significantly alter Nos2, IL-6 and Ptgs2 expression in RAW264.7 cells. In conclusion, extracts from Greek biodiversity plants regulate the expression of Nos2 and IL-6 in RAW264.7 macrophages. Hence, they may exert anti-inflammatory properties.

P-01.4-013
Erythrocyte membrane fatty acid profile and cardiometabolic features of testicular cancer survivors

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Given the young age at diagnosis, effective therapeutic strategies, and high survival rate, testicular cancer (TC) is a model malignancy for long-term survivorship. Nevertheless, chemotherapy-related toxicities and potential hypogonadism may intensify the risk of metabolic disturbances and cardiovascular sequelae. The aim of this study was to explore the associations between the fatty acid (FA) profile of erythrocyte membrane phospholipids, a robust, yet underutilized risk assessment marker, and the cluster of cardiometabolic features including abdominal obesity, atherogenic lipoprotein phenotype, and dysglycemia in patients successfully treated for TC. This cross-sectional study recruited a sample of TC patients undergoing post-curative follow-up care. FA panel was determined by gas chromatography and surrogate indices of desaturase enzyme activities were estimated based on product-to-substrate FA ratios. Omega-3 Index (%) was calculated as the sum of eicosapentaenoic (EPA) and docosahexaenoic acid (DHA). In the analyzed cohort ($n = 52$, age $\bar{x} = 36.44 \pm 8.48$ years), 59.62% of men were overweight or obese and 76.92% were dyslipidemic. An inverse correlation was found between the Omega-3 Index ($\bar{x} = 4.42 \pm 0.87\%$) and waist-to-hip ratio ($r = -0.395$, $P < 0.05$). LDL-cholesterol correlated negatively with delta-5-desaturase activity marker, EPA and Omega-3 Index ($r = -0.388$, $r = -0.395$, $r = -0.407$, all $P < 0.05$, respectively). Higher levels of HDL-cholesterol were associated with a higher total content of monounsaturated FAs ($r = 0.384$, $P < 0.05$), and a lower degree of delta-6-desaturase activity ($r = -0.370$, $P < 0.05$). Stearoyl–CoA desaturase-18 was associated with triglycerides ($r = 0.316$ and $P < 0.05$), while delta-6-desaturase correlated with glycated hemoglobin ($r = 0.282$, $P < 0.05$). Unfavorable FA composition and deregulation of desaturase enzyme activities underpin the need for the timely introduction of targeted measures in order to prevent adverse cardiometabolic health outcomes among TC survivors.

P-01.4-014
Influence of heparin on the immunodetection of cardiac troponin

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Ternary troponin complex consists of three proteins: troponin I (TnI), troponin T (TnT) and troponin C (TnC). Cardiac isoforms of TnT (cTnT) and TnI (cTnI) that are expressed exclusively in heart are actively used as biomarkers of heart disorders. Heparin is a highly negatively charged polysaccharide that is used to prevent blood coagulation in the treatment of acute myocardial infarction (AMI) and to prepare heparin plasma samples used for immunodiagnostics. The work aimed to study the interaction of heparin with ternary cTnI–cTnT–TnC complex (ITC) and its effect on the immunochemical determination of cardiac troponins. To reach the goal, we spiked ITC, cTnI–TnC complex (IC) or free cTnT to normal heparin and citrate plasmas and analyzed the samples by gel-filtration (GF). In resulting profiles, the peaks of ITC and free cTnT but not IC shifted to a higher molecular weight region in heparin plasma compared to citrate plasma. In the GF profiles of heparin and citrate plasma samples of AMI patients, the peak of the full-size ITC also shifted to a higher molecular weight region in heparin plasma whereas peaks of the low-molecular weight (LMW) ITC eluted in the same volume in heparin and citrate plasmas. Since LMW ITC contains only a C-terminal fragment of TnT, we conclude that heparin binds to cTnT via its N-terminal and/or central part of the molecule. The recovery of spiked ITC and free cTnT by some antibodies specific to cTnT was significantly lower in heparin plasma than in citrate plasma samples, which may indicate the sites of heparin binding. We identified four regions of cTnT that are affected by heparin, and antibodies specific to these regions should be carefully checked prior to use in immunochemical tests. To sum up, here we show that the binding of heparin to ITC is due to the interaction of heparin with N- and/or central part of cTnT in ITC and these interactions may have a profound effect on the immunochemical detection of cTnT. *The authors marked with an asterisk equally contributed to the work.

P-01.4-015
Characterisation of potassium channels in guinea pig cardiomyocytes

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According to WHO data, myocardial infarction is one the most common causes of death in humans. Protecting the heart muscle

against ischemia/reperfusion (I/R) damage is one of the greatest challenges of medicine. Nearly 95% of ATP in cardiomyocytes is generated by mitochondria. Moreover, mitochondria take part in cell protection. Potassium channels (mitoK) are present in the inner mitochondrial membrane (IMM). They play a key role in energy metabolism of the cell, synthesis of reactive oxygen species, transmembrane potential regulation, as well as in apoptosis. It has already been reported that activation of mitoK has a protective effect against I/R injury. Although, to bring this phenomenon from bench to bedside, it is necessary to understand the mechanisms governing it. Identification of mitoK in cardiomyocytes and their pharmacological characteristics is essential. In our research, using the patch clamp technique, we recorded the activity of individual channels present in the IMM. Recorded activities were not homogeneous. So far, in the heart of the guinea pig, which was the subject of the study, there were recorded activities inhibited by ATP with conductivity in the range of 100-130 pS, which indicated features of the mitoK$_{ATP}$ channels. Another group of channels was sensitive to Ca^{2+} concentrations and showed dependence on voltage. The high concentration of Ca^{2+} increased the probability of an open state, while the positive potential caused the channels to remain closed. These channels were activated by NS11021 and inhibited by paxillin, which suggests that we are dealing with the mitoBK$_{Ca}$ channel. Preliminary data confirmed the presence of the α-channel subunit by western blot and mRNA encoding α- and 4 β subunits of mitoBK$_{Ca}$ channel. Research was also carried out on the influence of IR light on the modulation of mitoK channel activity. This study was supported by the Polish National Science Center (grants No. 2019/34/A/NZ1/00352 to AS). *The authors marked with an asterisk equally contributed to the work.

P-01.4-016
Phenotypic and functional features of polarized N1 and N2 neutrophils in inflammation; their effect on macrophage polarization

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Following myocardial infarction, neutrophils are rapidly recruited to the ischemic region where they initiate the inflammatory response, attract monocytes and orchestrate post-myocardial infarction evolution. Recent evidence has revealed the existence of heterogeneity and versatility of neutrophils. We questioned whether N1-inflammatory or N2-anti-inflammatory neutrophils contribute to the generation of distinct macrophage phenotypes. Here, we investigated the phenotypic and functional features of polarized N1 and N2 neutrophils and search for the effect of factors released by N1/N2 neutrophils on macrophage phenotypes. Neutrophils were polarized towards N1 or N2 phenotype using IFN-g+LPS and IL-4, respectively, and RNA-seq, qPCR and cytokine profiler assay were used to investigate their molecular profile. Macrophages obtained from human THP cells were incubated with secretome of N1/N2 neutrophils released for 18h upon removing of IFN-g+LPS and IL-4. The results showed that in N1-neutrophils, a substantial number of up-regulated genes code for inflammatory cytokine, that were either unmodified or down-regulated in N2 neutrophils. Functional analysis revealed that, unlike N2-neutrophils, N1 neutrophils exhibited: higher oxidative burst and ROS levels, increased activity of MPO and MMP-9 and increased chemotactic response. Macrophages exposed to secretome from N1-neutrophils exhibited increased gene expression of IL-1β and TNF-α, and the secretome from N2 cells enhanced the expression of efferocytosis molecules Mertk, MGF-E8 and TGF-β. In conclusion, the data define novel specific markers, signaling mechanisms and functional features of N1 and N2-neutrophils and highlight the role of the cross-talk between neutrophils and macrophages on the specific macrophage polarization. Acknowledgements. This work was supported by a grant of Ministry of Research and Innovation, CNCS - UEFISCDI, project number PN-III-P4-ID-PCCF-2016-0172, within PNCDI III".

P-01.4-017
Ursolic acid reduces NADPH oxidase expression and oxidative stress in the atherosclerotic aorta of apolipoprotein E-deficient mice by inhibiting NF-kB and STAT1/3 signaling

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NADPH oxidase (Nox)-derived reactive oxygen species are important triggers of oxidative stress in atherosclerosis. Selective triterpenic acids display anti-oxidant and anti-inflammatory effects in experimental models of disease by molecular mechanisms that remain scantily elucidated. The study aimed at investigating the potential of ursolic acid (UA), a pentacyclic triterpenoid, to regulate Nox expression and oxidative stress in atherosclerotic mice. Male ApoE-/- mice fed a normal (ND) or a high-fat, cholesterol-rich diet (HD) were distributed into experimental groups to receive via intraperitoneal injection 1 mg/kg UA, or its vehicle, for 4 weeks. Human monocyte-derived macrophages (Mac) polarized to pro-inflammatory (M1) or anti-inflammatory (M2) phenotype were exposed for 24 h to 5 μM UA. Real-time PCR, western blot assay, and Oil Red O staining were employed. The expression of Nox1, Nox2, and Nox4 subtypes, p65/NFkB, STAT1, and STAT3, and the formation of 4-hydroxynonenale/4-HNE- and nitrotyrosine/NT-protein adducts were found significantly elevated in the atherosclerotic aorta of ApoE-/- (HD) mice as compared with ApoE-/- (ND) animals. Treatment of ApoE-/- (HD) mice with UA significantly reduced the up-regulation of Nox subtypes, oxidative stress markers (4-HNE, NT) and the protein levels of p65/NFkB and STAT1/3 pro-inflammatory transcription factors. UA suppressed the induction of Nox1, Nox2, Nox4, and Nox5 and reduced NF-kB and STAT1/3 signaling in cultured M1-Mac. In atherosclerotic mice, ursolic acid down-regulates the aortic expression of Nox enzymes and markers of oxidative stress, possibly by reducing the expression of p65/NFkB and STAT1/3, important transcriptional regulators of Nox subtypes. Ursolic acid or its pharmacologically active derivates could become important therapeutic tools to reduce oxidative stress and inflammation in atherosclerosis. Work supported by PN-III-P2-2.1-PED-2019-2497, PN-III-P2-2.1-PED-2019-2512, PN-III-P4-ID-PCE-2020-1898.

P-01.4-018
MicroRNA-210-3p contributes to inflammatory response in the atherosclerotic aorta of apolipoprotein E-deficient mice: potential role in human atherosclerosis

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Emerging evidence implicates miR-210-3p in the pathophysiology of atherosclerosis. The precise molecular targets and function of miR-210-3p in atherosclerosis remain poorly understood. The aim of this study was to investigate the potential role of miR-210-3p in regulating the expression of key pro-inflammatory mediators in experimental atherosclerosis. Human non-atherosclerotic (superior thyroid artery) and atherosclerotic (carotid artery) tissue specimens, ApoE-/- mice and human monocyte-derived macrophages (Mac) were employed. ApoE-/- mice fed a normal (ND) or an atherogenic (HD) diet were randomized to receive via intraperitoneal injection PBS or 10 mg/kg miR-210-3p LNA inhibitor/negative control miRNA inhibitor (NCI) for 6 weeks: (1) ApoE-/-(ND) + PBS, (2) ApoE-/-(HD) + PBS, (3) ApoE-/- (HD) + NCI, and (4) ApoE-/-(HD) + miR-210-3p inhibitor. Resting Mac were transfected with 50 nM NCI/miR-210-3p inhibitor and subjected to Mac polarization to pro-inflammatory (M1) and anti-inflammatory (M2) phenotype. Real-time PCR and western blot assays were employed. The level of miR-210-3p was found significantly elevated in human atherosclerotic lesions, atherosclerotic aorta of ApoE-/- mice and in M1-Mac. The uptake of FITC-labeled NCI by the atherosclerotic aorta and different organs of ApoE-/- mice was demonstrated by high-resolution fluorescence imaging. In silico prediction indicated that miR-210-3p may target negative regulators (SOCS1, TNIP1) of NF-kB pro-inflammatory transcription factor. Inhibition of miR-210-3p suppressed the up-regulation of inducible nitric oxide synthase (NOS2), MCP-1, and TNFα in the atherosclerotic aorta of ApoE-/- mice and in cultured human M1-Mac. MiR-210-3p expression is elevated in human and experimental atherosclerosis. Inhibition of miR-210-3p could become an important therapeutic strategy to reduce inflammation in atherosclerosis. Work supported by PN-III-P2-2.1-PED-2019-2512, PN-III-P4-ID-PCE-2020-1898, PN-III-P2-2.1-PED-2019-2497.

P-01.4-019
Multidisciplinary approach to reveal young athlete syncope

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Laboratory medicine, along with genetic investigations in sports medicine, is taking on an increasingly important role in monitoring athletes' health conditions. Acute or intense exercise can result in metabolic imbalances, muscle injuries or reveal cardiovascular disorders. This study aimed to monitor the health status of a basketball player with an integrated approach, including biochemical and genetic investigations and advanced imaging techniques, to shed light on the causes of recurrent syncope he experienced during exercise. Biochemical analyses showed that the athlete had abnormal iron, ferritin and bilirubin levels. Coronary Computed Tomographic Angiography highlighted the presence of an intramyocardial bridge, suggesting this may be the cause of the observed syncopes. The athlete was excluded from competitive activity. In order to understand if this cardiac malformation could be caused by an inherited genetic condition, both array-CGH and whole exome sequencing were performed. Array-CGH showed two intronic deletions involving MACROD2 and COMMD10 genes, which could be related to a congenital heart defect; whole exome sequencing highlighted the genotype compatible with Gilbert syndrome. However, no clear pathogenic mutations related to the patient's cardiological phenotype were detected, even after applying machine learning methods. This case report highlights the importance and the need to provide exhaustive personalized diagnostic work up for the athletes in order to cover the cause of their malaise and for safeguarding their health. This multidisciplinary approach can be useful to create ad personam training and treatments, thus avoiding the appearance of diseases and injuries which, if underestimated, can become irreversible disorders and sometimes can result in the death of the athlete.

P-01.4-020
Hydrogel from native tissue induces an anti-inflammatory and anti-fibrotic phenotype to cardiac fibroblasts

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Cardiovascular diseases (CVD), with myocardial infarction (MI) the most common CVD, are the leading cause of death globally. Our aim was to develop a myocardial-derived hydrogel from native myocardial tissue to be injected in the infarcted area, in order to reduce the inflammatory response, ensure the appropriate ECM and facilitate cardiac fibroblast (CF) infiltration for efficient myocardial repair post-MI. Here, we developed and characterized a native hydrogel obtained from porcine myocardium (MIGEL) and investigated the phenotype of CF grown on the hydrogel. MIGEL was obtained from decellularized myocardium by enzymatic digestion followed by gelling. The GAGs and extracellular matrix (ECM) proteins content were measured using ELISA and western blot, and MIGEL biocompatibility with CF was investigated by live/dead and MTT assays. The phenotype of CF was investigated by measuring expression of different inflammatory and ECM molecules by qPCR and western blot. The results showed that MIGEL contains GAGs and ECM molecules: collagen I and IV, elastin, fibronectin and laminin. CF grown on the MIGEL displays the specific morphology, form a well-defined cell network and over 95% viability. As compared with normal culture, CF grown on MIGEL exhibits a significantly decreased expression of α-SMA, CNN2, VEGF-2, collagen I and MCP-1 and an increased expression of IL-10, CNN5 and MMP-1. In conclusion, the data shown that MIGEL has a structure comparable to native myocardium, keeping in its composition GAGs and specific ECM proteins. By reducing the expression of α-SMA, collagen I, CNN2 and MCP-1, and inducing IL-10 and CNN5, MIGEL promotes a quiescent, anti-inflammatory and anti-fibrotic phenotype to CF, data that recommends it as a suitable hydrogel for therapeutic approach post-MI.

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Ageing

P-01.5-001
DNA polymerase Lambda: a new putative mitochondrial DNA polymerase?

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DNA polymerases are key proteins in genome replication and repair. There are 16 human DNA polymerases, and they are classified in 4 families, according to their amino acid sequence and structure, and PrimPol that belongs to the Archaea-Eukaryotic Primase family. Their main roles are in the nucleus, with exception of DNA polymerase gamma (Pol γ) that is responsible for mitochondrial DNA (mtDNA) maintenance, and PrimPol with functions within both compartments. However recently, additional DNA polymerases (specifically Pol ζ or REV3L, Pol β and Pol θ) have been found in the mitochondria, interacting with other mitochondrial proteins. The aim of this project is to identify if further additional DNA polymerases localise to the mitochondria and, if so, to understand if they influence positively or negatively the integrity of mtDNA, particularly in the acquisition of genome mutations and their impact in aging and life- and healthspan. To study the potential of further DNA polymerases localising to mitochondria, subcellular fractionation was performed after inducing different DNA lesions, followed by protein expression detection of native or epitope-tagged DNA polymerases. DNA polymerase lambda (Pol λ) was the main DNA polymerase targeted and in addition to its nuclear expression, Pol λ was also detected in the mitochondrial fraction. More studies are needed to assess and confirm Pol λ mitochondrial localisation, particularly confocal microscopy to determine the spatial location of Pol λ without possible contamination between organelles from fractionation approaches. Moreover, gene knockdown and mitochondrial metabolism assays will be performed to understand if Pol λ is involved in mtDNA physiological processes, and aging studies will be done using *Caenorhabditis elegans* to assess the influence of DNA polymerases in the mitochondrial genome. *The authors marked with an asterisk equally contributed to the work.

P-01.5-002
GABAergic system of subfornical organ in adult and aged rats

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The subfornical organ (SFO) is one of the circumventricular organs (CVOs) of the brain located in the roof of the third ventricle. Due to its high vascularization and lacking blood–brain barrier, SFO is affected by humoral signals and thus mediates blood–brain communication. Despite notable interest in the SFO, the effects of ageing which occur in one of the major neurotransmitter system of CNS here remain to be clearly established. In this study we examined GABAergic system of SFO using immunohistochemical methods. The brain of adult (4-6 months) and old (23 months) male Wistar rats was explored (n = 3 for each age). Serial frontal sections were prepared approximately at the 1.4 mm behind Bregma. Antibodies against glutamate decarboxylase 67 (GAD67) were used in order to visualize GABAergic structures. It was established that GABAergic structures are ubiquitous within SFO. GABAergic neurons tend to locate in the peripheral regions near ventricle and, moreover, one can notice sparse subependymal GABAergic neurons. GABAergic terminals are common in SFO and tend to form nets around not only individual cells, but also cell clusters and perivascular space of fenestrated capillaries and septal veins. There is also a considerable amount of axon terminals in subependymal area. In old rat's brain tissue, the intensity of immunohistochemical reaction is significantly lower. In the lateral part of SFO near septal veins we found GABAergic cells, which seem to be absent in SFO of adult animals. The results obtained suggest the estimated broadening of GABAergic cell population and general degradation of GABAergic innervation in SFO during aging. The reported study was funded by RSF according to the research project № 22-25-00105, https://rscf.ru/project/22-25-00105/.

P-01.5-003
Analyzing *Origanum vulgare* ssp. hirtum (Lamiaceae) essential oil for neuroprotective potential against scopolamine-induced zebrafish (*Danio rerio*) model

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Origanum vulgare ssp. hirtum has been used as medicinal herb promoting antioxidant, anti-inflammatory, antimicrobial and neuroprotective activities. We investigated the protective effects and the mechanism of *O. vulgare* ssp. hirtum essential oil (OEO) on cognitive impairment and brain oxidative stress in a scopolamine (Sco)-induced a zebrafish (*Danio rerio*) model of cognitive impairment. Our results showed that exposure to Sco (100 μM) leads to anxiety, spatial memory, and response to novelty dysfunctions, whereas the administration of OEO (25, 150, and 300 μL/L, once daily for 13 days) reduced anxiety-like behavior and improved cognitive ability, which was confirmed by behavioral tests such as the novel tank-diving test (NTT), Y-maze test and novel object recognition test (NOR) in zebrafish. Additionally, Sco-induced brain oxidative stress and increasing of acetylcholinesterase (AChE) activity were attenuated by the administration of OEO. The gas chromatography-mass spectrometry (GC-MS) analyses were used to elucidate the OEO composition, being thymol (38.82%), p-cymene (20.28%), and γ-terpinene (19.58%) as the main identified components. These findings suggested the ability of OEO to revert the Sco-induced cognitive deficits by restoring the cholinergic system activity and brain antioxidant status. Thus, OEO could be used as perspective sources of bioactive compounds, displaying valuable biological activities, with potential pharmaceutical applications. Acknowledgment: Authors are thankful to Romanian Ministry of Research, Innovation and Digitization, within Program 1 –

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P-01.5-004
Effect of hesperidin, hesperetin, rutinose, and rhamnose on skin aging

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Skin aging is a complex physiological process caused by internal (chronological) and external factors. Chronological aging is associated with spontaneous accumulation of modified biomolecules by non-enzymatic glycation and formation of advanced glycation end-products (AGE). AGE cause oxidative stress, tissue damage, inflammation, and the development of complications of diabetes. Therefore, it is useful to reduce their formation, and the use of natural substances is one of the possible prevention strategies. In our study, we focused on the molecular mechanism of the antiglycation effect and the comparison of hesperidin, hesperetin, rutinose, and rhamnose. Hesperidin and its aglycone hesperetin are classified as natural flavonoids that are compounds with anti-inflammatory and antioxidant potential. Hesperidin has also been shown to be a potent anti-photoaging factor by regulating metalloproteinases and inflammatory interleukins. Hesperidin is glycosylated by rutinose, which is composed of rhamnose and glucose. The cytotoxicity of our selected substances was evaluated using the MTT assay on primary cultures of normal human dermal fibroblast (NHDF). Subtoxic concentrations of hesperidin, hesperetin (1-25 μM), rhamnose, and rutinose (1-25 mM) were tested on the skin aging model. The antisenescent activity of the test compounds was evaluated by measuring the level of pro-inflammatory cytokines (IL-6, IL-8) and matrix metalloproteinases. The results of each method (ELISA and MTT) will be discussed in the poster presentation. Acknowledgements: This work was supported by the student grant: Skin anti-aging study of hesperidin, hesperetin, rutinose, and rhamnose: Comparative study (Registration number: DSGC-2021-0065). This student grant is funding from the OP VVV Project No. CZ.02.2.69/0.0/0.0/19_073/0016713, Improving Schematics of Doctoral Student Grant Competition and their Pilot Implementation. Project MEYS LTC20069 is acknowledged as well.

P-01.5-005
Molecular alterations in ageing sperm and their relevance for male fertility decline

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Male fertility is strongly affected by environment and lifestyle. Advanced paternal age has been linked with changes in testicular structure and function, impaired semen parameters and DNA integrity, lower pregnancy rates and decline in offspring fitness. The sperm quality decline with ageing has also been associated with an increase in oxidative stress. However, only a few studies reported the deregulation of sperm proteins or RNAs associated with this risk factor for male infertility. This work aimed to study the ageing-related alterations in human sperm protein and small RNA content possibly responsible for the age-associated decline in male fertility. To do so, 120 Portuguese men from the Aveiro region (Portugal), aged between 19 and 56 years old were included in this study. Basic semen analyses were performed on all sperm samples according to WHO's guidelines. To avoid contamination by somatic cells, density gradient sperm selection was performed. The proteome of 19 normozoospermic human sperm samples divided into four groups according to men's age was evaluated by quantitative proteomic analysis. The small RNA content of 16 human sperm samples was investigated using small RNA sequencing. Our data showed no correlation between paternal age (mean age 35.2 ± 6.32 years) and the seminal parameters examined. Proteomic analysis revealed 46 differentially expressed proteins (DEPs) between groups. Gene ontology analysis of all deregulated sperm proteins shows that response to unfolded protein, positive regulation of mitochondrion organization and apoptotic process, negative regulation of phosphoprotein phosphatase activity, and spermatogenesis are common biological processes affected. Transcriptomic analysis identified 5 differentially expressed miRNAs (DEMs) between groups. The DEPs and DEMs here identified could help to elucidate and/or became potential diagnostic markers for the age-associated decline in human sperm quality.

P-01.5-006
Collagenase, elastase, hyaluronidase inhibition by hesperidin and its structural part hesperetin, rutinose, and rhamnose

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Skin aging is a multisystemic degenerative process characterized by phenotypic and functional changes of cutaneous cells. These changes are accompanied by structural disturbances in extracellular matrix components such as collagen, elastin, and hyaluronic acid caused by the increasing activity of collagenase, elastase, and hyaluronidase. One of the most important ways to prevent skin aging is the topical application of dermatological preparations containing active compounds that inhibit collagenase, elastase, and hyaluronidase. For this purpose, naturally occurring compounds derived from plants have been investigated in dermatology. The flavonoid hesperidin and its aglycone form hesperetin, both abundant in citrus fruits, possess antioxidant, anti-inflammatory, and free radical scavenging activity. Hesperidin has also been shown to be an anti-aging and anti-photoaging agent. Rutinose, which is the structural component of hesperidin, consists of rhamnose and glucose. The anti-aging effect of rhamnose has been partially documented, but rutinose has not yet been studied. The aim of this study was to determine the ability of hesperidin, hesperetin, rutinose, and rhamnose to inhibit enzymes associated with skin aging (collagenase, elastase,

hyaluronidase) to better understand the mechanism of action of hesperidin. We tested the effect of hesperidin, hesperetin, rutinose, and rhamnose on the pure collagenase, elastase, and hyaluronidase activities using tube tests. Evaluation of these results will be discussed in our poster contribution. Acknowledgement: This work was supported by the student grant: Skin anti-aging study of hesperidin, hesperetin, rutinose, and rhamnose: Comparative study (Registration number: DSGC-2021-0065). This student grant is funding from the OP VVV Project No. CZ.02.2.69/0.0/0.0/19_073/0016713, Improving Schematics of Doctoral Student Grant Competition and their Pilot Implementation. Project MEYS LTC20069 is acknowledged.

P-01.5-007
Skin phototoxicity of ambient particulate matter mitigated by the protective effects of ascorbic acid and resveratrol

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Continuous exposure of human skin to air pollution can result in a range of skin conditions. Solar simulated light significantly increases the skin toxicity of fine particulate matter ($PM_{2.5}$) against human skin cells due to the photoproduction of free radicals and singlet oxygen. As it is hardly possible to minimize the exposure of skin cells to $PM_{2.5}$, strategies to reduce the damage are needed. We employed L-ascorbic acid and resveratrol as potential topical compounds against pollution-related skin impairment. Electron Paramagnetic Resonance (EPR) spin-trapping, DPPH assay, and time-resolved singlet oxygen phosphorescence were used to determine the scavenging activity of compounds. MTT assay was used to analyze the $PM_{2.5}$ cytotoxicity. Live-cell imaging was applied to examine wound healing properties of cells exposed to $PM_{2.5}$ and the modulatory effect of antioxidants. Immunofluorescent staining was used to demonstrate oxidative damage mediated by light-activated particulate matter. Iodometric assay and JC-10 assay were used to investigate the properties of antioxidants against $PM_{2.5}$-induced damage to cellular lipids and mitochondria. Both compounds effectively scavenged free radicals and singlet oxygen produced by $PM_{2.5}$, reduced cell death and prevent oxidative damage to the cytoskeleton, mitochondria, and cellular lipids, and promote wound healing. L-ascorbic acid and resveratrol, especially when applied together can protect HaCaT cells from negative effects of $PM_{2.5}$ dark and light-induced toxicity. This research was supported by the National Science Center (NCN) of Poland grant Preludium-2020/37/N/NZ1/01054.

P-01.5-008
Na,K-ATPase and ROS hormesis

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Na,K-ATPase is an ion pump that uses the free energy of ATP hydrolysis for the creation of a transmembrane ion gradient. It belongs to the P-type ATPase family. Na,K-ATPase is involved in almost all life cellular processes, and therefore it is important to know its molecular mechanisms and regulation by physiological regulators. In this study, we have studied the effect of H_2O_2 on the enzyme system. At low concentration H_2O_2 (< 100 μM) activates the enzyme system while at high concentrations inhibits it. H_2O_2 changes p-chloromercuribenzoic acids (PCMB) affinity. This is a necessary and sufficient condition to conclude that H_2O_2 modifies thiol groups of the enzyme system. Low, activatory concentrations of H_2O_2 (< 100 μM) changes kinetic parameters of the enzyme system. It alters the affinity for MgATP complex, Na^+ and K^+ and increases maximal velocity (Vmax) of the enzyme.

P-01.5-009
Investigating biochemical and molecular markers responsible for early aging in childhood cancer survivor

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Although survival rates of childhood cancer patients have improved over the past four decades, childhood cancer survivors (CCS) clearly show the risk of possible long-term clinical complications related to chemo/radiotherapy and consistent with early aging. However, the cellular/molecular basis of all described symptoms and signs remain missing. Among the alterations involved in the aging process, mitochondrial metabolism and the consequent increment of oxidative stress production play a pivotal role. Thus, using mononuclear cells (MNCs) isolated from CCS peripheral blood, the oxidative phosphorylation (OxPhos) efficiency, oxidative stress/ antioxidant defense balance, mitochondrial dynamics markers were evaluated. Analyses were performed on 196 CCS samples aged between 5 and 20 years, comparing the results with those obtained on MNCs of 154 healthy subjects aged between 5 and 106 years old. Data show that the CCS OxPhos efficiency decreased compared to the healthy age-matched samples but was similar to that observed in the elderly subjects. In addition, an increment of oxidative stress with respect to the healthy age-matched population was observed, despite antioxidant defense activation. In addition, applying a mathematical model predicting the age based on the glucose metabolism, CCS displayed a biological age significantly increased by decades compared to the chronological age. CCS but not healthy age-matched and elderly subjects showed a 5-fold downregulation of CLUH, PGC1-α, and SIRT6 gene expression, suggesting that the altered expression of these genes could not be linked with physiological aging. In addition, CCS MNCs showed

an unbalance between mitochondrial fusion and fission and altered mitophagy and autophagy pathways. In conclusion, our study identified some biochemical and molecular alterations possibly contributing to the pathophysiology of aging and metabolic deficiencies in CCS.

P-01.5-010
A thermosensitive hydrogel harboring CD146/IGF-1 nanoparticles for skeletal muscle regeneration

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Muscle atrophy is commonly observed in degenerative patient as a result of dysregulation in skeletal muscle. Muscle wasting with aging has some clinical consequences, such as not only decreased ambulation and exercise tolerance, but increased risk of pulmonary and thromboembolic complications. The aim of this study is to provide a beneficial tactic on muscle regeneration using intramuscular delivery systems with nanoparticles containing CD146. Herein, we construct an injectable, *in situ* hydrogel system consisting of CD146, IGF-1, collagen I/III, and poloxamer 407, termed CIC gel. The thermosreversible phase transition of the CIC gel at body temperature results in the sustained-release of CD146 and IGF-1 after intramuscular administration. The secreted CD146 then binds to VEGFR2 on muscle surface and effectively induces efferocytosis of neutrophils and macrophages. Consequently, these combined molecules activate muscle regeneration via autophagy and suppress muscle inflammation and apoptosis. In this report, we provide an applicable concept of the myogenesis-activating protein formulation, broadening the thermoreversible hydrogel to the protein therapeutics for damaged muscle recovery.

Host–pathogen interactions

P-02.1-001
A yeast-based screening assay identifies drug that inhibit SARS-CoV-2 main protease

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused high mortality and enormous economic loss. Thus, in addition to effective vaccines, there is an urgent need of effective drug molecules to fight against COVID-19. This necessity motivated us to explore potential compounds targeting main protease (Mpro) enzyme of SARS-CoV-2, which is essential for processing the polyproteins that are translated from the viral RNA. In this work, we have developed a highly permeable to drugs and extremely stable, inducible Mpro expression system causing a strong change in yeast phenotype. The yeast was successfully engineered with the CRISPR / Cas9 method both to introduce the gene encoding Mpro and to remove the genes responsible for multi-drug resistance. Our system has been screened for Mpro inhibitors using nearly 2000 thousand drugs, and 800 plant and fungal extracts. Our screening results showed only one drug called ebselen (also called PZ 51, DR3305, or SPI-1005) that was able to restore the growth of yeast cells expressing Mpro. The selection of only one drug from among several proposed Mpro inhibitors may indicate a very rigorous nature of our yeast-based screening assay, allowing for the selection of the drug with the strongest Mpro inhibitory effect, as well as the lowest cytotoxic effect. This work was supported by the research grant of Adam Mickiewicz University, Poznań, Grant Number 6/2020 "Research on COVID-19". *The authors marked with an asterisk equally contributed to the work.

P-02.1-002
Casein kinase 2 activity is a key regulator of transgene expression from AAV vectors

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Gene therapy has become an effective strategy for treatment of genetic diseases as well as a promising alternative to standard therapies in numerous disorders. Adeno-associated viral vectors (AAVs) are one of the most widely used transgene carriers, as they provide transduction of numerous cell types *in vivo*, with limited activation of immune response. Although more and more therapeutic strategies involving AAVs are developed, in some cases transduction efficiency is not sufficient to obtain meaningful improvement of the patient's condition. This issue seems to be the major obstacle in gene therapy for heart diseases, as clinical trials utilising AAVs revealed very low to undetectable transgene expression in the targeted tissue. In this study, we aimed to identify the main cellular barriers for successful transduction with AAV vectors and develop strategies to bypass them, using human induced pluripotent stem cells (iPSC)-derived cardiomyocytes (iPSC-CMs), epicardial fibroblasts (iPSC-CFs) and human aortic endothelial cells (HAECs) as a model. Our analysis revealed that inhibition of casein kinase (CK2) during transduction increased percentage of transgene expressing cells – 2-fold for iPSC-CMs and up to 15-fold for HAECs and iPSC-CFs. This effect was specific to CK2 inhibition, as compounds interfering with downstream CK2 signalling had limited impact on the transduction efficiency. Application of CK2 inhibitor was associated with degradation of Mre11 protein and transient induction of DNA damage response in the cells, evidenced by increase in p53 level and formation of ATM hotspots in the nuclei. Since Mre11 interferes with transgene expression from AAVs, we suggest that inhibition of CK2 activity prevents Mre11 interaction with vector genomes and thus, enables more efficient transduction. Our results indicate a crucial role of CK2 in AAV transduction process and provide a promising strategy to enhance transgene expression from AAVs in various human cell types.

P-02.1-003
Unravelling the impact of the 5′-3′ exoribonuclease XRN1 in the HIV-1 replication cycle

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RNA-based viruses must rely heavily on the cell's RNA machinery, as they require it for key steps in their replication cycle. The Human Immunodeficiency Virus type 1 (HIV-1) is no different

and establishes a tight grip over the ensemble of cellular RNAstasis. However, the RNA decay machinery has often been overlooked as a component in RNAstasis, and its interactions with HIV-1 are largely understudied. The present work focuses on its main effector: the 5'-3' exoribonuclease XRN1. XRN1 constitutes the finishing step of most RNA decay pathways, like mRNA quality control and mRNA turnover. Its influence in viral host–pathogen interactions has been the focus of attention in recent works, as evidence shows XRN1 as a target for many RNA viruses, as ways of protecting the viral genome from degradation. The HIV-1 genome (vgRNA) carries a dual role, encoding the main structural protein Gag that conforms the viral particle and as the virions genome. We therefore asked; is XRN1 influencing vgRNA's fate within the cell? We established a simple pipeline of experiments to assess XRN1 and the RNA decay machinery's impact on the vgRNA's fate. We evaluated HIV-1's expression under both transient overexpression of XRN1 and stable XRN1 knockdown cells. Viral genomic transcript levels were measured by RT-qPCR and Gag levels were assessed through Western Blot densitometry. The sub-cellular localization of vgRNA and Gag were also studied using fluorescence microscopy using the same overexpression and knockdown settings. Additional *in vitro* assays using recombinant XRN1 and synthetic genomic constructs were also performed to further elucidate the decay dynamics of vgRNA. Our results showcase an unexpected and complex interplay between HIV-1 and cellular mRNA decay. Intracellular amounts of vgRNA increased upon XRN1 overexpression and decreased under XRN1 knockdown, leading us to investigate a potential role beyond genomic decay and opening a new door in HIV-1 host-pathogen interactions.

P-02.1-004
Discovery, isolation and characterization of a novel potentially emergent pathogen

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Vibrio species are ubiquitous and can be found in salt water and estuarine environments throughout the world. The *Vibrio* genus is diverse and includes several species that are pathogenic for humans and marine animals. New species of *Vibrio* are still being discovered to this day. A motile bacterium that grows aerobically, forming yellowish colonies, was isolated from a water sample collected in Cold Spring Harbor, New York, USA. Comparison of its 16S rRNA gene sequence to databases suggested that it is a new species within the genus *Vibrio*. The highest *in silico* DNA–DNA hybridization value found was 23.4 and 21.4% for chromosome I and II, and their GC content was 50.4 and 49.7%, respectively. Optimum growth was achieved at 20°C in Tryptic Soy Broth with aeration. A microscopic observation was conducted in different growth phases and revealed a rod-shaped motile cell. The antimicrobial susceptibility was accessed by determining the minimum inhibitory concentrations of antibiotics from several classes. A deeper phylogenetic analysis was conducted using the 16S and several housekeeping genes. A prediction algorithm of pathogenicity gave a 70% probability of the species being pathogenic for humans. Since the novel species isolated came from Cold Spring Harbor, we have named it *Vibrio harborensis*. The medical community should be alert for this potentially emergent pathogen.

P-02.1-005
Biochemical parameters of serum during changes in the quantitative and species composition intestinal microorganisms *Cyprinus carpio* L.

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The aim of the work is to study the dynamics of biochemical parameters with changes in the quantitative and species composition intestinal microorganisms *Cyprinus carpio L.* Violation of the mechanism homeostatic regulation of biochemical parameters was accompanied by significantly significant changes ($p \leq 0,05$) in the total number and species composition of fish intestinal microorganisms during the identification matrix-activated laser desorption/ionization time-of-flight mass spectrometry MALDI-TOF. Of the 48 identified cultures microorganisms, 34,6 % were *Aeromonas hydrophila* O14, O11, O34, O81. The number virulent isolates of *Pseudomonas aeruginosa* also increased; *Pseudomonas putrefaciens*; *Pseudomonas alcaligenes*; *Escherichia coli*; *Klebsiella pneumoniae*; *Enterococcus faecalis*; *Enterococcus faecium*; *Staphylococcus aureus*; *Candida albicans*. Direct correlations were established, $r = 0,93$, between total protein concentrations and an increase in the number of isolates producing hemolysins, bacteriocins, β-polysaccharides – markers of biofilm formation. With a decrease in the level fibrinogen, hemoglobin, and hematocrit, necrosis epitheliocytes gill lamellae, dystrophy myocardiocytes, and diapadesis erythrocytes developed. Changes in glucose levels caused pathology of the endocrine organs, hepatopancreas, nephrotoxic syndrome. Violation lipid metabolism, changes in the concentration of cholesterol, triglycerides, potassium, sodium, magnesium characterized the overall level of physiological stress. An increase in the concentration urea and creatinine was accompanied by a syndrome acute renal failure. Serum biochemical parameters, quantitative and species composition of intestinal microbiomes are fast and economical markers body homeostasis stability for diagnosing and evaluating the effectiveness ichthyopathology therapy.

P-02.1-006
Serum levels of IGF system proteins change with the severity of COVID-19

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IGF (Insulin-like Growth Factor) system proteins, including their ligands IGF1 and IGF2, their receptor IGF1R and binding proteins (IGFBPs) that control their bioavailability, are involved in pulmonary homeostasis and in respiratory diseases, including COVID-19. However, their circulating levels have not yet been

studied comparatively between groups of patients with different degrees of severity of the disease in order to determine their possible value as biomarkers in this context. Serum levels of IGF1, IGF2 and IGF1R were determined by ELISA, and those of IGFBP2, IGFBP3, IGFBP4 and IGFBP5 by immunoblotting. Results were compared between three groups of patients with different degrees of severity of COVID-19, and with those of an uninfected control group (total n = 120): uninfected (n = 24), asymptomatic (n = 32), hospitalized (n = 32) and ICU (n = 32) controls. IGFBP3/IGFBP2 ratios were also quantified. While IGF1 and IGF2 levels decreased in hospitalized and ICU patients, IGF1R levels were increased in ICU patients. IGFBP2 levels were also elevated in ICU patients, and conversely, IGFBP3 and IGFBP5 levels and IGFBP3/IGFBP2 ratios tend to decrease progressively with the severity of the disease. IGFBP4 levels were only significantly increased in the hospitalized patients compared to the control group. Changes in concentration levels of IGF1, IGF2, IGFBP3, and IGFBP5 follow similar patterns with a downward trend with COVID-19 severity, and are opposite to those of IGF1R and IGFBP2. IGFBP4 shows a different profile, being higher in hospitalized patients. Serum levels of IGFs change with the degree of COVID-19 and decreasing IGFBP3 and IGFBP5 levels and IGFBP3/IGFBP2 ratios show up as candidate biomarkers of disease severity. (Funding: Grants PGC2018-097397-B-I00 (MICINN, Spain) and 6.FRS-ABC.019 (Government of La Rioja) for JMGP; Fundación Rioja Salud (Government of La Rioja) for JAO and PP-M; FEDER/FSE).

P-02.1-007
Identification of *Bacillus* spp. strains that inhibit the intracellular infection caused by *Listeria monocytogenes*

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Listeria monocytogenes is an opportunistic pathogen that may cause meningitis. This bacterium is widely present in the environment and usually infects humans through contaminated food, particularly dairy and meat-related products. Fetuses and neonates, older adults, and patients with an immuno-compromised status are particularly exposed to the most severe forms of listeriosis. The first *L. monocytogenes* strains found with antibiotic resistance were isolated in 1988. Since then, new antibiotic-resistant strains have been identified, making it necessary to search for novel treatment options and preventative strategies. In this work, we focused on *Bacillus* spp. as a potential source of probiotics that may be used to prevent infections caused by a wide range of gastrointestinal pathogens, including *L. monocytogenes*. We tested a set of *Bacillus* spp. strains in colonic tissue cells that produce antimicrobials active against *L. monocytogenes*. These strains were selected because of their potent antimicrobial activity against a wide range of bacteria that included several pathogens, such as S*taphylococcus aureus, Salmonella typhimurium, Enterobacter cloacae* and *L. monocytogenes*. In addition, these strains are able to survive at physiological concentrations of biliary salts and mildly acidic pH conditions. As a result, we identified a very promising *Bacillus* spp strain that is highly effective in stopping the listerial colonization of human intestinal epithelial cells. This potential probiotic strain could be the basis of a primary preventative intervention strategy focused on susceptible patients to listeriosis. *The authors marked with an asterisk equally contributed to the work.

P-02.1-008
The marine cyanobacterial metabolite gallinamide A and its analogues are potent inhibitors of SmCB1 drug target and effective anti-schistosomals

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Schistosomiasis, a parasitic disease caused by blood flukes of the genus *Schistosoma*, is a global health problem with over 240 million people infected. Treatment relies on just one drug, and new therapies are needed. *Schistosoma mansoni* cathepsin B1 (SmCB1) is a critical protease for digestion of host blood proteins and a validated drug target. Gallinamide A is a natural peptidic metabolite isolated from the extract of marine cyanobacteria. It interacts with cysteine proteases in a covalent irreversible manner as Michael acceptor. Here, we screened a library of more than 20 synthetic analogs of gallinamide A for inhibition of SmCB1 and identified inhibitors with low nanomolar potency. These compounds exhibited a strong suppression effect on live schistosomes in culture. Furthermore, we solved the high resolution crystal structure of SmCB1 in complex with gallinamide A and determined its binding mode. Our study provides a new structural template that can be exploited for the development of novel antischistosomal chemotherapeutics.

P-02.1-009
Schistosoma mansoni cathepsin C: from functional biochemical analysis to antiparasitic inhibitors

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Blood flukes of the genus *Schistosoma* cause schistosomiasis, a neglected parasitic disease that affects over 200 million people. Treatment relies on just one drug, and new therapies are needed. Our work is focused on the cysteine protease cathepsin C from *Schistosoma mansoni* (SmCC), which is involved in digestion of host hemoglobin, the most important source of nutrients. We demonstrated using functional proteomics that SmCC is present in blood-dwelling developmental stages of *S. mansoni* infecting humans (eggs, schistosomula, and adults). Gut association of

SmCC in adult parasites was shown by immunofluorescence microscopy. Further, we investigated regulation of SmCC activity by synthetic inhibitors. A library of peptidomimetics with a reactive tetrafluorophenoxymethyl ketone warhead was tested in a kinetic fluorescence assay against native and recombinant SmCC. The most potent inhibitors of SmCC activity were able to induce deleterious phenotypes in cultured schistosomes. Our results suggest that SmCC is a promising target for the treatment of schistosomiasis and SmCC inhibitors represent potential anti-schistosomal drugs.

P-02.1-010
Understanding iron hijacking by *Staphylococcus aureus*: a structural and mechanistic insight into the interaction of IsdB hemophore with human hemoglobin

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Iron is an essential nutrient for almost all living organisms and exerts central functions in the metabolism thanks to its favorable chemical properties. During infection, *Staphylococcus aureus* relies on host iron reservoir exploiting hemoglobin (Hb) heme as the preferred primary source. Hemophores deputed to heme retrieval and internalization are expressed on bacterial surface and IsdB performs the first step, intercepting free Hb and extracting heme. IsdB is a proven virulence factor highly expressed during staphylococcal infections, raising interest for the potential development of new antimicrobials. We exploited cryo-EM single particle analysis, Small (SAXS) and Wide (WAXS) Angle X-ray Scattering and time-resolved (TR) measurements to gain a deeper insight into IsdB:Hb complex formation and IsdB-mediated heme extraction mechanisms. To this aim, we exploited three Hb ligation states to isolate key steps in heme extraction: carboxyHb and oxygenated Hb, resistant to heme removal, and oxidized Hb, the physiological IsdB substrate. Two structures representing the pre- and after extraction IsdB:Hb complexes were solved at 2.9 Å and 5.8 Å resolution: data indicates a specific initial binding of IsdB to Hb β-subunits, followed by Hb tetramer dimerization and binding of a second IsdB molecule on α-chains (Previously published in De Bei O et al. (2022) PNAS in press). SAXS and WAXS static measurements confirmed the stoichiometry of the complexes, while TR-WAXS allowed the identification of the structural dynamic events occurring upon IsdB:Hb interaction and the definition of a sequential model describing the observed kinetics. Also, TR-spectroscopy revealed that heme extraction starts only upon IsdB binding on both Hb subunits. These results broadly improve our current understanding of IsdB structural and functional dynamics, thus supporting future studies aimed to impair *S. aureus* iron acquisition, a promising target for the development of a new class of antimicrobials.

P-02.1-011
Presence of endobacterial microbiome in clinical isolates of *Aspergillus fumigatus*

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Invasive fungal infections are being increasingly recognized as a major threat to human health, particularly for immunocompromised patients. Recent evidence suggests that endosymbiotic bacteria play an important role not only in fungal functional traits, but also in the enhancement of fungal virulence. Despite this, in a clinical context the effect of bacterial endosymbionts on fungal virulence is not known. The clear emerging clinical problem together with the evidence that the endobacterial microbiome can significantly impact its host, made us question if the virulence of Aspergillus spp., the most common cause of life-threating filamentous fungal infections, can be modulated by presence of intracellular microbiome. To confirm the presence of bacterial endosymbionts in clinical isolates of *Aspergillus fumigatus* we have performed an amplification of full length 16S rRNA gene of DNA extracted from fungal mycelium. The fungal cultured were derived from single asexual spore subjected to temperature and antibiotic pressure – to eliminate ephemeral bacteria associates. Analysis of the full-length 16S rRNA gene of *A. fumigatus* isolates revealed presence of a complex fungal bacteriome. Additionally, data of the fungal bacteriome show that *A. fumigatus* isolates have a 'core' bacteriome but each isolate has specific bacterial partners, in total disclosing a complex diversity of bacteria partners compared to that reported recently in very distinct fungal phyla including *Aspergillus*. Finally, the intracellular localisation of endobacteria in the fungal host will be confirmed by FISH microscopy, the protocol is currently under optimization. The preliminary data support the presence of bacterial endosymbionts in the clinical isolates of Aspergillus fumigatus tested so far. Our aim is to disclose *A. fumigatus* bacteriome in clinical context and, at the end, understand the bacteriome impact in virulence of Aspergilli.

P-02.1-012
Immunodetection of M-CSF in *Galleria mellonella* (Lepidoptera) hemocytes after *Conidiobolus coronatus* (Entomopthorales) infection and application of fungal metabolites

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Innate immune reactions in insects are based on evolutionary-conserved components such as cytokines, which are key factors in the immune response of both mammals and insects. One such type of cytokines, colony-stimulating factors (CSFs), play an essential role in the regulation of immune and inflammatory responses to infections. One such infectious agent is the entomopathogenic cosmopolitan soil fungus *Conidiobolus coronatus*. The aim of study was to determine the presence of a Macrophage Colony-Stimulating Factor (M-CSF)-like molecule in *Galleria mellonella* hemocytes (immunocompetent cells involved in defense responses against pathogens in insects) following infection or exposure to harman and norharman (metabolites of *C.*

coronatus). *G. mellonella* larvae were exposed for 24 h and 48 h to sporulating *C. coronatus* colonies. The harman and norharman were administered in three concentrations, topically and by mixing with food. M-CSF was detected in hemocytes using ELISA, immunocytochemistry and flow cytometry. Infection with *C. coronatus* increased M-CSF in *G. mellonella* hemocytes. Regarding topical application, harman did not increase M-CSF production in hemocytes while norharman demonstrates a stimulating effect only the lowest concentration. Regarding the dietary route, administration increased the production of M-CFS in hemocytes at all concentrations. Interestingly, the flow cytometry results suggest that this cytokine can be produced only by selected subpopulations of hemocytes. Our findings provide an insight into the mechanisms underlying the response of the insect innate immune system to entomopathagens. The high similarity between the innate immunity systems of insects and mammals suggests that insects may replace mammals as model organisms, thus reducing costs and avoiding ethical problems. As such, further studies are needed to better understand the immunological similarities between vertebrates and invertebrates.

P-02.1-013
Design, production and characterization of antiviral proteins targeting SARS-CoV-2

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The virus responsible for the current COVID -19 pandemic is SARS-CoV-2, which has caused >400 million infections and >5 million deaths (as of February 2022). Despite vaccination efforts, there is still an urgent need to develop strategies to control infection and treat patients. One of the proteins bound to the viral membrane is the spike (S) protein, which consists of two subunits: S1, which contains a receptor-binding domain (RBD) responsible for binding to the host cell receptor, and S2, which facilitates membrane fusion between the viral and host cell membranes, previously published in: Jackson CB et al. (2018) Nat Rev Mol Cell Biol 23, 3-20. Thus, this protein is primarily responsible for the ability of the virus to enter host cells, making it one of the most promising therapeutic targets of coronavirus, previously published in: Cao L et al. (2020) Science 6515, 426-431. The aim of this work was to design and produce antiviral proteins that could prevent the interaction between the two proteins and thus block infection by binding to the RBD region and blocking its interaction with the host receptor, angiotensin converting enzyme-2 (ACE2) protein. First, several antiviral proteins were computationally designed using the Rosetta program based on the interactions between ACE2 and the RBD. Next, six molecular dynamics simulations (MD) of 1 μs of three candidates were performed to test their interaction with the RBD. This was followed by experimental validation after expression and purification of the three candidates. The secondary structure and thermostability of these proteins were tested by far-UV circular dichroism spectropolarimetry. Surface plasmon resonance was used to evaluate the affinity of each candidate for RBD. Neutralization assays were performed to investigate the neutralization ability of the proteins. The experimental results show that one of the developed proteins is a promising therapeutic approach that will be further improved in the future.

P-02.1-014
The utility of complete blood count-derived markers for characterising pulmonary tuberculosis severity and prediction of treatment response

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Blood count-derived markers, namely thrombocyte-lymphocyte ratio (TLR), neutrophil-lymphocyte ratio (NLR), monocyte-lymphocyte ratio (MLR), and C-reactive protein (CRP), are of scientific interest since they have exhibited the ability to differentiate site and form of tuberculosis and predict treatment outcome in pulmonary tuberculosis (PTB) patients. However, the utility of these markers in the early identification of patients with severe PTB and risk of delayed treatment response is unclear. This study aimed to explore the association of baseline TLR, NLR, MLR, and CRP with disease severity and treatment response in patients with newly diagnosed PTB. Clinical data were obtained from medical records of otherwise healthy PTB patients (n = 54) admitted to the Riga East University Hospital, Centre of Tuberculosis and Lung Diseases. Chest X-ray and CT findings were used to evaluate disease severity at the time of admission. Biomarker levels were estimated from the complete blood count analysis performed before treatment onset. Time to sputum culture conversion (tSCC) was used to evaluate treatment response. Among patients, all four baseline markers fairly positively correlated with the number of affected lung lobes ($\rho = 0.31–0.40$, $P < 0.05$). A significant increase in NLR and MLR was observed in patients with bilateral lung lesions, but the presence of pulmonary cavitation resulted in the elevation of MLR and CRP. The tSCC was associated with CRP and NLR ($\rho = 0.34$, $\rho = 0.40$, $P < 0.05$) and the values of the markers were significantly higher in the slow responding patients (tSCC>60 days). In conclusion, selected biomarkers may be useful in characterising the extent of PTB, but the CRP and NLR have the potential of predicting treatment response. Further studies are required to specify biomarker cut-off values for different PTB severity grades and the risk of delayed tSCC. Acknowledgements: the study was supported by the Latvian Council of Science, project No. lzp-2020/1-0050.

P-02.1-015
Identification of neutralizing epitopes of the vaccinia virus H3L protein

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Vaccinia virus (VACV) is one of the most promising agents in oncolytic virotherapy. However, even a single application of an oncolytic virus can form antiviral immunity, including virus

neutralizing antibodies. To reduce the immune response without reducing the oncolytic properties of a virus, it is important to investigate the mechanisms of viral neutralizing immunity and determine the epitope composition of virion proteins. VACV H3L protein is a major immunogenic protein in humans. Taking into consideration a 3D structure, we developed a panel of H3L-truncated proteins. The ability of the truncated H3L proteins to be recognized by human antibodies was confirmed by western blot analysis with human serum samples from volunteers vaccinated with VACV. It was shown that human sera recognized four truncated p35 proteins: H3LΔ (1-282 aa), H3LΔ6 (128-282 aa), H3LΔ12 (1-239 aa), and H3LΔFuse (1-34 –(GGGS)3–228-239 aa). These four truncated proteins were used to immunize mice. BALB/c mice were administrated intraperitoneally with 50 μg of each truncated VACV H3L proteins emulsified with Freund's adjuvant three times with 14 days intervals. Two weeks after the third immunization, blood samples were collected from the facial vein. The ability of the obtained mice sera to recognize corresponding truncated H3L protein was examined by western blot analysis and ELISA. Plaque-reduction neutralization tests showed that VACV was neutralized by sera from mice immunized with H3LΔ, H3LΔ12, and H3LΔFuse. The sera of H3LΔ12-immunized mice showed maximal neutralization titer. The obtained results demonstrated that the neutralized H3L epitope is discontinuous and its constituent parts are located in the 1-34 aa and 228-239 aa regions. This study was supported by the Russian Scientific Foundation (Project #20-74-00135).

P-02.1-016
Functional analysis of a tobamovirus-inducible pepper ethylene-responsive factor gene

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Tobamoviruses are economically important pathogens of pepper. To gain a deeper insight into the resistance mechanisms of pepper against tobamoviruses we investigated changes of the transcriptome of pepper leaves after inoculation with two tobamoviruses by Illumina RNA-Seq. *Obuda pepper virus* (ObPV) inoculation resulted in hypersensitive reaction (incompatible interaction), while *Pepper mild mottle virus* (PMMoV) inoculation resulted in systemic spreading of the virus without symptoms (compatible interaction). ObPV inoculation markedly induced a large and diverse group of pepper genes encoding resistance proteins, signaling cascades, transcription factors, pathogenesis-related proteins as well as proteins participating in terpenoid and phenylpropanoid biosynthesis, ethylene metabolism, sulfur metabolism and energy production. In contrast, ObPV suppressed genes related to photosynthesis and carbon fixation. PMMoV inoculation exerted much less effect on the transcriptome of pepper leaves than ObPV[1]. We selected one highly ObPV-inducible pepper ethylene-responsive factor (*ERF*) gene for functional analysis. For transient overexpression of this *ERF* gene in *Nicotiana benthamiana*, it was inserted in a plasmid vector, transformed to *Agrobacterium tumefaciens* and infiltrated into *N. benthamiana* leaves. For control plants, *N. benthamiana* leaves were transformed by *Agrobacterium* containing an empty vector. Two days after the *Agrobacterium* infiltration mRNAs were isolated from *ERF*-overexpressing and control leaves and the transcriptional profiles of these samples were analyzed by Illumina RNA-Seq. Interestingly, the overexpression of *ERF* gene resulted in the up-regulation of numerous genes involved in terpenoid and phenylpropanoid biosynthesis, glycolysis, citrate cycle and protein degradation. These results showed that this *ERF* gene may play an important role in the regulation of pepper resistance mechanisms against tobamoviruses.
[1]Kalapos B et al. (2021) Sci. Rep. 11: 20680

P-02.1-017
Distribution of phytoplasma infection in weeds, insect vectors and tomato plants

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'*Candidatus* Phytoplasma solani' is a pathogen bacterium which infects phloem of many plant species, being endemic in European region. The aim of study was to demonstrate the presence of '*Ca.* P. solani' infection in possible natural reservoirs of perennial plants, in insect vectors and in tomato plants, and ultimately to determine the possible options to control the spread of phytoplasma infection in Moldova. Molecular diagnosis was carried out at 12 weed species, 4 local tomato genotypes and insects from order Hemiptera which can be potential vectors of phytoplasma. Analysis of perennial plant species *Convolvulus arvense*, *Calystegia sepium*, *Daucus carota*, *Chenopodium album*, *Setaria viridis*, *Solanum nigrum*, *Potentilla reptans*, *Artemisia vulgaris*, *Urtica dioica*, *Sonchus oleraceus*, *Polygonum convolvulus*, *Polygonum aviculare* demonstrated the presence of '*Ca.* P. solani' among two species: *Convolvulus arvense* and *Solanum nigrum*. Molecular analysis of potential insect vectors of phytoplasma revealed the presence of infection in more than 20% of analyzed insects. Also, the task was to analyze the spread of phytoplasma infection in tomato varieties of local breeding for two years. The proportion of infected with '*Ca.* P. solani' tomato plants was quite high at the end of both growing seasons. The total percentage of infected plants reached 70%. Thus, our research found that '*Ca.* P. solani' is a real danger to tomato in Moldova, the results of our work are also important for a successful and more targeted fight against phytoplasma infection, knowing intermediate hosts that can be vectors and reservoirs of infection.

P-02.1-018
Ultrastructural analysis of the late stages of SARS-CoV-2 infection in Vero cells

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Since 2019, the world is experiencing a COVID-19 pandemic period caused by the new betacoronavirus SARS-CoV-2. This pathogen

has led more than millions of people to death and many questions about the molecular mechanisms of interactions with the host cell are still unanswered. Viral entry and egress are important steps for the virus cycle. Although SARS-CoV-2 internalization has been largely studied, the egress steps of SARS-CoV-2 are still not fully described. In this study, we address the morphological characteristics of SARS-CoV-2 morphogenesis and egress by transmission and high-resolution scanning microscopy, with the aim of adding more information about the route of nascent virions towards the extracellular medium. Our results reinforce the role of small secretory vesicles as a vehicle to the individual egress, which could be the predominant via to the SARS-CoV-2 egress in Vero cells. *The authors marked with an asterisk equally contributed to the work.

P-02.1-019
New insights into dynamics and conformational changes of the influenza A matrix protein 1 upon oligomerisation

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The matrix protein 1 (M1) is the most abundant and highly conserved protein found in influenza A. This multifunctional protein mediates nuclear export of the viral ribonucleoprotein complex in its soluble monomeric form, while acting as a scaffolding for viral assembly and budding in its membrane-bound homopolymeric form. Mis-timed conversion from the water soluble form of M1 to the membrane-bound polymeric form would be detrimental to the virus and must therefore be tightly regulated. Previous work from our laboratory (Mohd-Kipli F. et al. (2021) J Biol. Chem. 296, 100316) hypothesised that conformational changes in loops at the oligomer interface regulate assembly of M1 into the membrane-bound 'stacked' oligomer. In this study, we confirmed that the M1 N-terminal domain loops exhibit extensive dynamics in solution across a range of timescales by NMR spectroscopy, and investigated the role of loop conformational changes in oligomerisation using a combination of mutagenesis, NMR spectroscopy, and an *in vitro* oligomerisation assay. M1 mutants were designed to perturb loop dynamics by restricting loop conformational changes while maintaining residues required for the interactions that directly stabilise the oligomer subunit interface. Using a light scattering-based *in vitro* assay with lipid-based membrane-mimetics, we show that the mutants significantly perturb oligomerisation of the M1 N-terminal domain. NMR spectroscopy of these mutants indicate large local perturbations in structure and dynamics but an unperturbed global monomeric structure. We propose that these residues form part of a membrane-binding 'sensor' used by M1 to ensure appropriate polymerisation at the membrane surface.

P-02.1-020
Non-RBD binding antibody neutralize SARS-CoV-2

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Since November 2019, the COVID-19 pandemic has been going on around the world, according to the WHO, more 5.5 million people have died. The main strategy for developing therapeutic antibodies is to obtain human viral neutralizing antibodies directed to the receptor-binding domains (RBD) of the SARS-CoV-2 S-protein. However, it is known that the immune response of humans and mice to different antigens is different, therefore, studies of B-cell epitopes of SARS-CoV-2 S-protein with mouse monoclonal antibodies may allow us to find new virus neutralizing epitopes. Eighteen monoclonal antibodies (mAbs) against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were obtained using hybridoma technology from mice immunized with inactivated SARS-CoV-2. ELISA demonstrated that selected 16 mAbs bound recombinant spike (S) protein and 2 mAbs bound recombinant nucleocapsid (N) protein. The equilibrium dissociation constants of the obtained mAbs against S protein ranged from 0.08 to 10 nM. Three mAbs bound recombinant RBD of S protein, the equilibrium dissociation constants of the mAbs against RBD ranged from 0.2 to 3 nM. Anti-RBD mAbs did not neutralize SARS-CoV-2 in the plaque reduction neutralization test. mAbs RS2 demonstrated a dose-dependent inhibition of plaque formation after infection with SARS-CoV-2. The kD and IC50 values for this antibody were 0.2 nM and 400 mcg/ml, respectively. To determine the S protein region responsible for binding to mAb RS2 S1, S2 and RBD subunit of S protein SARS-CoV-2 were expressed in CHO cells. Unfortunately, the localization of the epitope recognized by neutralizing mAb RS2 was not identified using ELISA or western blot analysis. Moreover, mAb RS2 do not recognized full sized recombinant S-protein in western blot analysis. The obtained results demonstrated that the epitope recognized by neutralizing mAb RS2 were discontinuous and have quaternary structure.

P-02.1-021
Crystal structures of TIR-domain proteins from Thoeris bacterial antiviral system

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Thoeris is a bacterial antiviral defense system composed of two genes thsA and thsB [1]. ThsB protein contains Toll/interleukin-1 receptor (TIR) domain which is a canonical component of animal and plant immune systems. Based on domain oraganization of ThsA proteins, Thoeris systems can be subdivided into two types: type I ThsA is composed of a sirtuin-like (SIR2) and STALD domains, type II ThsA is composed of transmembrane and Macro domains. Representative structures of the type I ThsA and ThsB proteins and their functional studies were published recently [2,3]. Type I ThsB protein recognizes phage infection and produces a signaling molecule variant of cyclic ADP-ribose (v-cADPR). v-cADPR is then bound by ThsA STALD domain and activates NAD hydrolysis by SIR2 domain resulting in NAD depletion and the host cell death [3]. In plants, intracellular pathogen sensing by immune receptors also triggers their TIR domains to generate v-cADPR. Type II ThsB presumably produces different unknown signaling molecule which could be recognized by type II ThsA Macro domain. Here we present crystal structures of two ThsB proteins from type II Thoeris systems. *In vivo* data show that functional ThsB is required for anti-phage activity of the Thoeris system. The ThsB structures show similarities with nucleoside ribosyltransferases, eukaryotic TIR

domains and ThsB from the type I Thoeris system. Differently from the most TIR domain proteins, ThsB protein is a monomer, however studies of eukaryotic TIR-domain proteins suggest that oligomerization may be required for ThsB activity. References: 1. Doron S et al. (2018) Science 359, eaar4120. 2. Ka D et al. (2020) Nat Commun 11, 2816. 3 Ofir G et al. (2021) Nature 600, 116–120. *The authors marked with an asterisk equally contributed to the work.

P-02.1-022
Molecular recognition of host-glycans by fimbrial adhesins of *Salmonella* Typhimurium

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Salmonellosis is a common foodborne human health concern. The emergence of antibiotic-resistant strains and the economic burden derived from the disease management call for the development of new therapeutic approaches targeting *Salmonella*. The adhesion of *Salmonella* bacteria to the host cells is crucial to initiate the infection process. This step is assured by the specific interaction between fimbrial adhesins from the pathogen and receptors from the host cells, which can be glycans from glycoproteins. Therefore, aiming for the interruption of these interactions is a potential strategy towards preventing the infection. In this sense, our objective is to study the atomic details of the adhesin/glycan interactions, to gain insights into the rational design of adhesin-blocking glycomimetics. This work focuses on the molecular recognition studies of three adhesins from *S. enterica* Typhimurium whose binding specificities have been slightly described, particularly FimH that recognizes terminal α-mannose residues, Std that binds α1,2-fucosylated glycans and Pef that binds the Lewis X blood group antigen (Rehman T et al. (2019) Microb Pathog 137, 103748). Using nuclear magnetic resonance techniques, the epitope maps of the ligands in presence of the proteins will be discovered, yielding key information to elucidate the molecular recognition events. Acknowledgments: the authors thank FCT-Portugal for the project EXPL/QUI-OUT/0069/2021, UCIBIO projects (UIDP/04378/2020 and UIDB/04378/2020), and Associate Laboratory Institute for Health and Bioeconomy-i4HB project (LA/P/0140/2020). The NMR spectrometers are part of the National NMR Facility supported by FCT-Portugal (ROTEIRO/0031/2013-PINFRA/22161/2016, co-financed by FEDER through COMPETE 2020, POCI and PORL and FCT through PIDDAC). F. M. and H. Co. also thank the CEEC contracts (2020.00233.CEECIND and 2020.03261.CEECIND, respectively).

P-02.1-023
3D structure of the NF-kB-targeting toxin AIP56 and characterization of determinants required for low pH-induced conformational changes, membrane interaction and translocation

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Bacterial AB toxins are major virulence factors that possess a modular structure organized in two distinct components: component A, providing enzymatic activity and targeting a cytosolic factor with crucial function to eukaryotic cells; and component B, which usually contains a receptor-binding (R) domain, conferring toxin specificity, and a translocation (T) domain that assists the translocation of component A to the cytosol. Apoptosis-inducing protein of 56 kDa (AIP56) is a short-trip, single chain, AB toxin secreted by the type II secretion system of *Photobacterium damselae* subsp. *piscicida*, a Gram-negative bacterium that causes a septicaemia in economically important marine fish. AIP56 has zinc-metalloprotease activity towards NF-kB p65, and targets fish phagocytes, mouse and human monocytes, macrophages and dendritic cells, leading to their elimination by apoptosis. In this work, the three-dimensional structure of AIP56 was solved and its domains, as well as the determinants required for low pH-induced conformational changes, membrane interaction and translocation were characterized. AIP56 has a three-domain structure including: (i) a catalytic domain; (ii) a small intermediate domain containing an unusually long linker peptide and (iii) a receptor-binding/pore-forming domain. Contrary to other single-chain toxins, AIP56 translocation is not carried out by an independent T domain but mediated by the middle and B domains. Furthermore, AIP56 has a helical hairpin-like structure at the end of the catalytic domain, which is dispensable for pore formation but contains protonable amino acid residues responsible for controlling the low-pH triggered conformational changes required for translocation. Altogether, these results clarified the structure-function of AIP56, and opened doors to understand the mechanism of action of a growing number of toxins that are homologous to AIP56 and are present in various prokaryotic and eukaryotic organisms.

P-02.1-024
Assessment of HPV16 viral oncogenes' role in genome-wide pattern deposition of MBD2/3 components of NuRD nucleosome remodeling complex

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Human papilloma virus (HPV) is the etiologic agent of cervical cancer and the third most commonly diagnosed type of cancer in women worldwide. The nucleosome remodeling and deacetylation complex (NuRD) is a group of associated proteins with ATP-dependent chromatin remodeling and histone deacetylase activities. MBD2/3 proteins from NuRD complex exhibit methyl-CpG-binding domains, which mediate an interaction with methylated DNA. In this purpose, we developed an experimental model HPV 16 E6/E7 oncogene silencing in CaSki cell culture. We performed ChIP-Seq (Chromatin Immunoprecipitation Sequencing) for MBD2, MBD3 genome wide DNA binding pattern (e.g. promoters, gene control region, transcriptional enhancers, etc.) in untreated and HPV16 E6/E7 shRNAs treated CaSki cell culture and the results were analysed using Base Space Illumina Apps. MBD2/3 proteins were localized at the level of intron, intergenic regions and TSS. After ChIP-Seq peak score analysis, a cut-off of 9 was established and 54 gene loci were identified. The corresponding genes were further analysed in qRT-PCR and their expression was found to be deregulated. When both oncogenes (E6 and E7) were silenced, we noted an enrichment of MBD2/3 proteins at EPCAM, ZFPM2, NRIP1, DCP2, miR-490 gene loci involved in metastasis and cancer progression. Another interesting gene loci involved in mRNA processing (EIF4G3) and ncRNA with unknown function (LINC02036, LINC0122, SEMA5A-AS1 and LINC01718) were identified. Viral oncogenes act synergistically on the gene transcription pattern by interacting with the MBD2/3 proteins of NuRD complex. Epigenetic gene control is a complex phenomenon that is guided by internal, cellular and external factors as well as viral infections. Acknowledgments: TE39/2020 *The authors marked with an asterisk equally contributed to the work.

P-02.1-025
Verification of markers for early stage of tick-borne encephalitis infection *in vitro* and *in vivo*

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Tick-borne encephalitis virus (TBEV) is a tick-transmitted pathogen from the genus Flavivirus which occurs in Europe and Asia. Although TBEV mainly infects small animals, such as rodents, wild or farm animals, humans can be also infected as accidental hosts. After the infection, immune cells were suggested to distribute TBEV via the lymph nodes into the blood, and the replication of TBEV occurs in various organs. The number of tick-borne encephalitis (TBE) cases has increased globally in the last years. Flu-like symptoms appearing after one or two weeks after the infection can be followed by the second phase of illness, namely meningoencephalitis, after one month. Unfortunately, an efficient treatment of TBE does not exist yet, vaccination is the only means of defence against TBE. Since the percentage of vaccinated people is low, it is important to identify specific markers for the early stage of TBE infection for diagnostics in the blood/sera samples a few days after the tick´s bite. In this study, primary immune cells were derived, infected, and RNA and proteins were isolated for transcriptomic and proteomic analyses, respectively. Transcriptomic analyses were performed and the up-/down-regulated genes were determined. Seven most upregulated genes, mostly members of IFIT and OAS families, were selected for further qRT-PCR, western blot, or indirect immunofluorescence verification in vitro. For *in vivo* verification, mice were infected with TBEV (strain Hypr) and bled to death at 0, 1, 2, 3, 4 days post infection. Leukocytes were isolated from blood, RNA and proteins were isolated from them. Finally, the functionality of specific selected markers from *in vivo* RNA/protein samples were performed using qRT-PCR and western blot analysis.

P-02.1-026
The analysis of differential expression and interactions of host and virus derived RNAs in human primary neural cells reveals candidate effectors of TBEV-induced neuropathogenesis

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Arthropod-borne pathogens have been gaining major attention of medical research with spreading distribution of their vectors in response to changing climate. The incidence of tick borne-encephalitis virus (TBEV), often causing severe neurological symptoms in humans, has also been increasing despite the existence of vaccines. Effective treatment and symptom alleviation of patients require a thorough knowledge of TBEV-induced neuropathogenesis, which has not been accomplished to date. Our study aims at a wide-range analysis of transcriptional dynamics and associated cellular processes induced by TBEV in neural cells. Addressing the differences in cellular response between different TBEV strains and cell types allowed us to identify the key factors that are involved in virus infection and the development of neuropathological symptoms. We performed screening and analysis of differential expression of polyA, miRNA/lncRNA and virus-derived small RNA (vd-sRNA) populations of all transcribed RNA molecules in primary human neurons and astrocytes infected by Neuderfl and Hypr strains of TBEV 24- and 72-hours post-infection. All differentially expressed RNA molecule species were submitted to an in silico analysis of interacting RNA networks which identified candidate regulatory miRNA/lncRNAs and vd-sRNAs and genes they potentially target to modulate their expression. The most significantly dysregulated were inflammatory and immune response pathways. Additionally, the regulation of neural development and mitosis were affected in primary neurons infected by a cytopathologically more potent Hypr strain. Our study successfully identified candidate pathways

involved in the development of neuropathogenesis of TBEV on the level of host and virus-derived RNAs. Our data provide the first comprehensive list of TBEV targeted cellular functions. Their further characterisation may contribute to complete the description of the TBEV neuropathogenesis mechanism.

P-02.1-027
The omics hunt for novel molecular markers of resistance to *Phytophthora infestans*

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Wild Solanum accessions are a treasured source of resistance against pathogens, including oomycete *Phytophthora infestans*, causing late blight disease. We analyzed *Solanum pinnatisectum*, *Solanum tuberosum*, and the somatic hybrid between these two lines, representing resistant, susceptible, and moderately resistant genotypes, respectively. Proteome and metabolome analyses showed that the infection had the highest impact on leaves of the resistant plant and indicated, among others, an extensive remodeling of the leaf lipidome. The lipidome profiling confirmed an accumulation of glycerolipids, a depletion in the total pool of glycerophospholipids, and showed considerable differences between the lipidome composition of resistant and susceptible genotypes. The analysis of putative resistance markers pinpointed more than 100 molecules that positively correlated with resistance. Putative resistance protein markers were targeted in an additional 12 genotypes with contrasting resistance to *P. infestans*. At least 27 proteins showed a negative correlation with the susceptibility including HSP70-2, endochitinase B, WPP domain-containing protein, and cyclase 3. In summary, these findings provide insights into molecular mechanisms of resistance against *P. infestans* and present novel targets for selective breeding.

P-02.1-028
Human MBNL1 gene is involved in the life cycle of echoviruses and coxsackie A viruses

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Echoviruses and coxsackieviruses are positive-strand (+)RNA viruses causing various human diseases [1]. Like other Picornaviridae, they use internal ribosome entry sites (IRESs) to direct translation of their mRNAs [2]. However, the molecular mechanisms underlying the synthesis of their proteins are poorly understood. To find human genes involved in the echovirus life cycle, we performed CRISPR/Cas knockout screen for the resistance of cultured HEK293T cells to cytopathic infection caused by Echo6, Echo11, Echo19, and Echo30 viruses. We identified MBNL1 (Muscleblind Like Splicing Regulator 1), a gene encoding an RNA-binding protein involved in alternative mRNA splicing, as necessary for the infection. The MBNL1 protein is a partner of another splicing regulator, PTBP1 [3], which is well known as the IRES trans-acting factor (ITAF) required for the activity of a number of picornavirus IRESs [2]. We hypothesized that MBNL1 is also an ITAF involved in the IRES-mediated translation. A monoclonal MBNL1 knockout (KO) cell line was prepared and infected with various enteroviruses. Echoviruses Echo6, Echo7, Echo14, Echo19, and Echo30, as well as coxsackie A viruses CVA16 and CVA9 showed a 3-4 day-long delay in the induction of MBNL1 KO cell death, as compared to the wild-type HEK293T cells, while CVA9 had no cytopathic effect on the KO cells at all. In contrast, coxsackie B viruses CVB3, CVB4, and CVB5, as well as polioviruses PV1, PV, and PV3 did not require MBNL1. The importance of MBNL1 for the enterovirus IRES-mediated translation was investigated with reporter constructs in mRNA-transfected cells and in a cell-free system. Its role in the virus life cycle will be discussed. The study was supported by the Russian Science Foundation (grant no. 20-14-00178). References [1] Lee et al. (2010) J. Clin. Virol. 49, 175-179. [2] Sorokin et al. (2021) Biochemistry (Moscow) 86, 1060–1094. [3] Gooding et al. (2013) Nucleic Acids Res. 41, 4765–4782. *The authors marked with an asterisk equally contributed to the work.

P-02.1-029
Directly induced neurons – novel neuronal aging model for HSV studies

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Herpes Simplex Virus 1 (HSV-1) is a double-stranded DNA virus, which is associated with a number of clinical diseases including oral and genital lesions, keratitis, and encephalitis. HSV-1 is able to establish lytic and latent infection in non-neuronal cells and neurons, respectively. Periodically, in neurons, the viral genome can reactivate in response to certain stimuli to induce lytic gene expression and produce infectious viruses. Data suggest that up to 95% of people worldwide have HSV-1 and more than 50% of aging population have HSV-1 DNA in their CNS. Despite the increasing knowledge about HSV-1 we know little about how latent infection affects the biology of infected neurons, especially during the cellular aging process. Commonly used mouse and human cell models likely do not recapitulate diseases associated with HSV-1, in adults and aged individuals. This is particularly important to understand given the link between HSV infection and the progression of late onset Alzheimer's disease. The research goal of this studies is to obtain a novel model system to investigate HSV infection using directly induced neurons (iNs). Our cell reprogramming strategy is based on direct conversion of somatic cells, which maintains age-related signatures. In our studies, we use patient-derived fibroblasts of different ages that we induce by transgenic expression of pioneer neuronal transcription factors supported by chemical modulation. So far, we have successfully generated series of induced neuronal human cells evaluated by immunofluorescence assay. Also, we found significantly decreased level of viral infection in young

versus old fibroblast, confirmed by FACS analysis. For this founding we used our novel Stay-put GFP HSV-1 virus model which prevents common cell-to-cell spread infection. Next steps include investigating the differential outcomes of HSV infection in between both the patient-derived fibroblasts and iNs of different ages using single-cell RNA sequencing (Drop-seq).

P-02.1-030
The influence of new nicotine products on periodontal inflammation and glycation end products level in saliva

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New nicotine products, especially electronic cigarettes (e-cigarettes), have become increasingly popular in recent times, but their influence on oral health have not been investigated yet, in spite of the fact that saliva collection is considered to be a non-invasive method to detect inflammatory markers in oral cavity. The aim of this study was to detect and compare correlation between periodontal inflammation index (CPI) and advanced glycation end products (AGEs) level in saliva of teenagers using electronic smoking devices using regression analyzes method. All the patients were divided on two groups: the first group (THG) was presented by patients who used tobacco heating devices for smoking, the second group (VG) was presented by vaping patients. Saliva samples were collected for all patients between 9-10 AM, in a sterile vial of 5 mL with the use of Saliva Collection Aid, by Salimetrics, USA. After collection, samples were frozen and transported to Central Scientific Laboratory of Kharkiv National Medical University. The AGEs level was 105.82 ± 30.17 pg/ml in the THG and 92.53 ± 16.52 pg/ml in the VG, CPI level was equal to 0.84 ± 0.31 and 0.98 ± 0.15 in the THG and VG accordingly. For estimating the relationships between CPI index and level of AGEs in the THG group, regression analysis was done. As R2 is equal to 0.73121, there is rather strong correlation between level of CPI level and AGEs. Based on regression model, we assume that the level of AGEs can be significant biomarker of inflammation in patients using tobacco heat devices; the level of dependence is 73%. The regression analysis in the VG group was also done. As R2 was equal to 0.364175, there is very weak correlation between level of AGEs and gingivitis according to CPI. Conclusions. In the group of patients who used tobacco heating devices there was a strong correlation between periodontal inflammation index CPI and AGEs level which proves damage effect for oral health.

P-02.1-031
Repurposing drugs to fight COVID-19: SARS-CoV-2 envelope targeting by clinically approved porphyrins and analogues

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Despite mitigation measures and vaccination programs, there are still very few medicines to treat COVID-19. Porphyrins and analogues (P&A) usually present broad-spectrum antiviral activity. Some are clinically approved for photodynamic therapy in cancer. Therefore, repurposing clinically approved P&A might be an alternative to treat COVID-19. In this work, we evaluate the ability of the clinically approved temoporfin, verteporfin, talaporfin and redaporfin to inactivate SARS-CoV-2 infectious particles, characterizing their mechanism of action. Loss of infectivity of P&A treated SARS-CoV-2 was assessed by plaque assay. P&A photoactivation successfully inactivated SARS-CoV-2 with very low concentrations and light doses. However, only temoporfin and verteporfin were able to inactivate SARS-CoV-2 in the dark, verteporfin being the most effective. Next, P&A dark antiviral mechanism was characterized starting from P&A interaction with membrane models. P&A partition, membrane-insertion depth, lipid-membrane disruption and changes in membrane ordering were investigated using fluorescent spectroscopy. Among all tested P&A, verteporfin presented the highest partition coefficient, *Kp*. Curiously, temoporfin and redaporfin presented similar *Kp* values, although redaporfin did not present dark antiviral activity. Noteworthy, redaporfin was located closer to the surface of the lipid bilayer and both temoporfin and verteporfin were located closer to the centre. Finally, only temoporfin and verteporfin induced reduction of GP (laurdan-generalized polarization), with transition from an ordered phase to a liquid-crystalline phase. Our results suggest that dark antiviral activity is dependent on P&A interaction with viral envelope. Membrane affinity, penetration, and destabilization are critical for P&A dark antiviral activity. Furthermore, dark anti-SARS-CoV-2 activity opens the possibility for off-label P&A application in the systemic treatment of COVID-19. *The authors marked with an asterisk equally contributed to the work.

P-02.1-032
Targeting brain-resident viruses across the blood–brain barrier *in vitro* using peptide-porphyrin conjugates

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The recent SARS-CoV-2 pandemic brought awareness to the permanent dangers of viral infections and outbreaks. Beyond its inherent infections, several viruses such as Dengue (DENV), Zika (ZIKV), HIV and even SARS-CoV-2 have the potential to infect the brain, causing more aggressive and irreversible injuries. These brain infections are particularly hard to treat not only because the number of efficient antiviral drugs against these viruses is scarce, but also due to the restrictive permeability of the blood–brain barrier (BBB) that hinders brain drug-intake. To overcome these issues, we designed peptide-drug conjugates formed by covalent attachment of a BBB peptide shuttle and a broad-spectrum antiviral porphyrin drug. We synthesized eighteen novel peptide-porphyrins conjugates (PPCs) and tested their activity *in vitro*, both in BBB-translocation and antiviral capacity against DENV, ZIKV, HIV and SARS-CoV-2. Cytotoxicity towards pharmacologic relevant cell lines was also studied. After careful fine-tuning of the on-resin synthetic chemistry, DIC/Oxyma

coupling has emerged as preferred method, bearing a 99% conjugation yield. Ten PPCs inactivate at least two different viruses *in vitro*, a selection criterion for further evaluation, with IC50s ranging between 0.5 to 33 μM. Although all ten PPCs efficiently translocate the cellular BBB model in vitro, a set of seven stand out as the most druggable since they are not cytotoxic towards all cell lines tested. Overall, peptide-porphyrin conjugation shows to be an innovative and promising strategy to treat viral brain infections.

P-02.1-033
Anti-Zika virus and anti-Dengue virus peptide-porphyrin conjugates able to translocate the bloodbrain and bloodplacental barriers – mechanism of action and pharmacokinetics

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Some enveloped viruses such as Zika virus (ZIKV) and Dengue virus (DENV) are able to cross cellular barriers such as the blood–brain barrier (BBB) and the blood–placental barrier (BPB), allowing them to reach the central nervous system (CNS) and cause severe neurological complications on adults and fetuses. To this day, there are no antivirals available against ZIKV and DENV. Also, both barriers have a highly selective and restrictive permeability to the majority of molecules that prevent the uptake of drugs to the CNS. Novel peptide-porphyrin conjugates (PPCs) have shown to act against enveloped viruses on an *in vitro* model of the BBB.[1] In this work, seven PPCs with proved activity against ZIKV and DENV were tested on their ability to cross a human *in vitro* model of the BBB and the BPB, composed by HBEC-5i and JEG-3 cells, respectively. First, their BBB and BPB *in vitro* translocation pharmacokinetics was characterized, and then the translocation mechanisms were studied. Using inhibitors of the main endocytic pathways and trafficking we were able to unveil the preferential mechanism of internalization used by the PPCs in both barriers. Our results show that all PPCs efficiently translocate the *in vitro* BBB model, preserving the integrity of the barrier, while only four maintained the same features for the BPB model. Despite this variability among the different PPCs, a common internalization mechanism dependence on the formation of clathrin pits and Golgi vesicle trafficking was found. The results show that PPCs are valid antiviral candidates against ZIKV and DENV infections on the CNS. Reference [1] Mendonça, D. A., Bakker, M., Cruz-Oliveira, C., Neves, V., Jiménez, M. A., Defaus, S., Cavaco, M., Veiga, A. S., Cadima-Couto, I., Castanho, M., Andreu, D., & Todorovski, T. (2021). Penetrating the Blood-Brain Barrier with New Peptide-Porphyrin Conjugates Having anti-HIV Activity. Bioconjugate chemistry, 32(6), 1067–1077 *The authors marked with an asterisk equally contributed to the work.

Looking for new antibiotics

P-02.2-001
Identification of genes involved in EPS-degrading activity of Erwinia phages E105, TT10-27 and Key

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Fire blight, a plant disease caused by *Erwinia amylovora*, leads to significant economic losses in the cultivation of fruit and ornamental trees. The genus *Erwinia* includes other species that cause fire blight-like symptoms: *E. pyrifoliae*, *E. piriflorinigrans*, *E. uzenensis* and *E. horticola*, the causative agent of beech black bacteriosis in Ukraine. A promising approach for fire blight biocontrol is the use of phages and phage-based products, such as phage exopolysaccharide depolymerases (EPSDs). Expanding halo zones around plaques indicate EPS-degradation activity of lytic *Erwinia*-infecting bacteriophages E105, TT10-27 and Key. The aims of this work were to identify and characterize genes associated with their EPSD activity. Phage genomes were sequenced using the Illumina HiSeq 2500 platform and assembled with DNASTAR's SeqMan NGen12 software. Structural annotation was performed using Glimmer and GeneMark.hmm. Functional annotation was conducted by the BLASTp, ExPASy, CDD and HHpred tools. Gene *47* of phage E105 encodes the 857 aa long EPSD that has a predicted molecular weight of 93.50 kDa. EPSD of phage TT10-27 is encoded by gene *83*. It consists of 881 aa with a predicted molecular weight of 95.5 kDa. The Key EPSD encoded by gene *141* is a protein of 1042 aa residues, corresponding to a molecular mass of 111.5 kDa. Having completely different amino-acid sequences, studied EPSDs share the amino acid sequence homology to EPS-degrading enzymes from other erwinia-phages. It should be noted that AmsF, involved in amylovoran biosynthesis of *E. amylovora*, has homology (26-27% of aa identity) for all studied EPSDs. The E105, TT10-27 and Key EPSDs share a similar structure with a conserved phage_tailspike_middle domain (cd20481) involved in virion binding. HHpred analysis revealed that the EPS-degrading activity of these enzymes originates from the C-terminal domain since it is related to the EPS biosynthesis protein WceF of *Pantoea stewartii*.

P-02.2-002
Abstract withdrawn

P-02.2-003
Aromatic heptaene macrolides as an initial "golden shot" towards systemic mycoses

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Systemic mycoses are fungal diseases attacking the body through an internal organ. They are the cause of increasing morbidity rates, especially among the immunocompromised population (e.g.

with HIV), but also exist as a co-infection in patients with COVID-19. Nowadays, the "life-saving/last chance" drug (and a poison to humans), Amphotericin B (AmB), is the representative of nonaromatic heptaene macrolides. However, previously obtained data indicate significantly higher antifungal activity of the aromatic heptaene subgroup (AHM), which is unfortunately correlated with their high mammalian toxicity. The undertaken studies concern Partricin and Candicidin complexes as the main representatives of AHMs. Three aromatic heptaene macrolides: Partricin A, Partricin B and Candicidin D were isolated from fermentation broths of *S. aureofaciens* and *S. griseus*, respectively, by initial purification, followed by selected chromatographic techniques such as centrifugal partitioning chromatography (CPC) and prep-HPLC. Later on, they were subjected to controlled chromophore geometry change (*cis-trans* to *all-trans*) via photochemical isomerization, using UV light of $\lambda = 365$ nm. The obtained all-trans isomers, regarded as aromatic analogues of AmB, were tested for *in vitro* selective toxicity indexes (STIs) towards *C. albicans* cells versus human erythrocyte model. Due to the diminished hemotoxicity of the *all-trans* isomers – which were maintaining most of their fungicidal power – the obtained STIs were very promising and far better than for AmB (previously published in: J.Górska et al. (2021) Int. J. Mol. Sci. 22). Hence, the *all-trans* AHMs bear a great potential to be weaponized on the antifungal battlefield. Nevertheless, since individual AHMs are commercially unavailable and difficult to obtain due to their complex nature, our studies will focus on facilitation of the isolation process and further improvement of their STIs, mainly through rational, structural modifications.

P-02.2-004
Modeling spatially structured environments with lyotropic liquid crystals for antimicrobial susceptibility testing

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Antimicrobial resistance (AMR) is among the top 10 global public health threats. Antimicrobial overuse in part because of the overload of ICU departments in the context of the COVID-19 pandemic facilitates the phenomenon. Designing novel effective antimicrobial strategies necessitates the development of new methodologies of antimicrobial susceptibility testing (AST) instead of routinely used laboratory approaches that do not consider recent findings concerning the crucial impact of structural complexity inherent for macroorganism tissues, and physical characteristics of the environment on microbial population behavior and biological properties. Liquid crystal (LC) materials combining properties of both the liquid and the solid phase offer tremendous potential for the development of various spatially structured liquid model environments and solid surfaces for studying tripartite system bacterium-antibiotic-bacteriophage. Our pilot study was aimed to examine the behavior of the population of *Proteus vulgaris* — the representative of the third most common etiological factor of nosocomial and catheter-associated urinary tract infections — in different microcosms based on a lyotropic nematic liquid crystal. The study design included examining the bacterial growth, motility, and morphology under the transition of pre-grown population from different isotropic nutrient media to anisotropic microcosms based on LC. Growth kinetics, motility pattern as well as morphotype conversion from swimmers to swarmers changed significantly after the transition of the bacterial population to different microcosms based on LC as compared to those in isotropic conditions. The significance of swarming motility and swarming-specific induction of the virulence factors of *Proteus* for its pathogenicity and AMR has been debated widely yet remains unclear. Our findings indicate the attractiveness of artificial spatially structured microcosms based on LC for the study of these phenomena. *The authors marked with an asterisk equally contributed to the work.

P-02.2-005
Exploring ClpC1 as a drug target against *Mycobacterium tuberculosis*

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ClpC1 has emerged as a highly attractive target for anti-tuberculosis drug development. Indeed, several natural product antibiotics (NPAs) target this system and display activity against non-replicating bacteria opening good perspectives for a faster and more efficient treatment. ClpC1 is a member of the class II AAA+ family of proteins, which contains a N-terminal domain (NTD) and two distinct ATP-binding modules, D1 and D2. While no full-length structure of ClpC1 is currently available, considerable structural work has been performed on the easy-to-handle NTD domain. Curiously, despite representing only a small portion of the full protein, all the NPAs have been shown to bind to the ClpC1-NTD domain and high resolution X-ray structures of the binding sites are available for cyclomarin, ecumicin and rufomycin. While this allows a proper mapping of the NPAs binding pockets, it is still not clear how binding to the NTD can translate into functional impairment of the remaining protein. Using EU instruct infrastructures; we have obtained exciting cryo-EM data that allow us today to have a model of full-length ClpC1. The information obtained improves our understanding of ClpC1 function and the NPAs mechanisms of action and may help in the development of more efficient drugs against tuberculosis.

P-02.2-006
Polyoxotungstates insights into anti-quorum sensing, antibiofilm, and antiviral activities

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Bacterial resistance to antibiotics has led researchers to find compounds with potential antibacterial action and/or with the ability to reverse antibiotic resistance. Polyoxotungstates (POTs) are inorganic based clusters that may fulfill that need [1,2]. Herein, we report the ability of the polyoxotungstates (POTs) with Wells-Dawson P2W18, P2W17, P2W15, and Preyssler P5W30-type structures to disturb microorganisms, either susceptible or resistant to antibiotics. The compound P5W30 showed the highest activity against the majority of the tested bacterial strains. The other tested POTs did not show inhibition zones for the Gram-negative bacteria, *A. baumanii*, *E. coli*, *K. pneumoniae* and *P. aeruginosa*. For P5W30, MIC values < 0.83 mg.mL-1 (100 μM) and 4.96 mg.mL-1 (600 μM), respectively for *S. aureus* and MRSA, were determined. It was verified by NMR spectroscopy, that the most promising POT, P5W30, remains intact under all the experiments conditions. P5W30 also showed a synergistic antibacterial effect in combination with the antibiotic cefoxitin and chloramphenicol against MRSA 16. Furthermore, anti-biofilm activity of P5W30 was more pronounced when used alone in comparison to its combination with the antibiotic cefoxitin. Finally, the antiviral activity of P5W30 using the coliphage Qß shows maximum inactivation at 750 μM (6.23 mg.mL-1). In sum, P5W30 shows anti-quorum sensing and anti-biofilm activities besides being a potent antibacterial agent against *S. aureus* and to exhibit antiviral activities against enteric viruses. [1] Bijelic, A., Aureliano, M. & Rompel, A. (2018) Chem. Commun. (Camb). 54, 1153–1169. [2] Aureliano, M. et al. (2021) Coord. Chem. Rev. 447, 214143 This research was funded by FCT – Foundation for Science and Technology through project UIDB/04326/2020 (M.A.); the Austrian Science Fund (FWF): P33927 (N.G.); P33089 (A.R.); the University of Vienna (N.G. and A.R.).

P-02.2-007
The use of Farnesol to increase the antifungal activity of some antibiotics against *Candida albicans*

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The aim of the work is to prove that Farnesol can increase the antifungal activity of some antimycotics. It is no secret that clinical strains of microorganisms, including pathogenic yeast-like fungi (YLF), are resistant to currently used antifungal agents. In this regard, it is relevant to study the combinations of existing antimicrobial drugs and a medicinal extract of plant origin – Farnesol (Far). In the present experiment we want to see: how much does the resistance profile of non-biofilm microorganisms change? We used 6 clinical isolates of *C. albicans* and one museum strain for the study of the interaction of Far with the most used antimycotics. To determine the sensitivity of YLF to antimycotic drugs nystatin (NS 50 μg), amphotericin-B (AP 10μg), ketoconazole (KT 10μg), clotrimazole (CC 10 μg), voriconazole (VOR 10μg), fluconazole (FU 25μg), miconazole (MIC 10μg), intraconazole (IT 10μg) the classical disco-diffusion method was used. The study showed that the resistance profiles of *Candida* strains are not improved by Far in all strains. If we consider Far in all three concentrations 100-50-25 μl, then in 23 cases out of 56 (7x8) we can definitely talk about a fungi static effect, i.e., an increase in sensitivity to drugs, which is 41% of the total number of experiments. The remaining 54% of cases displayed no changes in the structure of resistance to antifungal drugs or, in rare cases (5%), deterioration of indicators. If we consider the results of only the highest concentration of Far 100 μl, then the sensitivity improvement occurs in 33 out of 56 cases, which is 59 % more than half of the cases. This paper has been supported by the RUDN University Strategic Academic Leadership Program and Research project № 031622-0-000 of the Department of Microbiology and Virology RUDN Medical Institute (Moscow, Russia) under the direction of Podoprigora I.V.; and by the grant of the President of the Russian Federation for young scientists-doctors of sciences MD-1730.2021.3.

P-02.2-008
Synthetic flavonoid BrCl – a potential solution against fluconazole resistant *Candida* strains

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Candida pathogenic species represent a serious threat to public health worldwide. Many species such as *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. krusei* are responsible for 70% of the systemic fungal infections, causing death especially for immunocompromised patients. Anti-*Candida* chemotherapy is nowadays challenged by multidrug resistance phenomenon, especially in relation to fluconazole, making the treatment of *Candida* infections very difficult. Therefore, the design of new antifungal molecules is fundamental to face the alarming increase in the rate of *Candida* infections. In this context, we investigated the antifungal potential of a synthetic flavonoid with bromide as halogen

substituent at the benzopyran core (BrCl) by assessing the minimum inhibitory and fungicidal concentrations (MIC, MFC). The potential effect of flavonoid on biofilm formation and cell viability was also tested. The mechanism of action was assessed using fluorescence and scanning electron microscopy (SEM). Our flavonoid showed a very good anti-*Candida* activity with a MIC of 15.62 μg/mL and MFC of 31.25 μg/mL. In the presence of the tested flavonoid, *Candida albicans* yeast to hyphal transition was prevented and biofilm formation was inhibited. After only 6 hours of incubation, the flavonoid BrCl caused significant and irreversible damage to the cell membrane. Also, a synergistic effect with fluconazole against a *C. albicans* resistant clinical isolate was evidenced. The result suggest that BrCl flavonoid has a high potential to be used as an efficient antifungal agent. *The authors marked with an asterisk equally contributed to the work.

P-02.2-009
Antimicrobial and antimycotic activity of *Tripleurospermum perforatum* (Merat.) M. Lainz

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Currently, antibiotics are used to treat bacterial infections, the use of which is associated with a wide range of side effects. Herbal remedies containing a complex of biologically active compounds do not allow bacteria to acquire resistance, since not one compound has pharmaceutical activity, but their complex, which potentiates the action of the main components. The objects of the study were an aqueous extract of the herb *Tripleurospermum perforatum* (Merat.) M. Lainz, collected at the beginning of budding. To study the antimicrobial and antimycotic activity, strains of microorganisms *Staphylococcus aureus*, *Escherichia coli* and yeast fungi *Candida albicans* were used. The antimicrobial and antimycotic activity of herbal preparations was determined by the Kirby-Bauer method, the diffusion of filter paper discs into solid nutrient media "Endo", meat-peptone agar, "Saburo" for strains of *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, respectively. It was found that the zones of growth inhibition of *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* were 22, 10 and 18 mm, respectively, which indicates a pronounced activity of the herb *Tripleurospermum perforatum* in relation to the studied microorganisms. The study was supported by a grant from the President of the Russian Federation for young scientists - doctors of science MD-1730.2021.3 The publication was made with the support of the RUDN University Strategic Academic Leadership Program.

P-02.2-010
Antibacterial remedy «Art-dentale» for the treatment of inflammatory oral diseases cavity

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The prevalence of periodontal disease reaches up to 98% in the adult population. In this regard, the prevention and treatment of periodontal diseases are actual. Therefore, we have developed an herbal remedy consisting of extracts of folia *Eucalyptus vulgaris*, herba Serpylli, and pericárpium *Punica granatum*. The extracts were obtained by electropulse extraction using water-propylene mixtures as an extractant for each type of plant material: for folia *Eucalyptus vulgaris*, herba Serpylli, and pericárpium *Punica granatum*, the propylene glycol concentration was 50, 95, and 70%, respectively. Then the extracts were mixed in a ratio of 1:1:1. *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* were used to study the antimicrobial and antimycotic activity. The antimicrobial and antimycotic activity of herbal preparations was determined by the Kirby-Bauer method, the diffusion of filter paper discs into solid nutrient media "Endo", meat-peptone agar, "Saburo" for strains of *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, respectively. It was found that the zones of growth inhibition of *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* were 29, 18, and 21 mm, respectively, which indicates a pronounced activity of the remedy «Art-dentale» concerning the studied microorganisms. With a 1:1 dilution of the treatment «Art-dentale», the growth zones were 23, 15, and 19 mm, respectively. Thus, it was shown that the remedy «Art-dentale» exhibits a pronounced antibacterial and antimycotic activity both in its original form and when diluted with water in a ratio of 1:1. The study was supported by a grant from the President of the Russian Federation for young scientists - doctors of science MD-1730.2021.3 The publication was made with the support of the RUDN University Strategic Academic Leadership Program.

P-02.2-011
Antimicrobial peptidomimetics: antimicrobial activity, pro-proliferating properties, QSAR and ADME

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Uncontrolled use of antibiotics for both medical and non-medical purposes has led to emergence of multi-resistant bacteria. Its increasing resistance to conventional antibiotics has created a need for a new class of chemotherapeutics, preferably with different mechanism of action to known antibiotics. We have developed novel peptidyl derivatives based upon the structure of the N-terminal fragment (R8L9V10G11) of the human cystatin C protein. A derivative called Cystapep 1 has a strong activity

against Gram-positive bacteria [1,2], including MSSA and MRSA strains, at the same time exerting pro-proliferative effect on skin cells [2]. The investigation explores the *in vitro* activity of Cystapep 1 analogues against Gram-positive staphylococci as well as Gram-negative bacteria. Cystapep 1 analogues display strong inhibitory effect on *S. aureus*, *S. cohnii* and *S. schleiferi* and at the same time show poor influence on *P. vulgaris*, *P. aeuroginosa* and *K. pneumoniae*. It has a slight impact on *E. coli* growth. Since it is active against bacteria which are responsible for skin-related infections, its cytotoxicity on keratinocytes and fibroblasts has been evaluated. Its pro-proliferating potential has been investigated by LDH assay. The QSAR allowed to find structures crucial for the activity of the analogues, for which *in silico* ADMET profile has been assessed. References: [1] Jasir A. et.al., (2003); APMIS, 111, 1004-1010 [2] Dzierżyńska M. et.al., (2019); Prot Pep Letters, 26, 1-12 Acknowledgement: This work was supported by the grant no. UMO-2020/39/O/NZ7/00430

P-02.2-012
The relationship between the enhancement of the bacteriolytic activity of lysozyme and the productive sorption of the enzyme on living bacterial cells in the presence of glycine and charged amino acid

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Lysozyme is increasingly being considered as an alternative to antibiotics in the fight against resistant bacteria. New information has also emerged about the non-enzymatic role of lysozyme in regulating the functioning of the immune system [1]. In addition, it has recently been found that the antibacterial activity of lysozyme against Gram-negative bacteria is significantly enhanced in the presence of glycine and charged amino acids [2], but the molecular reason for this effect remains unclear. In this study, we investigated the physicochemical parameters of sorption of lysozyme on living cells in the presence of glycine and charged amino acids. The relationship between the increase in productive sorption and the increase in the bacteriolytic activity of the enzyme was revealed. All experiments were carried out at pH 8.5 and a temperature of 37°C. The substrate was a suspension of *E. coli* cells. The additives were Gly, Glu, Asp, Lys, His, and Arg. The increase in the rate of bacterial lysis in the presence of additives ranged from 1.4 to 1.9 times. The desorption constant of lysozyme on bacterial cells in the presence of additives decreased by 1.4–1.7 times, which indicates a stronger binding. The greatest effects were observed for Gly and Arg. Understanding the molecular nature of the enhancement of the antibacterial action of lysozyme in the presence of additives may help in the development of new highly-effective antibacterial drugs. This work was carried out with the financial support of Lomonosov Moscow State University: AAAA-A21-121011290089-4. 1. Ragland SA et al.(2017) PLoS Pathog, 13, e1006512 2. Levashov PA et al.(2019) FEBS Open Bio, 9, 510-518

P-02.2-013
In silico identification and exploration of a new non-toxic antimicrobial peptide of medicinal leech microbiome

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Antimicrobial agents are demanded to treat infectious diseases, one of the leading causes of death worldwide. Longstanding thoughtless use of antibiotics has led to the development and spread of drug-resistant microorganisms. This problem has become especially acute within the Covid-19 pandemic. Antimicrobial peptides (AMPs) are considered as effective therapeutic agents that are less prone to the development of bacterial resistance than antibiotics. In the present study, we identified antimicrobial non-toxic peptides from the leech metagenome protein database using a gradient boosting approach (CatBoost). The antimicrobial activity of the peptides was tested against Gram-positive and Gram-negative bacteria (*Bacillus subtilis, Staphylococcus aureus, Staphylococcus haemolyticus, Escherichia coli, Mycobacterium smegmatis*). One of the analyzed peptides, peptide HMAMP-2, showed the best antimicrobial activity against the tested bacteria at low concentrations. Viability and hemolysis evaluation of cells incubated with peptides demonstrated peptide HMAMP-2 did not affect cell viability at high concentrations. It was experimentally shown that the peptide killed pathogens due to the membranolytic mechanism of action. According to nuclear magnetic resonance analysis, the peptide adopted an α-helical structure in a membrane environment. In addition, we demonstrated that peptide Hm-AMP2 binds to LPS, which supported the immunomodulatory properties of the peptide. Thus, peptide Hm-AMP2 is characterized as a promising non-hemolytic and non-toxic antimicrobial compound. Peptide Hm-AMP2 is likely involved in the regulation of the inflammatory response of the innate immune system, which should be studied further. The developed algorithm for the identification of antimicrobial peptides with a reduced toxic effect can be used for the rational design of effective non-toxic antibacterial agents. The research was supported by the Russian Science Foundation (project № 20–15–00270).

P-02.2-014
Antimicrobial activities of phytochemicals from aromatic plants of Cyprus

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The usefulness of plants and their products for medicinal purposes has been known since ancient times. With the increase of antibiotic resistance, particularly in healthcare facilities, becoming more prevalent, there has been a renewal of interest in the use of these plants. In this work, we analysed the antimicrobial properties of extracts from aromatic plants of Cyprus. Specifically, we aim to investigate whether the different phytochemicals have the ability to interfere with the quorum sensing communication between the bacteria. Ten different species of aromatic

plants were selected, and their phytochemicals were extracted by ethanolic sonication. They were subsequently tested for antimicrobial activity by standard microbiology techniques against Gram-positive (*Staphylococcus epidermis*) and Gram-negative (*Escherichia coli*) bacteria. Those that did show any kind of activity were screened further to determine their Minimum Inhibitory Concentration (MIC) in both solid and liquid media. The results obtained indicated that among the plants screened, *Rosmarinus officinalis* L. (Rosemary) and particularly *Origanum vulgare* Oregano) have significant antimicrobial activity both in liquid and solid media at a concentration as low as 1mg/mL. They also showed the ability to prevent the formation of *E. coli* biofilms at a concentration of 1mg/ml as indicated by crystal violet staining. The results of this work indicate that these extracts could be used as alternatives to antibiotics to prevent and/or treat bacterial infections in both animals and humans. Keywords: quorum sensing, phytochemicals, natural products *The authors marked with an asterisk equally contributed to the work.

P-02.2-015
Wild animals provide pathogen control via potent probiotic *Bacillus pumilus* strains

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Global use of antimicrobials provokes intensive antimicrobial resistance selection. Hence, there is an urgent need to discover new antimicrobials. The production of antibiotics is an important mechanism maintaining biodiversity in natural microbial communities. The symbiotic microflora of wild animals living in aseptic conditions is naturally enriched in strains successfully competing with invasive opportunistic pathogens. Therefore, we consider wild animals as potent source of antibiotic-producing microorganisms. An ultrahigh-throughput microfluidic platform was implemented for the isolation of *S. aureus* antagonists from various animal species resulting in repeated isolation of different *Bacillus pumilus* strains. The isolated strains had a similar set of biosynthetic gene clusters (BGCs) encoding antibiotic amicoumacin (Ami), siderophore bacillibactin, and putative analogs of antimicrobials including bacilysin, class IId cyclical bacteriocin, surfactin, and desferrioxamine. Ami was discovered as a main antibiotic component of *B. pumilus* cultures, while the overall inhibitory potential was provided by the complex action of the discovered secondary metabolites. Genome mining revealed numerous Ami clusters with similar architectures in *Bacillus* and *Paenibacillus* species. These clusters represent three families, characteristic of the *B. pumilus*, *B. subtilis*, and *Paenibacillus* species. Genomic analysis of BGCs and metabolomics expand our understanding of probiotic strains, which is essential for metabolic reprogramming and development of designer probiotics for pathogen control. This work was supported by a grant from the Ministry of Science and Higher Education of the Russian Federation No. 075-15-2021-1049. Previously published in: Baranova MN et al. (2022) International Journal of Molecular Sciences 23, 1168 *The authors marked with an asterisk equally contributed to the work.

P-02.2-016
Engineering methylotrophic yeasts *P. pastoris* for heterologous AMP production

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The spread of multidrug-resistant strains of bacteria is an urgent threat to global health. In this regard, the need for new antimicrobials is of high demand. Antimicrobial peptides (AMP) are promising candidates for this role, since the mechanism of their action frequently involves bacterial membrane disruption. The direct killing mechanism results in an extremely low level of resistance. Chemical synthesis does not provide the universal option for AMP production. Moreover, it does not enable implementing ultrahigh-throughput technologies for new AMP discovery. Methylotrophic yeast *Pichia pastoris* is a convenient platform for the biotechnological production of AMPs, since it has a number of advantages, such as rapid growth, relatively low production costs, high yields of recombinant proteins and a variety of post-translational modifications. The most commonly used promoter for the heterologous expression of recombinant proteins in *P. pastoris* is the methanol-induced Alcohol oxidase I (AOX) promoter. Despite the advantages of high yields and improved production of toxic proteins, AOX promoter is not convenient for massive screening of antimicrobial activity. In this work, we implemented the Glyceraldehyde-3-phosphate dehydrogenase (GAP) promoter to generate yeast strains constitutively producing AMPs. The engineered yeast efficiently inhibited growth of the reporter *Escherichia coli* cells forming clear growth inhibition zones. The implementation of this approach will reinforce the biotechnological development of AMPs, their high-scale production and engineering by high-throughput screening techniques. This work is supported by Grant 21-14-00357 from the Russian Science Foundation.

P-02.2-017
Amicoumacin analogs for specific SARS-CoV 2 targeting

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The pandemics of SARS-CoV 2 dramatically influenced the field of virology challenging for new antiviral drugs with alternative modes of actions. Translation machinery represents an attractive target for new antivirals. In this study, the antibiotic amicoumacin (Ami) was used for the development of a prodrug against SARS-CoV 2. Naturally, Ami is produced by probiotic *Bacillus pumilus* strains mediating their antimicrobial activity. Ami is a particularly potent translational inhibitor both in pro- and eukaryotes. We hypothesize that delivery of inactivated Ami prodrug to infected cells will result in the release of the active molecule by cleaving the precursor by the Mpro protease resulting in the inhibition of translation. However, Ami is rapidly hydrolyzed into inactive products under physiological conditions.

A panel of Ami derivatives was synthesized to obtain stable Ami analogs. A panel of Ami analogs demonstrated increased stability in aqueous solutions while retaining antibiotic activity. The introduction of substituted amides and hydrazides increased the stability of the Ami molecule in aqueous solution, while the reasonable antibiotic activity was retained. Ami analogs provide a promising tool for translation machinery targeting and drug development. The work was supported by a grant from the Ministry of Science and Higher Education of the Russian Federation No. 075-15-2021-1049. *The authors marked with an asterisk equally contributed to the work.

P-02.2-018
Ionic liquid induction of novel natural products in *Neurospora crassa*

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Infections with no reliable medical therapy are emerging and are expressly concerning due to increasing numbers of drug-resistant pathogens. In 2019, the World Health Organization declared antimicrobial resistance as one of the top 10 global public health threats. Emergence of drug resistant pathogens impacts not only human and animal health but also agriculture. Thus, there is an urgent need for novel antimicrobials as therapeutics or as lead compounds for drug development. In this context, microorganisms constitute the most diverse and resourceful source for natural products. Fungi have the capacity to produce a large number of bioactive compounds; however, the biosynthetic gene clusters are often silent under normal cultivation conditions. Herein, we demonstrate that supplementation of the growth medium with sub-lethal concentrations of a biodegradable ionic liquid (IL) activates secondary metabolite production in the filamentous fungus *Neurospora crassa*. The antibacterial activity of exhausted media extracts was evaluated; IL-subjected extracts showed nearly 5- to 10-fold higher bactericidal efficacy compared to control (without IL). Analysis of the chemical landscape of the produced metabolites was done through untargeted mass spectrometry data analysis using the online platform Global Natural Product Social Molecular Networking (GNPS). A plethora of metabolites was putatively identified covering diverse compounds such as peptides (linear and cyclic), steroids, peptidomimetics (depsipeptides) and glycosides. Subsequent amino acid composition analyses highlighted the presence of rare non-proteinogenic amino acids. This feature has been reported in peptide-based metabolites displaying interesting bioactivity *e.g.* antiparasitic. Our results provide novel insights into the metabolomics of a fungus known to be a low metabolite producer and may shed light on new strategies for drug development exploring the physiological role of such metabolites.

P-02.2-019
Abstract moved to Short Talk SHT-02.2-5

P-02.2-020
Bioactive peptides "hidden" in toxins from the venomous fish *Thalassophryne nattereri,* an *in silico* driven study

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The therapeutic success of venom-derived molecules, such as bioactive peptides (BAPs), is linked to their increased specificity, stability and assessment of pharmacokinetics properties. The BAPs, such cell penetrating peptides (CPPs) and antimicrobial peptides (AMPs), share several physicochemical characteristics and have been investigated as potential alternatives to drug-delivery systems and antibiotic-based therapies, respectively. In the current study, CPPs and AMPs were predicted from the toxin natterins of the fish *Thalassophryne nattereri*, using *in silico* analyses. Using AMPA and CellPPD web servers, twelve AMPs and nine CPPs sequences were generated. To analyze physicochemical properties, all peptides were submitted to the ProtParam and PepCalc tools. The probability of membrane binding potential and cellular location of each peptide were estimated using the Boman index by APD3, and TMHMM web servers. All CPPs and two AMPs analyzed showed high membrane binding potential. Among all peptides analyzed, twenty had intra-membrane cellular localization. Peptides were examined for the immunogenicity, toxicity, allergenicity, and other ADMET profiles using different web servers. Only two AMPs were classified as toxic, with low potential for hemolysis. Finally, the secondary helix structure and the helical wheel projections were predicted by the PEP-FOLD3 and Heliquest web servers, respectively. Based on the parameters analyzed, two AMPs and two CPPs were selected and will be further tested experimentally *in vitro* to validate the virtual screening. Computer-aided design for predicting activity of peptides not only saves time and money, but also facilitates the designing of improved therapeutic peptides. Overall, this study confirms that toxins form a natural biotechnological platform still underexplored in drug discovery and the presence of CPP/AMP sequences among toxin families opens new roads in toxin biochemical research. *The authors marked with an asterisk equally contributed to the work.

P-02.2-021
Antimicrobial effects of molybdenum-based cluster compounds

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The leading topic of the current applied microbiology is a search for new ways to fight antibiotic-resistant bacteria. Moreover, bacterial cells naturally occur in the form of biofilm having protective abilities and enhancing their resistance to antimicrobial agents. The aim of this project was testing of the phototoxic effects of molybdenum-based cluster compounds on bacteria. The cluster complexes were synthesised by copper-free click chemistry between octahedral molybdenum cluster core

($Na_2[Mo_6I_8(N_3)_6]$) and 6 functionalized ligands. The mechanism of action was based on photodynamic therapy since the clusters possess photosensitizing properties under 400-450 nm light irradiation, resulting in reactive oxygen species formation, and causing oxidative stress to bacterial cells. Clusters were tested on several bacterial species including Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) and two Gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*). Phototoxic effects and dark toxicity of selected compounds were tested on planktonic bacterial cells as well as on biofilms. Methods used for assessment of toxic effects were mainly cultivation and quantification via The Misra and Miles Method or a confocal fluorescent microscopy. Selected clusters have shown potential phototoxic effects. Decimal differences in the numbers of colony forming units of bacteria were observed in comparison to control groups. In addition, these results were observed at concentration as low as 6 μM, which was observed as non-toxic on mammal tissue cells. However, more dramatic phototoxicity was shown on Gram-positive strains. In conclusion, these results are promising for further applications of Mo clusters acting as active surfaces resistant to bacterial biofilm development, which could be used in hospital and industrial settings. This work was supported by grant no. 21-16084J given by the Czech Science Foundation.

P-02.2-022
Identification of dual-targeted *Mycobacterium tuberculosis* aminoacyl-tRNA synthetase inhibitors using a machine learning approach

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According to WHO Global tuberculosis report in 2021, tuberculosis is the second deadliest infectious killer after COVID-19. The main problem of tuberculosis treatment is multidrug resistance of *Mycobacterium tuberculosis* to all current antibiotics. As a strategy to overcome resistance, we used a multitarget drug discovery approach since the probability of mutations simultaneously in several enzymes is negligible. Nowadays, aminoacyl-tRNA synthetases are considered as promising molecular targets for antibiotic drug design. The aim of our study was to develop dual-targeted *M. tuberculosis* leucyl-tRNA synthetase (LeuRS) and methionyl-tRNA synthetase (MetRS) inhibitors with a machine learning approach. The neural networks models were built using module nnet from R 3.6.1. ROC score for LeuRS models was in the range from 0.91 to 0.95, Matthews correlation coefficient (MCC) was in the range from 0.6 to 0.87. ROC score for MetRS models was in the range from 0.91 to 0.97, MCC was in the range from 0.75 to 0.83. The obtained ANN models were used for virtual screening of compound collection containing about 250,000 ligands. Among 200 tested compounds which were selected by a machine learning approach we have found 24 MetRS inhibitors, 10 LeuRS inhibitors and 7 dual-targeted inhibitors decreasing the activity of enzymes by more than 50% at the concentration of 100 μM in aminoacylation assay. Among 7 dual-targeted inhibitors three compounds belong to the derivatives of 2-(Quinolin-2-ylsulfanyl)-acetamide. We have investigated 10 derivatives of this chemical class in order to identify more potent dual-targeted inhibitors. It was found that the most active compound 2-[2-(6-Chloro-4-methyl-quinolin-2-ylsulfanyl)-acetylamino]-5-phenyl-thiophene-3-carboxylic acid amide inhibits M. tuberculosis MetRS and LeuRS with IC50 values of 33 μM and 23.9 μM, respectively. Therefore, 2-(Quinolin-2-ylsulfanyl)-acetamide scaffold can be used for further optimization and biological research.

P-02.2-023
Characterization of models of young and mature bacterial biofilms to study the activity of antimicrobial peptides

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The treatment of bacterial infections is facing unprecedented challenges due to the increasing prevalence of antibiotic-resistant bacteria and self-encasing of bacteria in biofilms. Bacterial biofilms represent a major healthcare problem since they are involved in the majority of microbial infections that occur in humans and are very difficult to treat. Because biofilm-associated bacteria exhibit reduced susceptibility to conventional antibiotics, there is an urgent need to find compounds that are active against bacterial biofilms. As an alternative to currently used antibiotics, there is an increasing interest in antimicrobial peptides (AMPs) due to their potential to kill bacteria regardless of their metabolic status. Nonetheless, the pharmacological development of anti-biofilm AMPs is hindered by lack of basic knowledge of the fundamental properties of biofilms. In this work, a strain of *Staphylococcus aureus*, which is one of the most clinically relevant pathogens commonly associated with biofilm infections, was used to develop biofilm models. First, the biofilm biomass at different stages of development was quantified by using a crystal violet binding assay. The results obtained allowed to distinguish 1-day and 7-days biofilms as young and mature biofilms models, respectively. The metabolic activity of 1-day and 7-days biofilm-associated cells was evaluated using a resazurin reduction fluorimetric assay, while the cell density was determined using a colony count assay. For mature biofilms, the metabolic activity was slowed by 2.4-fold, while the number of cells was reduced by 5-fold when compared to young biofilms. In the second phase of the work, we aim to investigate the activity and mechanism of action of a designed cyclic analogue of gomesin, [G1K,K8R] cGm, a potent antimicrobial peptide that has proven to be effective against Gram-positive and -negative bacteria, both in the planktonic and biofilm forms.

Molecular evolution

P-02.3-001
Ancestral NAT/NCS2 transporter is robust to reconstruction uncertainty

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Ancestral protein reconstruction (APR)—phylogenetic inference of ancient protein sequences, followed by gene synthesis, expression, and experimental characterization—has become a widely used strategy for studying the etiological relation of protein sequence, structure and function. We used APR to analyze the evolution of substrate specificities in NAT/NCS2 (Nucleobase-Ascorbate Transporter/ Nucleobase-Cation Symporter-2) family (Previously published in: Tatsaki E et al. (2021) J Mol Biol 433, 167329). We performed phylogenetic analysis of bacterial NAT/NCS2 homologs and reconstructed AncXanQ, the putative common ancestor of a monophyletic group represented by the xanthine-specific XanQ of *E. coli*. AncXanQ was analyzed functionally and found to be a broader-specificity permease which transports both xanthine and guanine and recognizes a range of analogs. To understand the difference between AncXanQ and XanQ, we subjected both homologs to rationally designed mutagenesis. Our results show that five residues outside the binding site are linked with the difference in specificity. Ancestral reconstruction, however, is a probabilistic method and involves uncertainties in the amino acid identities. AncXanQ was reconstructed with high-confidence. The mean posterior probabilities for most residues were >90%. Only 5 of 463 sites had a statistically plausible alternative reconstruction, all of them located outside the active site. To test whether these sequence ambiguities affect our results or not, we studied the five variants using site-directed mutagenesis. Our findings show that all engineered variants behave very similarly to AncXanQ. These results give us confidence in the validity of our approach. The project is co-financed by Greece and the European Union - European Regional Development Fund (ERDF) under the Operational Program "Competitiveness Entrepreneurship Innovation" (EPAnEK), NSRF 2014-2020 (MIS 5047236).

P-02.3-002
Whole genome sequencing provided additional insights into recent cases of acquired *M. tuberculosis* fluoroquinolone resistance

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Drug-resistant tuberculosis (TB) is a public health threat worldwide. Fluoroquinolones (FQ) are essential components of TB treatment regimens, and relatively common *M. tuberculosis* (Mtb) resistance to these agents is a significant concern. The aim of this study was to investigate 3 cases of possible Mtb FQ resistance development in TB patients. Whole genome sequencing (WGS) approach along with phenotypic drug susceptibility testing was used. Two Mtb isolates (initial FQ-susceptible and subsequent FQ-resistant) from each patient were analyzed. Two patients (A and B) experienced TB recurrence case, and the obtained Mtb isolates represented both disease episodes. Patient A had a history of noncompliance with therapy and withdrawal, while patient B experienced a long therapy duration of the first TB episode (24 months). In a third case (patient C), second Mtb isolate was acquired when patient resumed therapy after arbitrary discontinuation (time frame 6 months). WGS of Mtb DNA samples was conducted using IonTorrent technologies. SpoTyping (v2.1) software was used for spoligotyping. The results revealed that all isolates from patients B and C belonged to SIT1 spoligotype, while SIT42 pattern was identified for both Mtb samples of the patient A. Further bioinformatic analysis revealed a distance of only 1 (B and C) and 2 SNPs (A) between isolate pairs, minimizing plausibility of reinfection event for recurrent TB patients. In all cases, one acquired SNP was detected in *gyrA* gene: Gly88Cys mutation was found in SIT42 Mtb sample, while Asp94Gly mutation was detected in both SIT1 isolates. TBProfiler tool confirmed these SNPs as FQ-resistance-associated. To sum up, this study demonstrated the acquisition of genetic mutations in Mtb belonging to two different spoligotypes, and additional research is needed to decipher the reason of selective FQ-resistance development under the clinical circumstances of resistance. This study was supported by ERDF grant No. 1.1.1.1/20/A/046.

P-02.3-003
Polyketide synthases from non-bioluminescent fungi in hispidin biosynthesis

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Polyketide synthases are widely applied in biotechnology for effective biosynthesis of different bioactive and medical polyketides. One of them is hispidin, which is known to have antioxidant, anti-inflammatory and other potential therapeutic characteristics. Hispidin is also the metabolic precursor of the fungal luciferin and thus the key metabolite of the bioluminescence pathway in higher fungi. The biosynthesis of hispidin is catalyzed by hispidin synthases that are present only in glowing fungi (Kotlobay et al. 2018) and differ from other type I polyketide synthases both structurally and functionally lacking two out of seven catalytic domains. Noteworthy, the evolutionary events that have led to the emergence of hispidin synthases and bioluminescence in higher fungi in general still remain unexplored. In this work, we aim to study the evolution of hispidin synthases from ancestral proteins and to apply this knowledge for engineering polyketide synthases from non-bioluminescent fungi to produce hispidin. We analyzed the structure of polyketide synthases from three non-glowing fungi species, engineered their truncated versions and developed an assay to assess their ability to synthesize hispidin. Unexpectedly, two studied polyketide synthases carrying all seven domains were able to produce hispidin. Moreover, we showed that the loss of two C-terminal domains in these enzymes did not affect their ability to produce hispidin, while, surprisingly, in the case of the remaining polyketide synthase we observed the bioluminescence decrease after treatment with caffeic acid. The research was funded by RFBR and Moscow city Government according to the project № 21-34-70034.

P-02.3-004
Unveiling the evolution of ovothiol biosynthesis: focus on Cnidaria

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Ovothiols are histidine derived thiols originally found in marine invertebrates, protists and bacteria which are receiving increasing interest due to their peculiar redox properties, ecological role and therapeutic potential. The enzymes involved in ovothiols biosynthesis, the 5-histidylcysteine sulfoxide synthase OvoA and the pyridoxal phosphate-dependent lyase OvoB, have been recently characterized. Although this biosynthetic pathway is conserved in metazoa, ovoA gene loss events occured in ecdisozoa and in vertebrata, and two cases of ovoA acquisition through horizontal gene transfer (HGT) were discovered in bdelloidea rotifera and in hydrozoa (previously published in Gerdol M. et al., (2019) Proc. R. Soc. B, 286:20191812). All these events contributed to the complex evolutionary history of the biosynthesis of these molecules, whose diversification, distribution and biological roles remain still largely unknown. This study aims to get insight into the phylogenesis and spreading of this metabolic pathway within the metazoa lineage by focusing on the Cnidaria phylum. Thanks to the increasing availability of public genomic and trascriptomic data, we identified cnidarian species endowed with this secondary biosynthetic pathway. We reported another case of HGT in the hydrozoan *Clytia hemisphaerica*, which displayed a single polypeptide chain containing all the functional domains of the two enzymes responsible for ovothiols production. Through comparative trascriptomic analyses, we showed different ovoA expression levels during embryo and larval development of anthozoan and hydrozoan species, suggesting a functional role of these molecules in such a delicate phase of life cycle. Finally, we traced the occurrence of 5-thiohistidine derivatives in anthozoan and medusozoan animals through high performance-liquid chromotography analyses. Our work provides new knowledge to unravel the origin and diversification of ovothiols biosynthesis.

P-02.3-005
The role of YALI0B07117g and other erythrose reductse homologs in yeast *Yarrowia lipolytica*

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Erythritol is a four carbon polyol commonly found in fruits, in which it acts as a carbon storage. Due to its low caloric value in addition to high sweetness level it is used as a sweetener. One of the most important sources of erythritol is an oleaginous yeast *Yarrowia lipolytica*, which holds and unique ability to produce erythritol from glycerol as a sole carbon source. This valuable trait led to the increased interest in the metabolic pathways of erythritol production in *Y. lipolytica*. Erythrose reductase (ER) is an enzyme responsible for conversion of erythrose to erythritol, which is the last step of erythritol synthesis pathway in *Y. lipolytica*. Several genes with high homology encoding this enzyme were identified, yet the main enzyme is still up for debate. The work focused on analysis of the influence of deletion of selected ERs on erythritol production in erythritol synthesis medium. Based on the preliminary results, the *YALI0B07117g* was more intensively studied. The lack of this gene resulted in the shift from erythritol production towards mannitol and arabitol. Overexpression of this gene was obtained to increase the erythritol production parameters on a larger scale. The results of this study may shed a new light on the erythritol synthesis pathway and help to improve the production of erythritol on an industrial scale. This work was financially supported by the National Science Centre, Poland, project UMO-2018/31/B/NZ9/01025. *The authors marked with an asterisk equally contributed to the work.

P-02.3-006
WGS-based approach in studying first-line antituberculosis drug resistance-associated mutations in different *M. tuberculosis* genotypes

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Mycobacterium tuberculosis (Mtb) genotypes vary in multiple aspects, including geographical distribution, virulence, and drug resistance. While some Mtb genotype families (Beijing, LAM) are frequently associated with multidrug- (MDR) and pre-extensively drug resistant (pre-XDR) tuberculosis (TB) cases, the available data on resistance causing mutations in different genotypes are still limited. The aim of this study was to identify strain spoligotypes along with first-line anti-TB drug resistance-associated mutations in MDR and pre-XDR Mtb isolates using whole genome sequencing (WGS) approach. In total, 36 Mtb isolates (27 MDR and 9 pre-XDR) obtained from pulmonary TB patients in 2013-2014 were studied. Mycobacterial DNA samples were sequenced using Illumina MiSeq platform. SpoTyping (v2.1) software was used for strain spoligotyping, and resistance associated mutations were detected by TBProfiler tool. The most common spoligotyping patterns were SIT1 (N = 19) and SIT42 (N = 14), and a few samples belonged to SIT35 (N = 2) and SIT53 (N = 1). WGS data supported phenotypic DST results, confirming that all isolates were resistant to isoniazid (H) and rifampicin (R), while 35 were resistant to ethambutol (E), and 30 – to pyrazinamide (Z). In all cases, H resistance was related to *katG* Ser315Thr mutation, however, in all SIT42, SIT35, SIT53 and 4 SIT1 isolates SNPs in *fabG1-inhA* promoter region were also detected. Resistance to R and E was associated with 8 different SNPs in *rpoB* and *embB* genes, and 1 SNP in *rpoC* and *embA* promoter, respectively, while 14 mutations in *pncA* gene or promoter region were found among Z-resistant Mtb samples. For the studied medications the detected SNPs varied and appeared in different clusters depending on sample spoligotype. To conclude, this study highlighted heterogeneity in first-line anti-TB drug resistance-associated mutations between different Mtb genotypes. This study was supported by ERDF grant No. 1.1.1.1/20/A/046.

Microbial metabolism

P-02.4-001
The effects of some L-amino acids on the growth and hydrogen-oxidizing hydrogenases activity of *Ralstonia eutropha* H16

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The hydrogenases (Hyd) of chemolithoautotrophic *Ralstonia eutropha* H16 are attractive biocatalysts for operation of enzymatic fuel cell (EFC). The expression of O_2-tolerant Hyds was stated under energy limited conditions or in presence of gas mixtures (H_2, O_2, CO_2) and during bacterial heterotrophic growth [1]. It was shown that the supplementation of a small amounts (0.25 %) of yeast extract enhanced H_2-oxidizing Hyd activity ~20 fold. In this context, the effects of some L-amino acids (glycine (Gly), alanine (Ala), asparagine (Asn), proline (Pro)) on bacterial growth, oxidation-reduction potential (ORP) and pH kinetics, as well as activity of Hyds were studied. ORP was determined by using platinum (Pt) and titanium-silicate (Ti-Si) electrodes. The H_2-oxidizing Hyd activity of the cells was quantified by monitoring H_2-dependent methylene blue reduction at 570 nm. With the decrease of pH, the drop of ORP from positive (+240 ± 10 mV) to negative (-180 mV ± 10 mV) values was shown during bacterial 24 h growth in the presence of mentioned L-amino acids. After 72 h, in contrast to control (Fructose-Nitrogen (FN) medium), the supplementation of 7.0 μmol/ml Pro, Ala and Gly stimulated bacterial growth ~1.9, 2.8 and 3 fold, respectively. H_2-oxidizing Hyd activity did not observed in control samples, whereas addition of 7.0 μmol/ml Pro and Gly represented Hyd activity with maximum values of 14 ± 0.5 and 21.5 ± 0.5 U ($(g\ CDW)^{-1}$), respectively after 24 h. Our results suggest the correlation of pH, ORP and operation of Hyds in *R. eutropha* H16. Moreover, L-amino acids significantly enhance H_2-oxidizing Hyd activity of the cells. The obtained results are valuable for production optimization of O_2-tolerant Hyds, which can be used as anodic catalysts in EFCs. Published in: 1.Poladyan A et al. (2019) Growth of the facultative chemolithoautotroph *Ralstonia eutropha* on organic waste materials: growth characteristics, redox regulation and hydrogenase activity. Microb Cell Fact 18(1) *The authors marked with an asterisk equally contributed to the work.

P-02.4-002
Bioprospection of biosurfactant-producing microbes from Portuguese salterns

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Surfactants are tensioactive molecules with a wide range of applications, especially in the detergent, biomedical and petroleum industries. The global surfactant market moves billions, however most commercially available surfactants are petroleum-based and their extensive use may lead to significant environmental impact. This, together with the increasing environmental awareness, has prompted the search for new environmentally friendly surfactants, including the so-called biosurfactants, which are surfactants produced by microorganisms. Saline and hypersaline locations are an attractive source of microbial communities with unique features shaped by the extreme abiotic conditions where they live, constituting interesting hotspots for the discovery of new biosurfactants. In this work, we conducted sampling campaigns at two Portuguese salterns of marine and non-marine origin, located at Aveiro and Rio Maior, respectively. Chemical characterization of water samples revealed an interesting variability in terms of salinity, pH and heavy metal content among them. Culture-dependent techniques allowed the identification of halophilic microbes with remarkable surfactant-like properties, that can have promising industrial applications.

P-02.4-003
Exploring the *Cyberlindnera jadinii* carboxylate transporters for the improvement of microbial cell factories

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Platform chemicals, such as organic acids, are widely used in many industrial sectors. Once their production is still mainly sustained by fossil-based processes, novel technologies derived from renewable sources are required. Presently strategies for the bio-based production of organic acids are being developed, including the expression of carboxylate transporters in microbial cell factories. In this study, we focused on the identification and characterization of novel carboxylate transporters from *Cyberlindnera jadinii*, a yeast able to assimilate a broad diversity of carboxylic acids [1]. The predicted transportome was studied using two approaches. The *C. jadinii* homologs of the carboxylate transporters Jen1p (SHS family) and Ady2p (AceTr family) were expressed in a *Saccharomyces cerevisiae* strain without carboxylate transport systems. In a parallel approach, a pipeline of bioinformatics tools was developed to retrieve the putative carboxylate transporters present in the *C. jadinii* NRRL-1542 inferred proteome. In this method, multiple matches revealed the presence of homologs within a species and directly reflect the presence of orthologues. The putative transporters selected were cloned and expressed in *S. cerevisiae*. The functional characterization included the evaluation of growth on different carbon sources and the determination of the kinetic parameters for the uptake of radiolabelled carboxylates, i.e, acetate, lactate, succinate, and citrate. Phylogenetic studies and structural characterization, that included 3D-model prediction and molecular docking, was performed for the most promising transporters. The properties displayed by these transporters turn them into relevant targets to be explored as organic acid exporters in microbial cell factories. References [1] *Journal of Fungi* (2021), 7(1), 36

P-02.4-004
Preliminary structural and functional studies of fungal Class 3 asparaginases

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L-Asparaginases are enzymes of important medical and economic interests, in particular as therapeutics for the treatment of acute lymphoblastic leukemia or antibacterial and antifungal targets in agriculture. Asparaginases are classified into 3 classes: bacterial-type (Class 1), plant-type (Class 2) and Rhizobium-type (Class 3) [1]. The latter group is poorly characterized. The only structure of Class 3 asparaginase from *R. etli* (ReAV) was solved very recently [2] and revealed a unique fold with some resemblance to glutaminases and β-lactamases. Our extensive search of sequence databases revealed that Class 3 asparaginases are present in some pathogenic bacteria and fungi which cause severe plant and human infections. We found ReAV homologs with high (>50%) sequence identity in a wide spectrum of fungi, and selected five candidate species for further functional and structural studies, namely *Aspergillus candidus* (AcA), *Aspergillus fumigatus* (AfA), *Penicillium expansum* (PeA), *Colletotrichum fructicola* (CfA) and *Fusarium oxysporum* (FoA). All of the potential asparaginases were cloned and expressed in a bacterial system as His-tagged proteins. The proteins differ significantly in solubility, thermal stability and L-asparaginase activity. The highest enzymatic activity was observed for PeA and CfA, whereas the activity of AfA was undetectable. Comparison of CD spectra indicated that AcA, PeA, FoA have similar fold to ReAV, CfA has a structural distortion while AfA is not correctly folded, which may explain the lack of activity. We were able to crystallize the CfA, PeA and FoA proteins in different crystals forms. The successfully solved crystal structure of CfA confirms that the fungal enzyme has ReAV fold. Work supported by National Science Centre (NCN, Poland) grant 2020/37/B/NZ1/03250. 1. Loch J, Jaskolski M (2021) IUCrJ 8, 514-531 2. Loch et al. (2021) Nature Commun 12, 6717

P-02.4-005
Methanogenic archaea use different strategies to regulate their glutamine synthetase

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Spread across the three domains of life, glutamine synthetase (GS) is a key enzyme for ammonia assimilation [Cabello et al. 2004]. It catalyzes the ATP-dependent conversion of NH_3 and glutamate to glutamine. This reaction must be tightly regulated, especially in energy-limited microorganisms such as methanogenic archaea. While bacterial GS have been well studied, there is little known about archaeal GS, including their regulation [Pedro-Roig et al. 2013, Ehlers et al. 2005]. Here, we natively purified the dodecameric GS (*Mt*GS, 0.6-MDa) from *Methanothermococcus thermolithotrophicus*, a marine thermophile isolated from geothermally heated sediments. The enzyme activity is strictly dependent on 2-oxoglutarate (2OG, Ka = 170 μM) and structural characterization was performed to unveil its activation mechanism. Three crystal structures were obtained: an apo form (1.65 Å), a 2OG bound form (2.90 Å), and a 2OG-ATP-Mg bound form (2.15 Å). 2OG binds in an allosteric pocket and leads to conformational rearrangements that include the opening of the substrate-binding site, allowing catalysis to proceed. Interestingly, the 2OG binding residues are not conserved throughout methanogens. For instance, the enzyme in *Methermicoccus shengliensis* (*Ms*GS) does not share the binding motif. Accordingly, we showed that 2OG had no effect on *Ms*GS activity. Moreover, *Ms*GS structures solved in the apo (2.4 Å) and ATP bound state (2.6 Å) present no large rearrangements, contrary to *Mt*GS. However, the motif responsible for glutamine feedback inhibition in *Bacillus subtilis* [Murray et al. 2013] is present and *Ms*GS activity is reduced upon glutamine addition. These results offer an additional view on the different adaptive strategies used by archaea to regulate their N-assimilation. Regulation by 2OG is an effective energy-saving strategy as it is a cellular N-sensor and helps to adjust N-assimilation to the cellular needs in order to prevent energy losses [Müller & Wagner 2021].

P-02.4-006
Repression of L-methionine dependent fungal growth by L-penicillamine

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Invasive fungal mycoses are a serious threat to human health, especially to immunosuppressed patients. A significant role of fungal infections in the death toll of COVID-19 is also reported. Current antifungal therapies do not appear to be sufficient, therefore, identification of novel molecular targets is highly desirable. Enzymes participating in the biosynthesis pathways of essential amino acids like L-methionine (L-Met) seem to be promising. There are no enzyme counterparts in human cells which limits possibility of serious side effects appearance. Deletion of genes encoding relevant enzymes in this pathway led mostly to L-Met auxotrophy and reduced virulence of the pathogens in animal models of infection. The concentration of L-Met in the blood serum is ~ 30 μM and is probably too low to compensate for the effect of blocking the activity of fungal enzymes. We have examined L-homoserine O-acetyltransferase (Met2p) from *Candida albicans*, which catalyzes the first step in L-Met biosynthesis pathway and identified L-penicillamine (L-PEN) as its inhibitor. Unlike the first enzyme of the biosynthetic pathway in bacterial cells, a fungal enzyme is not inhibited by L-Met. L-PEN's antifungal effect could be observed mostly in *C. glabrata* and *S. cerevisiae* strains in the absence of L-Met in medium, proving that unlike most fungal species, *C. glabrata* and *S. cerevisiae* possess only one route leading to the biosynthesis of L-cysteine, which serves as a precursor for the L-Met production. In *C. albicans*, a bridge role between O-acetyl-L-serine and the transsulfuration pathway plays the Str2p enzyme that catalyzes the transformation of L-cysteine to L-Met via L-cystathionine. This route enables overcoming the auxotrophy and increases the adaptive capacity of this opportunistic pathogen. Our results show that to completely block the L-Met biosynthetic pathway, simultaneous inhibition of Met15p and Str2p is needed. The same substrate usage by these enzymes makes it probable.

P-02.4-007
Structural and biophysical characterization of ExtJ from the outer membrane electron transfer complex ExtHIJKL of *Geobacter sulfurreducens*

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Geobacter are Gram-negative bacteria capable of removing electrons generated by their cellular metabolism through extracellular electron transfer (EET). Their ability to exchange electrons between intracellular donors and extracellular acceptors is mainly attributed to the more than one hundred cytochromes disposed along the inner membrane, periplasm and outer membrane (OM) of these bacteria. Particularly, in the OM, five porin-cytochrome complexes responsible for the direct reduction of extracellular acceptors were identified. In this work, the periplasmic protein ExtJ from the OM porin-cytochrome complex ExtHIJKL was characterized by biochemical and biophysical techniques, including site-directed mutagenesis, MALDI-TOF-MS, CD and NMR spectroscopies. Recombinant ExtJ (8.3 kDa) forms homodimers *in vitro* via a C-terminal cysteine and is composed of five beta-sheets and one alpha-helix. Based on the model predicted using the AlphaFold neural network, ExtJ is attached to the periplasmic side of the ExtI porin through its C-terminal, indicating that the formation of homodimers *in vivo* is unlikely. Altogether, the results obtained constitute a stepping-stone towards the full understanding of the EET mechanisms in *Geobacter* bacteria. Acknowledgments: This work was supported by Fundação para a Ciência e Tecnologia (FCT) through the following grants: SFRH/BD/145039/2019 (TMF), SFRH/BPD/114848/2016 (LM), PTDC/BIA-BQM-31981/2017 (CAS), PTDC/BIA-BQM/4967/2020 (CAS). This work was also supported by national funds from FCT in the scope of the projects (i) UIDP/04378/2020 and UIDB/04378/2020 (Research Unit on Applied Molecular Biosciences - UCIBIO) and (ii) LA/P/0140/2020 (Associate Laboratory Institute for Health and Bioeconomy - i4HB). The NMR spectrometers are part of the National NMR Network and are supported by FCT (ROTEIRO/0031/2013 and PINFRA/22161/2016) cofounded by FEDER through COMPETE 2020, POCI, PORL and FCT through PIDDAC.

P-02.4-008
N2-fixation at the thermodynamic limit of life: characterization of N2-assimilation system and comparative transcriptomic profiling of thermophilic methanogen *Methanothermococcus thermolithotrophicus*

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Methanococcales are hydrogenotrophic methanogens which must rely on yet unknown metabolic strategies to perform the energetically costly N_2-fixation simultaneously with methanogenesis and overcome this energetic paradox, thriving at the thermodynamic limit of life (Previously published in: Thauer R et al. (2008) Nat Rev Microbiol 6, 579-591.). Here, we present physiological and structural studies complemented with a comparative transcriptomic analysis on a marine thermophile *Methanothermococcus thermolithotrophicus* as a model organism, elucidating unique metabolic capabilities and adaptations of this methanogen. Robust diazotrophic growth of this archaeon is strictly dependent on Mo and not inhibited by W, in contrary to other closely related diazotrophs. The complete N_2-fixation machinery was natively purified: the nitrogenase and its reductase, including a unique regulator NifI1,2. Structures of the nitrogenase reductase apo and holo were solved, capturing the different conformations of the thermophilic enzyme. NifI1,2 has a hexameric organization, an unprecedented oligomerization state for a PII-family protein (Previously published in: Leigh J and Dodsworth J (2007) Annu Rev Microbiol 61, 349-377.). Transcriptomic profiling revealed that 11.5% of the whole genome is differentially expressed in the cells grown under N_2-fixing conditions versus N-sufficiency. Besides *nif* and *amtB*/*glnK* operons known to be upregulated under nitrogen limitation (Previously published in: Veit K et al. (2006) Mol Gen Genomics 276: 41–55.), putative pathway for NO_3^-reduction, molybdate importers, components of CRISPR-Cas system and archellum, are all upregulated as well. The transcript levels of the genes for methanogenesis enzymes remain mainly unaffected, while components of both transcriptional and translational machinery are downregulated, illustrating intricate balancing and multiple strategies used by this organism to deal with energy constraints and nutrient limitation.

P-02.4-009
Potassium and proton ions transport during glucose fermentation in *Saccharomyces cerevisiae* under glycerol-induced osmotic stress at different pHs

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The maintenance of potassium and proton homeostasis is crucial for many cellular functioning and adaptation. *Saccharomyces cerevisiae* ATCC 9804 and ATCC 13007 strains were grown YPD (1% yeast extract, 2% peptone, 2% dextrose) or YPG (1% yeast extract, 2% peptone, 0.5 % glycerol) medium under microaerophilic conditions. Specific growth rate (μ), pH, and oxidation-reduction potential (ORP) change as well as K^+ and H^+ fluxes by whole cells in the presence or absence of F_oF_1-ATPase inhibitor dicyclohexylcarbodiimide (DCCD) were conducted. Glycerol inhibited μ of ATCC 9804 by 84.91% and ATCC 13007 strains by 60.15%. Survival of yeasts in YPG depends on pH and excess of oxygen: maximal biomass was obtained at pH 7 2. The changes of readings of ORP Pt electrode from positive ($+55.7 \pm 10$ mV) to negative (-154.6 ± 10 mV) values were observed at 24h upon the growth in glucose and at 72 to 96h of growth in YPG. Acidification of the medium has the same manner during growth in both conditions at pH 7.2 ($\Delta pH = 0.68$). Under stress conditions, in particular, when the mitochondrial H^+-ATPase is inhibited, K^+ transport is completely disrupted in *S. cerevisiae* ATCC 13007: the Trk2 transporter is activated, which, in addition to the Trk1 transporter, stimulates the K^+ efflux with 1.67 ± 0.05 mM/min per 10^{10} cells rate. Thus, obtained results indicate that mitochondrial ATPase and Trk2 transporter are crosslinked. H^+ flux by *S. cerevisiae* cells is directed to the extracellular environment, which indicates that in fermentative conditions the H^+ is pushed out of the cell generating

H^+-motive force. ATPase inhibition leads to H^+ uptake during glycerol utilization by both strains which correlated with K^+ efflux and indicates the role of Nha1 K^+/H^+ antiporter under stress conditions. The results will help to understand the role of ion transporters in regulating mechanisms for the assimilation of various carbon sources and let to find more efficient ways for waste, especially glycerol utilization.

P-02.4-010
Investigating role(s) of TOR1 complex in the regulation of plasma membrane activity, intracellular pH potassium homeostasis and ethanol tolerance in *Saccharomyces cerevisiae*

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K^+ is a key cation controlling various physical parameter bearing profound effects on cellular physiology. Target of rapamycin (TOR) is a conserved protein kinase in all eukaryotes responding to various nutrients which leads to the activation of plasma membrane ATPase for uptake of nutrients, pH regulation and K^+ uptake. However, little information is available on TOR, vis a vis Sit4, a phosphatase and Tco89 both non- essential components; regulation of Pma1 activity, K^+ transport and ethanolic sensitivity in yeast. Pma1 activity, K^+ and pHi are key determinants of ethanol tolerance. Therefore the present work is an attempt to study an interplay between TORC1, K^+ homeostasis, Pma1 activity, pHi and ethanol toxicity that limits its titer and latter's production and in yeast. *S. cerevisiae* 4741, sit4 and tco89 mutants were grown in YPD media till mid log phase. Washed cells with water twice and suspended in water overnight at 4°C. Pma1activity and H^+ efflux was done as reported by Serrano 1980. Potassium transport was measured by its analogue Rb^+ uptake by AAS as reported by Mulet et al 1999. pHi was determined by Portillo and Serrano 1989. Protein by Bradford 1976. Ethanol tolerance was evaluated on YPD agar media with ethanol. It was observed that sit4 had diminished level of Pma1 activity to 55% of wild type, while tco89 had almost no change. Extracellular acidification confirmed the same. Rb^+ uptake was decreased in mutant cells compared to that of wild type. However, the activity of Pma1 of mutants remained unaffected in the presence of rapamycin, which indicated that Sit4 mediates the effect of TORC1 on Pma1. The pHi of wild-type and mutants were 6.42, 6.24 and 6.20 respectively. sit4 and tco89 mutants were more sensitive to ethanol than their wild types. Conclusion: The findings suggest that mutation in the nonessential subunit of TORC1 decreased proton efflux, K^+ uptake, Pma1 activity, pHi and played an important role in ethanol tolerance.

P-02.4-011
Electron transfer reactions within the cytochrome network of *Geobacter sulfurreducens* monitored by NMR

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Electroactive bacteria rely on a network of cytochromes to transport the electrons from their metabolism through the cell's inner membrane, periplasm, and outer membrane. Remarkably, genes that code for cytochromes constitute more than 2% of genome of these bacteria. Several of the key cytochromes that are essential to transport electrons to the cell's exterior have been structurally and functionally characterized. This characterization is important; however, it is the study of the interactions between them that allows to identify the redox pairs, the order of the electron transfer events and, hence, to elucidate the respiratory pathways. We recently developed a strategy to monitor the electron transfer reaction between cytochromes by Nuclear Magnetic Resonance (Morgado L and Salgueiro CA (2022) Metallomics 10.1093/mtomcs/mfac012). Contrary to what is observed for other spectroscopic techniques, as UV-visible for which *c*-type cytochromes have identical spectral features, NMR explores the uniqueness of each protein heme resonances in both reduced and oxidized states. This permits a direct monitorization of the electron transfer reaction between *c*-type cytochromes that are abundant in electrogenic bacteria. Following the successful implementation of the new strategy to monitor the electron transfer reaction between cytochromes, the methodology was now further extended to identify periplasmic *c*-type cytochromes' redox pairs and to clarify the intricate redox networks of *G. sulfurreducens*. This work was supported by Fundação para a Ciência e Tecnologia through the grants SFRH/BPD/114848/2016 (LM), PTDC/BIABQM31981/2017 (CAS), PTDC/BIA-BQM/4967/2020 (CAS) and EXPL/BIA-BQM/0770/2021 (LM). This work was also supported by FCT within projects UIDP/04378/2020 and UIDB/04378/2020 (UCIBIO) and project LA/P/0140/2020 (i4HB). The NMR spectrometers are part of the National NMR Network and are supported by FCT (ROTEIRO/0031/2013 – PINFRA/22161/2016).

P-02.4-012
Can DapC be the missing aminotransferease in the arogenate route of L-tyrosine biosynthesis in *Corynebacterium glutamicum*?

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Corynebacterium glutamicum is well-known as an industrial workhorse primarily for the production of glutamic acid and lysine. Later, its metabolism has been engineered to extend it for the synthesis of a wide range of products, from different amino acids, value-added molecules to commodity chemicals. Despite its potential for the synthesis of different amino acids, it has never been exploited for the production of L-tyrosine. Available data suggest that among the two alternative routes for the post-chorismate branch of the pathway leading to L-tyrosine, this microorganism uses the arogenate route. In this route, an aminotransferase is required for the transamination of prephenate into arogenate (pretyrosine), which is then converted to L-tyrosine by the gene encoded by tyrA. Evidence shows that the n-succinyldiaminopimelate aminotransferases in actinobacteria, *Streptomyces avermitilis* and *Mycobacterium tuberculosis*, display prephenate aminotransferase activity (Graindorge M et al. (2014) J Biol Chem 289, 3198–3208). Furthermore, the fact that the 3-deoxy-7-phosphoheptulonate synthase and chorismate mutase of *M. tuberculosis* and *C. glutamicum* in aromatic amino acid synthesis share significant structural and mechanistic similarities (Burschowsky D et al. (2018) Biochem 57, 557-573) also suggested a link between the transaminases of these two microorganisms. When the functional transaminases encoded

by *C. glutamicum* (Marienhagen J et al. (2005) J Bacteriol 187, 7639-7646) were screened using n-succinyldiaminopimelate aminotransferase of *M. tuberculosis* (UniPrpt accession: O50434) as template, the n-succinyldiaminopimelates encoded by dapC in *C. glutamicum* was returned as a hit. Thus, we speculated that the protein encoded by dapC could constitute the missing transaminase in L-Tyr synthesis. To prove this hypothesis, dapC of *C. glutamicum* has been cloned and expressed in *E. coli* for characterization. Support by TUBITAK (120N728) is gratefully acknowledged. *The authors marked with an asterisk equally contributed to the work.

P-02.4-013
On the mechanism of ROS generation by cytochrome bc1

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Cytochrome bc_1 (Complex III, CIII) is a key component of the electron transport chain (ETC) coupling electron transfer to proton transport across the membrane in most bacteria and mitochondria. CIII operates according to the mechanism of the Q-cycle, in which two-electron oxidation of ubiquinol at the Q_o catalytic site leads to bifurcation – a transfer of electrons onto two distinct cofactor chains: high potential c-chain and low potential b-chain. The uncompleted bifurcation can lead to side reactions resulting in the formation of radical oxygen species (ROS). In this study we explore electron transfer sequences that potentially lead to ROS generation within cytochrome bc_1, benefiting from the newly-designed ROS detection method based on a reconstituted hybrid system of mitochondrial/bacterial cytochrome bc_1 coupled to mitochondrial aa_3-type cytochrome *c* oxidase, which enables measurements of enzymatic activity in uninhibited conditions. Towards this goal we analyze heme *b* knock-out *Rb. capsulatus* mutant strains with impaired electron transfer (at the level of hemes b_L and b_H, respectively), and *Rb. capsulatus* heme c_1 mutant strain mimicking redox equilibration of its mitochondrial equivalent (by lowering of heme c_1 redox potential). With this approach we examine how changes in the electron distribution along the cofactor chains impact on ROS production profile under various conditions. Comparative analysis of ROS generation of different mutational variants of cytochrome bc_1 facilitates discovery of the most prominent factors influencing ROS production within CIII and helps us understand the biology behind formation of ROS within CIII and ETC, contributing to future developments regarding regulatory functions of ETC complexes.

P-02.4-014
On the role and properties of heme-containing mobile domain of alternative complex III of *Flavobacterium johnsoniae*

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Proton motive force is utilized for ATP synthesis. It is achieved by coupling of electron transfer through the chain of bioenergetic enzymes with the formation of proton gradient across the membrane. The cytochrome *bc* family, which comprises e.g. cytochrome b_6f and mitochondrial complex III, is a representative class of such enzymes. Alternative complex III (ACIII) is a functional analogue of mitochondrial complex III, which operates according to a still elusive mechanism. The ACIII is present mainly in organisms that lack bc_1/b_6f complexes, such as *Flavobacterium johnsoniae* [1]. The molecular structure of ACIII, resolved recently by cryo-EM, revealed a highly intriguing spatial arrangement of cofactors. In particular, the membrane-anchored mobile domain of ActA subunit (mdActA) was suggested to serve as a mobile element functionally connecting ACIII with its partner, aa_3 oxidase [2]. Our recent work described the first genetic system for manipulations within ACIII of *Flavobacterium johnsoniae* [3]. Here we report a creation of a mutant that lacks mdActA (ΔmdActA) and its complementation, and examined whether and how the absence of mdActA alters the activity of the enzyme. The results show that in ΔmdActA mutant the electron transfer to the cytochrome aa_3 does not occur, and the consumption of oxygen by this strain is residual. Moreover, the ΔmdActA exhibits restricted bacterial growth. This revealed that heme of mdActA is the only donor of electrons to aa_3 oxidase. The ΔmdActA was complemented by a soluble version of mdActA, allowing us to isolate the mdActA and examine its biochemical properties. This study opens new possibility to study and define the electron transfer paths connecting ACIII with aa_3, and provide first insight into the molecular mechanism of action of the supercomplex. [1] Calisto F, et al., Chem Rev (2021), 121: 1804-1844 [2] Sun C, et al., Nature (2018), 557: 123–126 [3] Lorencik K, et al., Microbiol Spectr. (2021), 9: e00135-21

P-02.4-015
Effect of various carbon sources on the growth properties and photosynthetic pigments content of *Spirulina platensis*

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Among various species of cyanobacteria, *Spirulina platensis* is widely used in biotechnology, pharmaceutics, medicine, food industry as a source of valuable protein, essential amino acids and fatty acids, vitamins, as well as pigments, such as carotenoids, chlorophyll a and phycocyanin [1]. Chlorophyll and phycocyanin are used as colorants in the food and cosmetics industries. Moreover, phycocyanin has demonstrated high anti-inflammatory and anti-cancer activity. During this work the effect of various carbon sources – organic acids (succinate, malate, acetate) and sugars (glucose, fructose, lactate, lactose) on the morphology, growth properties of *S. platensis* IBCE S-2, and the contents of phycocyanin and chlorophyll a has been investigated. Glucose, fructose and lactate are more efficient substrates for *S. platensis*. The growth rate of *S. platensis* was 2-fold higher during the growth in the presence of glucose as a carbon source compared to the autotrophic control. Moreover, carbon source affects the amount of cyanobacterial photosynthetic pigments. The maximum production of phycocyanin and chlorophyll a occurred on the 4th day of cultivation in glucose containing

medium. The content of phycocyanin and chlorophyll a increased by ~2.2 and 1.7 fold, respectively. Morphological changes in *S. platensis* trichomes during the growth in the presence of carbon sources were observed. In the presence of glucose and fructose, cyanobacteria had many long filaments with complete, large cells rich in pigment. In contrast, in the presence of malate, nearly 20 % of the cells in trichomes were colorless and did not contain pigments. Thus, the carbon sources used affect the cyanobacterial growth, which is expressed in changes in the morphological parameters of filaments and cells and pigments content. The results obtained can be helpful for further optimization of cultivation conditions of cyanobacteria. [1] Lau NS et al. (2015) Biomed Res Int. 2015: 754934. *The authors marked with an asterisk equally contributed to the work.

P-02.4-016
Changes in some metabolic processes of yeasts *Candida guilliermondii* NP-4 under influence of microwaves with a frequency of 51.8GHz

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Ultra-high-frequency microwave radiation, which is now widely used by humans in everyday life, medicine, cell phones, and WiFi, harms living organisms. In humans, this effect is manifested by disruption of various organ systems, such as the nervous, immune, cardiovascular, reproductive, endocrine, etc. Living organisms develop and operate certain biochemical response mechanisms to survive in extreme conditions. Knowledge of these mechanisms is crucial for developing measures to protect living organisms from the extreme effects of the surrounding environment. Our work aimed to study the effect of ultra-high frequency microwave radiation (51.8 GHz) on deamination of purine compounds, lipid peroxidation, the activity of antioxidant enzymes catalase, SOD, peroxidase, and the ATPase activity of the yeast *C. guilliermondii* NP-4. It was shown that under the influence of microwaves, yeasts increase the rate of lipid peroxidation by ~70% compared to non-exposed cells at all stages of growth. Irradiation stimulates the activity of the antioxidant system so that the activity of the catalase enzyme in microwave-exposed yeasts increases by 97%, and the activity of the SOD enzyme by ~30%. This difference is probably due to an increase in the number of free radicals that can damage protein molecules and DNA. The peroxidase activity in exposed cells increases by ~40%. In microwave-exposed yeasts, an increase in the rate of deamination of purine nucleotides is observed, which decreases in the post-exposition recovery period in the case of adenine nucleotides, and for guanine nucleotides, it increases even more. The mitochondrial ATPase activity decreases by about 26% in exposed yeasts, and after post-exposition repair, it increases and exceeds that of non-exposed cells by 18%. Thus, the exposition of yeast cells to high-frequency microwaves (51.8GHz) increases the intensity of oxidation processes, which leads to an increase in the number of free radicals. As a protecting mechanism, the activity of antioxidant enzymes is stimulated. The ATPase activity increase contributes to the energy supply of the repairing cells. The results can be used to understand the response mechanisms of microorganisms to non-ionizing radiation, as well as to develop approaches to protect living organisms from it.

P-02.4-017
The effects of mycobacterial RmlA perturbation on *Mycobacterium smegmatis*

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Cell division and DNA synthesis has been extensively studied in eukaryotes, however the linkage between DNA replication and cell elongation in prokaryotes are unclear. Deoxythymidine triphosphate (dTTP), one of the canonical building blocks of DNA, is also used for cell wall biosynthesis in Mycobacteria. During this work we investigated the interrelationship between mycobacterial DNA and cell wall biosynthesis. We examined cellular dNTP pool, cell growth, cell morphology, and possible signs of stress in *Mycobacterium smegmatis* upon perturbation of rhamnose biosynthesys by RmlA overexpression. RmlA serves as a mycobacterial cell wall synthetic enzyme, that uses dTTP for cross-linking the peptidoglycan with the arabinogalactan layers by a phosphodiester bond. We found that RmlA overexpression changes cell morphology, causing cell elongation and disruption of the cylindrical cell shape. Furthermore we observed reduced cellular dTTP pool in RmlA overexpressing cells, without restricting cell growth. We found a significant overexpression of replication and cell wall biosynthesis stress factors upon RmlA overexpression. Using super-resolution microscopy, we observed that RmlA localizes throughout the whole cell length in a helical pattern in addition to the cellular pole.

P-02.4-018
Characterization of resin-associated Mycobiota

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Fungi are ubiquitous organisms and key soil colonizers playing critical roles in the ecosystem. They are able to grow in harsh biomes and evade defensive chemical-based barriers, namely resin. Resin builds part of the conifer's physiochemical upholder due to the broad bioactive potential of its diterpene components and derivatives. Resin-associated fungi are still seldom investigated, yet can constitute a rich source of unknown specialized metabolites. To address this knowledge gap, the mycobiota colonising a resin-contaminated soil was characterised combining both cultivation-dependent and independent methods. The resin-contaminated soil was collected inside a resin industry inactivated since the 70s located in the north-east of Portugal. The indigenous mycobiota found in the resin-contaminated soil was composed of 167 fungal OTUs, with taxonomic Shannon diversity index of 2.847. Nearly 80 cultivable fungal strains with distinct macroscopic characteristics could be isolated and taxonomically analysed (ITS-2). Chemical analyses revealed that the resin contaminated soil contains high C and low N levels compared to coniferous forest soil. The diversity of resin acids found in the resin-contaminated soil was distinctive of that found in gum resins collected in *Pinus pinaster* trees in the same location (NMR analyses). Overall, results show that the aged resin-

contaminated soil upholds a unique mycobiota (changed compared to that of a coniferous forest soil), comprising strains that have altered the archetypal signature of resin acids. The metabolic requirements of the isolated strains, including its ability to catabolise resin acids is under analysis. Better characterization of this mycobiota may reveal strains of relevant biotechnological potential, *e.g.* establishment of new biofunnelling strategies for valorisation of gum resin, a pivotal resource in Portugal.

P-02.4-019
Identification of redox partners involved in *Geobacter sulfurreducens*' extracellular electron transfer pathways by *in vivo* bacterial two-hybrid analysis

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The interest in *Geobacter sulfurreducens* has increased in recent years, as it has a high potential to be used in bioremediation and to change the way we generate energy. These applications are based on the bacteria's ability to perform extracellular electron transfer and reduce toxic/radioactive metals or electrodes outside the cell. Different cytochromes located in the inner membrane, periplasm or outer membrane were already identified to be involved on specific routes for reduction of each terminal electron acceptor. However, the pairs of redox partners were not identified, and consequently, the specific respiratory chains have not been defined. To understand these respiratory chains, it is necessary to identify the specific redox complexes. For this, we are using a β-galactosidase-based bacterial two-hybrid system to assess proteinprotein interactions. This assay is based on the activity of β-galactosidase in which two proteins of interest are fused to two non-functional but complementing galactosidase truncations. The level of complemented β-galactosidase activity, driven by the protein-protein recognition between both non-β-galactosidase parts of the chimeras, reflects whether the proteins of interest interact. To perform this assay, a series of plasmids were prepared for insertion in *E. coli* MC1061 (which lacks the entire *lacZ* locus) before measurement of the β-galactosidase activity by *o*-nitrophenol-β-galactoside (ONPG) hydrolysis. The results obtained for the interactions between the inner membrane associated proteins and the most abundant periplasmic ones will be presented. This work will be key to understand the electron transfer pathways of *G. sulfurreducens*. This work was supported by Fundação para a Ciência e Tecnologia through the grants PTDC/BIA-BQM/4967/2020 (CAS) and EXPL/BIA-BQM/0770/2021 (LM). This work was also supported by national funds from FCT within projects UIDP/04378/2020 and UIDB/04378/2020 (UCIBIO) and project LA/P/0140/2020 (i4HB).

P-02.4-020
Evaluation of microbiome of free-living and Zoo turtles by immunological methods and Raman spectroscopy

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One of the conservation strategies of threatened animal species is housing them in captivity, but many researchers had shown decreased or increased microbial diversity in captive hosts compared to free-living animals due to abnormal diets, artificial and sterile environmental conditions, antibiotic treatment, various stresses, etc. The knowledge of released organisms' microbiome presents an important research issue for conservation efforts. Therefore, the objective of the present study was to identify bacteria released in European pond turtle (*Emys orbicularis* L., 1758) skin microbiome on the Latvian territory as well cloacal swabs samples from Testudo (*Agrionemys*) horsfieldii, Testudo graeca, *Trachemys scripta*, *Emys orbicularis* (L.), Deirochelys reticularia living in Latgale Zoo and free-living were tested to Salmonellae. 26 swab samples skin and 16 cloacal swabs samples were collected. Bacteria were grown for isolation and differentiation of bacteria, CHROMagarTM Orientation and Chromogenic Salmonella Lab-AgarTM were used and immune sera. Individual colonies of bacteria were described based on size, color, texture, and morphology. Serotyping was performed by an immune serum to Salmonella O and H antigens by agglutination. The agglutination reactions were interpreted in accordance with the Kauffmann White protocol. The results have revealed that the Salmonella Agona was detected. The most commonly found bacteria were Pseudomonas spp. (40%) and Proteus spp. (27%). The microbiome was analyzed by Raman spectroscopy with 514nm lasers. Raman spectra were analyzed compared with standard spectral library These studies provide new basic information about the external microbiomes of the released *E. orbicularis* and are an important step in understanding their environmental roles. The project was supported by the Nr.14-95/2022/3 "The European pond turtle Emys orbicularis, L. 1758 microbiome investigation and analysis of the Latvian territory"

P-02.4-021
Hydrogen generation in sulfur-deprived green microalgae *Chlorella vulgaris*

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Molecular hydrogen is one of the most promising energy carriers that may facilitate a stable supply of energy in future. Application of green algae can decrease its cost by providing an opportunity for hydrogen production from water using solar power. Hydrogen evolution in green algae is linked to the photosynthetic electron transport in thylakoid membranes via reaction catalyzed by [Fe]-hydrogenase which is inhibited by oxygen produced from decomposition of water. Sulfur deprivation of algal cells is a solution to this problem. This work is an attempt to reveal the mechanisms of hydrogen photoproduction by sulfur-deprived *Chlorella vulgaris* Pa-023 (Microbial Depository Center, NAS, Yerevan, Armenia). For this purpose, all sulfates in algal growth media were replaced by chlorides. Hydrogen yield was recorded by the decrease of redox potential to low negative values during algal growth. The results showed ~ 2.4 times improved hydrogen yield in sulfur-deprived *C. vulgaris*, compared to algae grown on control Tamiya medium. To determine the contribution of photosystem (PS) II in hydrogen generation the effect of diuron, a known inhibitor of PS II activity, was investigated. Hydrogen generation in sulfur containing medium ceased in the presence of diuron and was suppressed ~5 times in sulfur-deprived conditions, suggesting PS II as a source of electrons for hydrogen generation during splitting of water. Analysis of the mechanisms of hydrogen photoproduction by green algae in sulfur-deprived conditions needs further research, however the results

obtained show a possibility of their use for improved hydrogen generation. *The authors marked with an asterisk equally contributed to the work.

P-02.4-022
Brewer's spent grain as a potential substrate for hydrogen production by *Parachlorella kessleri*

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Brewer's spent grain is a key by-product of the brewing industry and contains proteins, monosaccharides, the most abundant of which are xylose, glucose and arabinose [1,2]. Microelements, vitamins and amino acids can be found in brewer's spent grain making it an attractive substrate for the cultivation of microorganisms. During this work, the brewer's spent grain was applied as a substrate for hydrogen (H_2) generation in green alga *Parachlorella kessleri* MDC6524, isolated in Armenia. The most significant criteria for selection of a brewing industry waste as a substrate for algal H_2 production are its accessibility, carbohydrate content and cost. H_2 production by *P. kessleri*, cultivated on brewer's spent grain medium was started at 24 h growth, whereas in control cell, cultivated in Tamiya medium, H_2 yield was detected at 48 h. Moreover, the highest H_2 yield was observed in a 2-fold diluted brewer's spent grain medium, which was ~2 fold higher in comparison with control. HPLC analysis demonstrates that content of glucose, acetate and malate in brewer's spent grain decreased during algae growth, indicating that these compounds are used by algae as a carbon source for H_2 generation. To reveal the role of photosystem II in H_2 production during utilization of brewery waste, the effect of diuron on H_2 yield was studied. Diuron suppressed the H_2 yield in alga by ~60 % in 2-fold diluted brewer's spent grain medium, which indicates the important role of photosystem II in H_2 generation in *P. kessleri*. Thus, brewer's spent grain is a valuable substrate for H_2 production by green algae. 1. Jackowski M et al. (2020) Biomolecules 10, 1669. 2. Mussatto SI. (2014) J Sci Food Agric 94, 1264-1275. *The authors marked with an asterisk equally contributed to the work.

P-02.4-023
Exploring the degradation of selected non-steroidal anti-inflammatory drugs (NSAIDs) by *Cunninghamella* species, *in silico* toxicity studies of fungal transformation products

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Non-steroidal anti-inflammatory drugs (NSAIDs) widely used in human treatment belong to the main therapeutic class of molecules contaminating aquatic ecosystems worldwide and causing harmful effects on resident species. Fungi have a great capacity for degradation of chemical compounds, thus representing interesting components for the environmental cleanup practices. *Cunninghamella* spp., particularly *C. elegans*, *C. echinulata*, and *C. blakesleeana* are fungi that are widely studied in relation to their ability to transform xenobiotics in the same manner as mammals. The aim of the current study was to investigate the degradation of selected NSAIDs such as ibuprofen, naproxen and indomethacin in cultures of *Cunninghamella* strains. Finally, *in silico* toxicity assessment of fungal transformation products was carried out using Derek Nexus system. The biotransformation was carried out for 7 days, and its progress was monitored with liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The study indicated that main transformation products produced by *Cunninghamella* spp. were identical to metabolites obtained in humans. Ibuprofen was transformed mainly to 2-hydroxyibuprofen, naproxen to desmethylnaproxen, and indomethacin to O-desmethylindomethacin. Moreover, *in silico* toxicity tool indicated that the obtained *in vitro* transformation products do not exhibit any structural alerts for endpoints such as mutagenicity, carcinogenicity, teratogenicity, neurotoxicity, cardiotoxicity and irritancy. Acknowledgements The project was supported by the National Science Center Grant No 2020/37/B/NZ7/02546. *The authors marked with an asterisk equally contributed to the work.

P-02.4-024
Haem metabolism in *Campylobacter jejuni*

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Campylobacter jejuni is a Gram-negative bacterium and the most common cause of gastroenteritis worldwide. This pathogen is responsible of approximately 1/4 cases of diarrheal disease, which may be fatal in people with other comorbidities (1). Haem is an iron-protoporphyrin molecule that is essential to all living organisms. As such, this cofactor is required for survival of most pathogens within the infecting host, and several pathogens have dedicated haem biosyntesis pathways (2) as well as haem acquisition pathways (3). Our research focus on elucidating how *C. jejuni* produces haem intracellularly and how these systems maintain haem homeostasis. Our data shows that *C. jejuni* uses the so-called protoporphyrin-dependent pathway (PPD), in which we identify new proteins active in this microorganism, uncovering important protein–protein interactions involving these novel proteins. Therefore, this work sheds novel insights into *Campylobacter jejuni* haem biosynthesis, unravelling potential targets to fight infection. References (1) Eurosurveillance editorial team. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-Borne Outbreaks in 2011 Has Been Published. Euro Surveill. Bull. Eur. Sur Mal. Transm. Eur. Commun. Dis. Bull. 2013, 18 (15), 20449. (2) Lobo, S. A. L.; Scott, A.; Videira, M. A. M.; Winpenny, D.; Gardner, M.; Palmer, M. J.; Schroeder, S.; Lawrence, A. D.; Parkinson, T.; Warren, M. J.; Saraiva, L. M. Staphylococcus Aureus Haem Biosynthesis: Characterisation of the Enzymes Involved in Final Steps of the Pathway. Mol. Microbiol. 2015, 97 (3), 472–487. https://doi.org/10.1111/mmi.13041. (3) Ridley, K. A.; Rock, J. D.; Li, Y.; Ketley, J. M. Heme Utilization in Campylobacter Jejuni. J. Bacteriol. 2006, 188 (22), 7862–7875. https://doi.org/10.1128/JB.00994-06.

Human microbiome

P-02.5-001
Effect of DSS-induced ulcerative colitis and butyrate on the expression and activity of cytochrome P450 2A in GF and SPF mice

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The inflammatory bowel diseases (IBD) represent chronic and relapsing inflammation of gastrointestinal tract (GIT). The microbial community in GIT, also called the gut microbiota, significantly influences host physiology and disorders including IBD. Microbial metabolites participate in regulation of the host's metabolic and inflammatory pathways. One of these metabolites is butyrate, a short chain fatty acid produced by bacterial fermentation of non-digestible carbohydrates. Hepatic cytochromes P450 (CYP) are the most important enzymes responsible for the initial metabolism of drugs and other xenobiotics. Lines of evidence indicate that inflammation and microbial metabolites may influence drug metabolism in the liver affecting CYP expression. Here, the effect of butyrate and DSS-induced colitis on CYP2A expression/activity (an enzyme metabolizing metronidazole used to treat IBD in human) in female mice liver was investigated. Mice were either lacking microbiome (germ-free, GF) or the control ones (pathogen-free, SPF) and were all fed standard diet supplemented with or without sodium butyrate in drinking water. According to our results, DSS-induced colitis suppressed gene expression of Cyp2a5 and decreased enzyme activity of CYP2A in the liver of SPF mice. Interestingly, administration of butyrate prior induction of colitis affected the activity of CYP2A only in the presence of microbiota (SPF mice). Our results highlight the importance of butyrate pretreatment and microbiota presence under inflammatory condition and their interaction with hepatic drug metabolism. With regard to medications used in IBD and microbiota-targeted therapeutic approaches, it is important to deepen our knowledge of the effect of gut inflammation and followed therapeutic interventions on the ability of the organism to metabolize drugs. This work was supported by The Czech Science Foundation (grant no. 19-08294S) and by Internal Student Grant Agency of Palacky University IGA_LF_2022_025.

P-02.5-002
Influence of mental health medication on the microbiota of elderlies

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The improvement of economic, hygienic-sanitary conditions and the promotion of health through diet and moderate physical exercise has led to an improvement in living conditions in developed countries. This has resulted in an increase in life expectancy throughout the 20th and early 21st centuries. In Spain, people over 65 years of age constitute 19.58% of the population in 2020. Aging comes with several health issues, including mental health disorders and changes in the gut microbiota. A gut–brain axis consisting of a bidirectional network that links the central nervous system with gastrointestinal tract functions suggests that microbiota can influence an individual's health or lack thereof. On the other hand, physiological changes associated with aging affect the gut microbiota: taxa and their associated metabolic functions differ between younger and older people. Here we carried out a case-control approach to study the interplay between the gut microbiota and the mental health of elderlies. Fecal and saliva samples from 101 healthy volunteers over 65 were collected, of which 29 (EE-MH group) reported using antidepressants, anxiety, or insomnia medication at the time of sampling. The rest of the volunteers (EE-NOMH group) were the control group. 16S rRNA gene sequencing and metagenomic sequencing were applied to determine the differences between intestinal and oral microbiota of both groups. Significant differences were found in eight and five genera in the fecal and oral microbiota, respectively. Functional analysis of fecal samples showed differences in five ortholog genes related to the metabolism of tryptophan, the precursor of serotonin and melatonin, and six other significant categories related to serine metabolism, a precursor of tryptophan. Moreover, we also found 27 metabolic pathways significantly different between groups, including pathways regulating longevity, the dopaminergic synapse, the serotoninergic synapse, and two amino acids.

P-02.5-003
Comparative bacteriome analysis of tooth and cystic fluid in patients with apical periodontitis and radicular cyst – a pilot study

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Endodontic bacterial infection is the most common cause of apical periodontitis (AP). In some patients, an inflammatory odontogenic cyst of the jaw, so called radicular cyst (RC), can develop. Analysis of microbiota in a root canal is limited by its morphology; the transition of microbiota from apex into RC is, however, possible. The aim of this pilot study was to compare the bacteriome of dental plaques, teeth affected by AP, and cystic

fluids from RC. Swabs of dental plaque from teeth affected by AP and cystic fluid from 6 adult patients with radiographically and histopathologically verified RC were obtained. The teeth affected by AP that were extracted to allow the RC extirpation were crushed in the sterile capsule in a cryomill. Microbial DNA was isolated from all three matrices by QIAamp DNA Mini Kit and used for PCR amplification of the hypervariable 16S rRNA gene segments. Double-barcoded libraries for subsequent Next-generation sequencing on Illumina MiSeq instrument were prepared. Alpha diversity based on an amplicon sequence variant in cystic fluid was significantly lower than in the dental plaque and tooth with AP ($P < 0.05$, and $P < 0.01$, respectively). The bacterial abundancies in individual matrices also differed ($p \leq 0.001$), specifically in *Rothia*, *Streptococcus*, *Capnocytophaga*, *Leptotrichia*, and *Veillonella* sp. The relative abundancies of *Treponema*, *Tannarella*, *Prevotella*, and *Porphyromonas* sp. were higher in cystic fluids than in the teeth affected by AP and their dental plaques, respectively. In conclusion, non-sterile cystic fluid from RC contains DNA from anaerobic periodontal pathogens, which are also found in the tooth affected by AP in the same patient. This suggests the possible polymicrobial origin of RCs. The study was supported by the Ministry of Health of the Czech Republic, grant No. NU20-08-00205. Thanks to University Hospital Brno RVO (FNBr, 65269705), RECETOX RI (No LM2018121), CETOCOEN EXCELLENCE (No CZ.02.1.01/0.0/0.0/17_043/0009632). *The authors marked with an asterisk equally contributed to the work.

P-02.5-004
Anti-inflammatory effect of exopolysaccharides from *Lactobacillus brevis* on co-culture models of macrophage-Like and enterocyte

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Inflammation induced by dysbiosis or infection with pathogenic bacteria is considered one of the most common factors causing inflammatory bowel disease (IBD). However, beside the use of viable bacteria also their metabolites are gaining interest for the development of beneficial food supplements. Among these, exopolysaccharides (EPSs) have shown to present anti-inflammation, antioxidant, antitumor features (Angelin, J.et al. 2020). Thus, the aim of this study is to evaluate the effect of purified EPSs produced by *Lactobacillus brevis* (*L. brevis*), on the modulation of inflammatory response using an enterocyte based *in vitro* model. Specifically, enterocyte monolayers were incubated with *L. brevis* EPS alone (200 μg mL−1), and in presence of commercial lipopolysaccharides (LPS from *Salmonella minnesota*) (at 20 μg mL−1). TLR-4 and IL-6 expression was analyzed at transcriptional and protein level by qRT-PCR and western blotting. In addition, to assess the potential immune-modulation activity a gut inflammation model of Caco-2 and monocytes (THP-1 cells) was established (Di Guida et al 2021). Co-culture was used to perform the colorimetric THP1-Blue™ NF-κB-reporter assay. Results showed that both TLR-4 and IL-6 were upregulated, in presence of LPS, while significantly reduced when enterocytes were treated with the sole EPSs. Western blot analyses showed NF-κB activation following LPS treatment while EPSs slightly decreased its expression. The latter also reduced TLR-4 expression. Similar results were obtained by the colorimetric assay, LPS induced inflammation, and the level of NF-kB, was upregulated with respect to the CTR, while the combination EPSs/LPS significantly reduced NF-kB level. These results indicate that EPS possesses beneficial effects in modulating specific biomarkers (IL-6, NF-kB, TLR-4) tested on a gut inflammation model. References: 1- Angelin, J., & Kavitha, M. (2020).Int. J. Biol. Macromol.,162, 853-865. 2- Di Guida et al.(2021) Mar Drugs. (11):646. *The authors marked with an asterisk equally contributed to the work.

Cell division and cell cycle regulation

P-03.1-001
Division of root endodermal cells by auxin

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Nutrients absorbed through root epidermis are centripetally moved to xylems through apoplastic and symplastic transports. Related to this, the root endodermis plays a pivotal role as a barrier. The endodermis prevents the diffusion-based apoplastic transport, but facilitates the regulatory symplastic transport. Consequently, the endodermis selectively loads the nutrients to xylems. In this study, we showed that division of the endodermal cells is regulated by a plant hormone auxin in Arabidopsis. Unlike epidermal and vascular cells, the endodermis does not divide in Arabidopsis roots unlike epidermal and vascular cells, but auxin treatment provokes division of endodermal cells. Furthermore, the result of NPA (an inhibitor of polar auxin transport) further supported this. NPA treatment induced auxin accumulation in endodermal cells and strongly promoted the division of endodermal cells. These findings indicated that auxin plays an essential role in controlling division of endodermal cells, suggesting that polar auxin transport is a key biological process determining auxin accumulation and response in the endodermal cells. Interestingly, we also found that salt stress promotes the endodermal division as auxin does. Together with the result of the salt stress-induced endodermal division, we propose that environmental stresses are possibly involved in the auxin-dependent regulation of endodermal cell development.

P-03.1-002
Functional characterization of OsPAP3 in chloroplast biogenesis in rice

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In plants, chloroplasts are an essential organelle responsible for photosynthesis and the production of a variety of substances including phytohormones, amino acids, and lipids. Therefore, chloroplast development largely affects plant growth and productivity. Transcription of chloroplast genes, which is governed by Plastid-encoded RNA polymerase (PEP) is a key step for determining the chloroplast development. The transcriptional activity of PEP is tightly regulated by PEP-associated proteins (PAPs), suggesting that chloroplast development can be modulated by the expression of PAPs. In this study, we identified approximately 10 OsPAP genes in rice, whose expressions are regulated by light

conditions. Among them, we attempted to characterize the expression pattern and function of OsPAP3 focusing on rice chloroplast development. Expression of OsPAP3 was upregulated by light and predominantly expressed in leaves where chloroplasts form and photosynthesis occurs. The expression pattern of OsPAP3 was similar to that of the photosynthetic gene OsrbcL, whose expression is regulated by PEP. Together with the result that OsPAP3 proteins specifically localize at the nucleoid of chloroplasts, these findings suggested that OsPAP3 is deeply involved in chloroplast development. To characterize the function of OsPAP3 in rice chloroplast development, we generated Cas9-ospap3 transgenic plants and analyzed chloroplast development. Unlike wild-type plants, Cas-ospap3 transgenic rice exhibited an abnormal whitening phenotype in T0 generation. When the DNA sequence at the Cas9-target site of the OsPAP3 gene was analyzed, the whitening phenotype was tightly linked with the homozygous mutation of the OsPAP3. In T1 siblings, the whitening phenotype was observed at a 3:1 ratio (greening: whitening). These findings indicate that OsPAP3 plays a pivotal role in rice chloroplast development.

P-03.1-003
Do single and joint exposures of antineoplastic agents cause cytotoxicity to *Danio rerio* cell lines?

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In developed countries, the use of antineoplastic agents (AAs), whether individually or in mixtures, has increased up to 10% per year due to the high demand of chemotherapy. AAs are constantly released into hospital and urban wastewaters, where they, most commonly, undergo conventional treatment in wastewater treatment plants. Consequently, the residues of AAs have become emerging pollutants in aquatic environments where they may cause adverse environmental effects even at very low doses. In this study, to understand the potential risks and molecular mechanisms of AAs toxicity, the single and joint effects of two AAs with different mechanisms of action (trabectedin and doxorubicin) were evaluated. For that, zebrafish liver (ZFL) and zebrafish embryonic fibroblast (ZF4) cell lines were used as a model to test the hypothesis that AAs exhibit cytotoxicity. The two cell lines were incubated with a wide range of increasing concentrations of AAs along with the control treatments for 24 h. The viability of cells exposed was determined by a fluorometric assay using resazurin (commercially available as PrestoBlue®). Additionally, morphological changes were observed and assessed by microscopy. Since little is known on the effect of AAs on aquatic organisms, our results demonstrate that ZFL and ZF4 cells provide a relevant and sensitive tool to screen the toxicity of environmental pollutant in the frame of hazard assessment, highlighting the importance of investigating the potential of cell lines to serve as proxy to in vivo for the single and joint toxicity assessment of AAs. Therefore, in the future we intend to address the modulation of protein expression by AAs using a protein antibody microarray to unravel the molecular mechanisms underlying the cytotoxicity of AAs in aquatic organisms.

P-03.1-004
Development of heterocyclic azo compounds exhibiting antitumor activity using computational methods

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In the present work, we report a development of classification tree for substances with different chemical structures on the basis of docking data together with their *in vitro* affecting the poly amine oxidase (PAO) activity. The method of computer integration of ligands into the active center of yeast PAO was used to study the affinity of the tested compounds for the key enzyme of PA catabolism and predict their activity. In the Molegro Virtual Docker (MVD) version 4.0.2, the tested compounds were embedded in the binding site with the substrate in the yeast crystalline structure - Fms1 (1.5.3.17). This enzyme was chosen from the PDB (Protein DataBase, 1Z6L). The obtained data indicate that the key residues for ligand binding in the active center of the PAO molecule are His67, Tyr450, His191, Phe189, Gly487, Trp174, Cys488. According to their effect on PA degradation, the compounds in this study can be classified as presumably carcinogenic and antiproliferative. Substances that inhibit oxidative deamination show carcinogenic properties. Conversely, chemical compounds that activate PAO may become potential antitumour agents. These are aniline and azacrown ethers derivatives. Comparing the results of screening for PAO activity and docking data with PAO, a binary classification tree was proposed. It allows one to predict the nature of ligand activity from the partial binding energies of the 5 amino acid residues of the enzyme. Such model can serve as a basis for the synthesis of new modulators of PA metabolism.

Apoptosis

P-03.2-001
Manganese (II) complex with flufenamic acid and neocuproine and its effect on human endometrial cell lines

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Endometriosis is defined as the presence of a functional endometrium in the ectopic region, namely in an atypical area beyond its original region – the uterine cavity. This chronic disease is very widespread and occurs in up to 10% of women of reproductive age. It is well known that oxidative stress may be implicated in the pathophysiology of endometriosis, causing a general inflammatory response in the peritoneal cavity. This thesis aimed to study the effect of the manganese(II) complex with flufenamic acid [Mn(neo)(flu)$_2$] on endometrial tissue. For experiments, the immortalized human endometriotic cell line (12Z), normal mammary immortalized epithelial cells (HME1), considered as healthy

control, and cancerous cells human uterine leiomyosarcoma cell line (SK-UT-1) were used. Quantification of changes in gene expression of selected genes was carried out by qPCR. We observed elevated pro-apoptotic genes Caspase3 and Bax in endometriotic and cancerous cells compared to HME1 cells after treatment. This finding was also confirmed on the caspase enzyme activity level. In addition, we noticed decreased viability of the 12Z and SK-UT-1 cells compared to HME1 cells after treatment. In conclusion, treatment of 12Z and SK-UT-1 cells with $[Mn(neo)(flu)_2]$ induced apoptosis, as well as decreased cell viability. The effect of the complex has a more significant cytotoxic effect on cancerous cells and a pro-apoptotic effect on the endometriotic cell line. The studied complex can regulate immune functions, reproduction, and provide defense against reactive oxygen species, whereas flufenamic acid possesses antioxidant and inflammatory properties. Therefore, the combination of manganese and flufenamic acid in a complex structure could possess promising applications in the treatment of chronic inflammatory diseases such as endometriosis. This work was supported by VEGA 1/0620/19 and VEGA 1/0540/21. *The authors marked with an asterisk equally contributed to the work.

P-03.2-002

Abstract withdrawn

P-03.2-003

Red tattoo inks trigger IL-18 release from human keratinocyte cell line

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Over the past decade, the percentage of Europeans who has more than one tattoo increased 5% to 10% in 2003 and 12% in 2016. Despite increased demand, the number of patients visiting dermatologists was increased. The content of colorants in inks are variable but generally involved herbal components and heavy metal salts. This situation exhibits an important health risk. In this context, we evaluated three commercial red tattoo inks in human keratinocyte cell line (HaCaT). Tattoo inks were diluted as 0.1%, 0.01%, 0.001% and 0.0001% and applied to HaCaT cells for 24 hours. After incubation, cell viability assay was performed to determine IC50 values. Then, the levels of IL-18, a cytokine associated with inflammatory skin diseases, were evaluated by using IL-18 Human ELISA kit. The obtained data were statistically analyzed with ANOVA method in 95% confidence interval and bilateral relations between groups were determined by Tukey's post-hoc method. Our data showed that ink applications to the cells reduced viability in a dose-dependent manner. At 0.01% dilutions, cell viability significantly decreased in all three brand tattoos ($P < 0.05$). Furthermore, an elevated release of IL-18 was found in 0.01% groups when compared to untreated cells. In conclusion, our results suggest that tattoo inks including red inks may cause cytotoxicity and could trigger inflammatory response in skin. More toxicological studies are necessary to understand the effects of tattoo inks containing heavy metals and/or organic components. This research was funded by Ege University Research Foundation (BAP) under grant number TGA-2019-20818. Key words: Tattoo inks, cytotoxicity, inflammation, cell viability *The authors marked with an asterisk equally contributed to the work.

P-03.2-004

Characterisation of RNA binding activity of FAST protein in cell death regulation

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It is suggested that FAST protein, due to its ability to regulate Fas-induced cell death, is involved in several forms of autoimmune diseases. FAST acts as a survival protein - it enhances the expression of apoptosis inhibitors by inhibiting the activity of T-cell restricted intracellular antigen-1 (TIA-1), leading to reduction in apoptosis [1]. It is believed that the pro-inflammatory properties of FAST result from promoting the survival of inflammatory cells and/or preventing TIA-1 mediated repression of inflammatory mediators. Our research focuses on understanding of the role of FAST in apoptosis and alternative splicing that appear to affect each other at many levels of RNA regulation by this molecule. We report first successful protocols for obtaining purified recombinant human FAST after expression in bacterial system. Our *in vitro* experiments confirmed the ability of FAST to bind RNA. SELEX was utilized to identify the RNA sequence motifs recognized by FAST protein which eventually led us to the structural motif preferred by the protein. Based on the small angle X-ray scattering data and predicted 3D structure model, we propose a region in the protein involved in the RNA binding and we indicate residues responsible for these interactions. Misregulation of FAST can contribute to autoimmune syndromes by regulating Fas-induced apoptosis, which is a prelude to autoimmune disease in several experimental systems. Interestingly, FAST is overexpressed in patients with various diseases of this origin, including rheumatoid arthritis and autoimmune diabetes. In mice models, FAST KO reduces immune arthritis [2] and in the house dust mite allergic pneumonia model provides resistance to neutrophilic pneumonia [3]. We believe that understanding the structure of FAST may enable the design of drugs directed against autoimmune diseases. This work is financed by NCN Poland under grant #2020/37/N/NZ1/02348. [1]Li W. et al., 2004 [2]Simarro M. et al., 2015 [3]Simarro M. et al., 2010

P-03.2-005

Diet enrichment with *Thymus* spp. extracts as an approach to counter oxidative stress in the intestinal barrier

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The intestinal barrier (IB) acts as a semipermeable barrier, allowing nutrients absorption but also limiting the transport of harmful agents. However, an unhealthy diet or exposure to external aggressors, e.g. food contaminants, contribute to increased oxidative stress, playing a role in inflammatory status onset, associated

with several pathologies, or in tumour development. Lately, great relevance has been given to IB homeostasis and to the search for new strategies to counter its impairment. This work aimed to study the potential health-promoting effects of foods rich in antioxidant compounds, that when ingested will protect or reduce the IB susceptibility against oxidative damage. To meet this goal, aqueous extracts of *Thymus carnosus* and *Thymus capitellatus*, fully characterized by high-performance liquid chromatography, revealing high content in rosmarinic and salvianolic acids, and in luteolin and quercetin glycosidic derivatives, were used to expose Caco-2 cells as an IB *in vitro* model. Caco-2 cells were pre-exposed to non-cytotoxic concentrations of extracts and some of their main compounds, for 2 h, 4 h or 8 h, after which test solutions were replaced by culture media (additional 16 h incubation). Then, cells were exposed to the oxidative insult with *tert*-butyl hydroperoxide, for 4 h. Cell viability was assessed by Alamar Blue® and Annexin-V/PI double staining. Results show a dose- and time-dependent protection against a highly cytotoxic oxidative insult, and cells pre-exposed to extracts presented >90% cell viability whereas positive control presented < 10% cell viability. The mechanisms underlying the cellular protection of extracts were assessed by flow cytometry analysis of cellular reactive oxygen species and glutathione content, and by endogenous antioxidant enzymes activity. In conclusion, the inclusion of *Thymus* spp. in the diet presents a valid option to protect the intestinal cells against oxidative stress and may be considered as functional foods.

P-03.2-006
Activation of apoptic cascade via "zinc-finger-like" Zn-NSAID complex

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Endometriosis is a disease which can be defined as chronical inflammatory process which supports implementation of endometrial cells via aberrant angiogenesis and causes pathological manifestation of endometrium outside of the uterus. Non-steroidal anti-inflammatory drugs (NSAIDs) are well known and widely used to treat any type of inflammation as well as in the treatment of period pains of patients with endometriosis. Zn complex with niflumic acid (Zn-NSAID) which has similar structure as zinc-finger proteins was selected for studies of apoptosis induction in endometriotic cell line 12Z and healthy cell line hTERT HME1 (Rabajdova M et al (2021) Sci Rep 11, 19086). We measured calcium level in cytosol and H_2O_2 in mitochondria using Next-generation fluorescence live cell imaging on an inverted fluorescence microscope AnglerFish, analyzed inflammatory, anti-oxidant, and apoptic gene expression using PCR methods. We determined the increased calcium level in cytosol after 8 h of treatment with complex which corresponded with increased H_2O_2 level in mitochondria and increased gene expression of anti-oxidant enzymes *GPx1* and *CAT*. After 24h of treatment, we observed a decrease of Ca^{2+} in cytosol, increased gene expression of hypoxic factor *HIF-1α*, pro-apoptic *BAX*, and *Caspase 3/9* and decreased anti-oxidant enzymes gene expression. Intracellular Ca^{2+} concentration increases after onset of hypoxia and leads to open of voltage-gated Ca-channels and leads to overload calcium mitochondria which releases caspase cofactors, activates pro-apoptic Bad protein, at the same time blocks anti-apoptic Bcl-2 protein and increases pro-apoptic Bax protein. In cascade it turns into releasing of cytochrome c, formation of apoptosome and ends in apoptosis. We assume that the reason for triggering the apoptic cascade is the specific intercalation of Zn-NSAID complex into DNA and the regulation of this process at the gene level. This work was supported by Slovak grant agency project VEGA 1/0540/21.

P-03.2-007
Chemoresistance of osteosarcoma cells is independent of the level of mitochondrial fission protein DRP1

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Recent studies showed that perturbation of mitochondrial dynamics can sensitize various cancer types to anticancer drugs. Dynamin-related protein 1 (DRP1) is a crucial regulator of this dynamics, executing fission and promoting quality control of mitochondria. Here, we focused on the role of DRP1 in chemoresistance of osteosarcoma (OS) cells. OS is an aggressive pediatric neoplasm with overall survival rate of only 60%. Therapy-induced drug resistance of OS cells is a common cause of the treatment failure, leading to disease progression and fatal outcomes. Hence, strategies to re-sensitize drug-resistant tumors to chemotherapy are being intensively investigated. However, the role of mitochondrial dynamics in osteosarcoma drug resistance is not yet fully understood. To assess if DRP1 could serve as a therapy target, we utilized clones of post-therapy osteosarcoma derived cell line SAOS-2 with stably downregulated DRP1. Both DRP1-silenced (shDRP1) and control (shCTRL) clones were established using shDRP1 and scrambled shRNA lentiviral vectors, respectively. When compared to shCTRL, shDRP1 clones showed a significantly decreased levels of DRP1, including a dramatic downregulation of activating phosphorylation on Ser 616. However, cell viability assays did not reveal any DRP1-dependent differences in shDRP1 and shCTRL sensitivity to conventional chemotherapy (cisplatin, doxorubicin, vincristine, topotecan), targeted inhibitors (sunitinib, trametinib) and autophagy inhibitor (bafilomycin). Furthermore, the levels of other mitochondrial dynamics proteins (mitochondrial fission factor and mitofusin 2) were unchanged in shDRP1, which suggest that downregulation of DRP1 did not significantly affect mitochondrial dynamics. Overall, DRP1 was found to be a poor drug target in OS, as our results indicate that DRP1-idependent mechanisms of mitochondrial fission and quality control might compensate for DRP1 functions. Supported by the Czech Science Foundation (No. GJ20-00987Y).

P-03.2-008
Quantitative expression of BCL-2 and BAX, as parameters of apoptosis in colorectal cancer tissue

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Colorectal cancer is one of the most frequent neoplastic diseases in the human population and one of the most frequent causes of death. Bcl-2 and Bax are two of the most important proteins in the effector phase of apoptosis. They play a key role in tumor progression or inhibition of intrinsic apoptotic pathways and can determine the cellular fate. The aim of the present study was to assess the quantitative expression of Bcl-2 and Bax proteins in tumor tissue. The investigation was conducted in tissue homogenate of 42 primary colorectal cancers. As a control, the same amount of sample was collected from macroscopically unchanged colon regions of the most distant location to cancer. The sample was also collected from the closest healthy tissue adjacent to cancer. Bcl-2 and Bax expression was determined by method of indirect immunofluorescence. Our study demonstrated a statistically significant increase in the expression of BCL-2 compared to control ($P < 0.001$), and also an increase in surrounding tissue compared to control ($P < 0.01$). Expression of Bax was significantly lower compared to control ($P < 0.001$). Moreover, the Bax/Bcl-2 ratio was significantly lower in tumors resected from colon compared to healthy and adjacent tissue. These results suggest that colorectal carcinogenesis is associated with changes in apoptosis, which may be involved in the process of tumor invasion. This fact may be useful in determining the margins during tumor resection and individualisation of postoperative treatment. Bcl-2 and Bax might have predictive value as a potential molecular markers of colorectal cancer. Key words: Colorectal cancer, Bcl-2, Bax. This research was supported by the Science Fund of the Republic of Serbia, project number: 7750154 (NPATPETTMPCB).

P-03.2-009
Novel transcripts of human apoptosis-related BCL2-ovarian killer (BOK) question our knowledge about this gene and its products in human cancer cells

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BCL2-ovarian killer (BOK) is an intriguing member of the apoptosis-related BCL2 family. It has an ambiguous role that could be attributed to the presence of multiple BOK protein isoforms bearing different combinations of BCL2-homology (BH) domains and encoded by distinct transcripts resulting from alternative splicing. In this study, we used cutting-edge technologies to search for novel alternative mRNAs of the BOK gene in a panel of 37 human cancer cell lines. Briefly, total RNA was extracted from each cell line and reverse-transcribed using an oligo-dT adaptor. Next, we used nested PCR to determine novel coding regions and 3′ rapid amplification of cDNA ends (3′RACE) to identify unknown 3′ untranslated regions (UTRs), followed by Sanger sequencing, next-generation sequencing and nanopore (long-read) sequencing. The most frequent transcripts were confirmed by PCR and validated using single-molecule resolution RNA fluorescent in situ hybridization (smFISH). We found out that transcripts skipping exon 2 – which is currently considered to comprise the main translation start codon – are much more abundant than their counterparts containing this exon. In this case, the alternative translation start codon in exon 4 (or in exon 5) may be utilized. Moreover, transcripts also lacking exon 5 (or both exons 4 and 5) are expressed, as well. All of these transcripts may be combined with the two known 5′ UTRs or a novel one. Moreover, truncated exons 2, 4, 5 and 6 are frequently present. Exon 6 may also be split in two exons, or spliced with other exons located downstream (up to 11,400nt). Overall, our findings suggest that the human BOK gene spans a much larger genomic region, produces transcripts with multiple alternative 5′ and 3′ UTRs, encodes dozens of distinct transcripts in distinct cell lines with many of them having an open reading frame. These findings have the potential to change our current knowledge about the major mRNAs and protein isoform(s) produced by this gene.

P-03.2-010
The pore forming activity of Bax protein in mitochondrial like membranes upon oxidative stress conditions

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Apoptosis is crucial in embryogenesis, regulation of tissue homeostasis, and removal of harmful cells in higher organisms. The permeabilization of the mitochondrial outer membrane (MOM), followed by the release of cytochrome c, represents a point of no return in apoptotic progression. The crucial protein of the MOM permeabilization process is the pro-apoptotic Bax protein which upon stress-induced activation translocates to the MOM, inhibits there the pro-survival Bcl-2 protein, and induces pore formation. In the current model, the inactive Bax monomer is unable to permeabilize model lipid bilayer mimicking the MOM without the presence of Bax activation protein cBid, which also promotes active Bax dimer formation, a basal building block of MOM pores. It was found that the mitochondrial membrane system seems to play an active role with its lipids directly involved. Under oxidative stress conditions, oxidized lipids can be generated and directly involved in mitochondrial apoptosis. To characterize this phenomenon in more detail we added oxidized phospholipids into model MOM vesicles (GUVs, LUVs). Our vesicle leakage studies show, that the type and concentration of oxidized phospholipids in the model membrane has a significant effect on Bax-induced permeabilization of the membrane.

P-03.2-011
Fungal lectin CNL triggers atypical cell death selectively in Jurkat cells

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Lectins are carbohydrate-binding proteins with important molecular recognition functions in living organisms. Binding of lectins to glycans on the cell surface can lead to activation of receptors, their cross-linking into clusters destined for endocytosis, or

altered membrane permeability. Lectins can have either toxic, antiproliferative, or mitogenic effects on cells. The fungal lectin *Clitocybe nebularis* lectin (CNL) binds specifically to GalNAcβ1-4GlcNAc (LacDiNAc) and exhibits an exclusive antiproliferative effect on the leukemic T cell line Jurkat. CNL binds to the cell surface glycoproteins CD45 and CD43 and induces dose-dependent cell death and homotypic aggregation. Examination of characteristics of different pathways of programmed cell death reveals that CNL induces atypical cell death. CNL-induced cell death is characterized by condensation of nuclear chromatin, release of phosphatidylserine without activation of caspases, and leakage of the nuclear protein HMGB1 (damage-associated molecular pattern molecule high-mobility group box 1) into the cell culture medium. Interestingly, a plant lectin, *Wisteria floribunda* agglutinin (WFA), which also binds specifically to LacDiNAc, showed less selective cytotoxicity and triggered cell death in Jurkat cells, Tall-104 acute lymphoblastic leukemia, and Hut-87 cutaneous T-cell lymphoma cell lines with similar atypical features. Selective targeting of Jurkat T cells may reflect the potential applicability of CNL in novel strategies to treat and/or detect T-cell acute leukemia. [Perišić Nanut, M, Žurga, S, Konjar, Š, Prunk, M, Kos, J, Sabotič, J. The fungal Clitocybe nebularis lectin binds distinct cell surface glycoprotein receptors to induce cell death selectively in Jurkat cells. FASEB J. 2022; 36: e22215.]

P-03.2-012
URG7 and ER stress in neurodegeneration: a lucky intuition or a flash pan?

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The maintenance of protein homeostasis, defined as proteostasis, through the fine regulation and control of protein synthesis, trafficking, folding, secretion, degradation and signaling pathways is crucial for proper cellular functioning. There are several known pathological conditions caused by the perturbation of proteostasis, collectively referred to as 'proteinopathies' or PMDs (Protein Misfolded Disorders), all characterized by the accumulation of proteins that in an unusual way aggregate or misfold and also by oxidative stress and malfunction mitochondrial. The Unfolded Protein Response (UPR), protein ubiquitination and autophagy are cellular responses to protein misfolding stress, but if the stress is severe and prolonged, apoptosis is activated [Xicoy H. et al (2017), Molecular Neurodegeneration]. URG7 (Up-regulated Gene clone 7), an ER-resident protein whose expression is upregulated during HBV infection, is able to modulate the expression of UPR markers towards survival outcomes and reduces the ER stress by decreasing the amount of unfolded proteins in hepatic [Armentano MF et al. (2018) Biol Cell 110,147158] and in human neuroblastoma cell lines (SH-SY5Y), both stressed with tunicamycin. In light of these results, the activity of this protein in SH-SY5Y was further characterized. Our results showed a reduction of the cleaved form of PARP-1 (cPARP-1) and an increase of Beclin and LC3II proteins in cells stably infected with URG7 and treated with tunicamycin, suggesting the possible anti-apoptotic activity of URG7 and its potential involvement in the autophagic process. Furthermore, since calcium is one of the key regulators of cell survival and can induce ER stress-mediated apoptosis [Entaz B. et al (2016) Int J Mol Sci 17 (9), 1559], the change in calcium concentration under ER stress was evaluated. Our data support the hypothesis that URG7 is able to decrease Ca^{2+} release from the ER to the cytosol, confirming its hypothetical anti-apoptotic action.

Molecular immunology

P-03.3-001
Barnase interactions with barstar tune CART-cells activity against solid tumors

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Immunotherapy using T cells modified with chimeric antigen receptors (CART) is one of the most promising directions in the therapy of oncological diseases. CART therapy has shown impressive results in studies of hematologic malignancies. However, in light of the complications associated with uncontrolled hyperactivation and nonspecific cytotoxicity, there is a high risk of developing life-threatening side effects, most notably a cytokine storm. Therefore, the ability to regulate the activity of CART cells in real time during therapy is currently the number one task. Small protein mediators between CART cells and the antigen-recognizing moiety allow concentration control of CART cytotoxicity by abrogating their action when needed and targeting multiple receptors with a single CART. Here, we propose use of the barnase-barstar interface as a novel principle for switching on and off the cytotoxic effect of CART. We have developed fusion proteins of anti-HER2 DARPins in which barnase acts as a molecular mediator responsible for binding CART cells to HER2-positive tumor cells at a nanomolar concentration range. The DARPin barnase module has been shown *in vitro* to be a potent switch that activates BsCART against HER2 receptor-positive cancer cells. We have demonstrated dose-dependent cytotoxicity and cytokine release of CART against HER2-positive tumor cells. Finally, we demonstrated the efficacy of therapy with this modular approach against solid tumors in vivo. This study was supported by Ministry of Science and higher education of the Russian Federation project №. 2020-1902-01-302.

P-03.3-002
Hypersensitivity of iPSC-derivatives to autologous NK-cells is caused by increased expression of ligands for DNAM-1 and NKG2D family receptors

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The crucial problem of modern transplantation medicine is the donor organ shortage crisis. Patient-specific induced pluripotent

stem cells (iPSCs) offer the opportunity of personalized therapy without a lifelong regimen of immunosuppressive drugs. However, some reports have pointed to the immune response to syngeneic and autologous iPSC-derivatives. This issue raises concerns regarding the complete immunological tolerance of autologous iPSC-derivatives. Our study is dedicated to the study of NK-cell response against various iPSC-derivatives. According to the "missing-self" hypothesis, the primary function of NK-cells is the recognition of cells lacking self HLA class I molecules. Here we report that fibroblast- and chondrocyte-like cells differentiated from iPSCs provoked the degranulation of autologous NK-cell lytic granules. Isogenic iPSC-derivatives that do not express HLA I proteins due to knockout of the *B2M* gene elicited comparable high levels of NK-cell activation. At the same time, the initial isogenic skin fibroblasts used to obtain iPSCs nearly did not trigger NK-cell cytotoxicity. Thus, we assumed that the differentiation and following cultivation of iPSC-derivatives might negatively affect the balance of ligands for activating and inhibiting NK-cell receptors. We found that this balance in iPSC-derivatives was indeed violated. Compared with the initial somatic cells, the expression of HLA-I molecules in iPSC-derivatives was reduced, but the expression of ligands to the DNAM-1 and NKG2D activating receptors was increased. We showed that pre-treatment of iPSC-derivatives with the proinflammatory cytokine IFNγ increases the expression of HLA-I molecules, which leads to a decrease in the activation of NK-cells. In this regard, the balance of ligands for NK-cell receptors should be taken into account in iPSC-based cell therapies. This work was supported by the RFBR grants #20-315-90041 and #19-29-04113-mk.

P-03.3-003
Regenerative and inflammatory effects of anti-reflux surgery on human esophageal mucosa: what does the epithelium say even if the symptoms are gone?

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Reflux symptoms heal in most patients after laparoscopic anti-reflux surgery (LARC). Since LARC reduces contact with gastric contents such as acid, pepsin, and bile in the esophagus. The aim of this study was to investigate the effect of LARC on inflammatory process at molecular level in oesophagus epithelium. The esophageal biopsy samples were collected from 20 healthy volunteers and 22 patients with anti-reflux surgery (before and after the surgery). The post-LARC group was recruited approximately 6 months (2-14 months) after their operations. Panels of inflammatory molecules and related pathways were screened by RT-PCR and Multiplex-ELISA methods. NFkB, MEK1 and p38-MAPK levels were significantly increased in post-LARC. Also, in Post-LARC IL-1B, IFN-G, CXCL2, CCL25, CCL27, CXCL12, CCL17, CCL1, CCL20, MIF, IL-10, CCL1, CCL7, CCL8, CCL13, CCL26, CCL11, CCL17 and IL- 4 levels were significantly higher. Levels of CCL21 and CCL17 and CCL2 (-2.3-fold), EGF (-2.2-fold) genes were significantly increased in Pre-LARC compared to healthy subjects. mRNA expressions of AKT1 (-1.7-fold), FGF2 (-5.4-fold), HRAS (-2.2-fold), IL-1B (-2.2-fold), MAP2K4 (-2.4-fold) in post-LARC were also significantly decreased versus Pre-LARC. The presence of pro-inflammatory proteins such as IL-1B, IFN-G, CXCL2 in post-LARC indicated that the inflammatory response is still activated. In this group, the higher levels of homeostatic cytokines such as CCL25, CCL27, CXCL12, CCL17, CCL1, CCL20 and MIF demonstrated that cell homeostasis is maintained at approximately 6 months after the operation. The presence of anti-inflammatory response proteins such as IL-10, CCL1, CCL7, CCL8, CCL13, CCL26, CCL11, CCL17 and IL-4 showed that the inflammatory damage in patients with gastroesophageal reflux tried to be suppressed and the postoperative recovery process continues. We thank TUBITAK for their support of our study (1001 Project-ID:118S260).

P-03.3-004
Abstract withdrawn

P-03.3-005
Distinct profiles of allergic inflammation induced by Der p 2 allergen in BALB/c and C57BL/6 mice

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Der p 2 is one of the most clinically relevant house dust mite (HDM) allergens causing IgE-mediated responses in over 80% of the HDM allergic individuals, capable of activating both the innate and adaptive immune system (Batard T. et al.; (2016) J. Allergy Clin. Immunol 71, 220-229). It has been identified as a homolog of myeloid differentiation factor-2 (MD-2) the LPS binding co-receptor of Toll-like receptor 4 (TLR4) (Jacquet A. (2021) Front. Allergy 2, 1-8). Here we established a protocol for HDM allergy induction in two strains of mice, BALB/c and C57BL/6, to investigate the effect of mice phenotype on the profile of allergic inflammation to Der p 2. The recombinant Der p 2 was produced in *E. coli*, purified (IMAC) and characterized by circular dichroism spectroscopy and mass spectrometry. Female C57BL/6 and BALB/c mice were immunized with HDM extract and Der p 2 either subcutaneously or subcutaneously followed by inhalation of Der p 2 or HDM extract. After challenge, the mice were euthanized; blood, bronchoalveolar lavage (BAL), spleens and lungs were collected. Cells from BAL were identified by May-Grunwald Giemsa staining and microscopy and lung leukocyte populations were analyzed by flow cytometry. Cytokines (IL-4, IFN-γ and IL-10) and the presence of Der p 2 specific IgE and IgG antibodies were assessed by ELISA. The Th2 immune response was confirmed by elevated allergen-specific IgE and the allergic reaction was evidenced by infiltration of eosinophils and/or neutrophils into BAL. We noticed that BALB/c mice were inefficient in integrating local with systemic immune response, evidenced by almost no IgG or IgE production upon one subcutaneous injection and subsequent inhalation of Der p2; also the BAL infiltrate in these mice consisted of neutrophil infiltration, unlike C57BL/6 mice in which eosiniphilic infiltrate predominated. The differences between the mice strains could be exploited for generating different types of responses to the Der p 2 allergen.

P-03.3-006
CD45 knockout as a strategy for generation universal T and CART cells

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Treatment with T-cells modified by the chimeric antigen receptor (CARTs) technology remains challenging to attain cost reduction and protocol streamlining. A possible solution is to generate allogeneic universal CARTs (uCARTs), that do not develop GvHD. With that aim, we use CRISPR/Cas9 technology to generate a knockout (KO) of notable target – CD45 molecule. All leukocytes express this tyrosine phosphatase that is extremely important for regulating TCR activity and signaling but not CAR signaling. Therefore, it opens opportunities for creating active uCARTs. Our study aims to generate CD45KO T-cells to assess their survival, proliferation, behavior, and susceptibility to lentiviral transduction. We found suitable gRNA variants and optimized the conditions of the KO and further maintenance of CD45KO T-cells. The KO was triple confirmed: by cytometry, western blotting, and Sanger sequencing. We also compared RNA profiles in KO and non-KO T-cells. In addition, we evaluated the activity of TCR in KO T-cells. After establishing that the cells perfectly tolerate KO and their proliferation equals non-KO ones, we used them to create CD45KO CARTs by lentiviral transduction. *In vitro* analysis was performed to evaluate their activation and cytotoxic activity. These experimental data make it possible to verify that modified and conventional T-cells tolerate the CD45 gene KO. Despite the ambiguous literature data about T-cells survival with non-functioning CD45, KO does not affect T-cell survival. Furthermore, CD45KO CARTs are functionally active and provide the necessary level of CAR activation, which has been confirmed in vitro and in vivo. We have also demonstrated that TCR does not work in the case of a KO, which provides the possibility of using this technology to create uCARTs, greatly simplifying the pipeline and reducing the cost of therapy. This study was supported by the Ministry of Science and Higher Education of the Russian Federation project № 075152020773.

P-03.3-007
Candidates-biomarkers for the express test-system to diagnosis of SARS-CoV-2-induced immunopathology

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the virus that causes coronavirus disease 2019 (COVID-19), the respiratory illness responsible for an ongoing global COVID-19 pandemic. The clinical course of COVID-19 varies from mild symptoms to acute respiratory distress syndrome, hyperinflammation, and coagulation disorder. Gene expression analysis in peripheral blood cells (PBCs) is valuable to evaluate disease-associated and drug-response related genes. In this study, we aimed to investigate the gene expression profile of PBCs in patients with COVID-19. Whole blood samples were collected from patients with acute COVID-19 infection (n = 52) and healthy volunteers (n = 59). The gene expression of PBCs was determined by RT-qPCR. We investigated the expression of cytokines, interferon-stimulated, CD4, and coagulation genes in PBCs of the infected and healthy samples. Hyperexpression mRNA level of the *OAS1, RNASEL, MX1, EIE2AK2, IL8, IL6, IL10, F5* was found out in the blood of SARS-CoV-2-infected patients compared to the healthy sample. We also studied downexpression mRNA level of *CD4* in PBCs of SARS-CoV-2-infected patients compared to the healthy volunteers. We have identified the *OAS1, RNASEL, MX1, EIE2AK2, IL8, IL6, IL10, F5, CD4* genes in whole blood that classifies SARS-CoV-2-infected and healthy patients with good accuracy. Using the ROC curve we found out that *F5, MX1, RNASEL, CD4, IL10, EIF2AK2* genes are promising candidates-biomarkers for the express test-system to early diagnosis of COVID-19 immunopathologies (hyperinflammation, coagulation disorders, lymphopenia). These results suggested that the expression of cytokines, coagulation, CD4, interferon-stimulated genes in PBCs can be used for early detection of hyperinflammation, coagulation disorders, lymphopenia at COVID-19, and evaluation of efficiency treatment of this disease.

P-03.3-008
Identification of SARS-CoV-2 neutralizing antibodies using engineered bacteriophage bioconjugates

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Novel severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) infection, raised in late 2019 and unabated for more than two years, still makes challenges for modern society. New variants of SARS-CoV-2 tend to arise often and to displace previous ones, hence the development of a high-throughput and universal platform allowing the selection of antibodies with the highest neutralization and binding activity is of interest. Using our previously developed public platform for high-throughput B cell specificity screening, based on a combination of phage display and flow cytometry techniques, we constructed phage library with surface-exposed RBD (the major target for the development of neutralizing antibodies) peptides. We then constructed lentiviral scFv libraries in Jurkat cells. For these libraries, we used B cells from three donor groups: (i) SARS-CoV-2 recovered patients; (ii) Sputnik V vaccinated donors and (iii) healthy unvaccinated donors. We compared repertoires of RBD-specific antibodies in recovered and vaccinated donors. In addition, we compared the antibody repertoires selected with full-length RBD molecule and phage RBD peptides library. Selected clones with anchored scFvs were screened for neutralizing activity. The best clones from each sample were obtained as full-length immunoglobulins G and characterized for their neutralization activity. Our data indicate that the investigated technique can be successfully used for a comprehensive search for virus-neutralizing antibodies. This study was supported by Russian Science Foundation grant # 17-74-30019

P-03.3-009
Development of a competitive ELISA kit for evaluation of SARS-CoV-2 neutralizing antibody capacity in vaccinated individuals

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The COVID-19 pandemic, caused by the SARS-CoV-2 virus, has been devastating human lives since 2019.While each country carries out their vaccination program, many of them also continue to work on vaccine development. Determination of SARS-CoV-2 neutralizing antibodies offers important information for evaluating immune responses to the virus. Neutralization capacity can contribute to the understanding of subjects such as the immune status of individuals, the need for revaccination, relapse and recovery from the disease, as well as evaluation of vaccine efficacy. Neutralizing antibodies block the virus by preventing the interaction of the virus's S protein receptor binding domain (RBD) with human angiotensin converting enzyme-2(ACE-2). The most commonly used methods for the detection of neutralizing antibody titer are cell culture experiments with the virus such as the plaque neutralization assay. While these tests require biosafety level 3 conditions, ELISA tests can be performed in general laboratory without any sterile environment. Furthermore, while traditional methods take 2-3 days, ELISA stands out with its ease of application and can give results in just 90 minutes. In this study, we developed a prototype ELISA kit which is based on the principle of blocking the protein-protein interaction between RBD-HRP and ACE-2 with a possible neutralizing antibody in serum. For this purpose, the RBD protein was first labeled with horseradish peroxidase (HRP). Optimum use concentrations of RBD-HRP protein and human serum in the selected dilution buffer and the amount of ACE-2 protein to be coated on the ELISA plate were determined. As a result of repeated studies with a large scale of serum, the coefficients of variation(CV) for intra/inter-assay were calculated as 10% and 12%, respectively. Preliminary results of accelerated shelf-life studies showed that the ELISA kit maintained its shelf life up to 1 year at +4°C. This work is supported by TUBITAK (Project No 17H001 and 18AG020).

P-03.3-010
S-nitrosation orchestrates the appearance of cytoplasmic DNA in endothelial cells

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In mammals, DNA is generally confined to the cell nucleus and mitochondria. Its presence in the cytosol is abnormal and may indicate threatening conditions. Therefore, the detection of cytosolic DNA is fundamental to controlling cellular homeostasis. One of the pivotal post-translational modifications governing cellular fate is S-nitrosation. Our preliminary studies show that increased S-nitrosation in endothelial cells promotes the release of DNA into the cytoplasm, where it forms inclusions, which fully colocalise with protein aggregates. We aimed to elucidate the molecular mechanisms underlying this phenomenon. Therefore, we employed a wide range of molecular biology methods and a physical approach with atomic force microscopy. We observed that S-nitrosation causes DNA damage and alters nuclear envelope architecture and permeability. All these, together with actin-related nuclear confinement, enable the appearance of cytoplasmic DNA. Whereas it increases cytoplasmic DNases DNASE2A and TREX1, the canonical STING-driven response to cytoplasmic DNA is abolished. Conversely, we found that DNA is instantly secreted from cells, which can be further intensified by the induction of autophagy. Accumulation of protein aggregates on cytoplasmic DNA presumably serves as a signal for autophagy activation to enable the disposal of cytoplasmic DNA. To sum up, our data suggest a novel, mechanistic model of nuclear DNA release and of cellular response to self-DNA, which can be valid in conditions with aberrant S-nitrosation, like in inflammation. Moreover, these observations can give valuable insights into understanding the basis of tumorigenicity and autoimmune diseases.

P-03.3-011
Pyrazole derivative, immunosuppressant YM-58483, inhibits store-operated Ca^{2+} entry in peritoneal macrophages

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Store-operated Ca^{2+} entry (SOCE) is a ubiquitous mechanism of regulated Ca^{2+} entry in eukaryotic cells. It is activated upon depletion of intracellular Ca^{2+} stores and is involved in regulation of a wide range of cellular processes (exocytosis, gene expression, activation of immune cells, cell growth and proliferation, etc.) in normal and pathological conditions. After discovery of the important role of SOCE in the pathogenesis of human diseases (severe combined immunodeficiency, nasal polyposis, etc.), the researcher's interest in the development of low molecular weight blockers of SOCE increased. Thus, 3,5-bis(trifluoromethyl) pyrazole derivatives, specifically immunosuppressant YM-58483, have been proposed as potent inhibitors of SOCE. Taking into account the important role of SOCE in the functioning of immune cells, we elucidated the pharmacological characteristics of SOCE in rat peritoneal macrophages under the influence of YM-58483. To activate SOCE, we used two structurally different endoplasmic Ca^{2+}-ATPase inhibitors, thapsigargin and cyclopiazonic acid, which induced passive depletion of Ca^{2+} stores, as well as the disulfide-containing immunomodulators glutoxim® (disodium salt of oxidized glutathione with d-metal at nanoconcentration, PHARMA-VAM, Saint-Petersburg) and molixan® (complex of glutoxim and inosine nucleoside). Using Fura-2AM microfluorimetry, we demonstrated that the addition of 5 μM YM-58483 during already developed SOCE induced by thapsigargin, cyclopiazonic acid, glutoxim or molixan, led to almost complete suppression of SOCE. Thus, we have shown for the first time that in rat peritoneal macrophages, as well as in other cell types, pyrazole derivative YM-58483 effectively inhibits SOCE and is a useful pharmacological tool to study SOCE in macrophages. The results additionally confirm that Ca^{2+} entry induced by glutoxim or molixan is realized via the store-dependent mechanism.

P-03.3-012
Response of inflammatory cytokines TNF, IL-6 and neurotrophin 3 (NT-3) in patients with secondary peritonitis

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Peritonitis triggers an acute and simultaneous systemic release of pro-inflammatory cytokines and neurotrophic factors, such as neurotrophin-3 (NT-3), a member of neurotrophic factors, which can regulate inflammatory processes by direct interaction with the inflammatory cellular environment. Peritonitis is a surgical emergency. Our aim was to investigate the possible link between NT3 and the inflammatory cytokines TNFα and IL6 in patients with secondary peritonitis. We conducted a study of 60 hospitalized patients diagnosed with diffuse acute peritonitis caused by perforation. Their serum levels (pg/ml) of inflammatory cytokines TNF-alpha and IL-6, as well as NT-3 were determined preoperatively (D1) and postoperatively (D7) using commercial ELISA kits. Compared to preoperative time, postoperative TNF-alpha was significantly decreased [357 ± 255 (D1) vs 210 ± 94 (D7), $P < 0.05$] in survivors, but showed a threefold increase in deceased patients.[215 (D1) vs. 666 (D7)]. Survivors were divided into two groups according to NT-3 dynamics: group 1 showed a postoperative increase in mean NT-3 [105 ± 83 (D1) vs 217 ± 12 (D7) and group 2 a postoperative decrease with 40% of the mean NT-3 level [85 (D1) vs 51 (D7)]. In D7, the increase in NT-3 levels in group 1 was correlated with the decrease in TNF-alpha levels [333 ± 99 (D1) vs 253 ± 87 (D7), $P < 0.05$], and the decrease in NT-3 levels in group 2 was correlated with decreased IL-6 levels [1188 ± 105 (D1) vs. 43 ± 27 (D7), $P < 0.05$]. In deceased patients, a late response was found to be TNF-alpha [0 (D1) vs 52 (D7)] in 25% of cases or IL-6 [0 (D1) vs 427 (D7)] in 75% of cases. In conclusion, the favorable evolution of patients with acute peritonitis was characterized by a negative postoperative (D7) correlation between TNF-alpha and NT-3 and a positive one between IL-6 and NT-3. In contrast, the severe course of death has been associated with late TNFα or IL-6 responses which led to a loss of correlation with NT-3.

P-03.3-013
Autoantibodies against citrullinated adenosine deaminase at rheumatoid arthritis

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Rheumatoid arthritis (RA) is an autoimmune chronic disease accompanied with inflammation and destruction of joints. The discovery of new citrullinated proteins as a new autoantigens in blood plasma of RA patients and detection of anti-citrullinated protein antibodies developed against them increases the specificity of diagnosis, and opens new perspectives in the therapy of rheumatology. Adenosine deaminase (ADA) is one of key enzymes in purine metabolism. It is essential in differentiation and maturation of lymphoid cells, development of immune system in human. An increase of ADA activity is documented in such pathologies as tuberculosis, cancer, RA, etc. There are two molecular forms of ADA in tissues: the catalytically active protein, intracellular iADA, with Mm of 36-40kDa and its extracellular complex with DPPIV, eADA. (Mm of 260-300kDa). In this work, we present that in synovial fluids (SFs) of RA patients, unlike in gout, ankylosing spondylitis, juvenile idiopathic and reactive arthritis, with ADA activity 40-135U/ L the purified iADA is citrullinated up to molar ratio of 5-9. It should be noted that eADA at all investigated five arthritis types including RA isn't citrullinated. The specific antibodies (IgG) (67.5mg/ml) against purified citrullinated iADA (0.45 mg/ml) from SFs of RA patients were obtained by rabbit immunization which were characterized by ELISA assay. The binding between iADA (1.1mg/ml, as an antigen), with tenfold diluted of RA SF, as well with IgG (15mg/ml) isolated and purified from plasma of rabbit were revealed. The titer of obtained IgG was 4.5. The results indicate that IgGs contained in SFs of RA patients are identical the antibodies isolated from serum of immunized rabbit. Consequently, it may be assumed that obtained antibodies can be used in diagnosis to detect the modified enzyme as the new autoantigen at RA.

P-03.3-014
Antibody response to SARS-CoV-2 infection and Sputnik V vaccination

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Antibodies to the SARS-CoV-2 S-protein and its RBD fragment are considered the primary immune response markers against the SARS-CoV-2. We analyzed the content of antibodies against the S-protein and RBD of SARS-CoV-2 in the blood of employees of the SB RAS Institute of Chemical Biology and Fundamental Medicine. In 2020, some researchers were infected with COVID-19; in January-April of 2021, more than 80% of the Institute staff were vaccinated with Sputnik V. In May 2021, blood samples from 396 donors were collected. Using ELISA, we analyzed the presence of antibodies against the S- and N-proteins of the SARS-CoV-2 in the donor's blood plasma of four groups: COVID-19 convalescents, vaccinated with Sputnik V (group I); patients vaccinated with Sputnik V that were not infected with COVID-19 (group II); not vaccinated COVID-19 convalescents (group III); patients that were not vaccinated and infected with SARS-CoV-2 (group IV). The blood of the group I patients had the highest amount of antibodies to the S- and N-proteins of the SARS-CoV-2. The level of antibodies against S-protein in group II was higher than in group III. Next, electrophoretically homogeneous preparations of IgG were isolated from blood plasma by affinity chromatography on Protein-G-Sepharose. Further, using affinity chromatography on sorbents containing immobilized recombinant SARS-CoV-2 S-protein and RBD, we isolated antibody subfractions possessing affinity to these antigens (S-IgG and RBD-IgG, respectively). The content of RBD-IgG in the blood plasma of convalescent and vaccinated donors is about 1%. Antibodies of this subfraction have an affinity for other domains of the S-protein than RBD. The amount of these antibodies was less than 1%. In further studies, we plan to characterize S-IgG and RBD-IgG preparations and compare them with the total pool of IgG in vaccinated and/or recovered donors. The work was supported by the Russian Science Foundation 21-75-10105 (to Timofeeva A. M.)

P-03.3-015
Cystatin F expression, activation and glycosylation regulates NK cell cytotoxicity

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Cystatin F plays a central role in the regulation of cathepsins in cytotoxic immune cells. This type II family cystatin has distinctive features, such as its ability to be secreted and internalized into bystander cells via mannose-6-phosphate pathway. It is also located intracellularly in the endo/lysosomes, where it is activated from inactive dimeric form by proteolytic cleavage by cathepsin V, making cystatin F a strong inhibitor of cathepsin C. Cathepsin C is indispensable for the cytotoxic function of natural killer (NK) cells, as it enables the activation of granzymes, which mediate the cytotoxic effects on cancer cells. Cystatin F is normally expressed by immune cells, but its expression was found to be increased also in the tumor microenvironment. The increased levels of cystatin F may contribute to the immunosuppressive status of the tumor microenvironment. We have shown that recombinant cystatin F, added to cell media, was internalized to primary NK cells and decreased cathepsin C and granzyme B activities resulting in reduced cytotoxic capacity of NK cells. Incubation of NK-92 cells with selective reversible inhibitor of cathepsin V increased the amount of inactive dimeric cystatin F in NK-92 cells and consequently the cytotoxicity of NK-92 cells. Treatment of NK-92 cells with different N-glycosylation inhibitors affected cystatin F glycosylation. In NK-92 cells N-glycosylation inhibitor tunicamycin caused decreased localization of cystatin F in lysosomal compartments and increased cathepsin C activity. In contrast, increasing high mannose glycosylation with kifunensine resulted in increased localization of cystatin F in the lysosomes and decreased cathepsin C activity in NK-92 cells, which reduced their cytotoxic potential. By targeting the expression of cystatin F, the activating protease cathepsin V or modulating the glycosylation of cystatin F, we can reduce the detrimental effects of cystatin F on cytotoxic cells in the tumor microenvironment.

P-03.3-016
Role of monocyte chemoattractant protein-induced protein 1 in liver fibrosis and activation of hepatic stellate cells

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Monocyte chemoattractant protein-induced protein 1 (MCPIP1) is a negative regulator of inflammation, acting through cleavage of transcripts coding for proinflammatory cytokines. It was shown that Mcpip1 deletion in liver epithelial cells in Mcpip1$^{fl/fl}$Alb^{Cre} mice resembles features of primary biliary cholangitis. In this study, we analyzed MCPIP1 level in human fibrotic livers and hepatic cells isolated from CCl_4-treated mice. We also investigated MCPIP1 impact on activation of LX-2 hepatic stellate cell line. We analyzed liver biopsies isolated from 30 patients with fibrosis stage ranging from 0 to 3. To induce liver fibrosis during *in vivo* experiments, mice were injected with CCl_4 dissolved in corn oil (0.2 mg/g), 3 times a week for 4 weeks. *In vitro* experiments were performed on LX-2 cells treated with TGF-β (5/10 ng/ml) to induce their activation. Analysis of patients' liver biopsies revealed that MCPIP1 level in liver tissue correlates with the development of fibrosis. To elucidate in which type of cells Mcpip1 level is induced during fibrotic changes, we analyzed its level in hepatocytes, HSCs and Kupffer cells isolated from fibrotic murine livers. We found that treatment with CCl_4 induces Mcpip1 expression in hepatocytes and hepatic stellate cells. To evaluate role of MCPIP1 in activation of HSCs, we stimulated LX-2 cell line with TGF-β which resulted in increase of MCPIP1 level. Moreover, overexpression of MCPIP1 in LX-2 cells led to decreased mRNA expression of HSCs activation markers e.g. ACTA2, TGFB1, COL1A1 and α-SMA protein level. Contrary, MCPIP1 silencing in LX-2 cells resulted in their increased activation status. In conclusion, we demonstrated a potent MCPIP1 role in liver fibrosis and activation of hepatic stellate cells. MCPIP1 presence in HSCs can be essential to prevent their excessive activation. This study was supported by National Science Centre, grant numbers 2019/33/N/NZ5/00916 and 2017/27/B/NZ5/01440.

P-03.3-017
The role of Mcpip1 in cutaneous interaction of keratinocytes and immune cells

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Monocyte chemotactic protein-1-induced protein 1 (MCPIP1) is an RNase that negatively regulates stability of transcripts coding for pro-inflammatory cytokines. In human epidermis, MCPIP1 localizes predominantly in the suprabasal layers. Our previous study showed that keratinocyte-specific Mcpip1-deficient (Mcpip1^{EKO}) mice showed impaired skin integrity and an imbalance of keratinocyte proliferation and differentiation. While skin is mainly composed of keratinocytes, it also contains other types of cells, such as immune cells and melanocytes, which together provide skin homeostasis. The aim of this research was to study the role of Mcpip1 in the interaction of keratinocytes and immune cells, which is poorly understood. We first examined skin phenotype in mice with depletion of Mcpip1 in myeloid cells (Mcpip1^{MKO}), which revealed ongoing proallergic inflammation. We found infiltration of eosinophils and mast cells to the skin, in correlation with increased Th2-type cytokines and chemokines levels. Next, we generated double knockout mice with depletion of Mcpip1 in both keratinocyte and myeloid cells (Mcpip1^{EMKO}).

Those mice developed systemic inflammation earlier than each of the single knockout mice. RNA-seq analysis of skin from 12-week-old mice showed significant changes between all genotypes, pointing to a greater influence of keratinocyte- compared to myeloid-Mcpip1 in disturbances of skin homeostasis. Gene Ontology analysis assigned downregulated in Mcpip1^{MKO} mice genes to processes related to lipid metabolism and keratinization, while those processes were upregulated in Mcpip1^{EKO} mice. We identified 152 differentially expressed genes between Mcpip1^{EMKO} and Mcpip1^{EKO} mice and only 37 in case of the Mcpip1^{EMKO} and Mcpip1^{MKO}. In conclusion, our work revealed novel and distinct mechanisms by which keratinocyte- and myeloid-Mcpip1 ensures proper maintenance of skin homeostasis. This study was supported by the National Science Centre grant number 2020/37/N/NZ5/00575.

P-03.3-018
Inhibition of ER aminopeptidases leads to shifts in the immunopeptidome of cancer cells and the presentation of novel and potentially antigenic peptides

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ER aminopeptidases, ERAP1 and ERAP2, play key roles in the generation of antigenic peptides and can regulate adaptive immune responses and the immunogenicity of cancer cells. As a result, both enzymes are emerging as potential drug targets for cancer immunotherapy approaches. To better understand the effects of chemical inhibition of each enzyme on the antigenicity of cells, we utilized recently developed selective inhibitors targeting the active or allosteric sites of these enzymes, to analyze the changes in the immunopeptidome of the A375 melanoma cell line and MOLT-4 T lymphoblast leukemia cell line. We treated each cell line with inhibitors, isolated Major Histocompatibility class I molecules (MHCI) and sequenced bound peptides by liquid chromatography tandem mass spectrometry. In both cases, inhibitor treatment induced significant shifts on the immunopeptidome but with distinct patterns for each enzyme. ERAP1 inhibition led to changes in the length and motif of presented peptides, whereas ERAP2 inhibition primarily induced the presentation of many novel peptides. In both cases, most presented peptides were predicted to be optimal MHCI ligands and thus be potentially immunogenic. Our results suggest that ERAP1 is important for optimizing the length and sequence of the cellular immunopeptidome whereas ERAP2 primarily limits the presentation of some antigens. Inhibition of each or both enzymes in combination, could be used to enhance the immunogenicity of cancer and potentially synergize with immune checkpoint inhibitor immunotherapy. This work was supported by funds from the Special Account for Research Grants of National and Kapodistrian University of Athens (code 17454).

P-03.3-019
Immunological and biochemical changes in transgenic mice – a model of human multiple sclerosis

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In multiple sclerosis (MS) and other autoimmune demyelinating disorders, both autoreactive T lymphocytes and B lymphocytes play an important role in the pathological mechanisms. Different manifestations of demyelinating diseases are reproduced in animal models for investigation of pathological mechanisms and development of treatment. A widely used model is spontaneous or induced by immunization experimental autoimmune encephalomyelitis (EAE) in various strains of mice, such as autoimmune prone C57BL/6 line, transgenic 2D2 line with a specific to the peptide of myelin-oligodendrocyte glycoprotein (MOG_{35-55}) T cell receptor or Th mice with a specific to MOG_{35-55} IgH on the surface of B cells. In this work, we analyzed the changes in biochemical and immunological parameters during the spontaneous development of EAE in the 2D2 mice. The parameters were assessed in both males and females and then compared with the changes in male and female Th mice and males of the C57BL/6 line. Changes in the differentiation profile of hematopoietic stem cells from bone marrow, as well as the ratio of different populations of B and T lymphocytes from various organs, were shown to change the levels of IgG, binding MOG, myelin basic protein (MBP), histones, and DNA. Furthermore, an increase in the hydrolyzing activity of antibodies to these molecules was observed. In contrast to Th and C57BL/6 mice, in 2D2 males and females a significant increase was shown in the relative activity of IgG hydrolyzing MBP and DNA, but not MOG, which may also indicate features of the course of EAE in this line of mice and reflect different ways of the autoimmune response. The study was supported by a grant from the Russian Science Foundation, № 20-15-00162. * The authors marked with an asterisk equally contributed to the work.

P-03.3-020
A cell-free expression-based strategy for defining critical amino acid residues involved in antibody and tumor-associated transmembrane antigen interactions

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Developing novel antibodies directed against integral membrane proteins (IMPs) for diagnostic or therapeutic applications is still challenging due to the target complexity and limited antigen availability, caused by a lack of universal and cost-effective strategy for production. In our study, instead of using integral cell systems, a continuous exchange cell-free protein expression (CECFPE) platform derived from *E. coli* cellular extract was used. In the present study, we have demonstrated a robust and efficient approach for deciphering the impact of individual amino acid

residues involved in antibody binding to a certain immunogenic mimotope of model IMP. As the model antigen, we selected human transmembrane prostate androgen-induced protein (hTMEPAI), a 31.6 kDa single-pass α-helical protein, which is known as target and a negative regulator of TGF-b signaling and simultaneously played an oncogenic role in numerous cancers. To achieve the purpose of this study, we used the prokaryotic system, which has proven capability for the robust preparative production of IMPs with high yields. Along with a successful production of target proteins in precipitate mode, we had demonstrated a straightforward procedure of resolubilization in the presence of diverse detergents with immobilized metal affinity chromatography purification. Furthermore, the applied approach allowed us to obtain homogeneous protein samples suitable for further probing by antibodies. These were completed by the comparative screening in binding properties changes by prior developed rat mAbs against hTMEPAI mimotope (W298-L310) using surface plasmon resonance (SPR) within a created panel of single and multiple alanine mutants of the target protein. We were able to establish an excellent way for robust and high-throughput production of model transmembrane antigen combined with an in-depth biophysical and immunochemical characterization. *The authors marked with an asterisk equally contributed to the work.

P-03.3-021
Human CD4+ T-cell clone expansion leads to the expression of cystatin F

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The existence of CD4+ cytotoxic T cells (CTLs) at relatively high levels under different pathological conditions *in vivo* suggests their role in protective and/or pathogenic immune functions. CD4+CTLs utilize the cytotoxic effector mechanisms also utilized by CD8+CTLs and natural killer (NK) cells. During long-term cultivation, CD4+ T cells also acquire cytotoxic functions. In this study, CD4+ human T-cell clones derived from activated peripheral blood lymphocytes of healthy donors were examined for the expression of cytotoxic machinery components. Cystatin F is a protein inhibitor of cysteine cathepsins, that affects the cytotoxic efficacy of CD8+CTLs and NK cells by inhibiting the major progranzyme convertases cathepsins C and H as well as cathepsin L, which is involved in perforin activation. Here, we show that human CD4+ T-cell clones express the cysteine cathepsins involved in the activation of granzymes and perforin as well as both the inactive, dimeric and active, monomeric form of cystatin F. As in CD8+CTLs, cysteine cathepsins C and H were the major targets of cystatin F in CD4+ T-cell clones. Furthermore, CD4+ T-cell clones expressed the active forms of perforin and granzymes. The levels of the cystatin F decreased with time in culture concomitantly with an increase in the activities of granzymes. Therefore, our results suggest that cystatin F plays a role in regulating CD4+ T cell cytotoxicity. Since cystatin F can be secreted and taken up by bystander cells, our results suggest that CD4+CTLs may also be involved in regulating immune responses through cystatin F secretion.

P-03.3-022
Induction of myeloid-derived suppressor cells of monocyte origin (Mo-MDSCs) by colorectal cancer patient-derived extracellular vesicles carrying miRNA-21

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Our recent data showed that myeloid-derived suppressor cells (MDSCs) of monocyte origin play a crucial role in colorectal cancer (CRC) development and progression. On the other side, it was shown that melanoma-derived extracellular vesicles (EVs) can induce Mo-MDSCs from normal blood monocytes. In this study, we aimed to verify whether Mo-MDSCs may be induced from blood monocytes by CRC-derived extracellular vesicles (CRC EVs). EVs were isolated by sequential centrifugation from plasma of 32 CRC patients and 19 healthy donors (HD), followed by their quantitative and qualitative characterization, including the assessment of the miRNA-21 expression. Next, EVs were added to blood donor monocytes and after 24h of culture, the cells with phenotype of Mo-MDSCs were identified and isolated by FACS. Then Mo-MDSCs were co-cultured with polyclonally stimulated autologous T cells for assessing their immunosuppressive potential. The effects of EVs on cellular oxygen metabolism was evaluated in parallel (Seahorse, Mito Stress). To verify the role of miRNA-21 in generation of Mo-MDSCs, HD monocytes were cultured with CRC EVs transfected with miRNA-21 inhibitor. Peripheral blood of CRC patients contained significantly more EVs than blood of HD. CRC EVs possessed higher expression of EpCAM, and Her2/neu antigens and miRNA-21 in comparison to HD EVs. CRC EVs after culture with monocytes caused induction of suppressive Mo-MDSCs, positive for the expression of iNOS and PDL-1. Moreover, changes in the cellular oxygen metabolism were detected, indicating the induction of cells with regulatory potential. Inhibition of miRNA-21 in CRC EVs limited the induction of Mo-MDSCs from monocytes. CRC EVs may be responsible for the induction of Mo-MDSCs with strong suppressive activity from HD blood monocytes. We suggest that this process involves miRNA-21, transported in CRC EVs.

P-03.3-023
Der p 38, a novel TLR4-binding allergen, as a bidirectional regulator of eosinophils and neutrophils in allergy

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The house dust mite is the most common cause of allergic diseases, and TLR4 acts as a critical receptor of the allergic responses. This study examined a novel allergen that binds to

TLR4 in *Dermatophagoides pteronyssinus* (DP) and identified a new allergen, called Der p 38, which is similar to the NIpC/P60 family protein. Der p 38 was purified and characterized by FPLC and LC-MS/MS-based peptide mapping. Biolayer interferometry and structure modeling unveiled the TLR4-binding activity and structure of recombinant Der p 38. The allergenicity of Der p 38 was confirmed by a skin prick test, basophil activation, and dot blot. Natural Der p 38 was identified by an interaction with TLR4, which exists as the protein present in the midgut of DP. A skin prick test identified 22 out of 45 patients (48.9%) as Der p 38-postive allergic subjects. Der p 38-augmented CD203c expression was noted in the basophils of Der p 38-positive allergic patients. In animal experiments, Der p 38 stimulation induced the infiltration of neutrophils as well as eosinophils and exhibited similar clinical features of asthma via TLR4 in wild-type mice. In contrast, allergic effects caused by Der p 38 were not observed in TLR4-knockout mice. Der p 38 suppressed the apoptosis of neutrophils and eosinophils from normal and allergic subjects and enhanced cytokine production in human bronchial epithelial cells, which inhibited the apoptosis of neutrophils and eosinophils. These mechanisms include the MyD88-dependent pathway or independent pathway, including LYN, PI3K, AKT, ERK, and NF-κB. These findings show that Der p 38 is a bridge allergen between eosinophilic and neutrophilic inflammation, and the resolving key contributes to an understanding of the TLR4-mediated complex pathogenesis of allergic diseases.

P-03.3-024
Neurodegenerative effects of S100A8 and S100A9 in Parkinson's disease

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S100 proteins belong to a damage-associated molecular pattern (DAMP) with a variety of functions, such as inflammation and apoptosis. Parkinson's disease (PD) is a neurodegenerative disease characterized by the loss of dopaminergic neurons in the substantia nigra (SN). S100A8 and S100A9 enhanced the apoptosis of human neuroblastoma SH-SY5Y cells treated with 1-methyl-4-phenylpyridinium (MPP+), but they did not affect the survival and death of the cells on their own. S100A8 and S100A9 increased the upregulation of the Bax/Bcl-2 expression ratio induced by MPP+ and boosted the activation of caspase 9 and caspase 3 due to MPP+. SP600125, an inhibitor of JNK, and SB202190, an inhibitor of p38 MAPK, blocked apoptosis caused by the MPP+, MPP+/S100A8, or MPP+/S100A9 treatment. Both JNK and p38 MAPK were phosphorylated after MPP+ stimulation, and their activation was enhanced by the S100A8 or S100A9 treatment. S100A8 and S100A9 increased the expression of α-synuclein and Parkin and enhanced ROS production compared to MPP+ stimulation. An injection of 6-hydroxydopamine (6-OHDA) plus S100A8 or S100A9 caused greater loss of tyrosine hydroxylase (TH) immunopositive cell bodies from SN and microgliosis than an injection of 6-OHDA in a rat model of Parkinson's disease. The expression of S100A8 and S100A9 increased in the SN of PD-like mice administered 6-OHDA and patients with PD compared to normal controls. Overall, S100A8 and S100A9 may induce neurodegenerative effects and result in the aggravation of Parkinson's disease.

P-03.3-025
Molecular interplay between the transcription factors Ets-2 and Foxp3 in T cells

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Ets-2 is involved in various biological functions such as cell development, differentiation, and regulation of immunity. Foxp3 also plays an essential role in cell differentiation, as it is considered a crucial regulator of the development and function of T regulatory cells (Tregs). In this work, we investigated the effect of Ets-2 on the regulation of Foxp3 expression and vice versa, because *in silico* analysis revealed that Ets-2 and Foxp3 have binding sites at Foxp3 and Ets-2 promoters, respectively. Jurkat cells (T cell line) were transfected with increasing amounts of an Ets-2 (pCDNA3-ets-2) and a Foxp3 overexpressing vector (pRV.GFP FOXP3) in the presence (P/I) or absence (CM) of the mitogens PMA and ionomycin. Gene expression of Ets-2 and Foxp3 was examined at the transcriptional level by real-time qPCR and at the protein level by western blotting. ChIp analysis was performed to investigate the binding of Ets-2 to the Foxp3 promoter. Overexpression of Ets-2 resulted in a decrease in Foxp3 expression in both unstimulated and stimulated cells. Foxp3 also downregulated Ets-2 expression under both CM and P/I conditions. ChIp analyses showed that Ets-2 binds to the Foxp3 promoter under both CM and P/I conditions. In conclusion, Ets-2 downregulates the expression of Foxp3 by directly binding to its promoter. This may indicate a suppressive role of Ets-2 in the development and function of Tregs, possibly contributing to the development of autoimmune diseases. These results provide the basis for delineating the signaling pathways involved in this process.

P-03.3-026
Structural insight into the glycosylation-dependent interaction of CD69 with Galectin-1 by small-angle X-ray scattering

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CD69 is a C-type lectin-like transmembrane glycoprotein constitutively expressed on a variety of immune cells including neutrophils, T cells, B cells, and natural killer cells. Although the precise role of CD69 in immunity remains to be fully elucidated, compelling evidence has shown CD69 as a modulator of inflammatory responses. Putative protein ligands have been reported for CD69 in the last years. Galectin-1, a prototypical lectin characterized by a common structural fold and a conserved CRD with affinity for β-galactosides, has been identified as one of the novel binding partners of the receptor [1]. The biophysical and structural mechanisms of CD69:Galectin-1 complex formation are still lacking. We investigated the interaction by employing a combination of biophysical methods and have independently confirmed the binding as well as its dependency on N-glycosylation. In addition, we performed small-angle X-ray scattering experiments of CD69 with Galectin-1, where a preliminary structural insight into the complex was obtained. [1] de la Fuente, H. et al. The leukocyte activation receptor CD69 controls T cell

differentiation through its interaction with galectin-1. Mol. Cell. Biol. 34, 2479–87 (2014). This work was supported by the Czech Science Foundation (18-10687S), the MEYS CR (LTC20078), and BIOCEV (ERDF CZ.1.05/1.1.00/02.0109). CIISB research infrastructure project LM2018127, funded by MEYS CR, is gratefully acknowledged for the financial support of experiments at the CMS.

P-03.3-027
The influence of selected modulators of autophagy and lysosomal signaling pathway on the nuclear factor of activated T cells (NFAT) in relation to psoriasis

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Psoriasis (Ps) is a chronic autoimmune disease that affects approximately 3% of the world's population. The molecular mechanism underlying Ps is relatively poorly understood, but primarily it involves the interaction between keratinocytes (KCs) and cells of the immune system, primarily T helper (Th) lymphocytes. Activation of Th is a key component of a complex immune response. It requires the coordination of stimuli from the cell's environment with signaling pathways – immunological, metabolic and migratory. In recent years, increasing attention has been paid to the influence of lysosomal metabolism and autophagy on the regulation of the inflammatory process. There are also reports of autophagy and lysosomal signaling abnormalities in the context of Ps. In this study, the influence of selected modulators of lysosomal metabolism and autophagy on the nuclear factor of activated T cells (NFAT), regulating gene expression during activation and differentiation of T lymphocytes, processes characteristic for inflammation, was demonstrated. The studies were carried out on human Jurkat and Jurkat-Lucia^{TM}NFAT lymphocytes lines, as well as $CD4^+$ T cells from Ps patients and healthy volunteers. The cellular level and the localization of NFAT were determined by Lucia luciferase activity estimation with the use of fluorescence microscopy. The real time qRT-PCR test was performed to assess the level of gene expression. Ultrastructure of stimulated cells was visualized by transmission electron microscopy (TEM).

P-03.3-028
Elucidation of the structure of NKp30 receptor oligomers

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NKp30 is one of the main human natural killer (NK) cell activating receptors used in directed immunotherapy. The oligomerization of the NKp30 ligand binding domain depends on the length of the C-terminal stalk region, but our structural knowledge of NKp30 oligomerization and its role in signal transduction remains limited. We assessed that NKp30 oligomerization depends on its N-glycosylation [1]. Our results show that NKp30 forms oligomers when expressed in HEK293S GnTI$^-$ cell lines with simple N-glycans. However, NKp30 was detected only as monomers after enzymatic deglycosylation. We characterized the interaction between NKp30 and its ligand, B7-H6, with respect to glycosylation and oligomerization, and we solved the crystal structure of this complex, revealing a new glycosylation-induced dimerization of NKp30. Another challenge is to reveal the real structure of oligomers on the NK cell membrane. We approach this by comparing the structure of the oligomeric NKp30 in solution, which we solve by cryo-electron microscopy, with the results obtained by super-resolution microscopy. We provide new insights into the basis of NKp30 oligomerization and explain how the stalk region and glycosylation of NKp30 affect its ligand affinity. This furthers our understanding of the mechanisms involved in NK cell activation, which is crucial for the successful design of novel NK cell-based targeted immunotherapeutics. This work was supported by the Czech Science Foundation (18-10687S), the MEYS CR (LTC20078), and BIOCEV (ERDF CZ.1.05/1.1.00/02.0109). CIISB research infrastructure project LM2018127, funded by MEYS CR, is gratefully acknowledged for the financial support of experiments at the CMS. This work used the Integrated Structural Biology platform of the Strasbourg Instruct-ERIC center IGBMC-CBI supported by FRISBI (ANR-10-INBS-0005-001). [1] Skořepa O, et. al. Natural Killer Cell Activation Receptor NKp30 Oligomerization Depends on Its N-Glycosylation. Cancers 12(7):1998 (2020).

P-03.3-029
N-glycosylation of CD4+ T cells is altered in autoimmune thyroid diseases

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The immune system cells, involved both in acquired and innate immune responses, are largely controlled by glycosylation. CD4+ T helper cells, which were shown to be highly glycosylated, are the key elements responsible for the pathogenesis of autoimmune thyroid diseases (AITDs), including Hashimoto's thyroiditis (HT) and Graves' disease (GD). In HT, due to loss of immune tolerance to thyroid antigens, activated Th cells contribute to thyrocyte destruction, whereas in GD autoreactive CD4+ cells support the production of antibodies against follicular cell antigens. The aim of our study was to determine the changes of Th cell *N*-glycosylation in AITDs and to evaluate the effect of thyroid autoimmunity treatment on CD4+ cell *N*-glycome. The research material consisted of human blood samples from donors with the elevated antibodies anti-thyroglobulin (TgAb) and/or anti-thyroperoxidase (TPOAb) without hypothyroidism (n = 12, HT1), HT patients during L-thyroxine therapy (n = 23, HT2), GD patients with the up-regulated antibodies against thyrotropic hormone receptor before (n = 19, GD) and after (n = 6, GD/L) stabilization of TSH level following thyrostatic therapy (study groups), and healthy donors (control group, n = 18). CD4+ T cells were isolated from peripheral blood mononuclear cells (PBMCs) using an automated magnetic sorter. *N*-glycans were

released by PNGase F, 2-AA labelled, and analyzed by MALDI-ToF MS. The results showed that the amount of some fucosylated and monosialylated complex-type *N*-glycans, significantly increased in HT1 donors, and was normalized in response of the treatment. In GD we observed the significant reduction of *N*-glycan fucosylation on CD4+ cells. Our study implies that *N*-glycosylation of CD4+ cells is subject to changes in both AITDs. Taking into account the role of T helper cells these changes can be important in the course of thyroid autoimmunity. The study was supported by a grant from the Polish National Science Centre (grant no. 2015/18/E/NZ6/00602).

P-03.3-030
Changes in specific biomarkers indicate cardioprotective and anti-inflammatory effects of recreational SCUBA diving

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Recreational SCUBA (rSCUBA) diving is a specific type of underwater activity preformed in extreme environmental conditions. However, information on molecular events underlying (patho)physiological events that follow exposure to such conditions is still largely unexplored. To contribute to better understanding cardiac and immune system adaptation mechanisms triggered by repeated rSCUBA diving we measured copeptin, IgA, IgG, and IgM, complement components C3 and C4, and differential blood count parameters, including neutrophil-to-lymphocyte ratio (NLR), N-glycosylation of IgG and total plasma proteins (TPP), as well as cell-free DNA in blood samples of fourteen recreational male divers who performed five dives, one per week, at a depth of 20-30 m for 30 min, after a non-dive period of 5 months. Changes in measured parameters suggested that first dive triggered a stress response that was abolished with repetition of diving since its beneficial effects on cardiac system (copeptin level) and immune status (NLR, immunoglobulins level, as well as IgG and TPP N-glycosylation pattern towards anti-inflammatory status) were observed after the following dives. In conclusion, rSCUBA diving practiced on a regular basis induces a cardiac adaptive response and promotes anti-inflammatory status of the organism, thus conferring cardioprotection as well as multiple health benefits.

Cell signalling

P-03.4-001
Plasma membrane lipidome profile in HCC cell line: inhibition of some lipids mediated by Wnt/β-catenin signaling suppresses the tumor

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While it has been shown in our previous study (Sezgin E et al. The FEBS journal 284.15 (2017): 2513-2526.) that Wnt ligand preferentially binds to ordered membrane domains by lateral organization of plasma membrane components, the role of membrane lipid environment where Wnt-receptor complex forms is unknown. Here we identified changing lipid content by membrane lipidome profile using of shotgun lipidomics after Wnt ligand binds to plasma membrane in hepatocellular carcinoma (HCC) cell lines. HCC is a worldwide cancer with an increasing incidence and although the current drugs used for the treatment of advanced HCC provide an advantage in overall survival for up to only three months, the prognosis of the disease cannot be prevented due to developing resistance mechanisms and increasing heterogeneity. Therefore, it is necessary to discover new perspectives and methods in the treatment of the disease. Targeting of the Wnt/β-catenin signaling pathway by using inhibitors are among the treatment options since HCC has associated with aberrant mutations of the Wnt signaling pathway. Therefore, targeted therapy strategies that specifically target molecules or molecule–molecule interactions that cause signal deregulation in the pathway are prominent strategies in recent years. In this context, revealing the role of the lipid environment could contribute to specific targeting of cancer cells for therapeutic purposes. Based on the results obtained from analysis of lipidomics, we proposed that two lipids changed significantly after Wnt induction and inhibition of these lipids had a negative effect on proliferation and migration in cells. In this respect, we strongly believe that some lipids could be a potential target in the treatment of HCC.

P-03.4-002
PI3K/mTOR inhibition alters dysregulated lncRNA expression profile in leukemia stem cells and regulates downstream signaling

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Genetic/epigenetic changes in hematopoietic stem cells (HSC) contribute to leukemia stem cells (LSC) formation. We aimed to determine the lncRNA profiles that differ between HSC and LSC and define the changes in the lncRNA expressions by suppressing PI3K/mTOR signaling, which gives LSC selective advantage in proliferation. PI3K/mTOR signaling in LSC(CD44+/CD133+/CD38-) and HSC(CD34+/CD38-) cells (Celprogen) was suppressed by pathways' specific dual-inhibitor (0.6μM VS-5584) and confirmed by PathScan Antibody Array. LncRNA expressions were quantified by qRT-PCR. Bioinformatics analyses were performed using RAIN database. Activities of network-related transcriptional factors were investigated by dual-luciferase

reporter assay. The expressions of 26 lncRNAs were higher, 25 lncRNAs were lower in LSC compared to HSC. The most significant increases were in UCA1, GOMAFU, L1PA16(13.2, 10.4, 10.2-fold), and decrease was in anti-NOS2A(8.5-fold). PI3K/mTOR inhibition upregulated 16 lncRNAs and downregulated 12 lncRNAs in LSC and had less effect on HSC. Gene ontology results revealed that differentially expressed lncRNAs are associated with cell differentiation, pathways in cancer, transcription factor activity. Among the regulated lncRNAs; SRA(12.4-fold), UCA1(-2.4-fold), H19(-17.4-fold), L1PA16(-9.2-fold), PCGEM1(-9.8-fold) was predicted to interact with CREB1, CEBPA-AS1, Myc, STAT3. We confirmed that CREB1 transcriptional activity was repressed after PI3K/mTOR inhibition in reverse correlation with SRA level. As predicted with bioinformatics analysis, CEBP transcriptional activity was found to increase in reverse correlation with UCA1 level. Also, downregulations in H19, L1PA16 and PCGEM1 were found to be parallel with repressions in Myc/Max and STAT3 activities. LncRNAs identified to be dysregulated in LSC and associated with PI3K/mTOR signaling, a major oncogenic pathway in leukemogenesis, may contribute to the identification of novel therapeutic targets to eliminate LSC.

P-03.4-003
Seminal fluid induces epithelial mesenchymal transition (EMT) and enhances cell migration and inflammation in cervical cancer cells

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Squamous cell carcinoma of the cervix accounts for 70% of cervical cancer cases world-wide. Though there are various risk factors, the most common risk factor for cervical cancer development is sexual transmission and persistent infection of cervical epithelium with High-Risk HPV. Exposure of neoplastic cervical epithelial cells to seminal fluid (SF) has been shown to promote cell proliferation. Employing squamous cell carcinoma cell lines (SiHa and Me180), we investigated the effect of SF on epithelial mesenchymal transition (EMT), cell migration and inflammation. In this study, exposure of cervical cancer cells to SF caused morphological changes and the formation of stress fibres which was shown by Phalloidin staining and Confocal Microscopy Imaging. Using RT-qPCR we showed that SF upregulated EMT transcription factors Snail, ZEB1 and TWIST mRNA expression. EMT induction was further confirmed by the increase of N-cadherin and a subsequent decrease in E-cadherin protein expression. Transwell migration assays further showed that SF caused cell migration and invasion. In addition, we showed that SF upregulated proinflammatory and angiogenic genes in squamous carcinoma cell lines. This induction of inflammation was linked to NF-kB transcriptional signalling which we showed using the Dual Luciferase Reporter Assay. Activation of invasion and tumour promoting inflammation are two well-known hallmarks of cancer. Therefore, any factors that may exacerbate these hallmarks will play a role in the progression of the disease. Our results show that SF plays a role in enhancing the migratory and invasive potential of squamous cell carcinoma cells and that this associates with increased expression of transcription factors that have regulatory roles in EMT. These finding therefore implicate SF as a possible factor that may promote cervical cancer progression. *The authors marked with an asterisk equally contributed to the work.

P-03.4-004
CB1R as a potential therapeutic target in CRBN deficiency-associated mental retardation

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Cereblon (CRBN), a 442 amino-acid long protein, was first identified as a protein mutated in humans with an autosomal recessive nonsyndromic mental retardation (ARNSMR) syndrome. Specifically, deletion of the last 23 amino acids of the protein (R419X mutation) was responsible for this phenotype. Later, CRBN was described as the primary target of thalidomide and as a substrate receptor for the cullin-RING E3 ligase complex with DNA damage binding protein-1 (DDB1), Cul4A and Roc1. Recent studies have found selective cognitive impairments in CRBN knock-out mouse models similar to the alterations found in patients carrying the R419X mutation. Here, by using the Cre-LoxP technology, we have established three new mouse lines carrying selective genetic deletions of the Crbn gene in i) the whole body (CRBN-KO), ii) telencephalic glutamatergic neurons (Glu-CRBN-KO) or iii) forebrain GABAergic neurons (GABA-CRBN-KO), in order to study the differential contributions of CRBN subpopulations to memory and cognition. Interestingly, specific recognition memory shortfalls were found in CRBN-KO and Glu-CRBN-KO mice, but not in GABA-CRBN-KO animals, and they could be reverted by rimonabant i.p. administration, thus suggesting an enhanced cannabinoid CB1 receptor (CB1R) function as an underlying mechanism. Of note, previous wide-scale proteomic experiments in our laboratory had identified CRBN as a potential CB1R-interacting protein. A plethora of biochemical experiments support that both proteins interact, and cell signalling experiments have unveiled that CRBN binding to CB1R selectively impairs the CB1R-mediated inhibition of the cAMP pathway. Taken together, these findings support a plausible molecular link between CRBN deficiency, memory malfunctioning and CB1R hyperactivation.

P-03.4-005
Reciprocal HIF-1α modification by ERK1/2 and CK1δ kinases balances between the transcriptional and non-genomic HIF-1α functions

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Hypoxia inducible factor HIF-1 is the master transcriptional regulator of oxygen homeostasis and enables cancer cells to adapt to hypoxia. Apart from oxygen the HIF-1α subunit is also regulated by post-translational modifications such as

phosphorylation. We have previously shown that HIF-1α phosphorylation by CK1δ inside its heterodimerization domain impairs its activity by inhibiting its interaction with ARNT and formation of the HIF-1 heterodimer. Moreover, phosphorylation of HIF-1α by ERK1/2 at Ser641/643 increases HIF-1 activity by promoting its nuclear accumulation and allows HIF-1α to differentiate between its transcriptional role and its cytoplasmic non-genomic functions. To elucidate the significance of these two competing HIF-1α modifications we applied a CRISPR/Cas9 approach to generate a HeLa *HIF1A*$^{-/-}$ cell line. These cells were transiently transfected with GFP-HIF-1α constructs that carry a combination of HIF-1α mutations that either mimic (S247D, S641E) or abolish the phosphorylation (S247A, S641/3A) by either CK1δ or ERK1/2. All combination mutants were equally expressed but possessed different HIF-1 activity depending on HIF-1α mutant phosphorylation and subcellular localization. Moreover, HeLa *HIF1A*$^{-/-}$ cells were stably transfected with GFP-HIF-1α carrying mutations at the ERK modification sites to further analyze the role of this phosphorylation. Cells that expressed the WT or phosphomimetic SE HIF-1α form could thrive under hypoxia whereas cells expressing phosphorylation-deficient HIF-1α SA mutant could not survive. Ongoing proteomic and CHIP experiments performed by these stable cell lines under hypoxia, indicate that there is a distinct protein expression pattern that depends on HIF-1α phosphorylation. Overall, our results suggest that the reciprocal regulation of HIF-1α by these two kinases creates a gradient of HIF-1α distribution and activity in order to balance the transcriptional role of HIF-1α as well as its cytoplasmic functions.

P-03.4-006
Hypoxia induces HIF-independent alteration of nuclear architecture and function by ROS-mediated redistribution of nuclear matrix proteins

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Oxygen deprivation (hypoxia) elicits the induction of an adaptive machinery that primarily involves transcriptional reprogramming mediated by Hypoxia Inducible Factors. However, there are lesser-known transcription-independent processes occuring at the onset of hypoxia. These include epigenetic marking, alterations in mRNA processing and cellular architecture. The nucleus is structurally characterized by a filamentous network that comprises the nuclear matrix and lamina that are supported by proteins such as the Scaffold Attachment Factor B (SAFB) and Lamin B Receptor (LBR), respectively. We have observed that at the onset of hypoxia there is a significant change in nuclear plasticity coinciding with a swift rearrangement of SAFB and LBR along the nuclear scaffold as observed by microscopy and biochemical fractionation. Furthermore, these events are HIF-independent and reversible upon reoxygenation. Immunoprecipitation experiments demonstrate that hypoxia 1) alters SAFB1/2 interaction with vital components of splicing machinery and, 2) reduces LBR phosphorylation by SRPK1 and its association with nuclear envelope components. Moreover, these alterations are driven by the increased production of reactive oxygen species (ROS) occurring at hypoxia as a result of mitochondrial dysfunction. This ROS-dependent mechanism is also responsible for the remodeling of splicing machinery that relies on SRPK1 phosphorylation of SR-splicing factors such as SF2. Finally, hypoxia-induced SAFB1/2 and LBR dissociation from the nuclear scaffold, leads to 1) increased SAFB-dependent splicing of the proangiogenic *VEGFA* variant, 2) increased presence of cytoplasmic chromatin fragments, which were marked by the presence of LBR. Overall, our data suggest that cellular response to hypoxia involves ROS-driven changes on nuclear architecture that entails rapid rearrangement of nuclear periphery and matrix constituents that may be important for reprogramming gene expression and splicing.

P-03.4-007
Voltage dependent anion selective channel isoform 3 prevents mitochondrial impairment induced by oxidative stress

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Voltage Dependent Anion Selective Channels (VDACs) are pore-forming proteins localized in the outer mitochondrial membrane of all eukaryotes. They allow metabolite exchanges across the organelle and also participate in numerous cellular pathways thanks to the interaction with cytosolic enzymes and apoptotic factors. That's exactly what makes VDAC a key regulator of mitochondrial functionality and a well-established target for the treatment of many diseases (1). Among the three isoforms that exist in mammals, VDAC3 has long been the topic of our research as it represents the least abundant and the most enigmatic one. It features untypical biophysical properties that have been attributed to the specific oxidative status of its cysteine residues. Moreover, clues within literature have suggested VDAC3 as a putative redox sensor, stressing its involvement in mitochondrial ROS homeostasis (2). A direct proof, however, is missing. In this work, for the first time, we unambiguously demonstrate that VDAC isoform 3 prevents mitochondrial impairment induced by oxidative stress. Under physiological conditions, our human VDAC3 knock out model exhibited a severe down-regulation of mitochondrial biogenesis and a concomitant increase in the amount of reactive oxygen species (ROS) accompanied by a remarkable overexpression of main antioxidant enzymes. Treatments with drugs triggering mitochondrial ROS generation further evidenced the extreme vulnerability of cells lacking VDAC3 to oxidative damage. Accordingly, high resolution respirometry measurements confirmed that VDAC3 depletion significantly affects mitochondrial respiration upon exposure to low doses of rotenone. Transient transfection of VDAC3 knock out cells with a cysteine-null VDAC3 mutant ultimately proved that cysteine residues are responsible for the ability of VDAC3 to counteract mitochondrial ROS overload. 1. De Pinto V. et al. (2021) Biomolecules. 11:107. 2. Reina S. et al. (2016) Oncotarget. 7:2249–2268.

P-03.4-008
Interaction of the mitochondrial BKCa channel with natural compound and their derivatives

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Mitochondrial potassium channels in the inner mitochondrial membrane have been involved in cytoprotection during ischemic/reperfusion event. One of these channels is the mitochondrial large-conductance Ca^{2+}regulated potassium (mitoBK$_{Ca}$) channel. It has been reported that activation of the mitoBK$_{Ca}$ channel is important for protecting brain tissue against stroke damage as well as heart tissue against ischemic damage. In the mitochondria, the activation of mitoBK$_{Ca}$ channels seems to be a crucial mechanism to control the inner membrane potential, volume of the mitochondrial matrix and the level of reactive oxygen species. Studies carried out in our laboratory showed that substances of plant origin - flavonoid could activate the mitoBK$_{Ca}$ channels in various cells. The aim of the study was to identify new cytoprotective openers of mitoBK$_{Ca}$ channel. The mitoBK$_{Ca}$ channels are present in the mitochondria of human bronchial epithelial cells and the new stable transfected cell line of HEK293 expressing of the mitoBK$_{Ca}$ channel cardiac isoform. In current study, we performed patch-clamp experiments on mitoplasts to investigate interactions of the mitoBK$_{Ca}$ channel with plant derived compound and its synthetic derivatives. One of the tested substances was prenyl-naringenin. After the application, we observed activating effect, i.e. open probability of the mitoBK$_{Ca}$ channel increased by ~10%. Another synthetic substance that was tested was 15-en-18-Osuccinate. In this case, we observed channel inhibition. Detailed analysis of the mitoBK$_{Ca}$ channel regulation is crucial for a better understanding of cytoprotection induced by potassium channel openers, including substances of plant origin and their derivatives. This study was supported by a grant 2019/35/B/NZ1/02546 from the NCN, Poland (to PB), partially by Nencki Institute. Work implemented as a part of Operational Project Knowledge Education Development 2014-2020 cofinanced by European Social Fund; POWER.03.02.00-00-I007/16-00 (Sęk).

P-03.4-009
The role of sumoylation in the regulation and function of the exosome catalytic subunit Exosc10 under hypoxia

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Sumoylation is the covalent attachment of SUMO (Small Ubiquitin-like MOdifier) to target proteins. Recent data support that sumoylation of proteins controls the cellular response to different types of stress and especially in cancer. Solid tumors are characterized by low oxygen levels (hypoxia) because of the high oxygen demands by the proliferating cancer cells and the anomalous tumor vascularization. Using a SUMO-immunoprecipitation method combined with quantitative proteomics, we have identified proteins with significantly altered sumoylation status under hypoxia without changes in their expression levels (Chachami et al. (2019) Mol Cell Prot 18, 1197-1209). One such protein is exosome subunit 10, a 3'-5' exoribonuclease and one of the catalytic subunits of the RNA exosome. Mass spectroscopy and immunoprecipitation experiments showed that hypoxia decreased the sumoylation levels of Exosc10. *In vitro* and *in vivo* experiments were used to identify the main sumoylation site of Exosc10 and provide evidence that SENP3 is responsible for the desumoylation of Exosc10 under both normoxia and hypoxia. Interestingly, hypoxia also promoted the translocation of both SENP3 and Exosc10 from the nucleolus to the nucleoplasm, which, however, was not triggered by Exosc10 desumoylation. With the use of an inducible expression system of wild-type or sumo-deficient forms of Exosc10 in cancer cell lines, we are currently investigating the effect of sumoylation on the catalytic activity and function of Exosc10 as well as its impact on cell adaptation and survival under low oxygen. In addition to identifying new roles of sumoylation, this study aims to also provide new insights into the crosstalk between RNA degradation and hypoxia, which characterises diseases such as cancer, ischemia and inflammation. The identification of new control points and the understanding of the interrelated networks in the hypoxia response pathways can lead to therapeutic molecular interventions.

P-03.4-010
The role of TFAP2A and its sumoylation in the transcriptional response to hypoxia

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Hypoxia, a state of oxygen deficiency, enables events for the maintenance of homeostasis and adaptation of cells and tissues. Post-translational modifications play essential role in the hypoxic adaptation. Sumoylation is the covalent attachment of Small Ubiquitin-related MOdifier (SUMO) to a vast variety of proteins. Sumoylation regulates essential proteins of the hypoxic response machinery such as the Hypoxia Inducible transcription Factors (HIFs). Using a quantitative proteomic approach in combination with endogenous SUMO-immunoprecipitation in HeLa cells, we have identified transcriptional regulators that change their sumoylation under hypoxia (Chachami et.al. (2019) Mol Cell Prot 18, 1197-1209). One such protein is TFAP2A, a transcription factor implicated in a variety of cell processes including cell growth, differentiation, and apoptosis. TFAP2A is often found overexpressed in solid tumors that are characterised by hypoxia and its expression is correlated to oncogenesis and progression of metastasis. We have shown that the sumoylation level of TFAP2A is reduced under hypoxia, as suggested by the proteomic data. Interestingly, we could demonstrate that TFAP2A physically interacts with HIFs and the sumoylation status of TFAP2A affects the transcriptional activity of HIF-1. The involvement of TFAP2A in HIF-dependent gene regulation is currently under investigation using ChIP-Seq approaches. Using overexpression of mutant TFAP2A constructs that mimic or inhibit its

sumoylation and IP-proteomic techniques, we are also analysing the protein interaction networks between TFAP2A and HIF-1α and their involvement in the cellular adaptation to hypoxia. As both TFAP2A and HIFs are critical players in oncogenesis, understanding their interrelated pathways can be of value for the development of effective molecular therapeutic interventions.

P-03.4-011
Investigation of the functional role of neurotrophin receptor-associated death domain in neuronal development and microglia-glioblastoma interaction using the zebrafish model

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Wnt/β-catenin signalling is involved in proliferation and differentiation during development and maintenance of tissue homeostasis in adulthood. Dysregulation of Wnt signalling is associated with various cancers including glioblastoma (GBM), which is the most malignant primary brain cancer. GBM is mostly recognized as a heterogeneous tumour due to presence of different subpopulations within the tumour mass. This heterogeneity is derived from the interaction between tumour cells and stromal cells, such as microglial cells. While neurotrophins and their receptors are frequently misexpressed in malignant gliomas, how they are involved in glioma progression is largely unknown. We have identified neurotrophin receptor-associated death domain (nradd), a homologue of p75 neurotrophin receptor that regulates neuronal survival. Our data demonstrate that nradd is both a Wnt target and a negative feedback regulator of Wnt/β-catenin signalling. Moreover, we found that nradd is capable of inducing apoptosis in healthy and cancer cells, including hepatocellular carcinoma and neuroblastoma cells. Several studies have shown that Wnt signalling is abnormally activated in GBM, promoting tumour growth and invasion. Furthermore, microglia-GBM interaction contributes to an increase in tumour invasiveness, which is associated with Wnt signalling through changes in cell migration and invasion. Therefore, we need a better understanding of the interactions between GBM cells and the microglia. Since GBM-derived Wnt3a induces an M2-like phenotype in microglial cells, we hypothesize that nradd is a potential target to attenuate GBM progression through Wnt/β-catenin signalling. Hence, we aim to unravel the modulatory role of nradd in microglia-GBM interaction using the zebrafish model.

P-03.4-012
The activity of the MAPK and PI3K/AKT/mTOR pathways are decreased in patients with achalasia

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Idiopathic achalasia is a rare disease with an incidence of 1-10/100,000. The etiopathogenesis of the disease has not been fully elucidated. The aim of the study is to determine the activation states of important cell signaling pathways in achalasia. Thirty-seven achalasia patients and 15 healthy volunteers (HV) were included in the study. Esophageal biopsies taken by endoscopy were used to determine the expression levels of 180 genes and 7 phosphorylated proteins important in cell signaling. Gene expression levels were determined by the qRT-PCR method, protein levels were examined by the Multiplex ELISA method. There were 25 genes deregulated between the two groups. The gene expression levels of MEK1, ERK2, H-ras, N-ras (-2.09, -3.82, -4.01, -3.77 fold, respectively) molecules which responsible for activating the MAPK pathway were decreased in achalasia patients compared to HVs. Phosphorylated forms of proteins associated with the MAPK pathway (Erk1/2 (Th202/Tyr204), MEK1 (Ser217/Ser221), c-Jun (Ser63)) pathway were also low expressed in achalasia. EGF (-2,74 fold), which provides activation of the PI3K-AKT pathway, and the PI3K catalytic subunit PIK3CB (-3,73 fold) gene expression were less expressed in achalasia patients compared to HVs. Phosphorylated AKT phosphorylates the mTOR protein via the Rheb protein. In our study, mTOR (Ser2448), levels were found to be lower than HVs in achalasia patients. According to these data, it was concluded that MAPK and PI3K/AKT/mTOR pathways are less active in achalasia patients compared to HVs. On the basis of our results, examining the molecular data related to the signal pathways we determined in our study in other biological materials such as blood and esophageal muscle will be important in understanding the etiology of the disease.

P-03.4-013
Dissection of the role of IqgC domains in the cell-substratum adhesion

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Cell-substratum adhesion is important for motility, phagocytosis and cytokinesis of individual cells, as well as complex biological processes in multicellular organisms, such as development, tissue homeostasis and wound healing. In *Dictyostelium discoideum*, many proteins are involved in establishing the punctiform adhesion structures at the ventral cellular cortex, e. g. talin, paxillin, and Phg2. Protein IqgC is a RasGAP protein that negatively regulates large-scale endocytosis and binds and inactivates small GTPase RasG. We observed that cells deficient for IqgC are more loosely attached to the substratum than wild-type cells. IqgC possesses two main functional domains – a GRD (GAP-related domain) and an RGCt (RasGAP C-terminus). In homologous proteins, like human IQGAP1, GRD domain is important for binding small GTPases, while RGCt binds PtdIns4,5P2, E-cadherin, and β-catenin. We therefore set out to determine which

of these domains is important for the function of IqgC in adhesion. Cells overexpressing YFP-IqgC, YFP-GRD and YFP-RGCt were examined by confocal microscopy to inspect their localization to the ventral adhesion structures, whereas the IqgC KO cells transfected with deletion constructs lacking each domain were tested for substratum adhesion using a rotational agitation assay. We showed that the full-length IqgC localizes to the ventral adhesion structures and rescues the adhesion phenotype of IqgC KO cells, while only the RGCt domain localized to the adhesion structures. However, none of the tested truncated constructs was able to rescue the adhesion phenotype of IqgC KO cells. Thus, we concluded that the RGCt domain is sufficient for the localization of IqgC to adhesion structures but the GRD domain also contributes to the establishment of the interactions necessary for the IqgC activity in the cell-substratum adhesion.

P-03.4-014
IQGAP-related protein IqgC interacts with RasG and Rab5A on *Dictyostelium* macropinosomes

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Dictyostelium IqgC is a GAP (GTPase activating protein) specific for the small GTPase RasG, one of the main positive regulators of macropinocytosis (bulk fluid uptake). By deactivating RasG, IqgC negatively regulates macropinocytosis in amoeba *Dictyostelium discoideum*. IqgC strongly localizes to macropinosomes, where it colocalizes with active Ras. However, it remains on the internalized vesicle even after Ras has dissociated from the macropinosme (Previously published in: Marinović M et al. (2019) Proc Natl Acad Sci U S A 116, 1289-1298). This finding suggests that IqgC functions independently of RasG, probably in early macropinosome maturation. First, we examined the role of RasG in the recruitment of IqgC to forming macropinocytic cups. We observed the loss of IqgC localization to macropinosomes in *rasG* null cells. Mutant IqgC proteins unable to bind Ras and IqgC lacking its RasG-binding RasGAP domain also failed to localize correctly. These results show that interaction with RasG is indispensable for IqgC recruitment to forming macropinosomes. Next, we explored novel IqgC interactor(s) that could bind IqgC on the nascent macropinosome after Ras detached. Potential protein candidates were selected from the previously determined interactome, according to their known function in the early endosome maturation. Using Co-IP, and afterwards GST-pull-down assay with purified IqgC, we identified the early endosome marker, GTPase Rab5A, as a direct binding partner of IqgC. Furthermore, using confocal microscopy of live cells, we showed that both proteins colocalize on the primary macropinocytic vesicle. Consistently, a lipid dot blot using cell lysates identified phosphoinositides (PIs) involved in macropinosome formation and early maturation as IqgC membrane phospholipid interactors. Altogether, these results suggest that RasG is essential for IqgC loading to the forming macropinosome, but Rab5A and PIs likely mediate its retention on the RasG-vacated vesicle.

P-03.4-015
Oleanolic acid induces cytoprotective mitophagy in HCT116 human colon carcinoma cells

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Colorectal cancer is a disease that occupies a very high place in terms of its malignancy and mortality. Therefore, it is of high importance to discover compounds that can efficiently ameliorate the disease and reduce mortality. Oleanolic acid (OA), a natural triterpene compound, has been shown to possess beneficial health effects, including anticancer activity. However, the effect of OA on mitophagy in colon cancer cells is unknown. The aim of this study was to investigate the mechanism and the role of autophagy/mitophagy in the anticancer activity of OA in HCT116 human colon carcinoma cells, cell line commonly used in colon cancer studies, and therapeutic drug development. Additionally, we examined chemosensitization to 5-fluorouracil (5-FU). OA dose-dependently reduced viability of HCT116 cells, with IC_{50} = 29.8 μM. The expression of cleaved caspase-3 and poly (ADP-ribose) polymerase 1 (PARP) increased by OA treatment, suggesting that OA induces apoptosis in HCT116 cells. OA induced autophagy in cancer cells by increasing expression of Beclin-1, Atg5 and microtubule-associated protein 1A/1B-light chain 3 beta-II (LC3B-II), which was found to be protective. Induction of mitophagy was suggested by increased expression of p62 and PTEN-induced kinase 1 (PINK1) and reduced expression of translocase of outer mitochondrial membrane 20 (TOMM20), which colocalized with LC3B. OA induced nuclear accumulation of forkhead box O3a (FOXO3a) and phosho-FOXO3a. The cytotoxic activity of OA coincided by suppression of the phosphoinositide 3-kinase (PI3K)/Akt and extracellular regulated kinase 1/2 (ERK1/2) pathways and activation of AMP-activated protein kinase (AMPK), c-Jun N-terminal kinase 1 (JNK1), and p38. The results of the study show the cytotoxic activity of OA in HCT116 human colon carcinoma cells with concomitant induction of survival autophagy and mitophagy through modulation of key signaling pathways. Moreover, OA chemosensitized HCT116 cells to 5-FU. *The authors marked with an asterisk equally contributed to the work.

P-03.4-016
PKCε controls the fusion of secretory vesicles in mast cells in a phosphatidic acid-dependent mode

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PKCε is highly expressed in mast cells and plays a fundamental role in the antigen-triggered activation of the allergic reaction. Although its regulation by diacylglycerols has been described, its regulation by acidic phospholipids and how this regulation leads to the control of downstream vesicle secretion is barely known. Here, we used structural and evolutionary studies to find the

molecular mechanism that explains the selectivity of the C1B domain of PKCε by phosphatidic acid (PA). This resided in a collection of Arg residues that form a specific rim on the outer surface of the C1B domain, around the diacylglycerol binding cleft. In RBL-2H3 cells, this *basic rim* allowed the kinase to respond specifically to phosphatidic acid signals that induced its translocation to the plasma membrane and subsequent activation. Further experiments in cells that overexpress PKCε and a mutant of the PA binding site, showed that PA-dependent PKCε activation increased vesicle degranulation in RBL-2H3 cells, and this correlated with increased SNAP23 phosphorylation. Over-expression of PKCε in these cells also induced an increase in the number of docked vesicles containing SNAP23, when stimulated with PA. This accumulation could be attributed to the stabilizing effect of phosphorylation on the formation of the SNARE complex, which ultimately led to increased release of content in the presence of Ca^{2+} during the fusion process. Therefore, these findings reinforce the importance of PA signalling in the activation of PKCε, which could be an important target to inhibit the exacerbated responses of these cells in the allergic reaction.

P-03.4-017
Filarial worms and mammalian spermatozoa are likely to sense environmental stress in a similar way

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Voltage-dependent anion-selective channel (VDAC) is a protein located in mitochondrial outer membrane. It mediates the exchange of small metabolites and inorganic ions across the membrane it is in. VDAC is involved in many regulative interactions of mitochondrial and whole cell functioning. The most important role of that channel is transport of molecules necessary for cellular respiration and regulation of oxidative-reduction (redox) state. VDAC can be present in organisms as several paralogs encoded by separate genes. Each of the paralogs play distinct roles, however their features are not fully understood yet. It is assumed that such a diversity of VDAC-encoding genes might contribute to flexibility to cope with abiotic and biotic stresses known to affect mitochondrial functions. Moreover, cysteine residues located in the N-terminus of mammalian VDAC paralogs (highly expressed in the testes) may contribute to redox sensor function based on formation and elimination of disulfide bond, what can result in redox-sensitive VDAC (rsVDAC). Using representatives of currently available VDAC amino acid sequences we analyzed whether all paralogs of organism VDAC can be rsVDAC and whether rsVDAC property is possible if only one variant of VDAC is present in mitochondria. Obtained results indicate that possibility of all VDAC paralogs to be rsVDAc is very low, however rsVDAC can occur when organism have only one VDAC variant. Moreover, presence of rsVDAC may be associated with habitat conditions as rsVDAC appears to be prevalent in case of parasites. Thus, the channel may mediate detection and adaptation to environmental conditions. This work was supported by the research grants of National Science Centre, Poland, Grant Numbers 2017/26/D/NZ1/00075 and 2016/21/B/NZ4/00131.

P-03.4-018
Jen1 transporter endocytosis under alkali stress

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Saccharomyces cerevisiae cells modulate the extracellular pH, being able to alkalinize its surroundings when grown in the presence of alternative carbon sources. We have shown that, in *S. cerevisiae*, prolonged growth in lactate triggers external media alkalinization, which leads to Jen1 lactate transporter endocytosis. This phenomenon depends on a functional Jen1 transporter, on Bul1 arrestin and on active TORC1 complex (1). However, the regulatory networks that connect alkali stress to the Jen1 downregulation are still unclear. To further detail some of the regulatory mechanisms underlying the pH-dependent endocytosis of Jen1 lactate transporter, *S. cerevisiae* cells stable expressing *JEN1-GFP* were studied using aerobic continuous cultures in controlled bioreactors. In order to determine at which pH the Jen1 transporter starts to be internalized, a pH ramp was performed, in which the medium pH was automatically increased over time. The off-gas signal (CO_2 concentration) and optical density were also automatically monitored in the course of the experience. Samples for dry weight, HPLC (for external metabolites analysis) and fluorescence microscopy (to follow the cellular localization of JEN1-GFP) were taken overtime. These experiments enable us to determine in which specific pH the Jen1 transporter starts to be internalized and further degraded. With this information, individual bioreactors were programmed at relevant pHs (pH in which Jen1 is still at the plasma membrane, pH near the identified "switching point" and pH in which the lactate transporter was no longer at the plasma membrane). When the continuous cultures reached the steady state, samples were also taken for dry weight, microscopy and HPLC as well as for transcriptomic and potential enzymatic analysis. A global characterization of the regulatory networks underlying the Jen1 endocytosis under alkali stress will be presented. 1. Talaia G et al. (2017) J Mol Biol 429, 3678-3695

P-03.4-019
Mind the gaps: secrets of signalling specificity

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Plants are equipped with a myriad of membrane receptors to tune various endogenous and exogenous signals. A single plant cell must be capable of incorporating many independent signals to determine the outputs for growth, development, and immunity. Yet, many receptor pathways appear to share their main downstream signaling components. A long-standing question remains: how does a plant cell prevent inappropriate crosstalk and ensure specific outputs? We use the Arabidopsis root endodermis to compare two well-studied receptor pathways: one developmental pathway initiated by SGN3/CIFs, to establish the integrity of the Casparian strip (CS); and one immunity pathway that recognizes microbial pattern flg22 via the receptor FLS2.

Both pathways display strong similarities in the structure of signalling components but show distinct functional outputs: localised cell wall modifications that are key to root function - and immune responses against invading bacteria. The receptor SGN3 functions specifically in the root endodermis, while FLS2 expresses more highly in aerial tissues vulnerable to bacterial entry. To answer "if one could replace the other" and compare them in the same cell type, I established a plant line that allows stimulation of the two receptors exclusively in the endodermis (endogenous SGN3 and endodermal-expressed FLS2) via respective peptide treatments (CIF2 or Flg22). In this line, I directly compare their functional outputs using high resolution imaging and quantification and establish that endodermal-expressed FLS2 can be activated yet cannot functionally replace SGN3 pathway. Consistent with the functional phenotype, comparative RNAseq analyses reveal both common and pathway-specific transcriptional responses. Further, it suggests that the transcription factor MYB36 could be the hub that regulates SGN3-specific responses. Together, my work will elucidate mechanisms of how plants maintain signalling specificity on a single cell level.

P-03.4-020
Irisin stimulates the release of CXCL1 via upregulation of NFκB pathway from human neck derived differentiating adipocytes

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Obesity is at present a global epidemic. Brown and beige adipocytes function in utilizing excess fat to produce heat (thermogenesis), thereby counteracting obesity. Recent studies in rodents and humans indicated that these adipocytes also release cytokines, which may play a vital role in maintaining whole body energy homeostasis. Irisin is primarily released by myocytes during exercise and functions as a polypeptide regulator of beige adipocytes. We intended to characterize the effect of irisin on differentiating adipocytes derived from human subcutaneous neck (SC) and deep neck (DN) adipose tissue depots. Preadipocytes were isolated from SC and DN biopsies of the same donor, differentiated to adipocytes in the presence or absence of irisin. Global gene expression analysis was performed on nine independent donors. Irisin could not upregulate characteristic thermogenic genes, but upregulated genes related to several cytokines. Out of them, CXCL1 (the highest upregulated) was found to be released throughout the differentiation period, predominantly by SC and DN differentiated adipocytes. DN tissue biopsies showed a significant release of CXCL1 upon 24 hours irisin treatment. Gene expression data indicated upregulation of the NFκB pathway upon irisin treatment, which was validated by an increase of p50 and decrease of IκBα protein level, respectively. Continuous blocking of the NFκB pathway by SN50 (cell permeable inhibitor of NFκB nuclear translocation) significantly reduced the release of CXCL1. The released CXCL1 exerted a positive effect on the adhesion capability of endothelial cells. Together, our findings demonstrate that irisin stimulates the release of a novel adipokine, CXCL1, via upregulation of NFκB pathway in human neck area derived differentiating adipocytes, which plays an important role in improving tissue vascularization (Previously published in: Shaw A et al. (2021) Front Cell Dev Biol 9:737872).

P-03.4-021
Identification of new genes regulated by the anticancer p53 protein

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The p53 protein is a transcription regulator activated by various stress factors. Actinomycin D and nutlin-3a, which stimulate p53 in a different manner, when acting simultaneously (A+N), cause strong activation of p53 and consequently, a strong increase in the expression of many p53-regulated genes. The transcriptome sequencing (RNA-seq) of A549 cells with p53 activated by A+N revealed a very strong increase in the expression of hundreds of genes. Only some of them are the known targets of the p53. The objective of the research was to identify new connections between p53 and the genes it regulates. Based on our observations and according to the available databases on the p53 binding sites within the chromatin, 12 genes were selected (*TMEM52*, *CTSH*, *DRAXIN*, *FRMD8*, *RETSAT*, *CDH3*, *FAM13C*, *FBXO2*, *KANK3*, *BLNK*, *MR1* and *FRZB*) for a detailed analysis of the connection between p53 activation and gene expression. The gene promoters with potential p53 binding sites selected for detailed analysis were cloned with the use of routine methods. The regulation by p53 was assessed by means of luciferase reporter assays. The expression of the genes selected based on RNA-Seq results was measured by RT-PCR in control cells and in cells with the expression of p53 knocked down by two methods - lentivirus-delivered shRNA and by CRISPR/Cas9-mediated mutation of *TP53* gene. Moreover, the changes in global expression of proteins in cells as well as in culture medium of control cells and the cells exposed to A+N were measured with the use of the mass spectrometry and Western blotting. The key result of the project is the identification of new genes controlled by the p53 protein. Based on the known functions of the genes, we can expand our understanding of the mechanisms played by p53 in regulating the Wnt pathway, innate immunity, metabolism and functioning of the cytoskeleton. This work has been supported by grants no. 2017/27/N/NZ5/01079 to BŁS from the National Science Centre, Poland. *The authors marked with an asterisk equally contributed to the work.

P-03.4-022
PAPP-dependent IGFBP-4 proteolysis occurs in neurons and astrocytes derived from induced pluripotent stem cells

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Insulin-like growth factors (IGF-1 and IGF-2) play a pivotal role in cell motility activation and cell viability sustaining in many tissues and cell lines. One of the key mechanisms of IGF bioavailability

regulation is proteolytic degradation of IGF-IGFBP (insulin like growth factor binding proteins 1–7) complex by specific proteases. In nervous tissue specific proteolysis of IGFBP 2, 3 and 5 have been demonstrated, however, nothing is known about proteolysis of IGFBP-4. The only protease that cleaves IGFBP-4 in physiological conditions is PAPP-A (pregnancy-associated plasma protein A). IGFBP-4 proteolysis by PAPP-A occurs at a specific site resulting in formation of two proteolytic fragments: N-terminal (NT-IGFBP-4) and C-terminal (CT-IGFBP-4). In present study, we have developed a model system to investigate IGFBP-4 proteolysis in neurons and astrocytes derived from induced pluripotent stem cells (iPSCs). Using this model, we have measured specific IGFBP-4 proteolysis in neurons and astrocytes derived from iPSCs of 4 healthy donors and proved that IGFBP-4 is cleaved by PAPP-A. The level of IGFBP-4 proteolysis assessed by the concentration of NT-IGFBP-4 measured by proteolytic neo-epitope-specific fluoroimmune analysis is 2-3 times higher in astrocytes than in neurons. Also, IGFBP-4 proteolysis in conditioned media of astrocytes and neurons occurs only in the presence of IGF, confirming, that observed proteolysis is associated with PAPP-A. We also determined the concentration of PAPP-A in conditioned media of both cell lines, which in the case of astrocytes was 10-15 times higher than the concentration of PAPP-A in neurons, that correlates with the level of IGFBP-4 proteolysis in these lines. Thus, it can be concluded that IGFBP-4 proteolysis in nervous tissue mainly proceeds by PAPP-A, secreted by astrocytes. In this regard, astrocytes may potentially enhance the bioavailability of IGF for neurons, thereby maintaining their viability in the nervous tissue.

P-03.4-023
Localization of PAPP-A dependent proteolysis of IGFBP-4 in cardiomyocytes cultures

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IGF system plays crucial role in maintenance of cell viability. Insulin-like growth factors (IGF-1, IGF-2) interact with their receptors on cell surface and activate proliferation, migration and cell growth. IGF bioavailability for their receptors is regulated by IGF binding proteins 1-7 (IGFBP-1 – IGFBP-7) and various proteases, that cleave IGFBPs. Recently in our laboratory using primary culture of rat neonatal cardiomyocytes it was shown that proteolysis of IGFBP-4 occurs in cardiomyocytes and it is mediated by pregnancy associated plasma protein A (PAPP-A) (Previously published in: Serebryanaya DV et al. (2021) Biochemistry (Mosc) 86(11):1395-1406). In the present study we investigate the localization of IGFBP-4 proteolysis in cardiomyocytes, that still remains unknown. We used two models: primary culture of rat neonatal cardiomyocytes and culture of human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). Both cell cultures were characterized using immunostaining methods. The proportion of cardiomyocytes in the obtained cultures was up to 90% for the rat cardiomyocyte culture and up to 99% for the culture of human IPSC- derived cardiomyocytes. Using proteolytic neo-epitope-specific fluoroimmunoassay we have detected IGFBP-4 proteolytic fragments in cardiomyocytes medium and proved, that it is PAPP-A-dependent. Since PAPP-A is anchored in plasma membrane of fibroblasts with glucose amino glycan molecule (GAG), to investigate location of IGFBP-4 proteolysis we incubated cardiomyocytes with heparin that competes with GAG for PAPP-A binding. In contrast to control experiments in the absence of heparin, treatment of rat and human cardiomyocytes cultures with 25 μg/mL heparin leads to decreasing of IGFBP-4 proteolysis on the cell surface and its increasing in condition media. Thus, it can be assumed that IGFBP-4 proteolysis occurs on the surface of cardiomyocytes and PAPP-A is anchored to plasma membrane.

P-03.4-024
Examining the relationship of diabetes-related cytokine levels and EMT in STAT5-overexpressed Panc-1 and MiaPaCa-2 pancreatic cancer spheroid models in response to celastrol treatment

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Recent evidence has shown that cytokine levels have an important role in the development of pancreatic cancer by promoting cell proliferation and differentiation. Celastrol, which has anti-inflammatory and anti-diabetic effects, has been shown to promote apoptosis and inhibition of cell proliferation through regulation of cytokine levels in various tumors. In this study, firstly we examined the role of diabetes-related cytokines in the differentiation and proliferation of 3D spheroid formations using human pancreatic duct cell lines, Panc-1 and MiaPaCa-2 that STAT5 expression was modified. Secondly, the effect of celastrol treatment was investigated through JAK/STAT and PI3K/AKT/mTOR signaling on 3D spheroid models in relation to cytokine levels released from pancreatic cancer cells. Our study showed that increased expression of IL-6, IL-1β, TNF-α, PAI-1, c-Peptide, and insulin coincided with an increase in STAT5 and STAT3 expression in 3D spheroid models of Panc-1 and MiaPaCa-2 cells, determined by qRT-PCR and flow cytometry. Celastrol treatment reduced IL6/STAT3-mediated cell migration in Panc-1 and MiaPaCa-2 cells with increased STAT5 expression analyzed by immunoblotting. Furthermore, celastrol reduced PI3K/AKT/mTOR signaling as well as significantly increased spheroid diameters caused by STAT5 overexpression in MiaPaCa-2 cells. Taken together, these results show that targeting cytokine mechanisms by celastrol led to a significant reduction of aggressiveness of pancreatic cancer.

P-03.4-025
Growth hormone-releasing hormone receptors induce malignant transformation of the human prostate epithelium cell line RWPE-1

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Growth hormone-releasing hormone (GHRH) and its receptors have been implicated in the progression of prostate cancer. In this study, we analyzed the expression of GHRH receptors and

their carcinogenic potential on a non-tumour human prostate epithelial cell line (RWPE-1). The expression and cellular localization of GHRH receptors were analyzed by immunodetection and immunocytochemistry, respectively, with specific antibodies for p-GHRH and its splice variant, SV. These cells express both types of GHRH receptors that are located in the plasma membrane and cytosol. The carcinogenic potential was evaluated through the effects of 0.1 μM GHRH treatment on cell proliferation (BrdU assay and PCNA expression levels), migration (wound-healing assay), cell-cell adhesion and E-cadherin expression (western-blot). We found an increase in proliferation and migration, as well as a significant reduction of cell adhesion and E-cadherin levels, at 8 h. Thus, GHRH appears to act as a cytokine in the transformation of RWPE-1 cells by mechanisms that likely involve epithelial-mesenchymal transition (EMT), which reinforces a GHRH role in prostate tumorigenesis.

P-03.4-026
Myc-induced nuclear antigen: a cancer-associated protein hydroxylase linking cell growth to polarity?

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Hydroxylation is an emerging post-translational modification that is generally catalysed by a family of proteins called 2-oxoglutarate (2OG) oxygenases. Recent efforts to characterise these enzymes have mainly focussed on histone demethylation by lysine demethylases and the hydroxylation of Hypoxia Inducible Factor by the prolyl hydroxylases. Although these studies have underlined the fundamental roles of 2OG oxygenases in biology, the function of a large proportion of the proteins belonging to this family remains unknown. Myc-induced nuclear antigen (MINA) belongs to a poorly characterised subfamily of 2OG-oxygenases thought to catalyse stable protein hydroxylation. MINA is involved in important cellular processes such as cell growth and T-cell immunity and has been implicated in a variety of diseases including asthma and cancer. However, the molecular mechanisms by which MINA contributes to these processes are not well understood. MINA supports protein biogenesis via hydroxylation of its only known substrate, ribosomal protein Rpl27a, which may be consistent with positive roles in cell growth and cancer. Recent reports, however, indicate a paradoxical role for MINA in suppressing tumour development and progression, raising the intriguing possibility that MINA acts as a tumour suppressor in a context-dependent manner, possibly through as yet unidentified substrates. In order to better understand MINA's role in cancer, we applied a proteomic approach that allows substrate identification of protein hydroxylases based on enzymatic activity. Using this technique, we identified a novel candidate substrate complex for MINA. Here we present our work characterising the interaction of MINA with an orphan membrane complex. The work has potentially important implications for our understanding of hydroxylase biology and the role of protein hydroxylation in cell polarity/adhesion and cancer.

P-03.4-027
Regulation of cell stemness by the MCPIP1 protein

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The MCPIP1 (Monocyte Chemoattractant Protein-1 Induced Protein) protein is involved in the negative regulation of inflammation due to its RNase activity. A characteristic feature of MCPIP1 is presence of catalytic, PIN domain which allows mRNA degradation of pro-inflammatory cytokines such as IL-1β and IL-6. Recent reports suggest that MCPIP1 plays an important role in tumor development, skin diseases and non-alcoholic fatty liver disease. Here, we show that MCPIP1 may also regulates the markers involved in cell de-differentiation and stemness. We transduced normal epithelial cell line TCMK-1, to downregulate MCPIP1 or to overexpress its mutated, inactive form. We have shown that downregulation or mutation in PIN domain of MCPIP1 leads to increased clonogenicity of TCMK-1 cells. Moreover, cells with downregulation or D141N mutation of MCPIP1 protein, are characterized by higher levels of the stemness markers such as c-Met, CD44 and phosphorylated c-Myc. NGS analysis revealed that MCPIP1 mutation leads to changes in expression of genes involved in regulation of actin cytoskeleton, PI3K signaling pathway, oxidative phosphorylation and cell cycle. To confirm the role of MCPIP1 depletion in cell stemness, we used mice models with MCPIP1 knock out in hepatocytes $MCPIP^{fl/AlbCre}$. Our *in vivo* study showed that mice with MCPIP1 deletion in hepatocytes were characterized by increased expression of stemness markers *Cxcr4, Cxcl12, Myc* and *Cd133* in the livers compared to control mice. Our results indicate the importance of MCPIP1 protein in the changes of cell phenotype and levels of stemness markers. Moreover, depletion of MCPIP1 might be an important factor involved in liver physiology. This study was supported by National Science Center grants no. 2017/25/N/NZ5/03014, 2017/26/E/NZ5/00691 and MNS 15/2021.

P-03.4-028
The protective effect of alpha lipoic acid on iron sucrose-induced oxidative kidney damage in rats with unilateral nephrectomy by suppressing of p38 MAPK signaling and NFkB p65

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This study was aimed to investigate the possible protective effect of alpha lipoic acid (ALA), known as a powerful antioxidant, by focusing specific pathway, p38 MAPK and its downstream transcription factor, NFkB p65, which mediate the oxidative kidney

injury caused by iron treatment in rats with a CKD model. 35 Wistar albino rats were randomly divided into 5 groups; Control, ½ nephrectomy (Nx), Nx+ALA (100 mg/kg), Nx + Iron Sucrose (IS) (40 mg/kg) and Nx + IS + ALA. IS and ALA were injected intravenously on days 1, 7, and 14. Blood and kidney tissues were collected on the 15th day. The prussian blue staining showed that there was a significant iron deposition in the kidney tissues of the Nx+IS group compared to the control. Although, no significant changes were found by histological analyses, serum creatinine and urinary microalbumin levels were significantly increased in the Nx+IS group. However, ALA significantly reduced those changes. The protein expressions of the subunits of NADPH oxidase 4, which are NOX4 and p22phox were found to be significantly increased in the Nx+IS group. However, ALA suppressed their expressions in the Nx+IS+ALA group. The malondialdehyde level was reduced by ALA in that group while superoxide dismutase activity level was increased by ALA. Additionally, the mRNA expressions of TNF-α and IL-1β, that were related to inflammation, were decreased by ALA. Also, ALA resulted to improve changes in the apoptotic and antiapoptotic proteins, which are Bax and Bcl-2; respectively. The decreased level of kidney injury molecule-1 (KIM-1) was confirmed these changes. Morever, ALA suppressed the activation of p38 MAPK and NfkB p65, which plays major role in inflammation and apoptosis. In conclusion, our study suggests that ALA may be an effective candidate for inhibiting IS-induced oxidative kidney damage in CKD.

P-03.4-029
The disclosed mechanism of PARP1 activation by extracellular signals offers new therapeutic interventions

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The disclosed mechanism of PARP1 activation by extracellular signals and offered therapeutic interventions Recent findings revealed a new mode of PARP1 (polyADP-ribose polymerase) activation via a network of signal transduction mechanisms and in the absence of DNA damage. In this mechanism, PARP1 activation was induced by the binding of growth factors, transmitters, and hormones to their receptors in the cell membrane, as well as by membrane depolarization in excitable cells. PARP1 poly ADP-ribosylation lasted for more than an hour, and maintained a long-lasting activity of phosphorylated Erk (Extracellular signal-regulated kinases) in the chromatin, including Erk-induced gene expression implicated in synaptic plasticity, development, and proliferation. In this mechanism, PARP1 poly ADP-ribosylation was induced by intramolecular modifications in the protein, exposing the NAD+ (Nicotinamide Adenine dinucleotide) binding site in its catalytic domain, which resulted in its high affinity for NAD+. PARP1 polyADP-ribosylation affected gene expression through both direct and indirect effects on the post-translational modification of chromatin-bound proteins, including histone acetylation and polyADP-ribosylation of proteins implicated in DNA methylation. This signal-induced activation of PARP1 offers new opportunities for intervention in a variety of physiological and pathological conditions. Cohen-Armon M, Yeheskel A, Pascal JM. Signal-induced PARP1-Erk synergism mediates IEG expression (2019) Signal transduction and targeted therapy 4:8.

P-03.4-030
Bisphenol A affects Ca^{2+} homeostasis in mouse oocytes through GPR30 receptor and MAPK/ERK signalling pathway

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BPA is an organic compound widely used in the production of resins, polycarbonates, and plastics. As xenoestrogen, it can disrupt the functioning of animal (including human) organisms. Recent literature data indicate that BPA may affect fertility in mammals by disturbing several processes in oocytes and embryos, including epigenetic modifications, energy metabolism, and spindle assembly. However, its effect on Ca^{2+} oscillations, triggered in oocytes by a fertilizing sperm and key for activation of embryonic development, has not been yet examined. Here we showed that BPA (in a concentration typical for follicular fluid surrounding oocytes in ovaries) added to oocytes during their *in vitro* maturation decreased the percentage of oocytes that reached metaphase II stage and could be fertilized. Using time-lapse imaging and fluorescent Ca^{2+} indicator, we discovered that BPA shortened significantly the total duration of Ca^{2+} oscillations induced in metaphase II oocytes by fertilization. Additionally, it also increased frequency of the oscillations. As GPR30, G protein-coupled estrogen receptor, can bind BPA and is expressed in oocytes, we investigated whether it mediates BPA action in oocytes. G1, a GPR30 activator, added to the oocyte maturation medium instead of BPA, mimicked the BPA effect only partially: it shortened the total duration of Ca^{2+} oscillations but decreased frequency of Ca^{2+} oscillations. On the other hand, G15, a GPR30 inhibitor, alleviated the BPA-induced alternations. Moreover, inhibition of MAPK/ERK pathway also rescued BPA-induced changes in the duration and frequency of Ca^{2+} oscillations. In summary, our results indicate that BPA acts on Ca^{2+} homeostasis in oocytes at least partially through GPR30 and MAP/ERK pathway. As Ca^{2+} oscillations are crucial for activation of embryonic development and affect numerous processes in early embryos, our observations provide a novel mechanism of BPA action on mammalian fertility.

P-03.4-031
Does stimulation of PI3K signalling assist BRAFV600E melanoma cells in developing resistance to treatment?

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Overcoming the development of acquired resistance in response to vemurafenib and related $BRAF^{5600E}$ inhibitors is one of the major challenges in melanoma treatment (1, 2). One of these mechanisms of resistance involves hepatocyte growth factor (HGF), which is secreted by surrounding stromal cells in $BRAF^{5600E}$ inhibitor-resistant melanoma cells, resulting in the reactivation of the MAPK and PI3K-AKT signalling pathways (3). Our study showed that HGF or transforming growth factor α (TGFα) activated both the MAPK and PI3K-AKT signalling pathways in four melanoma (MM418-C1, C32, MM329 and D24) cell lines. We observed that PI3K-AKT is a possible upstream activator of ERK, whereby PI3K inhibition not only

reduced p-AKT expression, but also growth factor-stimulated p-ERK1/2 levels in melanoma cells irrespective of their BRAF status. While p-BRAF levels were reduced in cells transfected with BRAF siRNA, both p-AKT and p-ERK2 levels were increased when these cells were stimulated with HGF. This suggests that HGF can stimulate the PI3K-AKT pathway, which in turn activates ERK when its upstream activator BRAF is suppressed, and therefore contributing to the mechanism of vemurafenib drug resistance. 1. Chan XY, Singh A, Osman N, Piva TJ. *Int J Mol Sci*. 2017;18(7):1527. 2. Wagle N, Emery C, Berger MF, Davis MJ, et al. *J Clin Oncol*. 2011;29(22):3085-3096. 3. Straussman R, Morikawa T, Shee K, Barzily-Rokni M, et al. *Nature*. 2012;487 (7408):500-504.

P-03.4-032
Impact of lipid polyunsaturation on dopamine D2 receptor activation and signaling

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The dopamine D2 receptor (D2R) has been implicated in the etiology of several psychiatric disorders and is a main target of most antipsychotics. Interestingly, a "whole-body" decrease in long-chain polyunsaturated fatty acids (PUFA) levels – n-3 PUFAs such as docosahexaenoic acid (DHA) in particular – has been consistently described in these psychiatric disorders [1]. However, the mechanisms by which alteration in PUFA levels may contribute to pathogeneses and could alter the functionality and pharmacology of the D2R are unknown. Our project aims at unraveling the impact of membrane PUFAs on D2R pharmacological properties and conformation through biochemical, biophysical and modeling studies in both PUFA enriched cells and membrane model systems of controlled lipid composition. To this aim, we have investigated the impact of membrane PUFAs in the first stages of receptor activation, that is in the receptor/ligand interaction using fluorescence anisotropy and plasmon waveguide resonance (PWR) [2]. Moreover, PUFAs impact on the recruitment and activity of D2R signaling effectors and receptor internalization was investigated by energy transfer assays and live cell imaging approaches. Overall the data indicate that membrane PUFA composition impact ligand binding and receptor conformational changes. This effect could be related to a preferential interaction of PUFA-containing phospholipids with the D2R leading to the formation of microdomains around the receptor as revealed by molecular modeling [3]. Regarding the signaling cascades, PUFA cell enrichment does not impact cAMP production but enhances β-arrestin recruitment and impacts receptor internalization. The results could have a significant impact in the development of adjuvant therapeutic strategies for psychiatric disorders implicating the D2R. 1. Messamore and McNamara (2016) Lipids in Health and disease 15: 5. 2. Alves (2019) Acc Chem Res. 52: 1059. 3. Jobin et al. (2022) Molecular Psychiatry, *under revision*.

P-03.4-033
Autophagy regulation and microRNA-mediated signalling pathways are altered in HUVEC cells in the presence of extracellular histones

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Sepsis is a highly prevalent disease caused by an exacerbated response of the organism against an infection. Disease progression is divided into a first phase of hyper-inflammation and a second phase of immunosuppression. During the first one, a significant increase in pro-inflammatory molecules triggers multiple physiological changes that include the release, among others, of chromatin-related nuclear proteins (i.e. histones and HMGB1) into the bloodstream as a result of extracellular traps (ETs) formation, and/or as a result of necrotic processes. In this landscape, histones are known to have pro-inflammatory and procoagulant effects that significantly aggravate the symptoms of sepsis, especially when acting on the endothelium of blood vessels. However, many details remain unknown about how these effects are induced and mediated at the molecular level. In this work, we show that purified histones, when added to human umbilical vein endothelial cells (HUVEC), cause cytotoxicity mainly by inducing apoptosis in a dose-dependent manner, and that they affect autophagy regulation. Microscopy analysis allowed us to observe that cells incubated with extracellular histones show an increase in lysosomal markers, as well as a correlation between the location of immunofluorescence-labeled histone H3 and lysosomal particles. Since autophagy plays a crucial role in sepsis processes, we also wanted to assess the effect of histone-mediated cytotoxicity processes on the expression levels of some microRNAs related to inflammation and autophagy regulation, that have been proposed as biomarkers or putative therapeutic targets in sepsis. These preliminary results suggest that autophagy induced by extracellular histones might have a protective effect, and provide important insights into the relationship between the cytotoxic effects of histones, autophagy regulation, and microRNA-mediated signalling pathways.

P-03.4-034
The rapid electrotactic response of 3T3 fibroblasts to external direct current electric fields depends on inwardly rectifying potassium channels (Kir4.2)

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Direct current electric fields (EFs) occur in living organisms, being the part of some important physiological processes such as embryogenesis, wound healing, and tissue regeneration as they cause directional cell migration by process known as electrotaxis. Electrotaxis mechanisms have been extensively investigated but are still poorly understood, and no single 'sensor' for electrotaxis has been identified to date. As we expect that the dynamics of cell reaction to EFs is crucial for understanding of mechanisms of electrotaxis, we investigated the rate of electrotactic reaction

in murine 3T3 fibroblasts. 3T3 cells migrated cathodally at EF of physiological strength (1-3V/cm) with first symptoms visible after 1-2 minutes since EF application or reversal. Such a rapid response could be explained with the mechanism based on the opening of membrane ion channels rather than the redistribution of membrane proteins, such as chemoattractant receptors. Although the natural candidate in the process were calcium ions and channels, their participation in the response was excluded with various methods. Recently, it was shown that inwardly rectifying potassium channels (Kir) are involved in regulation of electrotaxis. We confirmed the presence of Kir channels in 3T3 cells as well as their involvement in the examined process. Both directionality of cell migration and rate of cell response were decreased after pharmacological inhibition of Kir channels (with barium ions) and down-regulation of the Kir4.2 subunit gene. A similar effect was observed after the impairment of natural regulation of Kir channels by endogenous polyamines depletion or magnesium ions elevation. Finally, we showed that prolonged stimulation with EF of 3V/cm leads to the directional migration restoration, which may suggest the biphasic mechanism of electrotaxis involving the delayed redistribution-based component. This work was supported by a grant from the National Science Centre 2018/31/B/NZ3/01750, Poland.

P-03.4-035
The regulation of interferon signaling system by p53 tumor suppressor protein

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The p53 protein functions not only as the tumor suppressor, but it also shows antiviral activity, e.g., by stimulating the expression of innate and adaptive immunity proteins, for this reason many viruses inactivate p53. Interferons are cytokines, which upregulate antiviral genes by activating phosphorylation of transcription factors from the STAT family. The best studied interferons belong to type I (e.g. interferon α - IFNα) and type II (interferon γ - IFNγ) groups. IFNα can be secreted by all cells, whereas IFNγ is secreted only by T lymphocytes and natural killer cells. Interferons differ in the sets of genes they activate. Considering antiviral activities of p53 and interferon signaling pathways, it is likely that the two systems interconnect. The goal of our research was to identify these points of cooperation and understand their functioning. The p53 and interferons share many target genes. Moreover, p53 stimulates the expression of SOCS1 protein, which inhibits interferon-stimulated activation of STAT1. By this mechanism p53 could attenuate the expression of genes controlled by interferons. We noticed that the enhanced phosphorylation of STAT1 in p53-deficient cells did not translate into increased expression of the majority of tested genes stimulated by IFNα, with the exception of *IFI6*. Moreover, we observed that p53 inhibited the phosphorylation of STAT1 stimulated by IFNγ, however, p53 attenuated the expression of only one (*TAP2*) of the tested genes stimulated by this cytokine. Unexpectedly, we noticed synergistic cooperation of p53 and IFNγ in activation of *CASP1* gene coding for caspase 1, which is a protein involved in pro-inflammatory death called pyroptosis occurring as a result to intracellular pathogen infection. Thus, it appears, that the major outcome of p53 activation and the exposure of cells to IFNγ is priming them to pyroptosis. This work has been supported by grants no. 2019/35/O/NZ5/02600 to MR from the National Science Centre, Poland.

P-03.4-036
Potential anticancer properties of a palladium (II) complex: protein targets and cytotoxicity

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Palladium (II) complexes have drawn much attention as potential anticancer drugs due to the similarity of their metal centre to platinum (II) complexes. Although cisplatin and other Pt(II) complexes are some of the most efficient chemotherapeutic drugs, such compounds are typically associated with serious systemic toxicity and acquired resistance. Even though the primary targets for these metallodrugs are DNA molecules, proteins are key components of the pathways involved in malignant transformation and therefore are likely to be identified as drug targets. In this work, the concentration-dependent cytotoxic effects of dichloro(1,2-diaminocyclohexane)palladium(II), [Pd(dach)Cl_2], on cervical cancer cell line (HeLa) were examined by Sulforhodamine B (SRB) colorimetric assay after 48 h and potential protein targets of the Pd(II) complex were found using a bioinformatics approach. The list of genes that encode HeLa cell baseline proteins was downloaded from Expression Atlas. Corresponding 9 462 protein sequences were revealed and screened for potential interactions with the Pd(II) complex using the Informational Spectrum Method. The method recovered 106 proteins that were analysed using the bioinformatics resource for gene enrichment analysis, DAVID. Functional Annotation Clustering revealed that terms with the highest enrichment scores and statistical significance were nucleoplasm, nucleus, and SOSS complex, which is involved in the maintenance of genomic stability. The Pd(II) complex, at a concentration of 50 μg/mL, showed a significant anticancer potential by reducing HeLa cells viability to 33.57%. Obtained results imply the anticancer ability of [Pd(dach)Cl_2] against the HeLa cancer cell line and point to its potential targets.

P-03.4-037
Involvement of ERK MAPK signaling pathway in cisplatin-induced mitophagy and autophagic cell stress in renal tubular epithelial cells

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Nephrotoxicity is the most common side effect and dose-limiting factor in patients receiving cisplatin (CP) chemotherapy. Due to high energy requirements, renal tubular epithelial cells are rich in mitochondria which are highly damaged by CP. Damaged mitochondria undergo their removal, mitophagy, but they are also strongly implicated in apoptosis induction. Mitogen-activated protein kinases (MAPK) are key participants in CP-induced nephrotoxicity. Extracellular signal-regulated protein kinase 1/2 (ERK1/2) has been shown to localize on mitochondria and is suspected to be involved in the regulation of mitophagy. It is currently unclear whether ERK 1/2 promotes generalized or mitochondria-selective autophagy, or whether mitochondrial localization of activated ERK is essential in CP nephrotoxicity. Here we show the timeline of events induced by CP in Human renal proximal tubular cells (HK-2) at 3, 6, 12, and 24 hour-treatment by monitoring the expression of various proteins

involved in injury (LC3B1/2, p62, Beclin-1, Atg5, Atg5-12, Pink, Parkin, Tom20, AMPK, PARP, Caspase -3, -9, and -8, Bax, Bcl-2 and CyclinD1). Mitophagy is activated in the early stages of CP damage as a protective event after which there is a turnover to apoptosis and autophagic cell stress. The role of Parkin in mitophagy turnover to apoptosis induction has also been clarified. ERK 1/2 is activated in the early stages of CP treatment and shifts its localization towards the nucleus and plays a role both in mitophagy, autophagy, and apoptosis. Additionally, we tested MEK inhibitor, Mirdametinib, and an ERK signaling pathway activator, Ceramide C6, and their influence on cell survival as well as the ERK 1/2 mitochondrial translocation under combinatory treatments. Smart targeting of molecules involved in CP nephrotoxicity should enable a new approach to nephrotoxicity prevention and potential application in the pharmaceutical industry.

P-03.4-038
Periventricular nucleus of the hypothalamus as a source of signaling molecules, dopamine and L-DOPA, in the cerebrospinal fluid

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Since the 1980s, the concept of dopaminergic centers of the brain as clusters of dopaminergic neurons has been fundamentally revised. In fact, it was shown that, in addition to dopaminergic neurons, most of these centers (arcuate nucleus, striatum, etc.) contain neurons expressing one of the enzymes of dopamine (DA) synthesis, tyrosine hydroxylase (TH) or aromatic L-amino acid decarboxylase (DAA). The final secretory products of these neurons are L-DOPA and DA; both play the role of a neurotransmitter and a neuromodulator. In this study, convincing evidence was obtained that the hypothalamic periventricular nucleus (PeN) is one of the largest dopaminergic centers of the brain, containing dopaminergic and monoenzymatic neurons. Using double immunostaining for TH and DAA, it was shown that PeN contains almost three thousand neurons – dopaminergic and many more monoenzymatic. Using HPLC, it was shown that the end secretory product in monoenzymatic TH-neurons is L-DOPA, while in monoenzymatic DAA-neurons and bienzymatic neurons it is DA. Importantly, in PeN, the L-DOPA synthesis predominates over the DA synthesis. Also, it was shown in a confocal microscope that the axons of bienzymatic and monoenzymatic neurons penetrate between ependymal cells into the 3rd ventricle, providing the secretion of DA and L-DOPA from the neurons of the PeN into the cerebrospinal fluid (CSF). And at last, HPLC has shown that CSF contains DA and L-DOPA in approximately the same proportion as PeN. This observation confirms our assumption that PeN is one of the most important sources of dopamine and L-DOPA in CSF. Thus, evidence has been obtained that PeN is one of the largest dopaminergic centers of the brain, containing, along with dopaminergic (bienzymatic) neurons, monoenzymatic neurons. In addition, DA and L-DOPA, secreted by the neurons of PeN into CSF, are considered as neurohormones involved in the regulation of target neurons of the brain. This project was funded by RSF 20-14-00325.

P-03.4-039
Does the brain have its own endocrine system?

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The goal of this work was to test our hypothesis that the brain at all stages of ontogenesis has its own endocrine system, which, like any endocrine system, consists of three functional blocks: (i) neurons that secrete neurotransmitters and neuromodulators (neurohormones) into the cerebral ventricles, (ii) cerebrospinal fluid (CSF) that provides transport of neurohormones along the ventricles of the brain in the rostrocaudal direction, (iii) neurons located in the periventricular region of the brain being targets for neurohormones contained in the cerebrospinal fluid. As markers of the endocrine function of the brain, we have chosen monoamines, which are widely involved in the regulation of neuron differentiation and brain development in the perinatal period, as well as in synaptic and volume neurotransmission in the brain in adulthood. We have obtained key evidence for the hypothesis of the existence of the endocrine system of the brain. Firstly, it has been shown that the CSF in adult rats and in the perinatal period - on the 18th embryonic day and on the 5th postnatal day, contains all the monoamines - dopamine, norepinephrine and serotonin. Secondly, when comparing the concentration of monoamines in the CSF and in the peripheral blood, it becomes evident that the monoamines contained in the CSF are of brain origin, and do not derive from the general circulation. Finally, it has been shown that monoamines are contained in the CSF at a physiologically active concentration at which they are able to control the development of individual neuron and the entire brain as morphogenetic or transcription factors, and to contribute to volume neurotransmission in adulthood. This project was funded by RSF 20-14-00325.

P-03.4-040
Osteconductivity and adhesion process of dental pulp stem cells are improved in the presence of open cell form 3D-printed titanium

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Titanium implants are currently considered safe and effective biomaterials thanks to their appreciable osseo-integration. The surface of titanium implants can be modified with selective laser melting (SLM) in order to improve the bioactivity and develop customized implants. Moreover, the design of macro-porous structures has become popular for reaching a durable bone fixation. 3D-printed titanium (Titanium A, B, and C), were cleaned using an organic acid treatment or with electrochemical polishing, and their surfaces were morphologically characterized by means of scanning electron microscopy. Then human Dental Pulp Stem Cells (DPSCs) were seeded and cultured on titanium surfaces with the aim of analyze cell adhesion capability, biocompatibility and osteoconductive properties. All tested specimens showed an appreciable biocompatibility due to the time-dependent increase of DPSC proliferation paralleled by the decrease of LDH released within the culture medium. Moreover, the obtained results disclosed that the open cell form with

interconnected pores of titanium A, resembling the inner structure of the native bone, improves cell adhesion inside the titanium specimen, being proteins related to cell adherence markedly expressed. Likewise, a better profile in terms of markers related to an early triggered cell osteogenic commitment is found mainly in titanium A, along with an ameliorated cell behaviour, plausibly ascribable to its open cell inner structure resembling the native bone trabecular structure. Proteins involved in cell adhesion and survival, as well as the cytokines released, are differentially modulated among the three different specimens, confirming the dynamic influence of the inner design and of post-production treatments on DPSC cell response.

P-03.4-041
The angiogenic cascade is down regulated by kinesin Eg5 inhibitors in gastric adenocarcinoma cell line

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Eg5 is a motor protein that allows bipolar spindle formation during cell division in an ATP-dependent manner; over the past years it has become an interesting possible target therapy for cancer, since it is overexpressed in different types of tumours. The aim of this work was to assess the anti-cancer effect of two newly synthetized Eg5 inhibitors 2 and 41, analogues of K858, on gastric adenocarcinoma cells (AGS), alone or in combination with the polyphenol Hesperidin (HSD), with an emphasis on angiogenic signaling regulation. Firstly, 2 and 41 confirmed their potential as antiproliferative molecules compared to untreated cells, more than K858, inducing a cell cycle arrest in G2 phase. In addition, they reduced AGS cell migration, negatively modulate the angiogenic event by counteracting the PI3K-Akt-VEGF pathway and Erk activation, evaluated by western blot analysis, and reduce VEGF secretion. Secondly, the combination of 2 with HSD improves the effect of 2 alone in terms of angiogenic signaling control, by reducing Akt activation, VEGF protein expression, AGS cell migration and inducing angiopoietin 2 (ANGPT2) gene expression. Conversely, the combination of HSD with 41 and K858 markedly emphasizes the antiproliferative effect of the compounds, reducing Erk activation and inducing cell cycle arrest. These findings reveal that Eg5 inhibitors successfully counteract angiogenesis molecular cascade by recruiting the PI3K/AKT/MAPK pathway, lowering VEGF activity and promoting ANGPT2 gene expression, which is even more effective when paired with HSD. Overall, Eg5 inhibitors could be a potential starting point for further *in vitro* and *in vivo* research aimed at developing a new gastric cancer prevention strategy.

P-03.4-042
NO signalling: the contribution of molybdoenzymes

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Cardiovascular diseases (CVD), metabolic syndromes and other related diseases are major concerns of our modern society. CVD, in particular, are a leading cause of morbidity and mortality in all areas of the World, except Africa, despite recent improvements in outcomes. A common denominator to these diseases is a compromised nitric oxide ($^{\cdot}$NO) bioavailability. NO is a signalling molecule that controls a variety of functions in humans, including the well-known vasodilation, but also platelet aggregation, neurotransmission, apoptosis, gene expression and many other. Its formation is mainly catalyzed by specific arginine, O_2-dependent NO synthase (NOS) isoforms and the NO life-time is controlled through its rapid oxidation to nitrate. In the last decade, a new nitrate-nitrite-NO pathway has emerged as a physiological alternative to the "classic" NOS pathway to support cell functioning under hypoxic conditions (when the O_2-dependent NOS is hampered). In this communication, we will show that the molybdoenzymes xanthine oxidoreductase (XD and XO) and aldehyde oxidase (AO) are able to reduce nitrate and nitrite to NO and describe the conditions under which the NO generation takes place. *In vitro* and *in situ* studies will be presented and discussed to show the relevance of these two enzymes on the NO metabolism in conditions associated with a reduced NO bioavailability.

P-03.4-043
Role PHF10 in migration of osteosarcoma U2OS cells

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PHF10 is a subunit of PBAF complex, playing a crucial role in chromatin remodeling and gene expression regulation. PHF10 is expressed as four isoforms in mammalian cells. Two of them have C-terminal DPF domain and named PHD10-P and the other two have PDSM motif instead and named PHF10-S. Isoforms alternatively incorporate in PBAF complex and affect its functions. We investigated a role of PHF10 and P/S isoforms in the motility of osteosarcoma U2OS cells using wound scratch assay. We performed knockdown of PHF10-P, PHF10-S and both isoforms in U2OS cells and validated the decreasing of PHF10 or P/S isoforms mRNA and protein levels by RealTime PCR and western blot analysis. For wound scratch assay, we incubated cells with siRNAs and after 24 h made a wound by scratching the cell monolayer in a straight line with and capturing the wound healing for 20h using the Zeiss Axiovision 7 system. Wound area of control U2OS was closed in 20 hours by 29.88% (25.28-36.66, n = 9). Wound closure area of U2OS with KD total PHF10 increased to 43.59% (23.94-82.67, n = 18). With KD of S and P-isoform after 20 h the wound was closed by 74.82% (55.08-96.23, n = 18) and 70.42% (44.41-86.99, n = 18), respectively. The median wound healing rate in control was 2840μm^2/h(2377-3448, n = 9). KD PHF10 leads to an increase in the rate of wound healing, the median rate is 5361μm^2/h (2302-8162, n = 18; statistically significant, Kruskal-Wallis test, $P < 0.0001$). Suppression of both isoforms leads to an increase in the rate of wound healing; for KD S and P-isoforms, the median rates are 5694μm^2/h (4687-7290, n = 18) and 5215μm^2/h (3756-6087, n = 18; statistically significant (Kruskal-Wallis test, $P < 0.0001$)), respectively. Thus suppression of the PHF10 protein and its isoforms leads to an increase in migration rate, but the mechanisms remain to be elucidated. This study was supported by the Russian Science Foundation [grant number 21-14-00258].

P-03.4-044
A novel, 7,8-disubstitiuted purine-2,6-dione derivative modulates TGF-β-induced epithelial-to-mesenchymal transition in alveolar epithelial type II cells

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Epithelial-to-mesenchymal transition (EMT) occurs in both physiological (e.g., during embryogenesis or wound healing) and pathological conditions (e.g., carcinogenesis). EMT may also contribute to tissue fibrosis due to epithelial cell involvement in the induction of pro-fibrotic phenotype in their mesenchymal counterparts and in the extracellular space. Recent studies indicate that intracellular cAMP-elevating agents, including phosphodiesterase (PDE) inhibitors, may exert anti-inflammatory and anti-fibrotic properties. Based on the structure of theophylline (1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione), we synthesized a group of its 7,8-disubstituted hydrazide derivatives with *N*'-arylidenebutanehydrazide moiety and characterized them as potent pan-PDE inhibitors. Here we investigated the *in vitro* safety profile and the effect of the selected compound 30, a 4-(8-(benzyl(methyl)amino)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7H-purin-7-yl)-*N*'-(2,3,4-trihydroxybenzylidene) butanehydrazide, on the transforming growth factor-β (TGF-β)-induced EMT in alveolar epithelial type II cells (A549 cell line). We demonstrated that tested pan-PDE inhibitor, had no neurotoxic, hepatotoxic, genotoxic, and mutagenic effect *in vitro*. Compound 30 diminished TGF-β-induced EMT-related (*VIM*, *FN1*, *SNAI*, *TGFB*, *MMP-9*, and *CDH2*) gene expression in alveolar epithelial type II cells. This was accompanied by observed changes in actin/vimentin cytoskeleton structure and morphology of TGF-β-induced A549 cell cultured in the presence of 30. The obtained data suggest an anti-fibrotic impact of the novel 7,8-disubstituted purine-2,6-dione derivative on A549 cells via modulation of epithelial-to-mesenchymal transition. This opens a new perspective in the search for new anti-fibrotic agents in the group of pan-PDE inhibitors. Acknowledgements: This study was supported by the National Science Centre, Poland, funded grants No. UMO-2017/27/B/NZ7/01633

P-03.4-045
Downregulation of miR29b-2 is associated with hypertrophic scarring

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Abnormal wound healing can lead to hypertrophic scarring. Hypertrophic scarring is a fibrotic pathology that continues to grow in the wound and its mechanism is not yet fully understood. It is characterized by increased collagen accumulation. Therefore, a targeted intervention in collagen synthesis could be the right treatment approach. There is increasing evidence for the efficacy of the miR29 family in fibrotic diseases, and extracellular matrix proteins dominate among the targets. In this study, we investigated the role of miR29 family in hypertrophic scars, investigating collagen maturation using HSP47 and LOX and collagen synthesis, via the TGF-b/Smad pathway. This study was approved by the ethics committee of the Faculty of Medicine, Dokuz Eylül University (283-SBKAEK). Hypertrophic scar and normal fibroblast tissues were collected from the department of plastic and reconstructive surgery. The expression of miR29 family members in the tissue was determined by real-time PCR. We found that the expression of miR29b-2 was significantly lower in hypertrophic scar cells than in control cells. Therefore, to prove experimentally the effect of miR29b-2 silencing, all experiments on primary cell cultures were performed by suppressing miR29b-2 with inhibitors using Hiperfect Transfection Reagent (Qiagen). We transfected silenced miR29b-2 into primary hypertrophic scar fibroblasts and found a significant increase in COL3A and HSP47 protein levels. TGF-b signaling pathway was also activated by suppression of miR29b-2. TIMP-1 gene and protein levels were also increased. These results suggest that miR29b-2 is effective in the formation of hypertrophic scars and that this activity is related to the expression level of miR29b-2.

P-03.4-046
The influence of ylNRG1 transcriptional repressor on polyol utilization and growth form in yeast *Yarrowia lipolytica*

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Yarrowia lipolytica is an unconventional yeast, and one of its distinguishing features is a preference for glycerol over glucose. It could also grow on another polyol – erythritol, however this carbon source is less preferable compared to both glycerol and glucose. Utilization of erythritol is activated by the specific transcription factor EUF1. Intriguingly, EUF1 is also up-regulating the homolog of NRG1 – a transcriptional repressor – which is associated with glucose repression in other yeast species. This raises the question about an actual role of yl-NRG1 in metabolism of *Y. lipolytica*. Thus, both the deletion and overexpression strains were created and tested for the ability of growth on different carbon sources. Yl-NRG1 overexpression resulted in the more efficient usage of glucose. Moreover, the modifications caused dramatic phenotypic changes, indicating a significant role of this repressor in regulation of filamentous growth. This work was financially supported by the National Science Centre, Poland, project UMO-2018/31/B/NZ9/01025.

P-03.4-047
Search for changes (markers) in the blood of patients at risk of developing Parkinson's disease at the prodromal stage and their validation in animal models of the disease

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The low efficacy of therapy of Parkinson's disease (PD) is due to its late diagnosis (according to motor symptoms) and the onset

of treatment. Therefore, the aim of this work was to develop a preclinical diagnosis of PD based on the search for molecular markers in the blood of patients at risk of developing PD at the prodromal stage and their validation in animal models of the preclinical and clinical stages of PD. The risk group used in this study was selected among elderly patients without manifestations of motor disorders, but with premotor symptoms, sleep and smell disorders. The control group included patients of the same age, but without premotor symptoms. Among 27 blood parameters (monoamines and metabolites, amino acids, urates, dopamine receptors, oxidative stress, toxic proteins) assessed in patients at risk, changes were found in 5 parameters in the form of a decreased level of L-DOPA, DOPAC and urates and an increase in the oxidative stress index. Since the premotor symptoms for which the risk group was selected are not absolutely specific for PD, we could not exclude that some of the detected blood markers are also nonspecific for PD. We assumed that specific markers for the preclinical (prodromal) stage of PD should be those markers that were found both in patients at risk and in animal models of the preclinical and clinical stages of PD. For this comparative analysis, we used mice treated according to a certain scheme with MPTP, a neurotoxin of dopaminergic neurons, which play a key role in the regulation of motor behavior. When analyzing in mice the same 27 blood parameters as in patients at risk, only a decrease in the content of L-DOPA and DOPAC was detected. Thus, the data obtained suggest that, at least, changes in the content of L-DOPA and DOPAC in the blood can serve as diagnostic molecular markers of PD at the prodromal stage. Research was funded by № 075-15-2020-795, № 13.1902.21.0027 of 29.09.2020, RF-190220X0027.

P-03.4-048
Oleanolic acid (OA) encapsulation with cyclodextrins for its application in wound healing *in vitro* models

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Bioactive terpenoid oleanolic acid (OA), has shown wound healing benefits. To decipher the molecular mechanisms behind this process, we studied OA cell migration in two epithelial cell lines: Mv1Lu and MDA-MB-231, by using wound healing scratch and migration front assays. Besides these, we analyzed pro-migratory proteins expression and subcellular localization. Mainly, we focused on epidermal growth factor receptor (EGFR), extracellular signal regulated kinase (ERK) and c-Jun transcription factor. These proteins compose a regulatory axis that leads into pro-migratory genes expression, activated by c-Jun. On the other hand, migration needs to be driven by dynamic changes in the actin cytoskeleton and focal adhesions remodeling, thus, we also focused on proteins that are involved in cell architecture: focal adhesion kinase (FAK) and paxillin. We previously shown that OA in DMSO (OA/DMSO) activated all these proteins. However, due to its lipophilic nature, OA *in vitro* delivery to cells is difficult. We have resorted to nanostructures that can improve molecule solubility and bioavailability in aqueous solutions, avoiding DMSO use: cyclodextrins (CDs). Hence, we tested OA/CDs complexes in epithelial cells, which strikingly improved OA in vitro application and increased cell migration, by recruiting a greater number of migrating cells than OA/DMSO. Furthermore, this was coherent with the fact that they induced c-Jun translocation and phosphorylation in epithelial cells at front migration assays. On top of that, EGFR/ERK/c-Jun axis was activated by OA/CDs. For their use, OA/CDs complexes have to be dehydrated into a lyophilizated powder for a few days. Nonetheless, we have performed a novel technology Spray-Dry, which achieved complexes dehydration within one hour. Subsequently, these complexes showed a significant activity on cell migration. All these results encourage us to pursue on OA/CDs as a therapeutic agent to applicate in wounds with difficult resolution. *The authors marked with an asterisk equally contributed to the work.

P-03.4-049
HGF prevents prolonged treatments with Ivacaftor from destabilizing Tezacaftor-rescued F508del-CFTR in cystic fibrosis airway cell

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Cystic fibrosis (CF), the most common inherited disease in Caucasians, is caused by mutations in CFTR, the most frequent of which is F508del. F508del causes ER retention and degradation of the mutant CFTR protein, but also defective channel gating and decreased half-life at the plasma membrane. Despite the recent successes with small-molecule CFTR modulator drugs, the folding-corrector/gating-potentiator drug combinations approved for CF individuals carrying F508del-CFTR have sometimes produced severe side effects. Previously, we showed that a prolonged, 15-day treatment of polarized bronchial epithelial monolayers with the VX-809+VX-770 (lumacaftor + ivacaftor) combination resulted in epithelial dedifferentiation effects that we found were caused specifically by VX-809. Moreover, prolonged VX-770 exposure also led to the destabilization of VX-809-rescued F508del-CFTR. Notably, co-treatment with the physiological factor HGF prevented VX-809-mediated epithelial differentiation and reverted the destabilizing effect of VX-770 on VX-809-rescued CFTR. Here, we show that prolonged treatment with VX-661 (tezacaftor), a second-generation corrector developed based on VX-809 structure, does not perturb epithelial integrity of polarized bronchial epithelial monolayers. Yet, its efficacy is still affected by co-exposure to VX-770, the potentiator present in all VX-661-containing combination therapies approved in the USA and Europe for treatment of F508del-CFTR carriers. Importantly, we found that co-treatment with HGF still ameliorated the impact of VX-770 in F508del-CFTR functional rescue by VX-661, without increasing cell proliferation (Ki-67) or altering the overall expression of epithelial markers (ZO-1, E-cadherin, CK8, CK18). Our findings highlight the importance of evaluating the cellular effects of prolonged exposure to CFTR modulators and suggest that the benefits of adding HGF to current combination therapies should be further investigated.

P-03.4-050
Molecular characterization of adipose-derived stem cells trans-differentiation to the neuronal lineage in the presence of graphene-oxide

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Stem cells are known for their tremendous potential and are widely used in the field of tissue engineering and regenerative medicine. In particular, human adipose-derived stem cells (hASCs) present multiple differentiation potential, including towards the ectodermal lineage. Lots of efforts have been put in exploring their capacity for stimulating the trans-differentiation of hASCs to neuron-*like* cells. Thus, the aim of our research is to offer a full molecular characterization of neuron-*like* cells derived from hASCs and to assess the potential of graphene oxide (GO) containing biomaterials to stimulate this trans-differentiation process. For this purpose, hASCs were seeded on gelatin based fibrous scaffolds enhanced with 0,5 and 1 % GO. Neuronal differentiation was induced for 12 days, using a specific cocktail of growth factors and supplements. Gene expression of neuronal markers was assessed by Real-Time PCR, protein expression was evidenced by immunolabeling and confocal microscopy and microRNA (miRNA) profile of neuron-*like* cells was determined by PCR array. Results have demonstrated the achievement of neuron-*like* cells, as the expression of neuronal markers nestin, β-III-tubulin, NeuN and MAP2 has been detected at both gene and protein level. Moreover, the presence of graphene oxide in the scaffold contributed to stimulating the differentiation process, as the expression of neuronal markers was significantly higher on the materials with GO content. The molecular analysis of miRNAs highlighted the upregulation of several molecules, such as miR-128, miR-9, miR-122-5p, which are actively involved in the trans-differentiation of mesenchymal stem cells. Based on these results, GO and hASCs can significantly contribute to the field of regenerative medicine and should further be investigated for their use in nervous tissue engineering. This work was supported by PN-III-P1-1.1-TE-2019-1191/MAGNIFICENT grant.

P-03.4-051
Correlation between epigenetic changes and cytoskeletal fibers distribution in human adipose-derived stem cells exposed to magnetic materials

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Magnetic biomaterials have recently emerged as efficient approaches in nervous tissue regeneration. Small intensities of magnetic field have been shown to have beneficial effects on stem cells' neuronal differentiation, also influencing cytoskeleton architecture and cell interaction with the substrate through focal adhesions. Changes in focal adhesion and cytoskeleton distribution can impact nuclear morphology, mediated via linker of nucleoskeleton and cytoskeleton (LINC) protein complex, which is located at nuclear membrane level and establishes contacts with both cytoskeleton proteins and nuclear lamina, suggesting a relationship between these changes and chromatin remodeling. Human adipose derived stem cells (hASC) are investigated for nervous tissue regeneration due to their capacity to transdifferentiate into cells with ectodermal origin, like neurons, given their mesodermal origin. The aim of our study was to investigate hASCs' interaction with magnetic nanoparticles (MNPs)-enriched gelatin scaffolds, in terms of cytotoxicity, cytoskeleton and focal adhesion changes, as well as to highlight LINC complex and possible histone modifications. Three compositions were tested and compared to pure fish gelatin scaffold. ROS production was quantified to investigate cytotoxic effects of the scaffolds. Immunolabeling and confocal microscopy allowed observation of focal adhesions, cytoskeleton and LINC proteins' distribution. Colorimetric screening was also conducted to assess histone methylation pattern at H3 level. No significant cytotoxicity was indicated, while focal adhesion and cytoskeleton distribution indicated favorable interaction between hASCs and magnetic scaffolds. Moreover, changes in H3 methylation pattern were observed in magnetic compositions compared to control. Thus, MNPs-enriched materials have beneficial effects towards hASCs, also suggesting regulation of molecular mechanisms. This work was supported by PN-III-P1-1.1-TE-2019-1191/MAGNIFICENT grant.

P-03.4-052
Pro-inflammatory cytokines trigger the overexpression of tumour-related splice variant RAC1B in polarized colorectal cells

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An inflammatory microenvironment is a tumour-promoting condition that provides sur- vival signals to which cancer cells respond with gene expression changes. One example is the alternative splicing variant Rat Sarcoma Viral Oncogene Homolog (Ras)-Related C3 Botulinum Toxin Substrate 1 (RAC1)B, which we previously identified in a subset of V-Raf Murine Sarcoma Viral Oncogene Homolog B (BRAF)-mutated colorectal tumours. RAC1B was also increased in samples from inflammatory bowel disease patients or in an acute colitis mouse model. Here, we used an epithelial-like layer of polarized Caco-2 or T84 colorectal cancer (CRC) cells in co-culture with fibroblasts, monocytes or macrophages and analysed the effect on RAC1B expression in the CRC cells by RT-PCR, western blot and confocal fluorescence microscopy. We found that the presence of cancer-associated fibroblasts and M1 macrophages induced the most significant increase in RAC1B levels in the polarized CRC cells, accompanied by a progressive loss of epithelial organization. Under these conditions, we identified interleukin (IL)-6 as the main trigger for the increase in RAC1B levels, associated with Signal Transducer and Activator of Transcription (STAT)3 activation. IL-6 neutraliza- tion by a specific antibody abrogated both RAC1B overexpression and STAT3 phosphorylation in polarized CRC cells. Our data identify that pro-inflammatory extracellular signals from stromal cells can trigger the overexpression of tumour-related RAC1B in polarized CRC cells. The

results will help to understand the tumour-promoting effect of inflammation and identify novel therapeutic strategies.

P-03.4-053
The effect of the combination of PI3K/AKT/mTOR pathway and histone deacetylase enzyme activity on cell cycle and cell death in acute myeloid cell lines

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Acute Myeloid Leukemia (AML) is characterized by the accumulation of immature myeloid cells in peripheral blood leading to hematopoietic malignancy. PI3K/Akt/mTOR signaling pathway has been found deregulated in AML cells. In addition, histone deacetylase (HDAC) is a very crucial epigenetic regulator for chromatin remodeling and any deregulation in the function of HDACs in cancer cells may result in suppression of genes involved in the regulation of important cellular process. In this study, we investigate the effect of PI3K pathway modulation and HDAC enzyme inhibition on cell cycle and cell death mechanism by using two AML cell lines; MOLM-13 and CMK. In previous study, we determined IC20 values (inhibitory concentration of 20% of the cell population) of PI3K inhibitor LY294002,and HDAC inhibitors, SAHA, PCI-3501 and Tubastatin A(Tub-A) and showed the cytotoxic effect of LY294002+SAHA, LY294002+PCI-3501 and LY294002+Tub-A combinations on mentioned AML cells with MTT viability assay (previously published in: Sansacar M et al.(2021)Clinical Lymphoma, Myeloma and Leukemia,21:S294).The determined IC20 values was used to check the effect of above-mentioned combinations on cell cycle and cell death mechanism using flow cytometry. Our results demonstrated that all combinations result in G1 phase arrest in MOLM-13 cells compared to untreated control cells. On the other hand, CMK cells treated with LY294002+SAHA, and LY294002+PCI-3501 were arrested in G2/M phase, while LY294002+Tub-A treatment result in G1 phase arrest in CMK cells.LY294002+SAHA and LY294002+PCI-3501 treatment increased apoptotic cell population compared with untreated control in both cells. In contrast, LY294002+Tub-A treatment didn't affect the apoptotic cell death compared to untreated control in both cells. In conclusion, especially because of the complexity AML, inhibition of the PI3K pathway and HDACs on different subsets may give an idea about the mechanisms that lead to the pathogenesis of AML.

P-03.4-054
Gemmotherapic extract from *Corylus avellana* potentiates the chrysin effect on activated hepatic stellate cells reversion

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Hepatic stellate cells (HSCs) represent the main players in liver fibrosis progress. They activate and overproduce extracellular matrix (ECM) proteins such as collagen type I and fibronectin, which accumulate and form scar tissue. Moreover, they start proliferating and the number of ECM-producing cells is highly increased. One of liver fibrosis therapies may target activated HSCs (aHSCs) and induce their apoptosis as a way to reduce the number of cells that secrete ECM components. Such treatments could be based on natural compounds such as gemmotherapy extract of *Corylus avellana (*CAG) and chrysin (CHR) coupled with cyclodextrins (CDs-RAMEB/HPBCD) in order to improve their bioavailability. The aim of this study was to investigate the potential of CAG-CHR/CDs nanocomplexes to induce the reversion of aHSCs and their apoptosis. HSCs were cultured and treated for 48 h with nanocomplexes CAG-CHR/CDs (RAMEB/HPBCD) 0-80 μM. Levels of cytotoxicity were evaluated by LDH test, and cell viability was evaluated by MTT and Live/Dead tests. HSCs were activated with TGF-β1 and treated with 40 μM of CAG-CHR/RAMEB. The apoptotic state of the cells after the treatment was evaluated both at gene (qPCR) and protein (immunofluorescence staining) level. The expression of fibrotic markers (*α-sma, col1a1, fibronectin*) and apoptotic markers (*caspases-3/7/9, bcl-2, bax*) was assessed. MTT and LIVE/DEAD tests indicated cell viability was not affected by CAG-CHR/CD. LDH assay indicated a higher cytotoxicity of HPBCD complexes compared to RAMEB complexes. 40 μM of CAG-CHR/RAMEB up-regulated efficiently gene and protein expression of apoptosis markers. CAG-CHR/CD stimulated the expression of *bax*, inhibition of *bcl-2*. Moreover, the cleavage of caspases-3/7/9 was induced. CAG-CHR/RAMEB showed antifibrotic effect by inducing the apoptosis of aHSCs. This work was supported by PN-III-P2-2.1-PED-2019-3609 grant (262PED/2020, GEMMODIALIVER).

P-03.4-055
Relief of renal damage by interfering the RAS/TGF-β axis in a model of diabetic nephropathy

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The progression of renal fibrosis in diabetic nephropathy (DN) has been linked to the Transforming Growth Factor-β1 (TGF-β) signaling and the local activation of the renin angiotensin system (RAS). The most relevant trigger of the TGF-β fibrotic cascade in DN is Angiotensin II (Ang 1-8), the main effector of the RAS, by inducing TGF-β and cross-talking with Smad3 signaling. Further, high plasma adenosine levels have been observed in DN patients and in experimental models of renal fibrosis. Consequently, in vivo treatment with MRS1754, an A2B adenosine receptor (A2BAR) antagonist, reduces renal fibrosis in diabetic rats. We aim to elucidate if the reduction of renal fibrosis by A2BAR antagonism may be related to blocking RAS activation and/or TGF-β signaling. Streptozotocin-induced diabetic rats (DM) were treated with the A2BAR antagonist MRS1754 (DM+MRS1754) for 8 weeks. Physiological parameters were measured weekly. After finishing the treatment, blood samples were taken and renal tissue sections were collected. Serum Ang 1-8, 2-7 and 2-8 levels were measured by ELISA. Aminopeptidase A (AP-A) and angiotensin-converting enzyme 2 (ACE2) expression was evaluated by western blot and immunofluorescence. MRS1754 treatment decreases proteinuria and

glomerulosclerosis in diabetic rats. Glomerular expression of AP-A and ACE2 was recovered and even induced in DM+MRS1754 rats. Consequently, in MRS1754-treated diabetic rat serum Ang 1-8 and 2-8 levels decreased, whereas Ang 2-7 was increased. Further, using immortalized podocytes as cellular model, we demonstrated that the activation of intracellular Smad2/3 in response to TGF-β was blocked by using MRS1754. Attenuation of renal fibrosis by using the A2BAR antagonist in experimental DN occurs by inducing AP-A and ACE2, affecting the RAS repertory of peptides, and by interfering with intracellular activation of Smads downstream of TGF-β signaling. Financed by FONDECYT 1211613 and 3180749

P-03.4-056
Effect of imatinib and dasatinib and their complexes with the drugs delivery system of supramolecular ribbon-like structures (on an example of Congo red) on bladder cancer cells

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One of the most innovative compounds that can be a drug carrier are supramolecular structures formed by self-association such as Congo red and Evans blue. These compounds are known as dyes. Their supramolecular structure and the ability to react with anticancer compounds is undoubtedly an innovative approach in cancer therapy. Bladder cancer is the second most frequently diagnosed cancer of the genitourinary system in Europe, characterized by frequent recurrences and a high risk of progression. Research shows that overexpression of the c-KIT receptor with tyrosine kinase activity may be responsible for the invasion and progression of this tumor. The aim of the work was to investigate the prevalence and significance of the c-KIT expression in bladder cancer cell lines with different malignancy potential (T24, RT4). To analyze the emerging complex between investigated c-KIT inhibitors (imatinib, dasatinib) and the selected carrier, along with the *in vitro* evaluation of the effects of the drug itself and the drug in a complex with the carrier on bladder cancer cells. The results of the conducted experiments revealed the expression of the c-KIT on tested cell lines. The experiments revealed that investigated compounds inhibited the proliferation of bladder cancer cells in a dose- and time-dependent manner. FACS analysis showed independently that examined inhibitors induced apoptosis. Further, the results of the study showed a decreased level of phosphorylation of the Akt and Erk1/2 kinases under the influence of the examined compounds. The results of the conducted study indicate that inhibitors of the c-KIT receptor with the selected carrier constitute an interesting and structurally diversified group of the potential anticancer compounds. We acknowledge the financial support from the National Science Centre, Poland (grant no. K/MNT/000232) and the international project (no. U1C/P03/NO/03.23) in the strategic program Initiative of Excellence at the Jagiellonian University.

P-03.4-057
Regulation of PKC by the phosphatase PHLPP as a potential therapeutic strategy in pancreatic adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) remains one of the deadliest and most aggressive forms of cancer, with a five-year survival rate of just 10%. Recent analysis of protein abundance in 105 PDAC tumours revealed that patients expressing relatively high levels of protein kinase C (PKC) and low levels of its negative regulator PHLPP1 had significantly improved survival rates (50% 5-year survival) compared with patients with low levels of PKC (0% 5-year survival). Here we address restoring PKC expression in PDAC by targeting PHLPP1/2, two phosphatases which dephosphorylate PKC to promote its ubiquitylation and degradation via the proteasome. Both catalytic inhibition and genetic silencing of PHLPP1/2 increased PKC protein levels in pancreatic cancer cell lines and PHLPP inhibitors showed modest efficacy to slow the growth of highly chemoresistant patient-derived PDAC organoids. In order to develop improved inhibitors against PHLPP1/2, we performed peptide overlay assays to determine the specific interaction interface between PKC and PHLPP1/2, which we confirmed by coimmunoprecipitation experiments. Identification of this interface can now be exploited as a unique druggable space to disrupt binding and stabilize PKC, which would be beneficial in patients with low PKC levels. We developed a NanoBRET assay to quantitatively measure protein-protein interaction between PKC and PHLPP1/2 in a high-throughput manner, which will be utilized to screen for disruptors of this interaction. The results obtained from this study will help the design and development of biomarker-driven therapeutics in PDAC.

P-03.4-058
Cellular redox homeostasis mediates contractility of isolated ileum of male Wistar rats

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Redox-mediated mechanisms play an important role in regulating smooth muscle contractility and depend on subtle redox homeostasis maintaining. Redox homeostasis in cells is obtained by the activity of antioxidant enzymes. Among them, glutathione reductase constantly reduces oxidized glutathione, providing a favorable cellular redox milieu. The aim of this study was to examine whether, to what extent and in what way changes in smooth muscle contractility occurred in conditions when glutathione reductase activity was changed. The experiments were performed on isolated ileum of male Wistar rats, electrically stimulated and treated with cumulative doses of carmustine, a glutathione reductase inhibitor. The activity of antioxidant enzymes

(catalase, glutathione reductase and glutathione peroxidase, as well as the amount of total SH groups and glutathione) was determined in treated ileum. The addition of carmustine inhibited glutathione reductase activity and decreased amplitude and frequency of electrically stimulated ileum. The results showed that glutathione reductase activity and maintenance of highly reductive cellular environment were essential to contractility processes.

P-03.4-059
JAK/STAT signaling during cellular senescence process in primary fibroblasts of blind mole rats

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Blind mole-rat (BMR), a long-lived subterranean rodent, is an exceptional model for aging, additionally, for cancer research since they are resistant to both spontaneous and induced tumorigenesis. The remarkable cancer resistance of BMR is suggested to be triggered by the cytokine-stimulated pathway with including secretion of interferonβ during increased population doublings which are named concerted cell-death (CCD). The JAK pathway has been well-known to have a crucial role in multiple cellular events. It is a cytokine-regulated pathway and its activation leads to cytokine secretion. On the other hand, cellular senescence is a stable cell-cycle arrest with a capacity to secrete various chemokines, growth factors, and cytokines. Therefore, the role of JAK signaling has recently gained an interest in cellular senescence studies. In this study, to uncover the expressional changes of the JAK signaling pathway during the aging process, we comparatively analyzed JAK1 and JAK2 mRNA expressions in BMR and mice. Then, we validated our results with a proteomics study. For this aim, we isolated primary fibroblasts from BMR and mouse and the aging process is achieved by the increased population doublings as a result of serial passaging. The senescent phenotype was validated by common cellular senescence biomarkers including the growth-curve, β-galactosidase staining, and IL-6 mRNA expression in young and replicative-induced senescent cells. Our results uncovered the elevated JAK levels in BMRs when compared to mice during replicative senescence. Besides, with proteomics analysis, we confirmed cytokine-mediated signaling activation in senescent BMR fibroblasts. These results suggest that the activation of JAK and cytokine-mediated signaling pathways could be a possible adaptation for the anti-tumor mechanism of BMR during the aging process.

P-03.4-060
The role of kappa opioid receptors in colitis-associated colorectal cancer in mice

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For centuries, opioids were known for their anti-nociceptive action in numerous types of pain, recently opioids were indicated as drugs with anti-inflammatory potential in experimental colitis as well as in animal models mimicking diarrhea-predominant irritable bowel syndrome due to alleviation of abdominal pain and inhibition of diarrhea. Among three types of opioid receptors (MOP, DOP, KOP) KOPs are not well characterized, however KOP agonists induced an anti-inflammatory response in experimental models of the rheumatoid arthritis and atopic dermatitis. The aim of this project was to determine if kappa opioid receptors may become a potential pharmacological target in colitis-associated colorectal cancer in mice. In our studies we used human (HCT-116, SW-480 and HT-29) and murine (MC-38) colorectal cancer cell lines to assess the effect of U50488H, a selective KOP agonist, on the cell viability and death. The effect of U50488H on proliferation of MC-38 cells was determined in xenograft model. A colitis-associated colorectal cancer was induced by azoxymethane and dextran sodium sulfate in mice and we verified the anti-cancer properties of U50488H. In both animal models U50388H was injected intraperitoneally at the dose of 1 mg/kg every third day. We found that U50488H in concentration-dependent manner inhibited the cell viability and death in human and murine colorectal cancer cell lines using MTT and LDH assays. We observed a decreased tumor weight and volume after treatment with U50488H in xenograft model. U50488H produced an anti-cancer effect in colitis-associated colorectal cancer model, as indicated by a decrease in tumor number and size in the distal colon and histological staining. An anti-cancer action of U50488H was confirmed at mRNA level (a decrease in relative expression of Nos2, Ptgs2, Tnfa, Il1b, Il6, Il12, Il18 and Ifng). To conclude we suggest that activation of KOP may constitute a pharmacological target in colorectal cancer in mice.

P-03.4-061
Cytostatic and cytotoxic effects of dovitinib (TKI-258) and its complex with the drug delivery system of supramolecular ribbon-like structures (on an example of Congo red) on bladder cancer cells

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Research shows that an enormous role in the development of bladder cancer may be played by increased activation of receptor tyrosine kinases (e.g. c-KIT) and the PI3K/ AKT/mTOR intracellular transmission pathway. Moreover, the invasion and progression of this neoplasm may be i.a. closely related to the overexpression of receptors with tyrosine kinase activity, e.g. c-Kit or PDGFR. For these reasons, tyrosine kinase inhibitors (e.g., dovitinib, TKI-285) are among the most advanced examples of personalized medicine. However, it should be taken into account that even this therapeutic approach has negative effects on healthy tissues and cells. Hence, a huge interest is aroused by carriers, i.e. techniques for the targeted introduction of drugs. The aim of this work was to determine the influence of selected inhibitor of c-KIT receptor tyrosine kinase (dovitinib) and its complex with the drug delivery system of supramolecular ribbon-like structures on growth, survival and migration of bladder cancer cell lines (RT4, T24) with different malignancy potential. The exposure of bladder cancer cells to investigated inhibitors resulted in dose-dependent suppression of proliferation compared to the control. FACS analysis showed independently that investigated inhibitor induce apoptosis of bladder cancer cells. The

percentage of apoptotic cells was increased in a dose-dependent manner by treatment with inhibitor. A wound-healing test/scratch assay showed a significant reduction in cell motility after exposure to investigated inhibitor and its complex with Congo red. Tyrosine kinase inhibitor such as dovitinib and its complex with the selected carrier represent a promising class of therapeutic agents for the treatment of bladder cancer. We acknowledge the financial support from the National Science Centre, Poland (grant no. K/MNT/000232) and the international project (no. U1C/P03/NO/03.23) in the strategic program Initiative of Excellence at the Jagiellonian University

P-03.4-062
Epigenetic traits related to vitellogenin gene expression reveal clues for organismal response to estrogen in *Cyprinus carpio*

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Epigenetic mechanisms orchestrate gene expression during development and lifelong adaptation to changing environments. In adult male fish estrogen exposure leads to feminization where vitellogenin (vtg) expression serves as robust marker for exposure to estrogenic endocrine disruptor compounds. With the aim to reveal related epigenetic traits and novel blood markers, we analyzed response of male carp to 17b-estrogen (E2). After three day E2 treatment, DNA methylation bisulfite analysis of 8 CpGs in vtg gene promoter in liver revealed diminished C-methylation in E2- respect to control carp, specifically in position 1 which correlated with increased vtg mRNA levels. We further studied male carp with high and low vtg expression in liver characterizing blood extracellular vesicles (EV), which seem to be released from different cells playing regulatory roles in an intricate communication network coordinating whole organism response. Small RNA libraries were prepared from EV enriched blood serum of male carp, presenting 50.1% miRNAs, 42.9% piRNAs, 7% snoRNA and snRNAs. From high versus low vtg carp, a set of 18 miRNAs differentially present in serum EVs was determined and target genes predicted. Comparison with *in silico* processed data sets from liver mRNAs of estrogen treated male zebrafish revealed 121 differentially expressed genes in liver in response to E2 in male fish. Finally, the integrative analyses of experimental and *in silico* data visualized a network of miRNAs and target genes involved in oxidoreductase pathways, response to chemical stimulus and small and lipid biosynthetic processes. These epigenetic markers might help to better understand organismal response to potential harmful exposure to endocrine disruptors in the aquatic environment. Acknowledgement: Conicyt 21130511 GV, 21150665 FS. This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska Curie grant agreement No 722634.

Proteins

P-04.1-001
Dynamics of the couple proteins in tip-links during hearing

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Discovery of mechanical transducers that help us to convert touch into nerve impulses earned Professor Ardem Patapoutian the Nobel Prize in Physiology or Medicine 2021. Hearing is similarly a mechanotransduction process, where a protein-couple, jointly known as tip-links, serve as gating-springs and convey the input mechanical force of varying intensities from sound-stimuli to electrical signal. Importantly, tip-links maintain the integrity of their marriage under periodic tension from input sound. With an overarching objective to decipher the force-responsive behaviors of tip-link complexes and their alterations with aging, here we probe the viscoelastic properties of the tip-links directly under mechanical stimuli at the single-molecule level. In this talk, I shall present how tip-links form counterintuitive 'catch-bonds' to overcome the mechanical pulses from sound, and as gear-box, accelerate the lifetime of the complex while conveying force for transduction. Further, tip-links serve as guardians to the non-regenerative hair-cells from the sudden impact of loud noise and at the cost of their marriage. Tip-links dissociate, however, re-engage fast. Towards the end, I shall discuss how tip-link proteins undergo phase separation on a cell membrane to accelerate the re-formation of tip-links and cell-cell adhesion.

P-04.1-002
Src-NADH dehydrogenase subunit 2 complex and recognition memory of imprinting in domestic chicks

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Src is a non-receptor protein tyrosine kinase that participates in a number of neuronal processes including synaptic plasticity. We have recently shown that the amounts of total Src and its two phosphorylated forms, at tyrosine-416 (activated) and at tyrosine-527 (inhibited) undergoes time-dependent learning related changes after visual imprinting in the domestic chick forebrain. These changes occur only in the intermediate medial mesopallium (IMM) – site of memory formation for visual imprinting. Src contains a region of low sequence conservation termed the unique domain, which interacts with mitochondrial genome coded NADH dehydrogenase subunit 2 (ND2). ND2 is a subunit of complex I in mitochondria and this interaction occurs outside this organelle at excitatory synapses in the brain. ND2 acts as an adapter protein anchoring Src to the N-methyl-D-aspartate (NMDA) receptor complex. In the present study we have inquired if Src-ND2 complexes are changed 1 h and 24 h after training in the chick IMM and posterior neostriatum (PN) – a control brain region not involved in imprinting. RIPA buffer solubilized membrane-mitochondrial P2 fractions were studied by immunoaffinity chromatography on Src antibodies with the following western immunoblotting. In the affinity eluates as well as in original P2 fractions ND2 and Src were measured. 1 h after

imprinting in the IMM of the left hemisphere the amount of ND2 is increased significantly with the strength of memory, whilst the Src protein is not changed. The ratio of linked ND2 to the content of the ND2 in P2 fraction follows the same pattern. No learning-related changes are observed at 24 h in the IMM or at both times of experiment in the PN. Thus, the amount of ND2 in the complex with Src is increased and not the total amounts of either ND2 or Src are upregulated. This increase is learning-related, time-dependent, region-specific and indicates the role of this protein complex in learning and memory of imprinting.

P-04.1-003
Characterization of MoaB2 from *Mycobacterium smegmatis*: a novel binding factor of mycobacterial SigmaA

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Mycobacterium is a genus of medicinally important bacteria with a number of human pathogens. Understanding the mycobacterial gene expression is necessary to develop new antibacterial approaches to fight diseases such as tuberculosis and leprosy. The central enzyme of bacterial gene expression is RNA polymerase (RNAP) whose core is catalytically active but to initiate transcription it needs a sigma factor. Factor A is the primary sigma factor (SigA) responsible for transcription of housekeeping genes but it is not known how exactly SigA levels are regulated in the mycobacterial cell. In stationary phase of growth, we identified a new protein associated with SigA. This protein, MoaB2 (17.9 kDa), is predicted to be involved in the biosynthesis of molybdopterin, an essential cofactor of a diverse group of redox enzymes. However, by several approaches, we demonstrated that MoaB2 binds to SigA, and pilot experiments suggest that MoaB2 affects the biological half-life of SigA, thereby regulating its intracellular level. Finally, we solved the 3D structure of *Mycobacterium smegmatis* MoaB2 by crystallography, providing a basis for its further studies. Taken together, MoaB2 represents a new binding partner of SigA in mycobacteria, and extends thus our knowledge of the architecture of the transcriptional machinery. This work is supported by grant No. 19/12956S from the Czech Science Foundation Keywords: MoaB2, SigA, RNA polymerase, Mycobacteria

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P-04.1-007
Mutational analysis of cystathionine beta-synthase from *Toxoplasma gondii* reveals the presence of a unique catalytically active conformation

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Cystathionine beta-synthase (CBS) is a key regulatory PLP-dependent enzyme of the reverse transsulfuration pathway, which catalyzes the condensation of L-homocysteine with L-serine to form L-cystathionine. Although the pathway itself is well conserved, properties of eukaryotic CBS enzymes vary greatly. Human CBS is activated by S-adenosyl-L-methionine (AdoMet) by inducing a conformational change involving the regulatory C-terminal Bateman region. Artificial removal (via protein engineering or proteolysis) or heat denaturation of the C-terminal regulatory domain activates the enzyme by permitting the substrates entry into the catalytic center. Herein, we have used deletion mutagenesis of the C-terminal Bateman domain, limited proteolysis, and thermal denaturation to extend our knowledge of the functional domain organization of CBS from *Toxoplasma gondii* (TgCBS). The crystal structure of TgCBS revealed that the enzyme exists in a basal-type folding which likely represents its sole conformational state and does not require additional conformational changes to develop its function, explaining why TgCBS is active and unresponsive to AdoMet. Accordingly, our present results showed that, in contrast to human enzyme, the removal of the C-terminal domain has no effects on the oligomerization and thermal stability of TgCBS and, most importantly, none of the three methods of human CBS activation can activate TgCBS in vitro. Overall, our findings support the hypothesis that TgCBS represents the first example of a non-allosterically regulated basal-like CBS enzyme and provide crucial information for understanding the intricate domain architecture and regulation of eukaryotic CBSs.

P-04.1-008
Mapping glycosaminoglycane binding sites on the surface of cathepsin B via nuclear magnetic resonance spectroscopy

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Cathepsin B (CatB) is a cysteine protease that is primarily located in the lysosomes of cells with duties ranging from nonspecific protein turnover to highly specialised tissue homeostases[Turk V et al. (2001) EMBO J 20, 4629–4633]. However, in pathological

situations CatB is found on cell surfaces, where it also degrades the extracellular matrix (ECM), which consists of elastins, collagens and proteoglycans. This activity can promote tumour growth in cancer. On the other hand, other members of the cathepsin family are regulated (i.e., facilitation of autocatalytic activation, modulation of activities or stabilisation under unfavourable conditions) by glycosaminoglycans (GAGs) as part of the ECM [Novinec M et al (2014) Biomed Res Int 2014, 309718]. The current project is about establishing a procedure for high level recombinant production of fully labelled (^{13}C, ^{15}N) CatB from *E. coli*, subsequent purification, refolding and activation steps sufficient to perform solution-state nuclear magnetic resonance (NMR) experiments. A full assignment of the ^{13}C and ^{15}N chemical shifts applying 3D correlation experiments is being performed, because NMR shifts are sensitive to secondary and tertiary structure as well as ligand binding and conformational changes. In a next step, the binding specificity of GAGs, like hyaluronic acid, chondroitin sulphate, dermatan sulphate, heparin or heparan sulphate, exhibiting different sulphation pattern, to CatB is investigated by ligand titration experiments to identify (specific) binding sites, as mainly electrostatic effects are expected to play key roles. The experimental work is supported by computational approaches to give answer to variable GAG binding propensities and recognition patterns, which was shown to vary significantly within the cathepsin family [Sage J et. Al. (2013) Biochem 52, 6487–6498]. The new findings about protein−GAG interactions could be used for new strategies in enzyme inhibition or tissue regeneration therapies.

P-04.1-009

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P-04.1-010

Characterization of the interaction between the Shank-PDZ and GKAP-GH1 domains

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Shank proteins are an abundant family of scaffolds in the postsynaptic density (PSD), a membraneless organelle found in glutamatergic synapses. The PSD can be described as a network of domaindomain interactions, whose individual dynamics all contribute to its larger scale behaviour. The PSD is associated with learning, memory, and overall, synaptic plasticity, and many of its proteins had been described in relation to conditions such as autism spectrum disorders. Shank1 and Shank3 are the more widely studied isoforms, both exhibiting the same domain composition, and the subject of choice for this study was the PDZ domains of both, which, out of its interaction partners, has the highest binding affinity to the C-terminal GH1 domain of GKAP. This interaction connects the platform formed by the Shank proteins to the "upper levels" of the PSD. Shank PDZ domain constructs of various lengths were designed and examined against a full-length GKAP-GH1 construct. ECD, ITC, affinity column assays and NMR spectroscopy were applied to characterize the structure and the interaction of the PDZ and GH1 constructs. Shank1 PDZ was produced and purified in ^{15}N and ^{15}N-^{13}C labelled forms and 2D ^{1}H-^{15}N and 3D ^{1}H-^{15}N-^{13}C correlation spectra were measured, including relaxation experiments. Resonance assignment of Shank1 PDZ was completed. T1 and T2 backbone amide relaxation rates were extracted. Shank1 PDZ was also titrated with the GH1 construct and changes in backbone amide chemical shifts were noted. The results provide insight into the dynamics of the interaction and pave the way towards answering questions regarding the stoichiometry and conformation of this complex.

P-04.1-011

Proteolytic control of preproteins stalled in human mitochondrial translocases

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Most of the mitochondrial proteins are synthesized as precursors in the cytosol. Such proteins require an effective import into a dedicated part of the organelle. To enter into the mitochondria, proteins cross mitochondrial membranes using translocation machinery. To pass translocation channels, such precursor proteins need to be largely unfolded. Yet, protein misfolding occurs even in physiological conditions. Misfolded proteins are capable of clogging translocases which impairs protein import and function of mitochondria. We investigated quality control mechanisms involved in unclogging of the translocases and their role in maintaining homeostasis in human cells. We used model fusion proteins that become blocked at an intermediate step of import, allowing investigation of the mechanisms that resolve this problem. Earlier studies in yeast identified the role of cytosolic factors like Cdc48 (p97/VCP) and proteasomes in removing stalled precursors. Using specific inhibitors, we confirmed the role of these cytosolic factors, but we also uncovered a paralleled clearance path that relies on mitochondrial factors. Importantly, our experiments, done in cells and isolated organelles, revealed the significant role of specific mitochondrial proteases in overhauling invalid translocation events. We also observed that stalled import intermediates cause significant changes to mitochondrial morphology. Activating the organelle-specific quality control mechanisms eliminated proteins that clogged mitochondrial translocases and reversed morphological deformity. Accumulating data substantiates the impact of transiting proteins on cellular protein homeostasis and human pathologies. Disturbance of protein import not only affects destination organelles but also deregulates protein synthesis and folding. Thus, we contribute to the broad field of proteostasis by improving the understanding of the molecular mechanisms that maintain the quality of mitochondrial proteome biogenesis.

P-04.1-012
How phosphorylation of a disordered region at HuR regulates its DNA binding

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Human antigen R (HuR) is an RNA-binding protein involved in maintaining the stability and controlling the translation of mRNAs, critical for cell survival, proliferation and apoptosis. HuR is recruited to stress granules (SGs), which are membraneless organelles involved in the regulation of translation, mRNA storage, stabilization and cell signalling during stress. HuR contains three RNA recognition motifs (RRMs) and its function and localization are regulated by posttranslational modifications, including phosphorylation by Janus Kinase 3 (JAK3) at diverse tyrosine residues within RRM1 (Tyr63 and Tyr68) and the linker between RRM2 and RRM3 (Tyr200). Functional studies revealed that Tyr200 phosphorylation is essential for HuR relocation into SGs. In this work, we used *in vitro* kinase assays to describe the JAK3-mediated phosphorylation of Tyr5, a residue contained in the disordered N-end stretch of HuR. Thus, we have designed a phosphomimetic protein of $HuR_{2\text{-}99}$-pTyr5 by replacing Tyr5 by the non-canonical amino acid *p*-carboxymethyl-L-phenylalanine (*p*CMF). Then, we have used thermal stability assays, Nuclear Magnetic Resonance (NMR) experiments and Molecular Dynamics simulations to characterize $HuR_{2\text{-}99}$-Y5*p*CMF. In addition, we have studied the impact of Tyr5 phosphorylation on DNA recognition processes by NMR and Isothermal Titration Calorimetry. Unveiling the molecular mechanisms underlying HuR posttranslational modifications may shed some light on the events regulating SGs assembly and aggregation during cell stress, and thus contributing to understand the basis of SGs-related pathogenesis.

P-04.1-013
Production of mutant Taq DNA polymerase

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DNA polymerases are widely used enzymes, which can amplify specific regions of DNA flanked by primers in the polymerase chain reaction (PCR). They have been used in basic research, industry and biotechnology. In our work we focused on the production of mutant Taq DNA polymerase. The high thermostability of Taq polymerase ensures its robust use in molecular biology techniques, biotechnology, as well as in clinical diagnostics and sequencing. We have used several expression vectors (e.g. pJexpres404, pET21a, pET28a) for construction of production *E. coli* strain with designed synthetic genes endoding mutant Taq DNA polymerase. We received successful recombinant strain construction in case we used pET28a vector. Amplification of target genes was performed using PCR and final construct was made by restriction cloning. We successfully transformed E. cloni 10G and StellarTM cell lines with the new construct and identified the target transformant from the grown colonies. We were able to produce high amounts of target protein by shake-flask expression, however the solubility was low. In the next work we will focus on the optimization of the production of soluble mutant recombinant Taq DNA polymerase.

P-04.1-014
Mistranslation helps *Escherichia coli* to adapt to oxidative stress

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Mistranslation can occur due to inaccurate amino acid selection by aminoacyl-tRNA synthetases (aaRSs). Although usually toxic, due to the production of aberrant proteins, there are a few examples of its adaptive effect on cellular responses to subsequent stresses. Isoleucyl-tRNA synthetase (IleRS) is one of aaRSs that possess editing domain and hydrolyze incorrect aa-tRNA product. In our experiments, we used *Escherichia coli* strain expressing IleRS with inactivated editing domain, which allowed us to induce mistranslation by incubating bacteria in the medium supplemented with amino acids structurally similar to isoleucine. We simulated variable mistranslation rates using different concentrations (0.25, 0.5, 0.75 and 1 mM) of valine (Val) or norvaline (Nva). Mistranslation induced the formation of elongated bacterial filaments, whose length correlated with increasing Val or Nva concentrations. Transmission electron microscopy showed morphological and ultrastructural changes in bacterial cells, such as numerous intracellular vesicles and bulge formation. Survival assays revealed a significant increase in the survival of bacteria grown with 0.5, 0.75 and 1 mM Val or Nva in the presence of 1 mM H_2O_2. Growth curve measurements showed that bacteria preincubated with Val or Nva grow better in oxidative stress conditions. Better growth was dependent on Val concentration, while preincubation with 0.5 mM Nva had the most prominent effect. If bacteria were exposed simultaneously to both mistranslation and oxidative stress, higher mistranslation rates were beneficial as well, however, the effect was only observed during early exposure to oxidative stress. The results show that there is mistranslation-induced adaptation to oxidative stress. Further work will focus on proteome analysis in order to clarify cellular mechanisms that increase survival and growth under oxidative stress due to misincorporation of valine or norvaline at isoleucine positions in proteins.

P-04.1-015
Modular assembly of hybrid GRM2 bacterial microcompartment shells

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Bacterial microcompartments (BMC) are bacterial organelles responsible for ensuring and/or enhancing a wide range of enzymatic reactions. Identified in around 25% of all sequenced bacterial genomes, BMCs are associated with both anabolic and catabolic reactions, all related to diversifying available carbon sources. Main components of BMCs are a semipermeable protein shell and enzymatic core. While the components of the enzymatic core can greatly differ depending on the catalyzed reaction, the

protein shell has relatively conserved structure, consisting of several thousand copies of three types of shell proteins hexamers BMC-H, pseudohexameric trimers BMC-T and pentamers BMC-P, together forming a polyhedral shell. Across known BMC loci, diverse variations of shell protein combinations can be found, with some loci missing BMC-T coding genes altogether, one such loci being GRM2 BMC encoding loci in *Klebsiella pneumoniae*. To explore the modular nature of bacterial microcompartment shell and analyze the possibility of incorporating BMC-T proteins in shells with no native BMC-T homologs, we tested eight BMC-Ts representing five different microcompartment types. Co-expression with GRM2 shell proteins and purification of bacterial microcompartment particles revealed four BMC-T proteins able to incorporate into BMC shell- RMM-T1, EUT-T1, GRM1-T and GRM4-T, confirming the modular structure of bacterial microcompartment shell and conserved nature of protein interactions employed in shell formation. Results also revealed increased proportion of large size particles for BMC-T incorporated particles, indicating a possible role of BMC-Ts in increasing the volume of BMC particles in addition to metabolite flow regulatory properties of both BMC-T and BMC-H proteins.

P-04.1-016
Readdressing the molecular basis of proteolytic cleavage of TLR8 Z-loop

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Toll-like receptors (TLRs) are transmembrane proteins that are key regulators of the innate immune response. TLRs trigger a pro-inflammatory immune response by recognising a variety of ligands, e.g. external pathogen-associated (PAMP) and internal damage-associated (DAMP) molecular patterns. In humans, ten TLRs have been identified. Each TLR contains three structural domains: a leucine-rich repeats (LRRs) motif, transmembrane helix (TM), and cytoplasmic Toll/IL-1 receptor (TIR) domain. The common mechanism of TLR signalling is that the interaction of a ligand with LRRs either induces the formation of a receptor dimer or changes the conformation of a pre-existing dimer. For some TLRs, in order to obtain an active dimer, the proteolytic cleavage of the long loop (Z-loop) located in the LRRs motif is a must. The molecular basis of this process remains unclear. In our work, we used homology modelling combined with advanced loop reconstruction to obtain complete structures for the individual domains of human TLR8. Additionally, we used a model from the AlphaFold server in order to compare the obtained results. The selected models were further used to build the TLR8 dimer containing LRRs, TM, and TIR domains altogether immersed in the lipid bilayer. Docking protocols merged with multiple molecular dynamics simulations were also incorporated in order to characterise the ligand-binding and protein-binding regions, especially the site where the proteolytic cleavage of Z-loop is performed. All of the above-mentioned protocols were also used to take an insight into the description of the dimerisation process of human TLR8. The work was supported by the Ministry of Science and Higher Education, Poland from the budget for science for the years 2019-2023, as a research project under the "Diamond Grant" programme [DI2018 014148].

P-04.1-017
14-3-3-protein regulates Nedd4-2 by modulating interactions between HECT and WW domains

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Neural precursor cell expressed developmentally down-regulated 4 ligase (Nedd4-2) is an E3 ubiquitin ligase that targets proteins for ubiquitination and endocytosis, thereby regulating numerous ion channels, membrane receptors and tumor suppressors. Nedd4-2 activity is regulated by autoinhibition, calcium binding, oxidative stress, substrate binding, phosphorylation and 14-3-3 protein binding. However, the structural basis of 14-3-3-mediated Nedd4-2 regulation remains poorly understood. Here, we combined several techniques of integrative structural biology to characterize Nedd4-2 and its complex with 14-3-3. We demonstrate that phosphorylated Ser342 and Ser448 are the key residues that facilitate 14-3-3 protein binding to Nedd4-2 and that 14-3-3 protein binding induces a structural rearrangement of Nedd4-2 by inhibiting interactions between its structured domains. Overall, our findings provide the structural glimpse into the 14-3-3-mediated Nedd4-2 regulation and highlight the potential of the Nedd4-2:14-3-3 complex as a pharmacological target for Nedd4-2-associated diseases such as hypertension, epilepsy, kidney disease and cancer. This study was supported by the Czech Science Foundation (Project 20-00058S), the Czech Academy of Sciences (Research Projects RVO: 67985823 of the Institute of Physiology).

P-04.1-018
Abstract withdrawn

P-04.1-019
L-Arginine in *Pseudomonas aeruginosa* controls c-di-GMP levels and biofilm formation

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Nutrients such amino acids play key roles in shaping the metabolism of microorganisms in natural environments and in host–pathogen interactions. Arginine is a nutrient able to dictate the fate of bacterial cells particularly in hostile setting, being a crucial metabolite for nitrogen, carbon and even ATP supply; in light of this, this nutrient is linked to chronic infections, biofilm / virulence and antibiotic resistance. The second messenger 3'-5'cyclic diguanylic acid (c-di-GMP) controls biofilm formation and dispersion in response to environmental cues. It has been demonstrated that Arginine can be perceived by the opportunistic human pathogen *Pseudomonas aeruginosa* [1] to control c-di-GMP via RmcA protein (Redox regulator of c-di-GMP). RmcA is a multidomain membrane protein harboring a Venus Fly Trap (VFT) domain devoted to periplasmic Arginine binding, a transmembrane helix portion and a cytoplasmic part displays 3 PAS and 1 LOV domains linked to GGDEF-EAL catalytic tandem,

where the hydrolysis of c-di-GMP occurs [2]. We analyzed various cytoplasmic truncated constructs of RmcA and we understood that RmcA is able to sense the metabolic status of the cell and to perform a redox-based switch depending on FAD / FADH2 levels. These data show that this nucleotide also plays a significant role in the remodeling of central metabolism. Furthermore, having tested the behavior of different cytoplasmic constructs we noticed some limitations in obtaining truncated constructs, so it is necessary to carefully evaluate which residues to cut in these domains to avoid false results [3]. [1]. Paiardini et. al. (2018) Proteins. 86(10): 1088-1096. [2]. Mantoni et al. (2018) FEBS J. 285(20): 3815-3834. [3]. Mantoni et al. (2021) Life 6;11 (1):31. IF: 2,991

P-04.1-020
A computational study on the catalytic mechanism of pyridoxal 5'-phosphate synthase: a new drug target against tuberculosis and malaria

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Pyridoxal 5'-phosphate (PLP) synthase is a key enzyme in the synthesis of PLP, consisting of two different subunits: Pdx1, the synthase subunit, and Pdx2, the glutaminase subunit. PLP synthase displays a Pdx1 dodecameric core assembled as two opposing interlocking hexameric rings, with one Pdx2 subunit attached to each Pdx1. The catalysis starts in Pdx2, which employs a non-canonical Cys-His-Glu triad to catalyze the deamination of glutamine to glutamate and ammonia. The ammonia molecule is then tunneled to the Pdx1 active site, where it will be the nitrogen source of PLP. Despite the ubiquity of PLP synthase in most plants, fungi, bacteria, and parasites that can synthesize PLP *de novo*, it is absent in mammals, which depend upon its uptake from their diet to survive. For this reason, PLP synthase is considered a novel and promising drug target against diseases such as malaria and tuberculosis. Therefore, the catalytic mechanism of Pdx2 (previously published in: Pina AF et al. (2021) ChemBioChem. DOI: 10.1002/cbic.202100555) and Pdx1 was studied with atomic detail using the computational ONIOM QM/MM methodology based on the DLPNO-CCSD(T)/CBS//B3LYP/6-31G(d,p):ff14SB scheme. The Gaussian09 and ORCA software were used to perform the calculations, whereas VMD and the molUP plugin were used to analyse the results. The computational results demonstrate that the catalytic mechanism of Pdx2 occurs in six steps divided into four main stages, whose rate-limiting step is the hydrolysis of the glutamyl-thioester intermediate (18.2 kcal.mol^{-1}), which closely agrees with the available kinetic data (19.1–19.5 kcal mol^{-1}). Regarding Pdx1, whose catalytic mechanism is currently under evaluation, the present computational results suggest a concordance with the available experimental data. The acquired knowledge can now help us in the rational drug design of transition state analogs as new inhibitors targeting the PLP synthase.

P-04.1-021
Unraveling the manifold ligand-binding activities of Megalin in a domain-wise manner

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Overt accumulation of small binding proteins in the kidneys is a well-known problem that causes high background in medical imaging and can lead to organ damage during therapy. Megalin-mediated endocytosis constitutes the main pathway for tubular reabsorption of such small proteins after passage of the glomerular filter, including antibody fragments as well as alternative scaffolds such as Anticalins. The ectodomain of the 600 kDa Megalin (LRP2) receptor consists of 4 cysteine-rich clusters. Each of these clusters comprises 7–11 LDLA repeats, which share a pattern of typically 6 conserved Cys residues, while otherwise showing high sequence variation. Little is known, however, about the mechanism of protein binding by Megalin during reabsorption. To map such physiological binding activities towards plasma proteins to functionally relevant Megalin domains a structural screening concept was developed. To this end, an expression method for individual receptor fragments in HEK293E cells was established employing the SpyCatcher protein as a fusion partner which was linked by a flexible PAS sequence. Real-time surface plasmon resonance (SPR) measurements with the second cysteine-rich LRP2 cluster showed a Ca^{2+}-dependent binding of the LRP2 chaperon Receptor-Associated-Protein (RAP), thus proving the functionality of the recombinant Megalin fragment. For a more detailed analysis, the receptor was genetically dissected into overlapping fragments, each consisting of three LDLA repeats, covering all four cysteine-rich clusters. After expression, Megalin fragments were immobilized on a microtiter plate coated with the SpyTag MBP fusion proteins directly from cell culture supernatant. Incubation with fluorescein-labeled RAP showed distinct signals for different fragments, thus indicating a region-specific binding profile. This strategy will now be used to assign specific Megalin domains responsible for the binding of physiologically or biomedically interesting proteins.

P-04.1-022
Molecular bases of AADC deficiency: what's hidden behind the asymmetry of an homodimeric enzyme

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Almost 20 years ago, the crystal structure of porcine holo Aromatic Amino Acid Decarboxylase (AADC) (90% sequence identity to human) was solved and, 10 years later, human apoAADC structure was published. AADC is a pyridoxal-5'-phosphate (PLP) dependent homodimeric enzyme responsible for the synthesis of two essential neurotransmitters dopamine

and serotonin whose altered levels are responsible for severe motor and neurological symptoms evident in the rare monogenic neurodegenerative inborn defect AADC deficiency (AADCd). In the past years, efforts on human recombinant AADC pathogenic variants were done to provide support to the research on AADCd by means of biochemical and biophysical approaches determining the impact of the amino acid substitutions on the enzyme features. Here, a further contribution to the comprehension of the AADCd is provided. At first, we solved the human holoAADC crystal structure in its native and ligand-bound form providing new insights on the mechanism of substrate binding and on the conformation of the active site mobile catalytic loop (CL). Then, molecular dynamic simulations together with SAXS analysis and limited proteolysis revealed that AADC is an asymmetric molecule in which the mobility of different domains of each monomer is related to the conformation of the CL and exerts effects on the facing monomer. Thus, all domains of the protein result connected to the active site and influence the catalytic competence. Following this view, we have acquired correlations between newly acquired structural measurements and functional data by analyzing more than 30 AADC pathogenic variants. This led us to obtain a key for interpreting the effect of the substitutions on the kcat and Km for the different substrates. The relationship among structural and functional approaches might represent the basis for severity prediction of AADCd with potential on future precision therapy such as gene therapy.

P-04.1-023
M-MuLV reverse transcriptase production in *E. coli* expression system

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Reverse transcriptases are important enzymes of retroviruses that convert single-stranded RNA into double-stranded DNA. In molecular biology, they are used for quantitative RT-polymerase chain reaction, generation of cDNA libraries for cloning and many more. M-MuLV (Moloney Murine Leukemia Virus) reverse transcriptase contained proposed point mutations that provide high thermostability and processivity. The aim of our work was to create new constructs suitable for expression and produce mutated recombinant M-MuLV reverse transcriptase in the *E. coli* expression system. In the first phase, we focused on gene amplification by PCR for mutated M-MuLV reverse transcriptase from the original commercially available pUC57 plasmid. We inserted sequences for restriction sites of chosen restrictases by specific primers on both side of gene coding M-MuLV to be able to insert it in right orientation into the pET21a / pJexpress404 vectors. We designed multiple primer sets with His-tag at the C- or N-terminus. We were able to create a target construct of the pJexpress404 vector with M-MuLV reverse transcriptase and a C-terminal His-tag. In the next part of the work we will focus on the creation of the remaining constructs and their subsequent production in the *E. coli* expression system. This research was supported by Slovak Research and Development Agency grants (Nos. APVV-17-0570, APVV-19-0196, and APVV-17-0333) and ERDF project: SmartFarm (No. ITMS2014: 313011V465).

P-04.1-024
NPM1 phase transition modulation via post-translational modifications and heterotypic interactions

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Nucleophosmin (NPM) is an abundant nucleolar protein involved in multiple functions in living cells, namely proliferation, genome stability, ribosome biogenesis and stress response, among others. NPM is integrated into the nucleolus, which is a membrane-less organelle (MLO) formed upon liquid-liquid phase separation (LLPS) of nuclear components. LLPS is thus a dynamic process that allows the cell to rapidly regulate metabolic reactions by controlling protein location and availability. In this context, post-translational modifications (PTMs), namely acetylation and phosphorylation, finely tune the phase separation properties and nucleoplasm redistribution of NPM upon DNA damage. Such a nucleolar-nucleoplasmic shuttling of NPM following DNA breaks is key to control the alternative reading frame (ARF)/p53 tumor suppressor pathway. In the nucleolus, NPM sequesters ARF, thus enabling p53 ubiquitination and subsequent proteasomal degradation. Upon genomic stress, NPM shuttles to the nucleoplasm and releases ARF, which in turn binds to the human double minute clone 2 protein (HDM2) to avoid p53 degradation. Noteworthy, DNA injuries trigger respiratory cytochrome c (Cc) migration to the nucleus. In this meeting, we present our most recent experimental findings on how Cc affects the interplay between NPM and ARF, as well as on how PTMs alter the nucleolar-nucleoplasmic shuttling of NPM so as to control tumor suppression.

P-04.1-025
Interaction of dengue virus capsid protein with nucleic acids may depend on its sequence

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Dengue virus (DENV) and Zika virus (ZIKV) are mosquito-borne flaviviruses, sharing structural features. The nucleocapsid core of the mature virion is formed by the 11 kb viral (+) single-stranded RNA condensed with multiple copies of the capsid (C) protein [1]. This is an essential protein, conserved among flaviviruses, which is involved in key steps of the viral life cycle, namely encapsidation and viral assembly [2]. One key step, essential for viral replication, requires DENV C specific binding to intracellular lipid droplets (LD), an interaction that was fully characterized. In addition to the interaction with LD, it was also demonstrated by us that DENV C interacts specifically with host very low-density lipoproteins (VLDL) and with the viral RNA

[2]. Those findings led to the development of pep14-23, a patented peptide based on the region comprising amino acids 14 to 23 of DENV C, which is able to inhibit DENV C binding to LD and VLDL [2]. Then, to characterize DENV and ZIKV C binding to the viral RNA, through biophysical approaches, we started examining locations within the viral RNA to which the protein has higher affinity, with specific RNA sequences being identified. Circular dichroism data show that some single-stranded DNA sequences analogous to those selected RNA sequences do indeed interact specifically with DENV C, causing changes in the protein secondary structure. Other biophysical approaches, such as dynamic light scattering, fluorescence and nuclear magnetic resonance spectroscopies will also be applied to better characterize this phenomenon, including the locations within DENV C to which the viral RNA is prone to bind. These data will allow to select and develop inhibitors against this interaction of DENV C with the viral RNA. This methodology might be applied to other related flaviviruses, as well as other human viruses. [1] Silva NM et al (2020) Trop. Med. Infect. Dis. 5 (4):150. [2] Faustino AF et al (2019) Int. J. Mol. Sci. 20(16):3870.

P-04.1-026
The effect of charge neutralization and motion restriction of the dengue virus capsid protein on the nucleocapsid assembly

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Dengue virus (DENV), a member of the Flaviviridae family, is formed by three structural proteins: capsid (C), membrane (prM/M), and envelope (E)[1]. C protein folds in a stable homodimer composed of four α-helices and a flexible and structurally disordered N-terminal region [1, 2]. Rich in basic residues, the protein interacts with viral RNA, forming the nucleocapsid (NC) [1]. Despite Cryo-EM studies revealing structural details on the organization of DENV proteins on the virus surface, structural information about NC is lacking [3]. In addition, the RNA-capsid complex has never been isolated from cells infected by DENV, suggesting a coordinate process between binding to RNA and NC's budding. To study C protein and NC assembly we designed an R85C mutant, in which arginine at position 85 was replaced by cysteine residue, forcing the formation of disulfide cross-link between α4 and α4' helices. We aim to understand how the presence of a disulfide bond can alter the motion of the protein, its stability, and consequently its interaction with other molecules in the cell. We established the expression, purification, and biophysical characterization of the R85C. This mutant self-assembles into nucleocapsid-like particles (NCLPs) in solution in the absence of nucleic acids, suggesting that the rigidity imposed by the α4/α4' covalent bond and neutralization of the positive charge at position 85 mimics what happens upon RNA binding. We observed the formation of regular particles by transmission electron microscopy. Dynamic light scattering, thermal and chemical denaturation experiments confirmed the formation of oligomeric particles compatible with NCLPs. This is the first study establishing an efficient *in vitro* self-assembly of DENV in solution without the need for a surface or nucleic acid. [1] Jones, CT et al. (2003) Journal of Virology, 77, 7143-7149. [2] Neves-Martins, TC et al. (2021) Current Opinion in Virology, 47, 106-112. [3] Kuhn, RJ et al. (2002) Cell, 108, 717-725.

P-04.1-027
Characterization of multiheme cytochromes involved in the electroactivity of *Desulfuromonas acetoxidans*

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Microorganisms that are able to exchange electrons with electrodes are termed electroactive and can be exploited in bioelectrochemical systems (BES) for sustainable applications, such as energy production and bioremediation. In electroactive organisms, extracellular electron transfer (EET) pathways composed by multiheme cytochromes (MHC) couple bioenergetic metabolism to the reduction of extracellular electron acceptors. Most of the EET processes have been studied in the two model organisms *Shewanella oneidensis* MR-1 and *Geobacter sulfurreducens* PCA. However, these organisms were isolated from freshwater environments, enabling BES devices to only operate at low ionic strength. This is energetically inefficient in terms of electrical conductivity within the system. Marine electroactive microorganisms such as *Desulfuromonas acetoxidans* DSM 684 represent a better alternative for the operation of energy efficient BES. The aim of this work is to identify, validate and study the EET pathway of *D. acetoxidans* to push forward the application of this organism in BES. Toward this aim, a putative EET pathway composed by MHC was identified, and these key MHC cloned, expressed heterologously, and purified for further characterization. N-terminal sequencing and mass spectrometry confirmed their identity and integrity, respectively. Functional characterization was performed by circular dichroism and EPR spectroscopies and by protein film voltammetry. In order to evaluate their electron transfer processes, interaction between partners is currently being probed by NMR. Altogether this study represents the first step towards the understanding the EET process in a marine microorganism, providing the necessary fundamental knowledge for the development of more energetically efficient BES.

P-04.1-028
Structure and dynamics of flavivirus capsid proteins: the role of the intrinsically disordered N-terminal region

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Even though the structure of flavivirus mature viral particles has already been solved by cryoelectron microscopy, there is no high-resolution information about the structure of the nucleocapsid (NC), which is formed by the viral genome and multiple copies of the capsid (C) protein. Flavivirus C proteins are stable homodimers. Each monomer has about 100 residues organized into 4 α-helices and an intrinsically disordered N-terminal region (IDR N-terminal). Quaternary hydrophobic contacts and salt bridges stabilize the dimeric interface. The binding between C protein and host's lipid droplets (LDs) is essential for infective

viral particle assembly. The C protein-LDs binding involves the hydrophobic cleft (α2/α2' helices) and the IDR N-terminal. The goal of the work is to establish the role of the IDR N-terminal in the structure and dynamics of flavivirus C proteins. For this, it was designed a C protein mutant without the IDR N-terminal (Δ1-19). The 3D structure was solved by nuclear magnetic resonance (NMR). Backbone 15N relaxation parameters (R1, R2, het-NOE) and 15N relaxation dispersion measurements (15N-CPMG-RD) were collected to establish the C protein dynamics. From the 15N-CPMG-RD data, thermodynamic parameters that drive the equilibrium between the ground and excited states were also extracted. To obtain information about the position and structure of the IDR N-terminal, the solvent exposure of each backbone chain amide was measured by solvent paramagnetic relaxation enhancement (sPRE), as well as the thermal susceptibility. The results show a partial protection of the α2/α2' helices in C proteins by residues from the IDR N-terminal IDR and/or α1/α1' helices, suggesting that the IDR N-terminal could be partially structured during an interaction with the hydrophobic cleft and server as a regulator of its exposure during the stages of the viral replication cycle.

P-04.1-029
Modulation of human serum albumin interaction with amyloid β peptide by ibuprofen, risperidone, serotonin and tryptophan

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Human serum albumin (HSA) is a depot of amyloid β peptide (Aβ), a key player in progression of Alzheimer's disease (AD). Replacement of HSA of AD patients with therapeutic HSA gave persistent positive results in clinical trials (previously published in: Boada M et al. (2017) J Alzheimers Dis, 56, 129-43). The directed shift of the HSA-Aβ equilibrium towards their complex is potentially able to exert similar effect. HSA affinity to Aβ can be improved by HSA loading with its ligands, as exemplified by linoleic/arachidonic acids (previously published in: Litus EA et al. (2019) BBRC, 510, 248-53). To explore the ability of HSA ligands to modulate its interaction with Aβ, we rationally selected the candidate HSA ligands via bioinformatic analysis: ibuprofen (IBU), risperidone (RIS), serotonin (SRO) and tryptophan (TRP). Their influence on HSA binding to monomeric Aβ40/42 was studied by surface plasmon resonance spectroscopy (SPR). Whereas 1 mM TRP did not affect the HSA-Aβ equilibrium, 100 μM RIS prevented the interaction. On the contrary, 1 mM SRO and 100 μM IBU decreased the equilibrium dissociation constant for HSA-Aβ complex by a factor of 7/17 and 3-5, respectively. 20 μM IBU did not affect Aβ40 fibrillation, but enhanced suppression of Aβ40 fibrillation by HSA, as evidenced by thioflavin T assay. 1 mM SRO inhibited Aβ fibrillation regardless of HSA. The HSA-induced suppression of Aβ40 fibrillation was unaffected by 1 mM TRP, but was reduced by 39 μM RIS, in accord with the SPR data. The revealed SRO/IBU-induced enhancement of HSA-Aβ interaction is in line with previous results of animal and clinical studies (previously published in: Cirrito JR et al. (2011) PNAS, 108, 14968-73; data of the American Academy of Neurology). The present study provides a mechanistic rationale of these observations, and indicates that the directed improvement of HSA affinity to Aβ may serve as a basis for AD prevention. *The authors marked with an asterisk equally contributed to the work.

P-04.1-030
Lessons from random mutagenesis of the prototypic *E. coli* Ntn-amidohydrolase EcAIII

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Bacterial L-asparaginases from Class 1 are used to treat acute lymphoblastic leukemia (ALL), but their administration is not free from severe side effects. Many experiments undertaken in the past to improve the properties of classical therapeutic L-asparaginases have been unsuccessful. Therefore, it seems reasonable to search for novel biotherapeutics among L-asparaginases from other structural classes. Potential candidates could be sought among Class 2 L-asparaginases, which belong to the family of Ntn-hydrolases. These enzymes, exemplified by the *E. coli* protein EcAIII, require a maturation autoproteolytic step to develop the L-asparaginase activity. However, Class 2 L-asparaginases have too low substrate affinity for clinical applications. Thus, protein engineering would be required to improve their catalytic properties for potential therapeutic applications. To better understand the maturation process and the catalytic mechanism, EcAIII variants were generated by random mutagenesis. The new variants of EcAIII were characterized by structural (X-ray crystallography), biophysical (nanoDSF, CD), and bioinformatic (AlphaFold2) methods. The observations and analysis of the experimental and molecular modeling data provide useful hints that can be applied to fine-tuned engineering of EcAIII, and of other Ntn-hydrolases in general. The findings were grouped into six lessons, focused on (1) adaptation of the EcAIII fold to new substitutions; (2) the role of Arg207 in EcAIII activity; (3) the network of residues necessary for autoprocessing; (4) the complexity of the autoprocessing reaction; (5) systematic conformational changes observed in enzymatically inactive variants; and (6) cooperativity of the EcAIII dimer subunits. Work supported by National Science Centre (NCN, Poland) grant 2020/38/E/NZ1/00035.

P-04.1-031
eSPC, an online tool to analyze biophysical data

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All biological processes rely on the formation of protein–ligand, protein–peptide and protein–protein complexes. Studying the affinity, kinetics and thermodynamics of binding between these pairs is critical for understanding basic cellular mechanisms. Many different technologies have been designed for this purpose, each based on measuring different signals (fluorescence, heat,

thermophoresis, scattering and interference, among others). Evaluation of the data from binding experiments and their fitting is an essential step towards the quantification of binding affinities. Here, we present a user-friendly online tool to analyze biophysical data from steady-state fluorescence spectroscopy, microscale thermophoresis, differential scanning fluorimetry experiments, Circular Dichroism and Mass Photometry. The modules of the data-analysis platform (https://spc.embl-hamburg.de/) contain classical thermodynamic models and clear user guidelines for the determination of biophysical parameters.

P-04.1-032
A novel regulator of the bacterial oxidative stress response identified in a human pathogen

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Bacteria have to cope with oxidative stress which is caused by distinct Reactive Oxygen Species (ROS), including superoxide O_2^-, hydrogen peroxide H_2O_2 and radical hydroxide $HO^\bullet$. High levels of ROS result in the oxidation of several biomolecules, and RNA is particularly susceptible to this chemical damage. Oxidation of RNA leads to translational defects and cell toxicity. Hence, this may indicate that RNA-binding proteins play an important role in the bacterial response to oxidation. In this work, we report the discovery of a new regulator of oxidative stress in *Listeria monocytogenes*, which is a Gram-positive bacterium and a major foodborne human pathogen. Through a screening of RNA-binding proteins that could be important for bacterial response to oxidative stress, we identified a major enzyme involved in RNA degradation, the exoribonuclease PNPase, as critical for *Listeria* survival under stress generated by different ROS. A deletion mutant of *pnp* (the gene encoding PNPase) is unable to grow under sub-minimal inhibitory concentrations (MIC) of different oxidation agents, like hydrogen peroxide (H_2O_2) and plumbagin (that generates O_2^-). Conversely, bacteria expressing PNPase fully recover growth after exposure to oxidation agents. Comparison with other regulators of oxidative stress revealed that PNPase-depleted cells exhibit a hypersensitive phenotype to ROS attacks. Infection assays further showed that the PNPase mutant strain is more susceptible to the ROS attack that occurs inside macrophages. This increased susceptibility of PNPase mutants to oxidative agents might be useful in the development of new antimicrobial strategies. Overall, this work highlights the expanding roles of RNA-binding proteins in the cell, revealing that they can be critical players in promoting bacterial resistance to oxidative stress. *The authors marked with an asterisk equally contributed to the work.

P-04.1-033
SARS-CoV-2 variants impact RBD conformational dynamics and ACE2 accessibility

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The coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has killed over 5 million people and is causing a devastating social and economic impact all over the world. The rise of new variants represents a difficult challenge due to the loss of vaccine and natural immunity, and increased transmissibility. These variants contain mutations in the spike glycoprotein, which mediates fusion between the viral and host cell membranes, via its receptor binding domain (RBD) that binds to angiotensin-converting enzyme 2 (ACE2). To understand the effect of RBD mutations, a lot of attention has been given to the RBD-ACE2 interaction. However, this type of analysis is limited since it ignores the conformational dynamics of the RBD itself. Observing that some variants mutations occur in residues that are not in direct contact with ACE2, we hypothesized that they could affect RBD conformational dynamics. To test this, we performed long atomistic molecular dynamics simulations to investigate the structural dynamics of wt RBD, and that of three variants (alpha, beta and delta). Our results show that in solution, wt RBD presents two distinct conformations: an "open" conformation where it is free to bind ACE2; and a "closed" conformation, where the RBM ridge blocks the binding surface. The alpha and beta variants significantly impact the open/closed equilibrium, shifting it towards the open conformation by roughly 20%. This shift likely increases ACE2 binding affinity. In the delta variant RBD simulations, the closed conformation was never observed. Instead, the system alternated between the before mentioned open conformation and an alternative "reversed" one, with a significantly changed orientation of the RBM ridge flanking the RBD. These results support the hypothesis that variants impact RBD conformational dynamics in a direction that simultaneously promotes efficient binding to ACE2 and antibody escape. *The authors marked with an asterisk equally contributed to the work.

P-04.1-034
Investigating the molecular mechanisms of the eukaryotic cytochrome c maturation system

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Cytochromes-*c* are ubiquitous proteins with enormous impact at the cellular level, being key players in metabolic processes such as electron transfer chains and apoptosis. The assembly of these proteins requires maturation systems that catalyse the formation of the covalent thioether bond between two cysteine residues and the vinyl groups of the heme. System III is the maturation system present in Eukaryotes, designated CcHL or HCCS. This system requires a specific amino acid sequence in the apocytochrome to be recognised as a substrate and for heme insertion. To explore

the recognition mechanisms of CcHL, the bacterial tetraheme cytochrome STC from *Shewanella oneidensis* MR-1, that is not a native substrate for System III, was mutated by site directed mutagenesis to be identified as a substrate. All mutants were also produced by the native bacterial maturation system. All proteins were analysed using UV-visible and Mass Spectrometry to address the spectral properties and number of inserted hemes, respectively. The results obtained show that it is possible to convert a bacterial cytochrome into a substrate for CcHL, but the presence of the recognition sequence is not the only factor that induces the maturation of a holocytochrome by System III. The location of this sequence in the polypeptide also plays a role in the maturation of the *c*-type cytochrome. Furthermore, CcHL appears to be able to catalyse the binding of only one heme per polypeptide chain, being unable to assemble multiheme cytochromes *c*, in contrast with bacterial maturation systems.

P-04.1-035
Response of the eutardigrades (Tardigrada) to high temperature-induced stress

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Tardigrada (water bears) are well known for their ability to undergo cryptobiosis and survival in extreme conditions. The best known type of cryptobiosis is anhydrobiosis i.e. response to the lack of water. In this state tardigrades are able to tolerate e.g. high pressure, very high and very low temperatures, harmful chemicals, space vacuum, solar radiation and extreme levels of ionizing radiation. Heat Shock Proteins (HSPs) are synthesized by tardigrades in the response to the environmental stress including changes in the environmental temperature. In this study, we tested expression of different HSPs in the tardigrade species: *Paramacrobiotus experimentalis* Kaczmarek, Mioduchowska, Poprawa & Roszkowska, 2020 under different temperature regimes (5°C, 20°C, 35°C, 40°C and 42°C). Tardigrada in four different life stages were tested: (I) fully active in culture medium, (II) entering anhydrobiosis (reduced activity), (III) inactive during anhydrobiosis and (IV) fully active after rehydration (post anhydrobiosis). This work provides new knowledge about selective activation of the HSPs genes in response to different temperatures and in various stages of tardigrades life. Keywords: Heat Shock Proteins; Tardigrada; Water bears

P-04.1-036
Nedd4-2 binding to 14-3-3 modulates the accessibility of its catalytic site and WW domains

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Neural precursor cells expressed developmentally downregulated protein 4-2 (Nedd4-2), a homologous to the E6-AP Carboxyl Terminus (HECT) ubiquitin ligase, triggers the endocytosis and degradation of its downstream target molecules by regulating signal transduction through interactions with other targets, including 14-3-3 proteins. In our previous study, we found that 14- 3-3 binding induces a structural rearrangement of Nedd4-2 by inhibiting interactions between its structured domains. Here, we used time-resolved fluorescence intensity and anisotropy decay measurements together with fluorescence quenching and mass spectrometry to further characterize interactions between Nedd4-2 and 14-3-3 proteins. The results showed that 14-3-3 binding affects the emission properties of AEDANS-labelled WW3, WW4 and, to a lesser extent, WW2 domains and reduces their mobility, but not those of the WW1 domain, which remains mobile. In contrast, 14-3-3 binding has the opposite effect on the active site of the HECT domain, which is more solvent exposed and mobile in the complexed form than in the apo-form of Nedd4-2. Overall, our results suggest that steric hindrance of the WW3 and WW4 domains combined with conformational changes in the catalytic domain may account for the 14-3-3 binding-mediated regulation of Nedd4-2. 1. Pohl P et al. (2021) *Commun Biol.*, 4, 899. 2. Joshi R et al. (2022), *Biophys. J.* accepted DOI: https://doi.org/10.1016/j.bpj.2022.02.025 This study was supported by the Czech Science Foundation (Projects 20-00058S), the Czech Academy of Sciences (Research Projects RVO: 67985823 of the Institute of Physiology) and by Grant Agency of Charles University (Project No.348421).

P-04.1-037
A single-molecule perspective on the RNA interactions of a Ser-Arg-rich splicing factor

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The boundaries between coding and non-coding regions within mRNAs are recognised by serine-arginine-rich splicing factors (SRSFs) that contain one or two structured RNA recognition motifs (RRMs) and a long intrinsically disordered domain consisting of many Arg-Ser repeats. These so-called RS domains are crucial for protein-protein and protein-mRNA interactions that regulate splicing as well as a multitude of other processes, and serine phosphorylation of RS domains modulates SRSF conformation, cellular localisation and function. Using single-molecule spectroscopy, we are aiming to understand how the intrinsically disordered character of RS domains and their phosphorylation patterns regulate the wide range of protein and RNA interactions previously discovered. First, we characterised the interaction of full-length SRSF1, the RRMs and the RS domain with ssRNA. The RRMs of SRSF1 interact specifically with an ssRNA containing a conserved binding sequence, while RS domain-RNA interactions are largely unspecific. Both domains individually exhibit low nanomolar affinity, whereas full-length SRSF1 binds the same ssRNA with high picomolar affinity. Surprisingly, even though the binding partners involved are highly charged, the interaction itself only shows a moderate dependence on ionic strength, hinting at a finely tuned balance between counter-ion release and uptake in the formation of this complex. Next, we examined how phosphorylation of the RS domain influences this interaction using synthetically and enzymatically phosphorylated proteins. An increase in phosphorylation correlates with a

decrease in affinity until the RS domain cannot interact with RNA any longer. That is, when SRSF1 localises to active transcription sites in its fully phosphorylated state, any protein-RNA contacts are likely mediated by the RRMs alone. Together, these results form the basis to quantify and understand the molecular mechanisms underlying the capacity of SRSFs to regulate splicing.

P-04.1-038
Structural and functional characterization of V120S variant of *E. coli* EcAIII Ntn-amidohydrolase

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L-Asparaginases, which catalyze the decomposition of L-asparagine to L-aspartic acid and ammonia, are a diverse group of enzymes used in biotechnology, and in medicine for the treatment of acute lymphoblastic leukemia. L-Asparaginases are divided into three Classes. Class 2 asparaginases (formerly known as plant-type) have too low substrate affinity for therapeutic applications. However, genetic engineering can be used to improve their catalytic properties. A characteristic structural feature of Class 2 asparaginases is the Na^+ binding loop, which stabilizes the active site. Some plant asparaginases (e.g. from common bean), but not the *E. coli* enzyme EcAIII, contain another alkali metal binding loop, which has high affinity for K^+ and activates the enzyme when this cation is present. Structural studies suggested that potassium-sensitivity can be introduced also in potassium-independent enzymes by a V→S mutation in the region of the activation loop. The aim of this work has been to examine the biophysical and structural properties of the V120S mutant of EcAIII. In general, the V120S mutant retained the thermal stability and activity of the WT protein. However, high-resolution crystal structure revealed that the mutation affected the structural properties of the activation loop region and the geometry of the active site. In particular, the activation loop is now capable of alkali metal binding. These observations suggest that mutagenesis in the region of the activation loop can be the first step towards the generation of EcAIII variants with controlled enzymatic activity. Work supported by National Science Centre (NCN, Poland) grant 2020/38/E/NZ1/00035.

P-04.1-039
Modulating FOXO3 transcriptional activity by low-molecular compounds

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FOXO3 is a member of Forkhead Transcription Factor family. Forkhead proteins share an evolutionarily conserved winged-helix DNA-binding domain (DBD), which recognizes specific DNA sequence. Through interaction with target DNA, FOXO proteins modulate various biological processes, such as cell death, cell-cycle arrest, DNA repair and energy homeostasis [1]. Due to FOXO3 ability to induce cell cycle arrest, it is considered a tumour suppressor. However, in certain cases, it has been shown that FOXO3 can promote tumour development and angiogenesis via maintaining cancer cell energy homeostasis. Moreover it also enhances tumour cell resistance to chemotherapeutic agents [2]. Therefore, targeting FOXO3 transcriptional activities by specific inhibitors can help to prevent drug resistance in cancer therapy. A pharmacophore screening identified a low-molecular compound, named S9, that interacts with FOXO3-DBD and modulates FOXO3 transcriptional programme in human cells. The mode of interaction between S9 compound and FOXO3-DBD was characterized using NMR spectroscopy and docking studies [3]. This compound was further modified to increase its inhibitory potency. In this work we tested a group of newly designed S9 derivatives. Their inhibitory potency and interaction with FOXO3-DBD was tested using NMR spectroscopy and native electrophoresis. Furthermore, the effect of these compounds on FOXO3 transcriptional activity was evaluated in cell cultures. We have shown that these new derivatives are able not only to bind to FOXO3-DBD but also to inhibit its interaction with the target DNA. [1] M. Hornsveld et al., Seminars in Cancer Biology, 50, (2018), 90–100. [2] S. Salcher et al., Mol. Cancer, 13, (2014). [3] J. Hagenbuchner et al., eLife, 8, (2019), e48876. This work was supported by the Czech Science Foundation (reg. No 21-02080S).

P-04.1-040
Insights into the dynamics of human neuropeptide Y type 4 receptor (hY4R): a solid-state NMR perspective

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It is well-known that G-protein coupled receptors (GPCRs) play fundamental roles in regulating and modulating a plethora of physiologic functions, which makes them very attractive biological targets for drug-based therapeutic approaches. However,

there is still a big gap in understanding the intricated molecular mechanisms, signalling pathways and structure–function relationships, particularly when it comes to differentiate between receptor subtypes of the same GPCR class,[1,2] thus posing serious challenges for the development of subtype-specific drugs. Here, we present our latest studies on the structural dynamics of the human neuropeptide Y type 4 receptor (hY_4R), a class A peptide-binding GPCR, primarily involved in the regulation of anorexigenic effects, and understood to play a key role in the insurgence of pathophysiological disorders such as obesity. An optimised cell-free expression system, followed by reconstitution in a synthetic lipid bilayer membrane of DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine) and DHPC (1,2-dihexanoyl-*sn*-glycero-3-phosphocholine),[3] allows us to obtain the wild-type hY_4 receptor in high yield (milligram scale). We use a multitude of methods from the biophysical toolbox to assess structural integrity and receptor functionality, including fluorescence polarisation, light scattering techniques and liquid chromatography-coupled mass spectrometry. Finally, via solid-state NMR spectroscopy of isotopically labelled receptor samples we investigate structural dynamics of the different receptor states, namely its apo-, active and inactive forms. References: [1] Jones AJY, Gabriel F et al. (2020) Molecules 25, 4729. [2] Kaiser A et al. (2020) Molecules 25, 4724. [3] Krug U. et al. (2020) Angew Chem Int Ed 59, 23854–23861; Angew Chem 132, 24062–24070.

P-04.1-041
Molecular basis of the interaction between tumor suppressor p53 and Forkhead box O (FOXO) 4 transcription factor

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Transcription factor p53 protects cells against tumorigenesis when subjected to various cellular stresses [1]. Under these conditions, p53 interacts with transcription factor Forkhead box O (FOXO) 4, thereby inducing cellular senescence by upregulating the transcription of senescence-associated protein p21 [2]. However, the structural details of this interaction remain unclear. Here, we characterize the interaction between p53 and FOXO4 by NMR, chemical cross-linking, and analytical ultracentrifugation. Our results reveal that the interaction between p53 TAD and the FOXO4 Forkhead domain is essential for the overall stability of the p53:FOXO4 complex. Furthermore, contacts involving the N-terminal segment of FOXO4, the C-terminal negative regulatory domain of p53 and the DNA-binding domains of both proteins stabilize the complex, whose formation blocks p53 binding to DNA but without affecting the DNA-binding properties of FOXO4. Therefore, our structural findings may help to understand the intertwined functions of p53 and FOXO4 in cellular homeostasis, longevity, and stress response. 1. Boutelle et al. (2021), Trends in Cell Biology, 31, 298-310. 2. Baar et al. (2017), Cell, 169, 132–147. Recently this work was accepted in the journal of Protein Science (DOI: 10.1002/pro.4287). This study was supported by Czech Science Foundation (grant number 21-02080S) and the Grant Agency of the Charles University (grant number 1002119).

P-04.1-042
A structure-function study of the newly identified *B. subtilis* LonBA protease and its isolated fragments

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ATP-dependent (AAA+) Lon proteases represent a family of highly selective enzymes that are key members of the cellular protein quality control system. They are present in all domains of life. Their substrates are abnormal proteins as well as short-lived regulatory proteins. The subunits of homooligomeric Lon proteases include the central ATPase module – the characteristic core of all AAA+ proteins, the C-terminal protease domain (Ser–Lys peptide hydrolase), and an extra domain located either in the enzyme N-terminal region or in its ATPase module. Based on differences in the environment of the active residues of the peptidase center, as well as of the architecture of the ATPase modules, Lon proteases are divided in MEROPS into subfamilies A (in prokaryotes and eukaryotes), B (in archaea), and C (in bacteria). However, some enzymes from Firmicute bacteria have been also placed in the LonB family. Analysis of their primary structures showed that, although the ATPase module of these enzymes has more similarity to its counterparts in LonB, the protease (P) domain is similar to the corresponding domain of LonA, thus establishing a new, "hybrid" LonBA subfamily. B. subtilis LonBA was cloned and expressed in *E. coli*, leading to isolation of BsLonBA. Chymotrypsin digestion showed that BsLonBA consists of stable domains. The ATPase module and P domain of BsLonBA were cloned, expressed, and purified, enabling a comparative structure-function study of BsLonBA and its truncated forms. Individual AAA+ module and P domain were shown to exhibit a reduced oligomericity degree compared to LonBA protease. Isolated P domain has no proteolytic activity and does not undergo autolysis, but is still capable of hydrolyzing low molecular weight substrates. The full-length enzyme exhibits only a low level of ATPase activity, whereas the isolated ATPase fragment practically loses the ability to bind and hydrolyze ATP. Supported by RSF project 21-74-20154. *The authors marked with an asterisk equally contributed to the work.

P-04.1-043
Enzymatic synthesis of new fluorine-containing and lipophilic nucleoside analogues

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Chemically modified nucleosides mimic natural nucleosides in terms of uptake and metabolism in live organisms. Reactions

that are similar to the biosynthesis of nucleosides in a cell due to high stereospecificity and mild conditions can be considered as a prospective area of "nature-like" manufacturing of biologically active compounds. Enzymatic transglycosylation is based on the transferring a carbohydrate residue from one heterocyclic base to another. Transglycosylation catalyzed by nucleoside phosphorylases (NPs) is the two coupled consecutive equilibrium reactions of phosphorolysis of nucleosides leading to outcome a new nucleoside using α-*D*-(2-deoxy)ribose-1-phosphate as intermediate and the corresponding heterocyclic base. Earlier, we proposed approaches optimizing transglycosylation reaction using 7-MedGuo as an initial substrate for the preparation of 5-substituted 2'-deoxyuridine derivatives and cladribine [Drenichev M.S. *et al. Adv Synth Catal*. 2018, 360 (2), p. 305-312]. In addition, we proposed a mathematical model of transglycosylation process, which can be used to quantify the effect of initial conditions on the result of transglycosylation [Alexeev, C.S. *et al. Adv. Synth. Catal*. 2018, 360 (16), p. 3090–3096]. In this study, we performed an optimization of NPs catalyzed transglycosylation and developed a three-step procedure for biosynthesis of perspective EV71 inhibitors based on fluorine-containing purine deoxyribonucleosides from more accessible corresponding ribonucleosides. In new scope of our work a library of cladribine derivatives containing a chiral hydrocarbon fragment at position 6 of purine base have been obtained for the first time. They may be considered as multifunctional drug prototypes due to their ability to act on several targets, *e.g*., DNA polymerases, adenosine receptors, *etc*. Biological activity of the obtained compounds is now under study. This work was supported by the Russian Science Foundation (project No. 21-14-00346).

P-04.1-044
Biochemical characterization of His6-tagged adenylosuccinate synthetase from *Helicobacter pylori*: first step towards design of new inhibitors

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Bacterium *Helicobacter pylori* is involved in development of several gastrointestinal diseases, including gastric cancer. Since *H. pylori* is estimated to infect ~50% of world's population, and is increasingly evading currently used therapies, new ways of its eradication are urgently needed. Unlike most other bacteria, *H. pylori* does not have genes for enzymes involved in *de novo* purine synthesis. Adenylosuccinate synthetase (AdSS, EC 6.3.4.4) is one of the enzymes involved in purine recycling and is therefore critical for this bacterium's survival. It catalyzes the synthesis of adenylosuccinate from IMP and aspartate, paired with hydrolysis of one phosphate group from GTP. Previously, we have purified and characterized native *H. pylori* AdSS (Bubić A et al. (2018) J Enz Inh Med Chem 33, 1405–1414), and here we present results obtained on His_6-tagged variant of the enzyme, which is the first tagged enzyme amongst bacterial AdSSs. Nucleotide sequence coding for 6 His residues was inserted at the C-terminal end of the gene by inversed PCR. Enzyme was overexpressed in *E. coli* BL21-CodonPlus(DE3)-RIL cells, with IPTG induction. After two purification steps (affinity and size-exclusion chromatography), electrophoretically homogeneous AdSS-His_6 was characterized by means of circular dichroism, enzyme kinetics measurement (regarding all three substrates) and stability (in respect to pH and temperature). Its properties were compared to those of native variant, and found to be very similar. For crystallization trials, AdSS-His_6 was incubated with excess of IMP, GTP and hadacidin (inhibitor competitive towards aspartate). X-ray diffraction data from several crystals were collected at the synchrotron and 3D-structure solved by molecular replacement with *Campylobacter jejuni* AdSS (PDB kod 3R7T) as a model. Interactions in the active site were identified. Our findings represent first step towards design of new AdSS inhibitors, potential drugs for treatment of *H. pylori* infections.

P-04.1-045
The texture of cheeses with modified alpha-s-casein to beta-casein ratio

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Cold microfiltration (MF) of milk enables partial removal of beta-casein from milk. The cheese milk with normal (control) (C), higher (H) and lower (L) content of beta-casein was produced (Iwaniak et al. 2021, doi.org/10.3390/ijms22062949). The beta-CN content in cheese milk was 31.93%, 35.65% and 22.11% for C, H, and L milk, respectively. The texture parameters were determined using TPA test. The hardness of C and L cheeses increased in the first stage of ripening (for C up to 30 days, for L up to 14 days), and then it decreased. The hardness of H cheese decreased from the beginning of maturation (maximum hardness on the first day). In the first phase of ripening (day 1-14) and in the final phase (day 45-60), the cheeses with the modified proportion of casein fractions were characterized by higher hardness than the C cheese (for which the highest hardness was recorded on the 30th day of ripening). At the end of maturation (60 days), H and L cheeses were characterized by a comparable hardness. In general, the adhesiveness of the control cheese increased during ripening, however the maximum was observed on the 14th day of ripening. The sensory analysis was carried out using the profiling method by a trained expert panel. The evaluation was made using the descriptors established in the preliminary tests for Dutch-type cheeses. The cheeses had the right consistency and structure as well as the correct eye formation. The control cheese (C) and cheese with a reduced beta-casein content (L) were characterized by a slightly higher hardness than H cheese. The obtained results do not fully correspond with the data obtained with the use of instrumental analysis (TPA test). The cheeses were characterized by the typical taste and smell of a Dutch type cheese. Project financially supported by Minister of Education and Science within the program entitled "Regional Initiative of Excellence" for the years 2019-2022, Project No. 010/RID/2018/19, amount of funding 12.000.000 PLN

P-04.1-046
Biological form of bacterial-type L-asparaginases, enzymes used as anti-leukemia drugs

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Bacterial-type L-asparaginases (ASNases) are broadly classified into three sub-groups, type I and II, each homologous to their respective enzymes in *E. coli*, and extremophilic ASNases

(exemplified by the enzyme from *H. pylori*). For over 40 years, EcAII from *E. coli* and ErAII from *D. dadantii* have been major components in treatment of acute childhood lymphoblastic leukemia (ALL). All structurally-characterized type I and II ASNases have been reported to be homotetramers, suggesting that this was the biological form of these enzymes. However, the importance of tetramers for their enzymatic activity was never satisfactorily addressed, since dimers of ASNases satisfy all chemical and structural requirements for catalysis. In this work we initially focused on finding answers to this problem, but found that this question may have been misplaced. The traditional approaches, such as size-exclusion chromatography or dynamic light scattering that utilize a high concentration of ASNase indicated homotetramers as the dominant form of the enzymes. However, techniques requiring much lower protein concentrations (analytical ultracentrifugation or mass photometry) showed that, at low-to-medium nanomolar (nM) concentrations, the predominant form of ASNases is a homodimer. Significantly, most of enzyme kinetics studies of ASNases are conducted at nM concentrations. Furthermore, in therapeutic applications, as well as in vivo analyses, concentrations of ASNases also fall into the low nM range. Using type I ASNase from Y. pestis as an example, we showed by combination of crystallography and biophysical techniques that its dimeric form is nearly indistinguishable from the tetrameric form, and that the thermal stabilities of both forms are comparable. We conclude that a long-held assumption of the need of tetramerization for activating the enzymatic activity of ASNases is incorrect, and that dimers represent the biologically relevant forms of these enzymes.

P-04.1-047
BTB domains of Tramtrack group are Arthropoda-specific multimerization modules

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BTB-domains, in conjunction with different types of DNA-binding domains, are frequently found in transcription factors, providing homodimerization and protein–protein interactions. The certain BTB-domains containing N-terminal specific sequence FxLRWN, where x is hydrophilic residue, belong to the 'ttk-group' and were firstly described in *D. melanogaster*. Our bioinformatics analysis revealed that the 'ttk-group' is not found beyond Arthropoda and is the prevailing group of BTB-domains found in *D. melanogaster* transcription factors since of 27 BTB-containing DNA-binding proteins 23 belong to this group. It was previously shown that several BTB-domains of 'ttk-group' exist as multimers and demonstrate heterodimerization capacity. Using small-angle X-ray scattering (SAXS) and microscopy approaches, we have confirmed the multimeric architecture of two 'ttk-group' BTB-domains – Lola and CG6765. According to SAXS, the molecular weight of multimers corresponds to octamer, although the atomic force microscopy and negative-staining CryoEM experiments for Lola BTB domain suggest that the multimer consists of at least six subunits. In yeast two-hybrid assay we demonstrated broad heteromeric interactions within 'ttk-group'. On the other hand, we detected only a few interactions of the non-TTK BTB domains with the BTB domains of the TTK group. This indicates that TTK-motif is involved in heteromeric interactions. To test whether the TTK-like motif determines multimer formation double mutations within the motif of Lola and Mod(mdg4) were designed and proteins were tested in the size exclusion chromatography. The mutations resulted in complete loss of multimer fraction, shifting the peak of elution profile to size of dimer. The results suggest BTB domains of the 'ttk-group' are specific to Arthropoda transcription factors and TTK-motif drives their heterodimerization and multimer formation. The study was supported by the Russian Science Foundation project 19-74-30026.

P-04.1-048
Structure of DNMT3B ADD domain suggested an absence of DNMT3A-like autoinhibitory mechanism

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De novo DNA methylation in early mammalian development depends on activity of DNMT3 methyltransferases family: DNMT3A, DNMT3B, and catalytically inactive DNMT3L. Compared to DNMT3A, DNMT3B has more strict tissue- and stage- specific expression profile with slightly different site-specificity lacking cooperative DNA methylation activity. Besides catalytic domain, these methyltransferases contain additional domains ADD and PWWP. ADD is a zinc-coordinating histone-binding domain. The autoinhibitory mechanism involving interaction between ADD and catalytic domains of DNMT3A has been described: ADD domain of DNMT3A bound to K4-unmethylated histone H3 tail dissociates from catalytic domain switching the enzyme to catalytically active state. We obtained the crystal structure of DNMT3B ADD domain, which demonstrates the extended conformation of auto-inhibitory loop even in the absence of H3 tail. The same structural re-arrangement creates the novel surface with protein–protein interaction potential, which is reflected in trimerization of DNMT3B ADD in crystal. The overall fold and conformation of histone-binding pocket remain unaffected. The lack of interaction between DNMT3B ADD and methyltransferase domain was confirmed using in vitro pull-down assay. Our results suggest that DNMT3B in comparison to DNMT3A have different modes of regulation of its activity independent of H3K4 methylation status. The novel protein-protein interaction surface can be involved in DNMT3B recruitment to the transcription factor binding sites in early development even in the actively transcribing regions bearing H3K4 trimethylated nucleosomes. The study was supported by the Russian Science Foundation project 19-74-30026.

P-04.1-049
Some properties of human small heat shock protein HspB7

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HspB7 belongs to the large family of small heat shock proteins forming the first line of stress defense controlling cell proteostasis. HspB7 is selectively expressed in cardiac and skeletal muscles and in adipocytes. Although this protein was described more than twenty years ago, many properties of HspB7 remain poorly characterized. Therefore, by using affinity chromatography on immobilized anti-HspB7 antibodies we obtained human cardiac HspB7 (tissue HspB7, tHspB7) and recombinant HspB7(rHspB7) expressed *E. coli*. In contrast to its recombinant counterpart, tHspB7 is acetylated on the N-terminal Ser and does not contain any other posttranslational modifications. We also analyzed expression of different small heat shock proteins (HspB1, HspB5, HspB6 and HspB8) in human heart and have found that the level of HspB7 expression is about 10 times lower than that of HspB1. Since HspB7 is predominantly expressed in skeletal and heart muscle it was supposed that this protein can somehow modulate assembly of actin filaments. In agreement with the earlier published data we found that in dot-blot rHspB7 interact with actin. However, co-sedimentation failed to reveal specific interaction of rHspB7 with F-actin or F-actin decorated by tropomyosin. The data of intrinsic fluorescence and native gel electrophoresis indicated that rHspB7 does not interact with G-actin and does not affect heat induced aggregation of either G- or F-actin. Comparison of the structure and properties of tHspB7 and rHspB7 requires further detailed investigation. Acknowledgement. This investigation has been supported by Russian Science Foundation (grant N 20-74-00013 to L.K.M). This research has been supported by the Interdisciplinary Scientific and Educational School of Moscow University "Molecular Technologies of the Living Systems and Synthetic Biology". *The authors marked with an asterisk equally contributed to the work.

P-04.1-050
Probing the catalytic mechanism of *Rhizobium etli* inducible asparaginase

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L-Asparaginases are a diverse group of enzymes divided into three Classes. Some Class 1 bacterial asparaginases are used in acute lymphoblastic leukemia therapy but their administration is associated with serious side effects. New sources of therapeutic asparaginases have been sought, and the inducible ReAV Class 3 enzyme from *Rhizobium etli* emerges as an interesting candidate. ReAV differs significantly from other bacterial asparaginases, indicating a different catalytic mechanism of asparagine hydrolysis. The crystal structure of ReAV [1] shows a dimeric enzyme with some similarity to β-lactamase fold. The active site of ReAV contains two Ser-Lys tandems, with a curiously hydrated Ser48 residue, located in the close vicinity of a Zn cation with an unusual coordination sphere created by two cysteines, a lysine and a water molecule. The Zn cation is unique to ReAV but it is not necessary for catalysis. To dissect the catalytic mechanism, five residues implicated by the crystal structure, Ser48-Lys51, Ser80-Lys263 and Cys135 (a Zn ligand), were subjected to site-directed mutagenesis and substituted with Ala. The five muteins were studied using biophysical and structural methods. The Nessler method clearly showed that all mutants were incapable of L-asparagine hydrolysis, confirming the significance of those residues for catalysis. Additionally, the replacement of Ser48 and Ser80 by Ala affected protein stability and folding, as indicated by CD spectra and low expression yields. We were able to crystallize mutants: S48A, K51A, K263A and C135A, and solve their X-ray structures. The crystal structures reveal some intriguing variations in the active site area. The C135A mutant is unable to bind Zn, and the coordination site fell apart. The K51A mutation disrupted the network of H-bonds in this region and modified the hydration of Ser48. Work supported by National Science Centre (NCN, Poland) grant 2020/37/B/NZ1/03250. 1. Loch JI et al. (2021) Nature Commun 12, 6717

P-04.1-051
The interplay between protein synthesis and proteasomal degradation in neuronal proteostasis

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Most catalysed chemical reactions inside cells depend on protein levels, and fine-tuning protein concentrations is key to ensure proper cellular function. With the aim to understand how the protein synthesis machinery and the ubiquitin-proteasome system (UPS) interact to effect neuronal proteostasis, we developed two complementary experimental paradigms. First, we assayed the effect that protein synthesis inhibition has on UPS activity. Our data suggest that protein synthesis blockade decreases the levels of cellular proteins tagged with K48-Ubiquitin chains, a bona-fide degradation signal. We also found that the levels and the activity of the 26S and 30S proteasomes were increased in response to protein synthesis inhibition. This increase in 26S and 30S proteasome levels was not at the expense of cellular 20S proteasomes, thus suggesting that protein synthesis blockade drives assembly of all 3 mature proteasome species (i.e. 20S, 26S and 30S). We are now in the process of probing the role of different chaperones involved in proteasome assembly and how they may be involved in the observed phenotype. In a second, complementary approach, we are asking how the protein synthesis machinery responds to selective destabilisation of a single protein substrate important for synaptic function. We combined the recently developed dTAG system with the photoconvertible fluorescent protein Dendra2 to visualise drug-inducible degradation of CaMKIIα in primary neurons. In parallel, we optimised a protocol that allows for the differentiation of mouse embryonic stem cells (mESCs) into complex neuronal cultures. We are now engineering the dTAG system at the genomic CaMKIIα locus of mESCs to achieve on-demand destabilisation of endogenous CaMKIIα protein and study the response of the protein synthesis machinery. By combining these two approaches we hope to better understand how the protein synthesis machinery and the UPS act coordinately to ensure neuronal proteostasis.

P-04.1-052
Amuλett: super-fast targeted multi-site mutagenesis based on uracilated single-stranded transient template

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Targeted Multi-Site Mutagenesis is a very valuable tool for many aspects of protein research, ranging from functional study to protein engineering but also for gene optimization. Tailor-made libraries based on structural information, consensus or phylogenetic analysis, as well as outputs of algorithms trained on directed evolution deep sequencing data, all benefit from such methods to introduce either pre-defined or random mutations at identified distal sites. This step can be challenging and time-consuming, limiting the speed of projects and sometimes their feasibility. Researchers need to resort to fast, efficient and inexpensive techniques to explore the vast landscape of protein sequence. Several methods exist with various efficiency and complexity but rely mostly on the use of mutagenic primers. Annealing of these primers on ssDNA templates shows some of the best performances. Generation of ssDNA uses two main strategies. In the first one, an exonuclease degrades the nicked strand of a plasmid while the second one uses uracilated phagemids produced in bacteria. Both templates can be removed after assembly of their mutated version either by enzymatic degradation or isolation of the produced mutated strand. Importantly, the removal of this template strand drastically decreases the number of wild-type contaminants. Here we propose a fully *in vitro* multi-site targeted mutagenesis method based on PCR-generated uracilated ssDNA transient templates. The output of the Annealing of Mutagenic oligonucleotides on Uracilated λ-exonuclease generated Transient Template (Amuλett) is a fully mutated dsDNA ready to be cloned by any techniques. The preparation of the transient template takes only one day and getting the mutated dsDNA, less than 4 hours. The process does not require any nicking site or phage production and uses only regular T4 Polynucleotide Kinase phosphorylated primers. The Amuλett thus provides a fast, cheap and efficient alternative to existing methods.

P-04.1-053
Non-canonical reactive site against serine proteases evolved on plant Kunitz inhibitors

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Protease inhibitors from the Kunitz family I3 are 20-25 kDa proteins widely distributed in the plant kingdom. They share a conserved β-trefoil fold in which variable loops are involved in interactions with proteases. Kunitz inhibitors target serine proteases using the canonical (Laskowski) mechanism based on a single binding loop with a conserved structure. Here, we present a set of high-resolution crystal structures of two potato Kunitz inhibitors in complex with serine proteases trypsin and chymotrypsin. The formation of binary and ternary complexes was analyzed by analytical ultracentrifugation. We identified a new, non-canonical type of reactive site that binds serine proteases with both trypsin and chymotrypsin specificities. It is formed by two separate loops interacting with the S1 and S1′ pockets of the enzyme. Through this structural mechanism, the non-canonical reactive site is stabilized against proteolysis by the target proteases, providing a functional advantage over the canonical design. The evolution of multiple reactive sites against serine proteases in the Kunitz family is discussed. *The authors marked with an asterisk equally contributed to the work.

P-04.1-054
Investigation of CFTR molecular evolution – a striking difference between two key orthologues

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CFTR chloride channel mutations cause the lethal and incurable disease cystic fibrosis (CF). Phosphorylated CFTR exhibits 'bursting' pore gating – 'bursts' of openings separated by short 'flickery' closures are flanked by long 'interburst' closures – driven by ATP binding/hydrolysis at the two nucleotide-binding domains. The human (hCFTR) and zebrafish (zCFTR) orthologues, which represent roughly the two ends of CFTR molecular evolution, possess different gating properties in line with structural differences revealed in cryo-EM studies. The R117 side chain, conserved across evolution, forms a crucial H-bond in outward-facing ('open') hCFTR which stabilizes the open state, lack of this bond in the R117H mutant causes CF. In the outward-facing zCFTR structure this H-bond is not noticeable, and we found that introducing the R117H mutation into zCFTR has no functional effect. Instead, we discovered a H-bond between the N120 and S109 side chains of outward-facing zCFTR. This bond is absent in inward-facing ('closed') zCFTR and in all available hCFTR structures in which the asparagine is replaced by an isoleucine. Moreover, a clear evolutionary trend is observed regarding the latter amino acid change. We thus aim to investigate the role of the N120–S109 interaction, a potential stabilizer of the 'flickery' closed state, and its relationship to the stabilizing effect of R117. In hCFTR, introduction of the asparagine caused a large decline in open probability (P_o) and mean open time (τ_o), but an increase in the mean flickery close time (τ_c) and the mean burst duration (τ_b). In contrast, elimination of the H-bond in zCFTR by truncation of the asparagine side chain (N120A) increased P_o and τ_o but decreased τ_c and τ_b. In conclusion, the N120-S109 interaction indeed stabilizes the 'flickery' closed state in zCFTR, and might have played a key role in the development of channel mechanics and pathophysiology.

P-04.1-055
Investigating the properties of rare, cystic fibrosis causing mutations of the CFTR chloride channel

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The currently incurable disease, cystic fibrosis (CF), is caused by the mutations of the CFTR chloride channel, which is located on the apical surface of epithelia and plays a role in salt-water transport. Gating of phosphorylated CFTR channels is driven by ATP binding and hydrolysis at the two cytosolic nucleotide-

binding domains. CFTR channelopathies are classified into six main groups, from which we study the four most significant classes. Class I-II mutants lack proper expression or trafficking, while Class III-IV mutants are deficient in gating or conductance. Five rare, previously unobserved, CF causing missense mutations (G126D, I336K, T465I, T582I, D984V) have been identified in the Hungarian CF population. Our aim is to better understand the functional consequences of these mutations and to propose appropriate treatments. We apply *in silico* homology modeling and experimental approaches to characterize the mutations. For class I-II mutants protein expression is assessed by western blot and immunofluorescence techniques, while for correctly expressed and trafficked variants (Class III-IV) channel gating properties are quantitatively studied in macroscopic and single-channel inside-out patch clamp recordings. Using *in silico* homology modeling, we detected no structural damage in the G126D and I336K mutants but both introduce charges onto the membrane-facing surface, which may impact on folding and/or gating. Mutation T465I targets one of the ATP binding sites, the Thr-to-Ile replacement eliminates a H-bond that stabilizes the β-phosphate group of ATP in the wild-type channel; this may decrease ATP affinity and cause gating defects. Mutations T582I and D984V also result in H-bond disruptions in both closed and open structures, and might lead to altered folding or gating. Together with experimental data, these findings may contribute to identifying appropriate therapeutic approaches for a cohort of Hungarian CF patients.

P-04.1-056
The thermostability of fungal luciferases

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Luciferase from *Neonathapanus nambi* (nnLuz) is a membrane enzyme emitting light as a result of luciferin oxidation reaction. Although the bioluminescent system of fungi was described several years ago, the molecular structure and reaction mechanism of luciferase remains unknown. The stable purified luciferase suitable for structure determination or mechanism experiments hasn't been reported. However, it is the second bioluminescent system that was genetically encoded for autoluminescence alone with the bacterial one. The aim of our work was an estimation of the thermal stability of different native luciferases for the identification of amino acid residues increasing the stability. Fifteen native luciferases from various fungal species were expressed in *Pichia pastoris* and isolated as membrane fraction. Their thermal stability was screened via the imaging of luciferases' luminescence during melting of membrane fraction or detergent-solubilized membrane fraction containing lucerase. The bioluminescence reaction and melting conditions were optimized for the composition and pH of the buffer, concentration of enzyme and detergents. Here, we revealed a pattern of changes in the parameters of the obtained melting curves. We also identified the group of closely related luciferase homologues (*Mycena sanguinolenta, Mycena lamprospora, Panellus stripticus*) with improved stability compared to other luciferases. Sequence alignment analysis of the luciferases revealed the key residues that could be mutated to further improve thermal stability of nnLuz. The reported study was funded by RSF, project number 22-44-02024, https://rscf.ru/project/22-44-02024/

P-04.1-057
Biochemical analyses of bacterial antiphage defense proteins, ThsA and ThsB

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The Thoeris system is an antiphage defense system found in over 2000 prokaryotic genomes and consists of two genes, *thsA* and *thsB*. We report the crystal structures and functional analyses of the two Thoeris proteins, ThsA and ThsB. ThsA exhibited robust nicotinamide adenine dinucleotide (NAD^+) cleavage activity, hydrolyzing the nicotinamide–ribose bond. The ThsA structure displayed a two-domain architecture containing N-terminal sirtuin-like and C-terminal Smf/DprA-LOG (SLOG)-like domains. Mutation analysis suggested that NAD^+ cleavage by ThsA is crucial for the antiphage function of the Thoeris system. ThsB exhibited a structural resemblance to Toll/interleukin-1 receptor (TIR) domain proteins such as eukaryotic Toll-like receptors and bacterial nucleotide hydrolases. Structural and functional analyses of ThsB suggested that it functions as a sensor in the protection against phage infection. Taken together, these results further our understanding of the molecular mechanism underlying the Thoeris defense system, highlighting a strategy for bacterial antiphage resistance via NAD^+ degradation. Previously published in: Doron S et al. (2018) Science 359 eaar4120. Ka D, Oh H et al. (2020) Nat Commun 11, 2816. *The authors marked with an asterisk equally contributed to the work.

P-04.1-058
Structural and biochemical characterization of anti-CRISPR AcrIF7

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Clustered regularly interspaced short palindromic repeats (CRISPRs) and CRISPR-associated (Cas) proteins constitute a bacterial immune system against invading bacteriophages. As a counter-defense mechanism, bacteriophages have evolved anti-CRISPR proteins to neutralize the host CRISPR-Cas system. AcrIF7 was discovered from prophages of *Pseudomonas aeruginosa* and effectively rescues CRISPR-sensitive phages in *P. aeruginosa* strains with type I-F CRISPR-Cas activity. Here, we determined the NMR structure of AcrIF7 and identified its target Cas component in the type I-F CRISPR-Cas system. AcrIF7 adopts a novel α/β fold with negatively-charged surface residues and interacts with the Cas8f subunit of the type I-F CRISPR RNA-guided surveillance complex. Extensive mutagenic analyses revealed that AcrIF7 associated with the highly conserved dsDNA binding site of Cas8f, primarily via electrostatic interactions. Our study provides structural and mechanistic insights into the function of AcrIF7, expanding the knowledge of Acr inhibitors against type I-F CRISPR immunity. *The authors marked with an asterisk equally contributed to the work.

P-04.1-059
Improved histological approach for localization and expression analysis of (non-)membrane proteins in zebrafish cryosections

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Ever increasing use of zebrafish model for studying different biomedical and toxicological issues linked to the physiological/ecotoxicological function of various (non-)membrane proteins, makes development and/or optimizing existing methods for studying protein localization/expression in this model extremely important. Compared to whole-mount preparations and/or paraffin-embedded tissue sections, zebrafish cryosections are still poorly used in this context. In this study, we used zebrafish embryo/larvae (24-120 hpf; N = 20 individuals/group) to: 1) determine key factors for preparation of high-quality zebrafish cryosections, 2) optimize the protocol for localization/expression of a nonmembrane F-actin protein using fluorescently-labeled phalloidin (FITC-Phalloidin), 3) determine the use of optimized protocol for immunofluorescence analyses of Na^+/K^+-ATPase membrane protein, and 4) investigate stage development-related changes in F-actin and Na^+/K^+-ATPase protein expression. The results revealed the highest quality of zebrafish cryosections after samples underwent 1 h-fixation in 4 % paraformaldehyde (PFA), incubation in 2.5 % bovine gelatine/25 % sucrose-mixture, followed by cutting to 8 µm thickness at -20°C. Fluorescence microscopy analysis of phalloidin-labeled zebrafish skeletal muscles revealed that 1 h-4% PFA fixed samples enabled optimal phalloidin-to-F-actin binding. Further (immuno)fluorescence analyses revealed detailed localization of F-actin and Na^+/K^+-ATPase in different zebrafish organs/tissues and their respective stage-dependent expression increase in somatic muscles and pronephros. Finally, staining in zebrafish cryosections and whole-mount samples revealed organ-specific and zone-dependent localizations of Na^+/K^+-ATPase α1-subunit. Project financed by Croatian Science Foundation, DANIOTRANS (HRZZ-IP-2019-04-1147). *The authors marked with an asterisk equally contributed to the work.

P-04.1-060
Characterization of a novel DyP-type peroxidase from a marine actinobacterium

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Dye-decolorizing peroxidases (DyP) were first discovered in fungi and were found to be capable of decolorizing a variety of industrial dyes. Depending on the size of the substrate and the availability of radical transfer pathways in the enzyme, DyPs catalyze the oxidation of a range of substrates. These enzymes bind hydrogen peroxide leading to the formation of the so-called Compound I, the key intermediate for catalysis. This then decays into Compound II, yielding back Fe(III) at its resting state. A new dye-decolorizing peroxidase (DyP) was discovered to have a different conserved motif (EXXDG) compared to the motif known for DyP-type peroxidases (GXXDG). We performed multiple assays, in order to electrochemically study and characterize this enzyme and its activity, with different techniques (CV, CA) and in different conditions, thin layer and adsorbed on the electrodes, w/o surface modifiers. The positively charged modifier, neomycin, was found to have no effect on improving the electron transfer. The redox potential dependence of pH and T was studied. Preliminary assays using hydrogen peroxide were attained at different concentrations and scan rates, at room temperature. Acknowledgments This work was supported by the project OXY-MOD of the Centre for Digital Life Norway (DLN) under Research Council of Norway grant number 269408 as well as SINTEF internal funding. Also, this work was supported by the Associate Laboratory for Green Chemistry - LAQV which is financed by national funds from FCT/MCTES (UIDB/50006/2020), Portugal. References 1. Cordas *et al*, J. Inorg. Biochem. (2022) 226, p.111651. doi:10.1016/j.jinorgbio.2021.111651 2. Catucci *et al*, Biotechn. Appl. Biochem., (2020) 67, p.751-759. doi:10.1002/bab.2015.

P-04.1-061
ACRC/GCNA protease is essential for DNA protein crosslink repair during embryonic development

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DNA-protein crosslinks (DPCs) are the second most common DNA lesions caused by byproducts of cellular processes such as formaldehyde and reactive oxygen species. These bulky lesions are an obstacle to all DNA transactions: replication, transcription, chromatin remodeling, recombination and repair. Therefore, the consequences of impaired DNA–Protein Crosslink Repair (DPCR) are severe. At the cellular level, aberrant DPCR leads to the formation of DSBs, genomic instability and/or cell death, whereas at the organismal level, impaired DPCR is associated with cancer, aging, and neurodegenerative phenotypes. Despite the severe consequences of DPCs, the mechanisms of the DPCR pathway are still largely unknown. Because proteases play a central role in DPCR, we set out to investigate the mechanism of function of the putative protease ACRC/GCNA (ACidic Repeat Containing/Germ Cell Nuclear Antigen) which has recently been linked to DPCR. In contrast to previous hypotheses that ACRC plays a critical role only in germ tissue, we found that ACRC is also expressed in the zebrafish kidney, intestine, and liver, suggesting a more general function. We also show that ACRC is a dominant protease during early embryonic development. Using newly created zebrafish strains with (1) a mutation in the protease core and (2) with a C-terminal deletion, we have shown for the first time that the proteolytic function of ACRC is critical for DPCR during early development and that when this function is impaired, severe accumulation of DPCs leads to cell death. To determine the mechanism of action of ACRC protease, we examined the function of specific protein domains by injecting different protein constructs into ACRC-deficient embryos and quantifying DPC levels and embryonic phenotypes. Overall, our results represent a detailed study of the ACRC protein and its function in DPCR *in vivo* and will help to understand how

defective DPC repair translates into disease phenotypes. *The authors marked with an asterisk equally contributed to the work.

P-04.1-062
Biophysical characterization of full length recombinant TAR DNA-binding protein (FL TDP-43) phase separation and aggregation

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The TAR DNA-Binding Protein (TDP-43) is a nuclear protein that is hypothesised to form reversible condensates via Liquid-Liquid Phase Separation (LLPS).[1] Disruption of TDP-43 homeostasis, and consequent aggregation, is strictly linked to ageing diseases, mainly Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Lobar Degeneration (FTLD).[2] Recently, a correlation between condensate formation and misfolded pathological inclusion in the cytoplasm has been suggested. Here, we carried out an *in-vitro* biophysical investigation of FL His-TDP-43, applying a pH jump method previously proposed,[3] and trying to define the physical state of droplets. We found out a tight dependence of droplet formation on pH, temperature, NaCl and protein concentration. Indeed, increase of these parameters, led to a stonger tendency of FL His-TDP-43 to undergo self-assembly, but interestingly, each condition showed a sort of "inversion point" where the trend changed. Then, we investigated the physical properties of FL His-TDP-43 phase separation through Fluorescence Recovery After Photobleaching (FRAP), under certain experimental conditions, and found out that FL His-TDP-43 phase separated by making droplets with spherical morphology, low fluorescence recovery and no coalescence behaviour and also by making aggregates in a droplets chain-like arrangement. These results reveal that TDP-43 can make droplets that might have hydrogel-like state behaviour, where the diffusion of the molecules is slow within the condensates and coalescence of distinct droplets is not allowed. We also revealed that self-assembly of FL His-TDP-43 may be tuned by external buffer conditions, due to change of intramolecular and intermolecular interactions of hydrophobic and hydrophilic residues. Bibliography: 1. McGurk et al. (2018), Molecular Cell, 71(5), 703-717.e9. 2. Neumann Manuela et al. (2006), Science, 314 (5796), 130-133 3. Van Lindt et al. (2021), Communications Biology, 4(1),77. *The authors marked with an asterisk equally contributed to the work.

P-04.1-063
Secondary structure of the NY25 peptide from influenza A(H1N1)pdm09 hemagglutinin at the pH of membrane fusion

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The secondary structure of the second polypeptide region of influenza virus hemagglutinin (Asn117–Tyr141) at pH = 5.0 is similar to the one at pH = 7.4 according to the results of X-ray crystallography (PDB ID: 1HTM), while the same region has not been recognized by the electron microscopy (PDB ID: 6Y5L). This work aimed to estimate the secondary structure of the corresponding NY25 peptide in a saturated solution at pH = 7.4 and pH = 5.0 by the HATR-FTIR method in 0.01M phosphate buffer after the evaporation of unbound water. Spectra were recorded by Thermo Nexus 670 FT-IR ESP Nicolet (Thermo Scientific) equipment. The position of the amide I band in the spectrum of NY25 peptide at pH = 7.4 is at 1655 cm^{-1} that is consistent with the typical one for alpha helix (1648–1657 cm^{-1}). Two components of the beta structural amide I band were revealed by the second derivative analysis of the spectrum by PeakFit v.4.12. The position of the low-frequency band is at 1639 cm^{-1}, while the high-frequency band is at 1686 cm^{-1}. The presence of the high-frequency component indicates that the beta structure is rather antiparallel than parallel, while the position of the low-frequency component at a wavelength higher than 1628 cm^{-1} indicates that the beta structure should not be intermolecular. The secondary structure of the NY25 peptide at pH = 5.0 is similar to the one at pH = 7.4. Namely, the amide I band is located at 1655 cm^{-1}. The absence of any shift of its position indicates that the length of the alpha helix should be the same at both pH = 7.4 and pH = 5.0. In contrast, the hidden beta structural band at pH = 5.0 has shifted maxima (1633 cm^{-1} for the low-frequency component and 1684 cm^{-1} for the high-frequency component) indicating that the lengths of beta strands in this beta hairpin might become different. The data obtained indicate that the corresponding part of H1 hemagglutinin does not change its overall secondary structure at pH = 5.0. This study was supported by the BRFFR grant X22KI-022.

P-04.1-064
Studying deubiquitinase regulation in inflammation

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Activation of nuclear factor kappa B (NF-κB) transcription factor-mediated gene expression is key for coordinating innate immune response and cell survival. A feature commonly shared amongst most inflammatory response pathways is their dependence on ubiquitin, a small modifier that can be post-translationally conjugated to target proteins. The formation of ubiquitin chains of different types is an essential signal in inflammatory response and subject to tight regulation by ubiquitin editing enzymes. Amongst them is the zinc finger protein A20 an inhibitor of NF-κB signalling with linear ubiquitin chain binding capacity that has previously been linked to chronic inflammatory diseases. Despite A20's pivotal role during NF-κB negative feedback regulation, previous studies lack fundamental experiments on the biochemical and structural level. In this study, I will investigate how phosphorylation regulates specificity of the ubiquitin-editing enzyme A20 and how A20 is regulated by A20-binding inhibitors of NF-κB (ABINs). To achieve this, I will provide the missing biochemical and biophysical characterization of the A20/ABIN complex. By filling in the gap on how A20/ABIN regulates inflammatory responses, I aim to contribute towards deciphering how dysregulation of the ubiquitin machinery leads to inflammatory pathologies.

P-04.1-065
Structural studies of *Bacillus subtilis* LonBA, member of a newly identified subfamily of Lon proteases

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ATP-dependent Lon proteases are bifunctional, multidomain, homooligomeric enzymes that belong to the system of cellular protein quality control. They are responsible for degradation of abnormal, misfolded, and some regulatory proteins. Lons are currently grouped into three subfamilies, named LonA, LonB, and LonC. Based on comparative analysis of their sequences, we identified a potential new subfamily, tentatively named LonBA, which shares certain characteristics of both LonA and LonB subfamilies. We carried out the cloning and developed optimized schemes for expression and isolation of the recombinant *Bacillus subtilis* LonBA (*Bs*LonBA). The domain organization of purified *Bs*LonBA was proven by limited proteolysis with chymotrypsin and the ATPase and peptidase domains were also expressed individually. The enzyme exhibits reduced ATPase and peptidase activity compared to *Escherichia coli* LonA. Coupling between the ATPase and peptidase centers was not found. We determined crystal structure of the proteolytic domain of *Bs*LonBA. Although the structure is similar in overall to its counterparts in other subfamilies, it contains a unique 12-residue insert. This structural fragment makes a β hairpin that, in turn, creates in a functional enzyme a barrel comprising six such hairpins. This element faces the inner chamber of *Bs*LonBA hexamer, occupied by the substrate during proteolysis. The role of this structural feature, unique to LonBAs, is not yet clear. We also found that a model of the ATPase domain of *Bs*LonBA, calculated with AlphaFold2, contains elements that are different from the corresponding regions of either LonA or LonB, thus supporting the notion that LonBAs, found so far only in firmicutes, represent a previously unrecognized subfamily of these biologically important enzymes. Supported by intramural NIH funds and by RSF project 21-74-20154.

P-04.1-066
Improving energy production by tuning redox proteins' properties

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Geobacter bacteria rely on a vast network of multiheme cytochromes to efficiently deliver electrons to its extracellular space and reduce soluble and insoluble compounds. This mechanism – extracellular electron transfer (EET) – has been explored in bioremediation of contaminated waters with Cr(VI), U(VI), V(V) acting as electron acceptors, and in the production of electrical energy using electrode surfaces as terminal electron acceptors [1]. An approach to improve this technology relies on the manipulation of the redox properties of *Geobacter*'s proteins engaged in EET. One of the most promising targets is the conserved PpcA family of periplasmic triheme cytochromes, which are fundamental in EET and in controlling *Geobacter*'s redox response [2]. Comparison between PpcA homologs of *G. sulfurreducens* (*Gs*) and *G. metallireducens* (*Gm*) revealed that, despite 80% of sequence identity, the proteins worked in a very different redox range, constituting an interesting system to directly study amino acid individual contribution to the protein's redox properties [3]. We replaced eleven non-conserved residues in PpcA *Gm* with PpcA *Gs*' counterparts and assessed their impact in the redox properties of the mutants combining UV-Visible redox titrations and 2D-EXSY NMR experiments. The results obtained for single [3], double, triple and quadruple mutants will be presented. This approach lays the path for development of cytochromes with rationally designed redox properties, that ultimately might contribute for improvement of EET efficiency in *Geobacter*. [1] Lovley, DR et al. (2011) Adv Microb Physiol 59, 1-100 [2] Liu, Y et al. (2011) ChemPhysChem 12.12: 2235-2241 [3] Portela, PC et al. (2021) J Biol Chem, 296 Work supported by: PTDC/BIA-BQM/31981/2017, PTDC/BIA-BQM/4967/2020, 2020.04717.BD (FCT Portugal); UIDP/04378/2020, UIDB/04378/2020(UCIBIO). NMR spectrometers are part of PTNMR funded by: ROTEIRO/0031/2013 — PINFRA/22161/ 2016 (FCT-MCTES); COMPETE 2020, POCI, PORL (FEDER); PIDDAC (FCT).

P-04.1-067
Identification of distinct mechanical unfolding pathways of protein in individual and coupled geometry

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In physiology, protein-based force-sensors commonly exists in two configurations, independently or as a protein pair in handshake geometry. Independent proteins sense and transmit the force through the one end to other and their force-responsive behaviour is studied extensively using single-molecule force spectroscopy (SMFS). Whereas protein in paired/complex form considered mere as surge-protectors and evolutionary importance of such protein complexes in force-transduction is still elusive. In this work, we aimed to investigate the significance of both these protein configurations. We studied the mechanoresponsive behaviour of a force-sensing protein, cadherin-23 in both these geometries using SMFS. Cadherin-23 interacts with Protocadherin-15 in handshake-type geometry and form the tip-link in inner ear. Accordingly, we pulled the Cadherin-23 individually and in handshake geometry with its partner protein Protocadherin-15. We found that in partner-assisted pulling, cadherin-23 is more resilient to force and follows different potential energy landscapes for unfolding compared to the individual one. Further, network analysis of *in-silico* studies explains this distinct behaviour by revealing the more force-dissipation with a dense network throughout the protein in handshake geometry as compared to the sole protein. Overall, our study identifies the distinct response to mechanical force in protein complexes compared to independent force-sensors. The distinct and superior mechanical response of cadherin-23 in handshake geometry than single protein geometry highlights a probable evolutionary drive of protein-protein complexes as force-conveyors over independent ones. *The authors marked with an asterisk equally contributed to the work.

P-04.1-068
AlphaFold driven biased biomolecular simulations

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AlphaFold is a novel method for prediction of 3D structure of proteins and protein–protein complexes based on deep learning. It uses a multiple sequence alignment of the modeled protein with its homologues to infer some important features of its 3D structure, in particular residue–residue distances. Residue–residue distances are predicted and further converted into an acurate structure model by AlphaFold. However, despite its success, AlphaFold has a limited capability to model the outcome of mutations, conformational changes, interactions with other types of molecules and other important phenomena. This provides a space for biomolecular simulations. Here we used the output of AlphaFold in the form of inter-residue distance probability profiles to guide biomolecular simulations by the metadynamics method. This approach was tested on folding of a series of fast-folding mini-proteins. With parallel tempering metadynamics, we were able to simulate multiple folding and unfolding events and to predict the temperature-dependent free energy profile in agreement with biophysical studies and reference simulations. The project was supported by Czech Science Foundation (22-29667S).

P-04.1-069
Tweety family members form tetrameric complexes at the cell membrane

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The family of Tweety homologs (TTYH) consists of three paralogs in vertebrates, displaying a ubiquitous expression pattern. Although considered as ion channels for almost two decades, recent structural and functional analyses challenged this role. While their molecular function has yet to be determined, previous studies demonstrated that members of this family are involved in several pathophysiological processes, including brain colonization of gliomas, status epilepticus, and various cancers. Here, we determined the stoichiometry of intact mouse TTYH (mTTYH) complexes in cells. Using *in situ* cross-linking and single-molecule fluorescence microscopy techniques, we show that mTTYH1 and mTTYH3 form tetramers at the plasma membrane. Intriguingly, these results are in contrast to the recent cryo-EM studies, revealing that members of the TTYH family share a dimeric stoichiometry following detergent solubilization. To uncover the molecular basis for this discrepancy, we used blue-native PAGE and fluorescence-detection size-exclusion chromatography, showing that detergent solubilization results in destabilization of tetramers, leading to their dissociation into dimers. Moreover, by employing hydrogen-deuterium exchange mass spectrometry (HDX-MS) analysis of purified mTTYH1, we demonstrate that the prominent extracellular domains are stabilized in the context of the tetrameric complex. Finally, crosslinking analyses of isolated mTTYH3 extracellular domains revealed the emergence of higher-order oligomers, culminating in the size expected for a tetramer, further supporting their involvement in TTYH tetramerization. Together, our efforts to unveil the stoichiometry and assembly mechanisms of TTYH proteins advance the structural understanding of this enigmatic family in the context of intact cells, and may also shed light on their cellular functions in health and disease.

P-04.1-070
Anthrax recombinant antigens for vaccine development

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Anthrax is one of ancient diseases that still occurs in the world. It is caused by a Gram-positive spore-forming bacterium *Bacillus anthracis*. Anthrax outcomes are extremely harmful for northern agricultural holdings. Moreover, *B. anthracis* spores could potentially serve as a biological weapon. Thus, the development of effective anthrax vaccines with a long shelf-life is desirable. Anthrax protective antigen (PA) is a main anthrax toxin component. It is an 83 kDa protein that consists of four domains. PA is a key component of existing and currently being developed anthrax vaccines. The main problem associated with using recombinant PA (rPA) in vaccines is its low stability. rPA instability is mainly due to deamidation of certain asparagines and to presence of furin and chymotrypsin cleavage sites in the amino acid sequence. We designed a recombinant protein rPA1+2 containing PA domains I and II with genetically inactivated furin and chymotripsine cleavage sites (^{162}NSRKKR^{167} sequence was substituted with ^{162}QSSNKE^{167} and ^{313}FF^{314} fragment was deleted) and a recombinant protein rPA3+4 containing PA domains III and IV with the most deamidation-prone Asn^{713} and Asn^{719} replaced by Gln. Previously published in: Ryabchevskaya EM et al. (2021) Hum Vaccin Immunother 17(2):560-565. Both rPA3+4 and rPA1+2 were demonstrated to remain stable for at least 19 days at +37°C and for at least 160 days at +25°C. Given the fact that PA's protectivity is not only attributed to a full-size protein, rPA1+2 and rPA3+4 should be an appropriate basis for a stable modern anthrax vaccine. To support this idea, the vaccine formulation containing tobacco mosaic virus-based spherical particles (SPs), which are innovative platform-adjuvant, was developed. The possibility of simultaneous adsorption of rPA1+2 and rPA3+4 to the SPs surface was proved. We propose SPs-rPA1+2-rPA3+4 compositions as a new generation stable anthrax vaccine candidate. Funding: Russian Science Foundation (Grant 18-14-00044).

P-04.1-071
LPMO direct electrochemical characterization

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Lytic polysaccharide monooxygenases (LPMOs) are a group of carbohydrate-active enzymes, classified in different families

(AA9, AA10, AA11 and AA13). These metalloenzymes, bearing a copper in the active site, can perform the oxidative cleavage of polysaccharides such as cellulose and chitin [1], being of great industrial interest. The mechanistic details are still under debate, but evidence points to an important role of hydrogen peroxide, both for initiating the catalytic reaction and for being formed during the catalytic cycle [2]. In the current work, the electrochemical behaviour of immobilized LPMO was studied (catalytic center redox potential, pH and T dependence) by direct electrochemistry, and the influence of different residues in the copper center (e.g. Ala to Gly, etc) was evaluated. Acknowledgements This work was supported by the Associate Laboratory for Green Chemistry—LAQV which is financed by national funds from FCT/MCTES (UIDB/50006/2020) and the Research council of Norway, through the project OXYMOD of the Centre for Digital Life Norway (DLN) Research Council of Norway grant number 269408. References 1. Forsberg, Z. *et al.* Comparative Study of Two Chitin-Active and Two Cellulose-Active AA10-Type Lytic Polysaccharide Monooxygenases. Biochemistry 53, 1647–1656 (2014). 2. Bissaro, B. *et al.* Oxidative cleavage of polysaccharides by monocopper enzymes depends on H_2O_2. Nature Chemical Biology 13, 1123–1128 (2017).

P-04.1-072
Novel inhibitors of TET1 protein based on the structure motif of iron chelators

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Targeting of epigenetic mechanisms such as the hydroxymethylation of DNA is intensively studied, with respect to the treatment of many serious pathologies, including oncological disorders. One possible way to modulate the hydroxymethylcytosine level could be based on the regulation of TET1 protein activity. The TET1 protein (ten-eleven translocation methylcytosine dioxygenase 1) is an iron(II)- and α-ketoglutarate-dependent dioxygenase and converts 5-methylcytosine to 5-hydroxymethylcytosine. Dioxygenase activity of TET enzymes is highly dependent on the presence of Fe(II) and 2-OG and key catalytic residues that bind Fe(II) and 2-OG are present within the DSBH domain. This indicates an important role for Fe(II) chelators in targeting TET1 and thereby its inhibition. Therefore, we tested various type of iron chelators for the inhibition of TET1 protein. In the present time we found suitable structure motif with high affinity for iron ions and potent inhibition activity. For better understanding mechanism of interaction with catalytic enzyme domain docking studies will be made. Obtained results strong implies, that iron chelator represents promising group of agents for the inhibition of TET1 protein. The research was funded by the Ministry of Health of the Czech Republic (grant no. NU21-08-00407). The authors also thank Operational Programme Research, Development and Education, within the project Center for Tumor Ecology—Research of the Cancer Microenvironment Supporting Cancer Growth and Spread (reg. no.CZ.02.1.01/0.0/0.0/16_019/0000785). *The authors marked with an asterisk equally contributed to the work.

P-04.1-073
Mechanism of coelenterazine photoinactivation of *Beroe abyssicola* photoprotein

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Bioluminescence, or glowing of living organisms, is very common among marine species. Most of them utilize Ca^{2+}-regulated photoproteins. Ctenophore photoproteins lose their bioluminescent activity when exposed to visible light. Berovin photoprotein from *Beroe abyssicola* contains coelenterazine in a preoxygenated form – 2-hydroperoxycoelenterazine. During bioluminescence reaction 2-hydroperoxycoelenterazine is converted into coelenteramide with the emission of light. Coelenteramide could be further hydrolyzed into coelenteramine. If the active photoprotein is irradiated with white light, a number of compounds with unknown structure is formed, apart from coelenteramide and coelenteramine. We isolated and identified previously unknown products of coelenterazine photoinactivation and elucidated their structure. The structures of purified compounds, that turned out to be substituted hydantoins, were confirmed by total synthesis. We also proposed a mechanism of berovin photoinactivation, similar to the autooxidation of GFP-like chromophores. This work was supported by Russian Science Foundation grant № 18-74-10102, https://rscf.ru/en/project/18-74-10102/. *The authors marked with an asterisk equally contributed to the work.

P-04.1-074
Identification and characterization of proteases secreted by Yarrowia clade

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For several decades, *Yarrowia lipolytica* was considered as the only member of the *Yarrowia* genus. However, rapid development of sequencing technologies resulted in finding, that several yeast species share numerous morphological and physiological features, such as lipid accumulation capacity, polyhydroxyalcohol biosynthesis or dimorphism. These species have been later classified to form the *Yarrowia* clade. While lipolytic activity is the subject of extensive research on the *Yarrowia* clade, studies related to the proteolytic enzymes are very limited. In this work, we conducted a comprehensive analysis of *Yarrowia* clade proteases, including proteolytic activity, zymography and proteomic analysis. We also proposed some potential future applications of these proteases, such as the production of spent grains hydrolysates (Brewery Spent Grain - BSG), including the analysis of the degree of protein hydrolysis as well as the antioxidant activity of the hydrolysates. The *Yarrowia* clade species varied in the proteolytic activities as well as they produced different proteases. The highest proteolytic activity was detected in the cultures of *Y.*

lipolytica W29 (2.47 U/ml), *Yarrowia galli* CBS 9722 (1.96 U/ml) and *Yarrowia alimentaria* CBS 10151 (1.38 U/ml). By proteomic analysis, the highest protein sequence coverage to the Alkaline Extracellular Protease (AEP) encoded by the XPR2 gene was detected for the *Y. lipolytica* W29 and *Y. alimentaria* CBS 10151 proteases. The antioxidant activities of the obtained hydrolysates were high and strongly correlated with the degree of protein hydrolysis. The results showed that yeast from the *Yarrowia* clade may be used for commercial alkaline protease production.

P-04.1-075
Functional characterization of the cytochrome-hemoglobin fusion protein in cable bacteria

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Cable bacteria (CB) are centimeters-long filamentous organisms that can electrically couple the oxidation of sulfide, in deeper sediment layers, to the reduction of oxygen near the sediment–water interface using an internal wire system for long distance electron transport[1]. This remarkable cellular organization extends the known length scale of biological electron transfer (ET) and implies that CB have found a mechanism to efficiently conduit ET over centimeter-scale distances, providing a prospect for new materials and technologies. The most intriguing finding in CB's genomes was the absence of conventional terminal oxidase, since CB revealed one of the highest oxygen reduction rates observed in nature[2]. Instead, the genomes encode a unique periplasmic protein to which the conductive properties might be the key to understand the CB properties. This protein is composed of 3 domains – a *c*-type pentaheme cytochrome domain and 2 truncated hemoglobins (trHbs) domains. Each trHb domain contains a *b*-type heme and is part of a family of small O_2-binding proteins[3]. Each domain was expressed separately and their functional redox range determined by potentiometric redox titrations followed by ultraviolet-visible spectroscopy (UV-Vis). Additional biophysical characterization was obtained by UV-Vis, small-angle X-ray scattering (SAXS), synchrotron radiation circular dichroism (SR-CD) and nuclear magnetic resonance (NMR) spectroscopy. The results obtained for this unprecedented fused protein will be presented and establish the foundations to unraveling the functional role of this unique protein in CB. [1]Bjerg JT et al. (2018) PNAS 115, 5786 [2]Scilipoti S et al. (2021) Sci Adv 7, eabe1870 [3]Kjeldsen KU et al. (2019) Proc Natl Acad Sci USA 116, 19116–19125 This work was supported by Fundação para a Ciência e Tecnologia (FCT) through grants PTDC/BIA-BQM/4967/2020 (CAS) and 2020.04717.BD (PCP). It was also supported by FCT projects UIDP/04378/2020, UIDB/04378/2020 and LA/P/0140/2020 (i4HB)

P-04.1-076
The eukaryotic cell-free expression system as a vehicle to obtain GPCRs of class F

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The members of human Frizzleds (FZD) belongs to the class F of the G protein-coupled receptors (GPCRs) superfamily and are involved in the Wnt signaling cascade via intricate interaction with transducer proteins including heterotrimeric G or Dishevelled (DVL) proteins. The crucial role of FZDs in human pathology bring them to foremost positions as a drug targets, particularly in anticancer therapy. Due to the lack of comprehensive understanding of FZDs structural arrangements and molecular mechanisms of activation, there are not many drugs have been developed for efficient pharmacological modulation of them. Here we focused on a preparative-scale production of human FZD-4 and FZD-7 using HEK293-based eukaryotic continuous exchange cell-free protein expression (CE-CFPE) system. It is revealed that the production rate of target proteins synthesis in the prescence of detergents gradually increases during incubation time within reaching a plateau after 14-16 hrs of incubation at 27°C. The production yields of studied proteins were averagely estimated to ~0,77-1,22 mg/ml of reaction mixture. Using metal chelate affinity chromatography to purify the target proteins, overall loss was estimated to be less than 8% of the total amount, but it was strongly dependent on the incubation time and temperature during batch adsorption of resolubilized protein with NTA-agarose beads. The structural properties and homogeneity of the purified target protein samples in the presence of selected detergents were further analyzed using a classic combination of straightforward biophysical approaches (such as SEC, CD, SPR). Overall, the described approach of recombinant FZDs preparation provided milligram amounts of correctly-folded proteins from several milliliters of the cell-free reaction mixture within a time span of fewer than 48 hours, which makes it suitable for further implementation. *The authors marked with an asterisk equally contributed to the work.

P-04.1-077
Diverging enantiopreferences: insights into the phenylalanine aminomutase mechanism and evolution

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Phenylalanine aminomutases (PAMs) are lyase-like enzymes using a special post-translationally formed residue, the 5-methylene-3,5-dihydro-4*H*-imidazol-4-one (MIO) as the catalytic electrophile. They catalyze the reversible ammonia elimination from phenylalanine and the interconversion between α- and β-phenylalanine. PAMs have diverse physiological functions in both plants and bacteria, they are responsible for the production of several secondary metabolites such as antibiotics and pigments. The structure and mechanism of MIO-containing enzymes have

been investigated thoroughly in the previous decades, however, there are still questions that need to be answered regarding the differences in the function of different PAMs. PAMs originating from either eukaryotic or prokaryotic source exhibit different enantiopreference for β-phenylalanine, eukaryotic PAMs convert l-α-phenylalanine to enantiopure (*R*)-β-Phe, while prokaryotic PAMs transform Phe to enantiopure (*S*)-β-Phe. It was showed in our previous study (Bata Z et al., (2021) ACS Catal. 11, 8, 4538–4549) that the enantiopreference of (*R*)-PAMs can be influenced with a tunnel engineering approach. It was also proved that all MIO containing scaffold harbors the potential to catalyze reactions with different enantiopreference. Our current investigation focuses on the kinetic analysis of PAMs to uncover the background of the mechanistic differences in PAMs of different origin. Our further goal is to determine the 3D structure of PAMs with non-hydrolyzable ligands in the active centre to clarify their disparate functional features.

P-04.1-078
Dampening of cross-correlations in β-strand of tip-link protein with aging induces hearing loss

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Aging is an inevitable and involuntary process, associated with gradual deterioration of health. Age-related hearing loss (ARHL) is one of the most frequent sensory disabilities which advances with age. ARHL is a multifactorial aging disorder. These factors irreversibly and slowly damage the integrity of the auditory machinery present in the inner ear. Auditory machinery is composed of tiny hair cells along with the stereocilia which are projected vertically in a stairway manner. The two consecutive stereocilia are held together by the interaction of two proteins, protocadherin-15 (Pcdh15) and cadherin-23 (Cdh23). Out of these two proteins, the Cdh23 is mapped with the ARHL phenotype, and mutations in the Cdh23 are marked with congenital and progressive hearing loss (PHL). PHL is an accelerated state of ARHL that marks the early onset of hearing. The lack of any biophysical models is the major bottleneck in designing the precise therapeutic remedy for ARHL. Here, in search of molecular origins for ARHL, we dissect the conformational behavior of Cdh23 along with the PHL mutant (S47P) that progresses the hearing loss drastically. Using an array of ensemble and single-molecule experimental, and computational approaches, we highlight lower thermodynamic stability, significant weakening in the hydrogen-bond network, and loss of inter-residue correlations among β-strands, due to mutation. This loss in correlated motions adverse the force adaptations from mechanical stimuli and slow down the folding in the mutant. As an antipode to the PHL mutant, we measured superior thermodynamic stability and correlated motions in another natural variant of tip-link protein, where serine is replaced by valine (S47V), found in vertebrates with a superior organ of corti and presumably no ARHL. We thus propose that loss in correlated motions within cadherin-23 with aging may trigger ARHL, a molecular feature that likely holds true for other disease mutations in β-strand-rich proteins. *The authors marked with an asterisk equally contributed to the work.

P-04.1-079
Investigating the role of the P97/Vcp segregase in DNA–protein crosslink repair (DPCR*) in vivo* using the zebrafish

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DNA-protein-crosslink repair (DPCR) is a crucial, specialized DNA damage repair pathway required for proteolytic cleavage and degradation of proteins irreversibly covalently crosslinked to DNA. We are currently investigating the specific roles of several enzymes in DPCR including the P97/Vcp segregase which extracts proteins from various cellular compartments and targets them for degradation, using the zebrafish as a model for *in vivo* studies. First, we found that the *p97* gene is duplicated in zebrafish. Interestingly, *p97* is ubiquitously expressed whereas its paralog *zgc:136908* is expressed only in skeletal muscle tissues, which suggests that *p97* is the true ortholog of the ubiquitous mammalian *P97/VCP*. To study the role of *p97* and *zgc:136908* in DPCR, we injected morpholinos in zebrafish embryos to specifically knock-down the expression of target genes. DPC levels were quantified in P97-deficient embryos before and after treatment with formaldehyde (a model DPC inducer) using RADAR (rapid approach to DNA adduct recovery) assay. By co-injecting the P97 morpholino with an mRNA encoding either WT P97 or K524A-mutated P97, we created P97-deficient zebrafish embryos to study the role of P97 specifically in DPCR since this mutation reduces, but does not abolish the ATPase activity of P97. To determine the epistasis of *p97* and *sprtn*, a protease known for its role in DPCR, we co-injected morpholinos targeting *p97* and *sprtn* in zebrafish embryos and performed RADAR assays. Taken together, our results will unravel the role of P97 during the orchestration of DPC repair *in vivo*.

P-04.1-080
Using synthetic antibodies(iBodies) to probe orphan receptors of natural products

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For centuries, natural products have been a source of biologically active compounds. Despite known bioactivity of these compounds, the mechanisms that cause these phenotypic changes are often undescribed. Our goal is to identify previously undescribed ligand-protein interactions, through the use of iBodies, which can help explain these mechanisms. iBodies are synthetic polymers with a N-(2-Hydroxypropyl)methacrylamide (HPMA) backbone decorated with different moieties. Previously published in: Sacha P et al. (2016) Angew Chem Int Ed Engl 12;55(7):2356-60. We have already created iBodies, via amine click chemistry, with a biotinylation-machinery and a ligand of plant origin. iBodies with biotinylation machinery will be able to identify specific ligand-receptor bindings due to the proximal labeling and

subsequent proteomic analysis. To establish our natural product model, we are using capsaicin and the transient vanilloid receptor protein (TRPV1). Capsaicin-TRPV1 binding is highly specific and well characterized. We have synthesized iBodies that contain both capsaicin and a riboflavin moiety that allows for biotinylation through diazirine chemistry of a biotin-tyramide substrate. We have carried out small-scale reactions using only the riboflavin catalyst, biotin substrate and a peptide, with analysis by high-resolution mass spectrometry. Results confirmed the modification of peptides with biotin and that our modifications do not hinder riboflavin's photosenziation ability but actually increases riboflavin's stability. In addition, we are using whole-cell patch clamp analysis with capsaicin iBodies and HEK293-TRPV1 stably transfected cells to determine the binding ability of iBodies to the receptor. We also plan on expanding the current setup with resiniferatoxin, another potent agonist of TRPV1. Upon completion of the model system, future experiments will be expanded to other natural products whose receptor partners are not fully described or unknown.

P-04.1-081
Interaction of murine cathepsin B and DARPin and its prospects

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Cysteine cathepsins are lysosomal proteinases that influence many cellular processes. They express as preproenzymes, get processed to proenzymes and are activated in the acidic milieu of lysosomes through cleavage of the propeptide by other active cathepsins. In healthy tissue, they are important for homeostasis and are involved in processes, such as bone remodelling, antigen processing and its presentation, thyroglobulin processing and protein turnover in general, including extracellular and adhesion proteins. They are known to overexpress and to be involved in the progression, invasion and metastasis of solid tumours as well, but it is arduous to ascribe exact individual roles to individual members owing to the heterogeneity of tumours. Cathepsin B (CtsB) has been most studied in the context of cancer which can be located at the surface of invadopodia of tumour cells (association with annexin A2) as a proenzyme and in membrane invaginations (caveolin-1) where it was linked with extracellular degradation. Designed ankyrin repeat proteins (DARPins) are genetically engineered antibody mimetic proteins based on natural ankyrin proteins that are used for binding and are involved in numerous cell functions. DARPins can be used as diagnostic or therapeutic agents due to their high specificity and affinity for the selected target. DARPin 4m3 shows a high affinity and selectivity for murine CtsB. Currently, the structure of murine CtsB remains unresolved due to a lack of success in crystallization efforts but DARPin 4m3 could be used for chaperone-assisted crystallization since its binding could sufficiently stabilize murine CtsB for crystallization purposes. Due to similar biochemical and physiological properties between human and murine CtsB, it makes sense to resolve the structure of murine variant for structure-based drug design, especially if we consider the role of mice in the development of drugs.

P-04.1-082
Polyionic vesicles containing therapeutic methionine γ-lyase: physicochemical parameters, embryo toxicity and targeted delivery

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Therapeutic enzyme-loaded polyionic complex vesicles created relatively recently, have permeability and biocompatibility and can function as nanoreactors. Earlier we have shown that encapsulation of antitumor methionine γ-lyase (EC 4.4.1.11, MGL) in polyionic nanocapsules formed by polyaspartic acid chain and polylysine chain with different number of chain links leads to the retaining of enzyme activity and improved pharmacokinetic characteristics compared to the native and pegylated MGL. For the targeted delivery of nanocapsules to the surface of the cancer cell, polylysine chain was decorated with the natural phytoestrogen genestein. The nanocapsules with included mutant form V358Y MGL (V358Y-PICsomes) were obtained and characterized by dynamic light scattering. The degree of inclusion of enzymes and physicochemical parameters of nanoreactors were determined. The embryotoxic effect of V358Y-PICsomes and their individual components was evaluated *in vitro*. It was found that all components of nanoreactors do not have a toxic effect on the development of early embryos. Nanoreactors based on enzymes encapsulated in polyionic nanocapsules are still at the beginning of their application. The lack of detailed studies concerning the encapsulation of therapeutic enzymes in PICsomes, as well as its targeted delivery to the surface of cancer cell, makes our research relevant. Acknowledgements The work was supported by the Russian Science Foundation (project No. 20-14-00258).

P-04.1-083
DUB module of SAGA complex may be involved in transcription regulation independently of the SAGA

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We have previously shown that SAGA complex is involved in the transcription of not only Pol II-, but also Pol III-dependent genes including snRNA genes. In this work, we verified whether DUB module of SAGA complex is involved in transcription regulation independently of the SAGA. To this end, we performed ChIP-seq analysis of Sgf11, Gcn5 and Brf1 proteins to identify sites of binding of DUB and HAT modules of SAGA complex and Pol III-dependent transcription machinery. We found that Sgf11 and Gcn5 were present on the set promoters of Pol III-dependent genes, together with Brf1. On promoters of Pol II-dependent genes, Sgf11 and Gcn5 are present without Brf1. We found that the DUB module is mostly recruited to promoters as part of the SAGA complex, but there are also DUB module binding sites independent of the SAGA. To identify the pool of SAGA-dependent genes we analyzed the differential gene expression in RNA sequencing data of Drosophila lines with mutations in the DUB module (Sgf11 and Nonstop subunits), HAT module

(Gcn5) and Ada2b which were obtained from BDSC. We found that DUB and HAT modules of SAGA complex regulate a common set of genes. In addition, we identified genes which transcription depends on either the DUB or the HAT module. Thus, DUB module of SAGA complex is involved in transcription regulation both as part of the SAGA complex, and independently of it. This work was supported by the Russian Science Foundation (Grant No. 20-14-00269).

P-04.1-084
Cytolysin A (ClyA) in *E. coli*: a tool for outer membrane vesicles (OMVs) surface decoration with chimeric immunomodulatory proteins

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One of the emerging trends in therapeutics is the development of novel drug delivery systems, allowing to convey drugs to a specific cellular target and minimizing adverse effects. Outer Membrane Vesicles (OMVs), small bacterial proteo-liposomes (20–250 nm), hold a great potential to be used as engineerable carriers. In this work we first characterized size and proteomic profile of the OMVs obtained from *E. coli* BL21(DE3), a commercial strain suitable for optimized recombinant protein production. Based on previous literature reports [1], we overexpressed a mutated form of Cytolysin A (ClyA) monomer, to be engineered as a membrane scaffold for the surface-decoration in OMVs of recombinant proteins with immunomodulatory potential. ClyA sequence with a C-terminal linker and a His-tag was cloned and recombinantly expressed in BL21(DE3) cells. ClyA enrichment in OMVs was verified by western blotting. ClyA C-terminal exposure on OMVs surface was then investigated by proteolytic degradation of ClyA C-terminal His-tag on intact OMVs, and immunofluorescence experiments using BL21(DE3) cells expressing ClyA. As a proof of concept, we produced two chimeric proteins of different length fused to ClyA C-terminus. The first (63 kDa) bearing the light variable chain of the AntiCD19 scFV (variable regions of the light and heavy chain) and the second (88 kDa) with both chains of the scFV portion. Both chimeras were recombinantly expressed and OMVs were purified. Our data showed the presence of the partial construct in OMVs. However, the whole scFV fusion protein failed to be encapsulated in the outer membranes, as confirmed by a cell fraction experiment. Data suggested a lowered OMVs encapsulation efficiency with increasing mass of the fusion protein. Further studies will foresee the addition of a N-terminal leader sequence to improve OMVs encapsulation of longer chimeric constructs. [1] Kim JY et al. (2008) J Mol Biol 380(1), 51-66.

P-04.1-085
Cold denaturation of the phase-separating fused in sarcoma protein

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Fused in sarcoma (FUS) is a complex prion-like ribonucleoprotein which undergoes liquid-liquid phase separation (LLPS) in stress conditions, leading to the formation of biomolecular condensates *in vitro* and *in vivo* (previously published in Chen C et al. (2019) Molecules 64, 1622). The mediation of formation of these liquid compartments is usually attributed to the low-complexity and disordered domains of FUS (previously published in Burke KA et al. (2015) Mol Cell 60, 231-241). In our work we questioned the role of the FUS folded domains in the LLPS mechanism and studied the influence of overall conditions on the phase separation of the full-length protein. Using a combination of turbidimetry, differential interference contrast and fluorescence microscopy, and nuclear magnetic resonance spectroscopy, we show that full-length FUS LLPS is highly responsive and tightly regulated by the surrounding condition (e.g. ionic-strength, salts, temperature and pH) and that intrinsic disorder is crucial for the LLPS process. Our results reveal that the folded FUS RNA-binding (RRM) and zinc-finger (ZnF) domains undergo cold denaturation above 0°C, a process that is determined by the conformational stability of the ZnF motif. We hypothesize that under cold shock conditions, cold denaturation might promote FUS self-assembly and LLPS, acting as a fast cellular response to cold stress. Our study provides the first insight into the influence of cold stress on the FUS protein and how phase separation can be structurally regulated in stress conditions.

P-04.1-086
Allostery and dynamics in nuclear hormone receptor transactivation

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The role of allostery in nuclear hormone receptor (NR) signaling is of growing significance. NR proteins relay cellular signals through distinct multiprotein assemblies. These molecular signals produce specific structural changes within NRs that determine the composition of the interacting proteins. NRs have a modular structural topology that includes an intrinsically disordered N-terminal domain (NTD) followed by DNA-binding (DBD) and hormonal ligand-binding domains (LBD). Agonist ligand binding results in conformational changes associated with a transcriptionally active state where the LBD rearranges to create a docking site for coactivators such as the steroid receptor coactivator 1 (SRC1). With biophysical (simulation and experiment) and cell-based transcription assays we are developing models for allostery that address the following questions: (i) What role do ligands play in the stoichiometric assembly of the NR:coregulator complex? (ii) How do discrete sites control the activity of these receptors? and, (iii) How do the DBD and LBD communicate to regulate coactivator and DNA recognition? Here, we report (1)

that agonist ligands can potentiate transactivation through both coactivator-binding sites; (2) on the structural basis for negative cooperativity between the NR heterodimers; and (3) evidence of direct, DNA-dependent, communication between the DBD and LBD. We also propose a novel 'frustrated fit' mechanism of allostery.

P-04.1-087
Deciphering rare opening of gates in ligand-transport tunnels of enzymes using enhanced molecular dynamics simulations

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Biochemical reactions occur in the active sites of enzymes, secured by several essential catalytic residues, which are mostly buried inside the protein core and connected to the bulk solvent through molecular transport pathways called tunnels. Moreover, the engineering of tunnel residues can produce mutants with enhanced properties, and tunnels also represent very interesting drug targets. Since most of these tunnels are transient in their nature, the preferred method to study them is molecular dynamics (MD) simulations. However, given the rare opening of tunnels, their identification and analysis often require extensive and time-demanding sampling. Here, we have explored to what extent Gaussian accelerated MD simulations (GaMD) models are suitable for the analyses of the rare opening of gates in transient tunnels in three model systems, wild-type haloalkane dehalogenase (DEHAL) enzyme along with its two mutants. These simulations revealed that GaMD models managed to identify all experimentally confirmed tunnels. Additionally, we observed that, indeed, GaMD simulations tend more frequent detection of tunnels, which were observed only exceedingly rarely in the Classical MD (cMD) simulations, but still on the biologically relevant time scales. Overall, the presented research highlights GaMD models as a promising approach to investigate tunnels in proteins, without compromising the geometrical properties of these ligand transport pathways. As such application of GaMD opens up new possibilities for the identification of complex networks of transient tunnels in large protein systems efficiently. The work was supported by the National Science Centre, Poland (2017/26/E/NZ1/00548), and the calculations were performed at the Poznan Supercomputing and Networking Center.

P-04.1-088
Looking for the brightest one: comparison of the effectiveness of *in silico* and library optimization methods

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Synonymous substitutions in first 11 codons of coding DNA sequence (CDS) affect probability of mRNA to form secondary structures capable for disrupting ribosome binding and decreasing protein yield. Probability of secondary structure formation can be quantified as value of minimal free energy (MFE) which can be calculated *in silico*. Studies show significant correlation between level of protein expression and calculated value of MFE. That's why optimization of 5'-CDS is widely used in codon optimizing algorithms *in silico*. An alternative approach is design of library of synonymous sequence variants fused to GFP with subsequent screen for best variants according to fluorescence intensity. In this work we attempted to show that sequence variants with calculated highest possible value of MFE can still have lower expression level that variants obtained with library methods. Three model proteins were used: canine cystatin C (CCC), human cardiac troponin I (hcTnI) and human BAG3 (BAG3). Libraries of 5'-CDS randomized protein::GFP fusions were created by overlap extension PCR with degenerate primer. Colonies with the highest expression level were selected based on fluorescence intensity. The expression level of non-fused optimized variants was measured after removal of GFP. Variants of sequences with higher possible MFE value (using synonymous mutations in same region as in libraries) were calculated by program viennaRNA and then created by PCR mutagenesis. As a result, in case of BAG3 and CCC both library and *in silico* methods showed significant increase in expression. In case of hcTnI only library method showed significant increase of protein expression while max-MFE variant showed similar expression level to original sequence. These results demonstrate that that *in silico* optimization still remains "trial and error" and in case of codon optimization, library methods suffer less from incomplete understanding of expression mechanisms and produce more practical results.

P-04.1-089
Functional and structural properties of tropomyosin isoforms involved in cancer transformation

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Tropomyosin (Tpm) is a widespread actin-binding protein that regulates properties of actin filaments. Tpm determines actin cytoskeleton functions and participates in the regulation of cell transport, division and migration. About 30 Tpm isoforms express in various human tissues. Tpm plays important role in the cell malignant transformation and metastasis. The Tpm isoform composition alters during bladder, kidney, breast and neuronal tissue cancer. In our work, we obtained structural and functional information about the interaction between actin filament and tropomyosin isoforms, which participate in cancer progression. In this study, we characterized the structural and functional properties of Tpm1.6, Tpm2.1, Tpm 2.2, Tpm3.1, Tpm4.1 and Tpm4.2. Circular dichroism and differential scanning calorimetry were used to obtain structural data. Tpm2.1, Tpm4.1, Tpm2.2, Tpm 1.6 had low thermal stability. The main temperature transition on the melting curves for them was in the temperature range 40-42°C. Tpm3.1 and Tpm4.2 had much higher thermal stability. Tpm isoforms with a lower stability showed higher affinity to F-actin. Tpm2.2 and Tpm1.6 had the highest affinity with $K_{50\%}$ values 0.33 μM and 0.45 μM, respectively. Tpm3.1 presented the lowest affinity to F-actin with $K_{50\%}$ values 2.61 μM. The stability of the Tpm-F-actin complexes was directly dependent from stability of Tpm molecules. Tpm4.2 had the highest stability of the complexes with Tpm dissociation temperature - 50°C. Tpm isoforms differently affected actin filament disassembly under the action of cofilin-1. For example, Tpm1.6 practically prevented disassembly, and Tpm4.2 accelerated this process. Our results indicated that Tpm

isoforms largely determine the characteristics of the actin filament and may underlie the regulation of the interaction of actin with its partner proteins, which plays an important role in cancer cell transformation and metastasis. The work was supported by President grant MK-5708.2021.1.4.

P-04.1-090
Analysis of the structure and filament assembly of human septin complexes

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Septins are GTP binding proteins that are considered as a fourth element of the cytoskeleton. These proteins are capable of forming apolar filaments, composed of hetero-oligomeric complexes, which are important for cytokinesis and a variety of other cellular processes. The complexes present a specific subunit arrangement and the interaction between septins are made by two different interfaces, NC or G. However there is a variability in size and composition among species. In humans, these can be either hexameric or octameric complexes, in which two copies of three or four different septin groups, respectively, assemble into a specific order. The assembly mechanism and the polymerization of the septin complexes are still unknown. X-ray crystallography technique has been widely used to study the septin monomers or dimers, on the other hand, it's not feasible to obtain crystals and high resolution structures of the full septin heterocomplex. Recently, using Cryo-EM, it was possible to provide a complete model for a human septin hexameric complex, with a global resolution of ~3.6Å. Previously published in: Mendonça DC et al. (2021) J Mol Biol 433, 167096. We observed a bending at the centre of the hexamer that can be related to their ability to interact with membranes. Furthermore we have better understood the NC interface between SEPT6 and SEPT7, where an internal cavity is present with a floor formed by two anchored α0 helices including their polybasic regions. It's known that filaments can be organized in higher order structures in the cell. Our current work aims to explore the regions responsible for the interactions between paired filaments. These interfaces are believed to be stabilized by the formation of coiled-coils from the septin C-terminal domains. The optimization of the sample preparation is in progress and first cryo-EM analysis will be presented. Altogether, this should give us a more complete understanding of the septin structure-function relationship.

P-04.1-091
Comprehensive analysis of bactofilin in *Caulobacter crescentus*

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Similar to eukaryotes, bacteria utilize filamentous proteins and filament systems that are collectively known as "cytoskeletons" to spatiotemporally organize cellular components. A widely conserved member of the bacterial cytoskeleton is the protein bactofilin. Bactofilin homologs are characterized by a conserved bactofilin domain that adopts a β-helical architecture and polymerizes spontaneously into large polymers such as bundles and 2D crystalline sheet (1). In the model organism *Caulobacter crescentus*, two bactofilin paralogues, BacA and BacB, form a sheet lining the stalked pole which recruits the peptidoglycan remodeling protein PbpC for stalk elongation (2). Using mutagenesis, subcellular localization studies and in vitro crosslinking, we identified several residues in BacA that are critical for its polymerization. It appears that the formation of protofilaments is mediated by hydrophobic amino acids at the end of bactofilin domain, whereas charged residues on the surface contribute to lateral interactions between protofilaments. Moreover, we confirmed that the membrane association of bactofilin is via a conserved N-terminal motif, as suggested by Deng et. al (3). A systematic mutational screening demonstrated that phenylalanine and lysine residues within this motif are indispensable for membrane binding. Finally, our study provides insight into the interaction between BacA and PbpC. Both *in vivo* and *in vitro* experiments suggest that the first 13 amino acids of PbpC are required for the interaction with BacA. We further narrowed down the PbpC-binding region of BacA by hydrogen deuterium exchange (HDX) analysis to the last winding of bactofilin domain. Together, our findings expand previous knowledge about bactofilin and shed new light on the mechanisms that underlie its biological function. References: 1. Vasa S et al. (2015) Proc Natl Acad Sci USA 112, E127–136. 2. Kühn J et al. (2010) EMBO J 29, 327–339. 3. Deng X et al. (2019) Nat Microbiol 4, 2357–2368.

P-04.1-092
Bringing *Ciona intestinalis* septins to light

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Septins are cytoskeleton proteins involved in many important cellular processes, such as cytokinesis, cell polarization and morphogenesis. Septins are ubiquitous in fungi and animals, and different subunits associate in the cell to assemble filaments, which build other sophisticated architectures. The number of genes coding for septins in different organisms is not homogeneous. *Ciona intestinalis* is a sea squirt largely used as a model system to explore the evolutionary origins of the chordate lineage and it possesses only four genes encoding septins. Interestingly, these genes represent one homologue for each group found in mammals: SEPT2, SEPT6, SEPT7 and SEPT9, predicting that *Ciona* septins are organized similarly to that observed in humans. Aiming to evaluate this, we produced and characterized the recombinant *C. intestinalis* septins (CiSEPT) and their complexes. Complexes containing three septins assemble as hexamers, but that with four septins (CiSEPT2-6-7-9) were non-homogeneous in length, showing the coexistence of hexamers and octamers. Using Transmission Electron Microscopy, CiSEPT2 was located at the end of the oligomers, agreeing with the positioning of the human SEPT2. Furthermore, we observed that the CiSEPT2-6-7-9 hetercomplex can assemble into higher order structures, similar to those already described for septins in other animals and yeast. Noteworthy, cryo-EM images show that, hexamers and octamers

can be clearly distinguished by the presence of two or three NC-interfaces, respectively. From the structural model fitted into the density map for the hexamer, the NC-interfaces can be seen to be involved in the formation of a cavity between subunits, similar to that described in the recent human structure. These results allow us propose *C. intestinalis* as a promising alternative model to study septins, satisfying the requirements for a complete but simpler model to understand the mechanisms behind the assembly of septin complexes and filaments.

P-04.1-093
Characterization of Kaiso binding sites in human kidney cancer cells

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DNA methylation is one of the key mechanisms involved in the regulation of transcription. Methylation of promoter regions often correlates with the inactivation of gene transcription. Methylated DNA attracts methyl-DNA binding proteins that are involved in transcriptional repression. Their depletion may result in gene activation and may serve as the basis of target therapy. Here, we investigated target genes of methyl DNA binding protein Kaiso in human kidney cancer cells. We performed ChIP-seq analyses for Kaiso binding sites and determined differentially expressed genes in Kaiso depleted cells obtained via CRISPR/CAS9 genome editing. We identified around 16000 regions bound by Kaiso. We confirmed that Kaiso may function as both transcriptional repressor and activator. Among up-regulated genes, we found PRKAR1B, RIOX1. SERPINF1, HSPB1, GABPB1-AS1, EML2 were downregulated. Also, we analyzed how the deficiency of Kaiso influences its target site's methylation. The methylation of the majority of Kaiso binding sites remained unchanged. However, we identified around 90 Kaiso binding sites in regulatory regions, that changed their methylation after Kaiso depletion. In most cases changes in DNA methylation did not lead to variation in the expression level of the target genes. Further, we introduced mutation on E535 amino acid in Kaiso that is abolished its ability to interact with methylated DNA but retained the ability to bind CTGCNA sequences. This mutation resulted in the loss of Kaiso's ability to bind to two-thirds of its binding sites. So, we can conclude that most of Kaiso DNA binding activity is related to interaction with methylated DNA. We identified new Kaiso target genes that can be activated or repressed by Kaiso. This research was funded by the Russian Science Foundation, project no. 19-74-30026 (Kaiso ChIP-seq), and by the Russian Foundation for Basic Research, № 19-29-04139 (E535 analyses).

P-04.1-094
Genetically controlled iron-oxide nanocompartments in mammalian cells based on encapsulin proteins

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We have demonstrated that spherical proteinaceous nanoreactors called encapsulins naturally occurring in bacteria such as *Quasibacillus thermotolerans* can be expressed in mammalian HEK293T cells. These encapsulins represent a two-component system consisting of a nanoshell and a natural ferroxidase cargo, enabling import and sequestration of up to 60000 iron atoms inside the nanocompartment. Genetic constructs and experimental details are given in Sigmund *et al*., ACS Nano 2019, and Efremova *et al*., Pharmaceutics 2021. We have identified that a small fraction of HEK293T cells grown in a Fe-containing medium and expressing these encapsulins can be isolated by commercially available columns for magnet-assisted cell sorting. Transmission electron microscopy and Energy Dispersive X-Ray Spectroscopy investigations revealed that each sorted cell contains thousands of electron-dense, Fe-containing nanoparticles with an average diameter of 30 ± 3 nm. Vibrating sample magnetometry proved the ferrimagnetic response of the sorted cells at 5-250K, which suggests the presence of magnetic phases different from antiferromagnetic ferrihydrite. Finally, we performed a proof-of-principle experiment showing the potential of encapsulin expression for cell manipulation when the cells were plated in the presence of a permanent magnet. According to the optical microscopy results, 24 hours later, the sorted cells were predominantly attracted to the edges of the magnetic cube immersed into the cell medium. The present study is the basis for a thorough understanding of the ferrimagnetism in mammalian cells via genetically encoded iron-storing proteins. We acknowledge support by the Federation of European Biochemical Societies (FEBS) via a Long-Term Fellowship, an Alexander von Humboldt Research Fellowship for Postdoctoral Researchers, an Add-on Fellowship for Interdisciplinary Life Science provided by the Joachim Herz Foundation (M.E.), as well as the Helmholtz RSF Joint Research Group (HRSF0064).

P-04.1-095
Preliminary identification of novel protein substrates for actin-histidine N-methyltransferase (SETD3)

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The SETD3 protein is widespread in multicellular eukaryotes and was recently identified as a protein histidine methyltransferase that catalyzes the Nτ-methylation of histidine 73 residue in actin. This post-translational modification is important for maintaining cytoskeleton integrity and is the only well characterized

biological effect of SETD3 activity so far. However, SETD3 was also postulated to play a role in the regulation of processes that are not directly related to actin homeostasis such as response to hypoxic conditions, and enterovirus pathogenesis. Several studies showed that human SETD3 physically interacts with ≈100 different intracellular proteins, implying that some of these interactors may actually be its protein substrates. The identification of those proteins might help to fully disclose the biological importance of SETD3 and was the objective of this investigation. Three different human SETD3-knockout cell lines (HAP1, HeLa, HEK293T) were generated employing CRISPR/Cas9 method and used as a source of SETD3 substrates in fluorography experiments. The SETD3-dependent methylation of proteins present in cell lysates was performed with [3H]SAM or [2H]SAM. Labeled proteins were separated by SDS-PAGE, soaked in a fluorographic reagent and enclosed in a hypercassette for 1 month at -70°C, and developed. Pieces of the dried SDS-PAGE gel containing labeled proteins were cut out, digested with trypsin and analyzed by hybrid mass spectrometry (Q-TOF). Fluorography revealed the presence of six protein bands that were labeled by SETD3. The Q-TOF analysis of these bands identified quinone oxidoreductase and glyoxalase domain containing protein 4 as potential novel substrates for SETD3 in human cells. They will be further investigated to verify their physiological importance. Acknowledgements: This work was funded by the Opus 14 grant (UMO-2017/27/B/NZ1/00161) from the National Science Centre, Poland.

P-04.1-096
Aspects of septin polymerization influenced by the presence of the Borg3 protein

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Septins are characterized by binding guanine nucleotides and forming heterocomplexes that can associate into filaments that, in turn, can form higher-order structures such as bundles and rings. These structures are considered the biologically active form of septins and, thus, it is crucial to understand how and under what conditions this process occurs. Families that regulate this process have been highlighted in the literature, among them the BORG's protein family has been described as a possible regulator of septin cytoskeleton formation and its architecture. In this work, we studied the influence of the C-terminal region of the proteins SEPT2, SEPT6 and SEPT7 on the formation of septin filaments, varying the ionic strength of the medium, the protein concentration and the presence of the region predicted to interact with septins of the Borg3 protein, called the BD3 motif. The polymerization assays were carried out *in vitro* under the desired conditions and then the samples were adsorbed onto glow-discharged ultrathin carbon film supported by lacey carbon on a copper grid (Ted Pella) and the contrast was carried out using 2% of uranyl phosphate. Images were obtained by transmission electron microscopy. Our results suggested that the ionic strength and the concentration of proteins are relevant factors for the formation of filaments and that, although the C-terminal regions of all proteins play a stabilizing role, only the C-terminus of SEPT2 is crucial for this process. The BD3 motif of the Borg3 protein, in turn, was able to induce the polymerization of septins under different conditions from those observed for septins alone, suggesting this to be potentially a key regulatory protein in this process. It was also demonstrated that the BD3 region is only able to recognize septin hexamers and assist in their polymerization when the C-termini of SEPT6 and SEPT7 are present.

P-04.1-097
Biochemical investigation of anti-CRISPR AcrIE4-F7 reveals a common inhibition strategy against divergent CRISPR-Cas types

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To defend invasions of bacteriophages and foreign plasmids, bacteria and archaea employ the clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated (Cas) systems. In turn, bacteriophages have evolved anti-CRISPR (Acr) proteins to neutralize the CRISPR-mediated immunity. AcrIE4-F7 is a fused Acr protein of AcrIE4 and AcrIF7 that inactivate type I-E and I-F CRISPR-Cas systems, respectively. Here, we report the NMR structure of AcrIE4-F7 and its target Cas components. The N-terminal AcrIE4 domain adopts a novel α-helical fold and binds to the PAM-interacting residues in the type I-E Cas8e subunit. The C-terminal AcrIF7 resembles the αβ fold of the native AcrIF7 that targets the PAM recognition site in the type I-F Cas8f subunit. Conserved negatively-charged residues in each Acr domain are essential for interaction with their respective Cas8 target components. Our study demonstrates that PAM recognition sites are the primary targets of AcrIE4-F7 to counter type I-E and I-F CRISPR-Cas systems, highlighting a common inhibition mechanism against divergent CRISPR-Cas types. Previously published in: Hong S., Lee G. et al (2022) Nucleic Acids Res 50(4), 2363-2376. *The authors marked with an asterisk equally contributed to the work.

P-04.1-098
Characterization of CPRs derived from hot pepper (*Capsicum annuum* L. cv. Bukang) and tomato (*Solanum lycopersicum* cv. Micro-Tom)

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Cytochrome P450s (CYP) play important roles in development and defense system in plant. CYPs perform their functions by NADPH-cytochrome P450 reductase (CPR) enzyme, which transfers electrons from NADPH to cytochrome P450. There are two CPR genes in the hot pepper (*Capsicum annuum* L. *cv. Bukang*) and tomato (*Solanum lycopersicum*. cv. Micro-Tom) genome each, which are *Ca*, *SlCPR1* and *-CPR2*. The *CPRs* expression levels were measured by quantitative real-time PCR in various development stages and stress conditions (jasmonic acid, salicylic acid, drought treatments). The *CaCPR1* expression level was gradually increased during fruit ripening. The *CaCPR2* gene was constitutively expressed in all the tested tissues but the expression

level was lower than the *CaCPR1*. Expression level of *SlCPR1* was higher in the leaves and flowers than in the fruit, also *SlCPR2* expression was highest in the leaves and flower, especially opened flower. As CPRs-EGFP were detected in endoplasmic reticulum (ER) of tobacco plants through intracellular localization analysis, that means all CPR proteins were localized in ER. Under the stress conditions, both of the *CPR1s* and *CPR2s* expression levels were changed possibly because more CPR enzymes are needed against stresses. To investigate the enzymatic properties, four CPRs were isolated from each plant (hot pepper and tomato) and heterologously expressed in *Escherichia coli*. The enzymatic activities were assessed using chemical substrates such as MTT and ferricyanide. *In vitro* assay showed that all CPRs mediated electron transfer from NADPH to the substrates. These results suggest that in hot pepper *CaCPR2* may plays minor enzyme under normal conditions, but under stress conditions, both *CaCPR1* and *CaCPR2* may be actively involved. In tomato, both *SlCPR1* and *SlCPR2* may work in normal condition, but they may work in different tissues, and the possibility was suggested that *SlCPR2* could be involved in the response of plants under stress conditions.

P-04.1-099
Development of the *Metridia* luciferase-based reporter for protein complementation assay

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Bioluminescent proteins are widely applied as reporters for various *in vitro* and *in vivo* biomedical assays due to high signal-to-noise ratio and non-toxicity. Metridia luciferases cloned from marine copepod *Metridia longa* represent a group of isoforms which catalyze the oxidation of coelenterazine with emission of blue light. Due to small molecular weight (16.5-22.0 kDa), high activity, thermostability and natural secretion, Metridia luciferase holds promise as a prospective protein for bioluminescent assay development [1]. Here, Metridia luciferase MLuc164 has been used to develop protein complementation assay (PCA). PCA is applied for detection of protein–protein interactions, where proteins of interest are fused with N- and C-terminus of the reporter. Once the proteins are getting close sterically, complementation of the reporter parts occurs resulting in the recovery of the reporter function. For the model PCA we constructed chimeric proteins on the base of two parts of MLuc164 fused with GCN4 leucine zipper (LZ). The cut site between N- and C- parts was designed by genomic data analysis, where G143 resided in the site of the intron's insert of the *mluc164* gene. Interestingly, this cut site corresponds to the empirically found G93 for complementation of Gaussia luciferase [2]. The constructs LZ-MLucN and LZ-MLucC were separately expressed in *E. coli* in a soluble form. The complex formation between leucine zipper domains was proved by SEC, seminative SDS-PAAG and by increase in bioluminescent activity. Thus 10% activity was recovered at luciferase parts complementation. The research was supported by RSF (project No. 21-74-00027). 1. Markova SV, Larionova MD, Vysotski ES. Shining light on the secreted luciferases of marine copepods: current knowledge and applications. Photochem Photobiol, 95(3), 705–721 (2019). 2. Remy I, Michnick S. A highly sensitive protein-protein interaction assay based on Gaussia luciferase. Nat Methods, 3, 977–979 (2006).

P-04.1-100
Comparison of the catalytic activity of butyrylcholinesterase and lipases using 4-mu palmitate as substrate

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Cholinesterase enzymes (ChEs), which classically hydrolyze the neurotransmitter acetylcholine, belong to the enzyme class of carboxylic ester hydrolases. Therefore, ChEs have the same catalytic mechanism as lipases. Based on studies of lipids and the similarity of the catalytic domain of lipases to butyrylcholinesterase (BChE), we investigated whether BChE is capable of hydrolyzing 4-methylumbelliferyl (4-mu) palmitate by using lectin affinity, inhibition kinetics, and molecular modeling experiments. Our results show that BChE is able to use 4-mu palmitate as a substrate. We compared the kinetic parameters of hydrolysis with the model lipase enzyme pancreatic lipase. Docking results showed that 4-mu-palmitate localized and interacted with the substrate binding site of both BChE and pancreatic lipase. 4-mu palmitate competitively inhibited BChE, and the IC50 and Ki values of 4-mu palmitate were reported to be 987.2 μM and 448 μM, respectively. Hydrolysis experiment, docking, and kinetic analysis of the inhibition of 4-mu-palmitate showed that BChE utilizes this molecule as a substrate. These results, together with lipases, suggest that BChE may be involved in lipid hydrolysis, which is the first report in the literature. However, further studies are needed to support our findings and to better understand the role of BChE in lipid metabolism and lipid hydrolysis.

P-04.1-101
Experimental and *in silico* studies on the interaction of a rhamnolipids with *Candida rugosa* lipase

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Lipases, a class of ester hydrolases, are an important group of biotechnologically relevant enzymes with important applications in the food, pharmaceutical and detergent industries. Commercial lipases are largely produced from microorganisms, particularly yeast and fungi. Here we explore the impact of the anionic biosurfactant rhamnolipids (RLs) on the structure and stability of commercially used *Candida rugosa* lipase (CRL) using

fluorescence spectroscopy, circular dichroism (CD) spectroscopy, surface tension analysis, and *in silico* studies. Fluorescence spectroscopy showed that RLs interact with CRL through a static quenching mechanism, resulting in a conformational change in CRL confirmed by CD. The negative ellipticity value for CRL was reduced upon the addition of the RLs. *In silico* computer modelling did not identify a single binding site for either mono-RL or di-RL based on the root mean square deviation (RMSD) criteria used. However, it did show that both mono-RL and di-RL had a high probability of binding in the same general region of the molecule and bound to some of the same amino acids. The binding region for the rhamnolipids was not in the known lipid binding pocket in CRL. RLs had a positive effect on CRL activity which was enhanced 1.8- and 2.7-fold at monomeric and micellar concentrations of RLs, respectively. The effect of RLs on CRL functionality was demonstrated through an extensive study of the emulsification properties of RLs + CRL mixtures. We conclude that RLs, despite their anionic charge, are surfactants that do not compromise the structural integrity of CRL. This makes RLs a promising alternative to current synthetic anionic surfactants in a wide range of commercial applications. This work was financed by the National Science Centre, Poland, project 2020/37/B/NZ9/01519.

P-04.1-102
Structure and function of a dodecameric machine: the human RuvBL1/RuvBL2 and its role in the large macromolecular complex PAQosome

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RuvBL1 (R1) and RuvBL2 (R2) are highly conserved eukaryotic proteins of the AAA+ ATPases family. They are the core engine of the R2TP complex and were described to participate in other large molecular complexes, such as Ino80 and TIP60. The R2TP complex – R1, R2, RPAP3, and PIH1D1 – together with the Prefoldin-like complex – URI1, PFDN2, ASDURF, UXT, PFDN6, and PDRG1 – form the PAQosome, which assists HSP90 in the assembly and maturation of different complexes that are essential in many cellular processes. However, the assembly mechanism and how the PAQosome function as a multi-unit chaperone complex remains unclear. In addition, different studies suggest that the PAQosome is also involved in ciliogenesis and in the development of cancer and ciliopathies, making it a potential attractive drug target. In this study, we aimed at characterizing the different components of the PAQosome by combining functional studies with structural data to better understand the importance of this molecular machine. TurboID is a novel approach used to study protein localization and interactions in living cells, also allowing the detection of weak and transient interactions with high sensitivity. We applied the methodology to R1/R2 to analyse their interactome and identify interactors in the context of important large cellular complexes. We were able to detect two proteins known to be part of the PAQosome, namely, URI1 and UXT. Their interaction with R1/R2 was confirmed by Surface Plasmon Resonance (SPR), using a recent method developed by our team which allows the kinetic characterization of an interaction regardless of the purity of the immobilized ligand (Extract2Chips). We also optimized the production process of R1/R2/URI1 and R1/R2/UXT ternary complexes and characterized them with other biochemical and biophysical tools. This will hopefully lead to homogeneous and monodisperse samples that will be vital to solve the 3D structures and shed light on such important biological processes.

P-04.1-103
Engineered oligomerization of calmodulin

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The evolution of proteins relies on a combinatorial assembly of a limited number of preexisting modules, known as protein domains. Genetic engineering provides tools to mimic a natural concept of modular protein domain assembly and design artificial proteins exhibiting desired properties for applications in biotechnology or medicine. Here, we report the biochemical and biophysical characterization of a novel fusion protein Ex/CaM. Chimeric target Ex/CaM consists of an oligomerization domain derived from ameloblastin (Ex) and a well-characterized monomeric protein calmodulin (CaM). By fusing Ex with CaM, we have induced the oligomerization of the originally monomeric CaM protein. The oligomerization status of Ex/CaM depends on its concentration and oligomers disintegrate below a critical concentration. Substitution of a crucial "oligomerization residue" to glycine prevented the self-assembly of Ex/CaM and confirmed that its oligomerization is driven by the specific oligomerization motif in Ex subunit. CaM forms the basis of numerous Ca^{2+} responsive biosensors and its oligomeric form offers versatile application in the field of nanotechnology.

P-04.1-104
identifying sequences within Sup35 N domain that drive the formation of biomolecular condensates

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Compartmentalization is a major regulatory mechanism that cells employ to control biochemical reactions. Alongside with membrane-enclosed compartments, reversible membraneless assemblies, also known as biomolecular condensates, are formed via liquid–liquid phase separation (LLPS). LLPS is driven by intermolecular interactions of multivalent proteins, the latter may contain either multiple folded interaction domains or intrinsically

disordered regions (IDR). IDRs also play an important role in protein aggregation, suggesting there is a connection between condensation and aggregation. The goal of this study is to identify specific sequences within IDR responsible for condensate formation. Sup35, the eukaryotic translation termination factor eRF3, is able to form both biomolecular condensates and amyloid fibrils. Unstructured NM domains of Sup35 play major role in these processes, but the exact mechanism of condensation is not quite clear. To identify specific sequences required for the LLPS, truncated Sup35NM variants were fused with yellow fluorescent protein (YFP) and then overproduced in *S. cerevisiae*. Then we investigated the ability of Sup35 truncated variants to form biomolecular condensates during stationary phase and after hyperosmotic shock. Here we show the role of Q/N rich region (a.a. 1-39), and the OPR region (a.a. 40-97) of Sup35 unstructured N domain in driving liquid–liquid phase separation of Sup35-derived constructs in yeast cells. Regions identified overlap with regions involved in Sup35 amyloid aggregation. Proteins with unstructured regions are abundant in eukaryotes, however, the link between the (un)structure and function of IDRs is poorly understood. Unveiling the mechanisms of condensates formation will help to bridge this gap. This work is supported by grant № 20-14-00148 from Russian Science Foundation, by grant № 817976 from NSF and by the St. Petersburg State University, project № 93025998.

P-04.1-105
Skeletal troponin I detection in the blood of patients with skeletal muscle injury

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Troponin I (TnI) together with troponin T (TnT) and troponin C (TnC) forms the troponin complex, which regulates muscle contraction. In humans TnI is presented by three forms – cardiac isoform (cTnI) and fast and slow skeletal isoforms (fsTnI and ssTnI, respectively). FsTnI and ssTnI present only in skeletal muscle but not in cardiac muscle, therefore it was suggested to use them as highly specific markers of skeletal muscle injury with different etiology. FsTnI and ssTnI are specific to fast- and slow-type muscle fibers, respectively, which could provide additional instrument to differentiate skeletal muscle pathologies. The aim of the study was to develop immunoassays that are capable of measuring of endogenous skeletal TnI. About 100 mouse antibodies recognizing different epitopes on the skeletal TnI were elaborated during the study. Several different sandwich-type fluoroimmunoassays were designed. These assays detect fsTnI, ssTnI separately and both molecules and did not cross-react with cTnI. Serial serum samples of patients that underwent surgical intervention during bone reconstruction were used to confirm the ability of test-systems to recognize endogenous skeletal TnI. Gel filtration techniques with subsequent immunochemical detection were used to determine that both fsTnI and ssTnI exist in blood of patients in complex with other components of troponin complex – TnC and TnT. Parts of skeletal TnI molecule shielded for interaction with antibodies by other components of troponin complex were mapped. As a result, test-systems for skeletal TnI measurement were developed and these systems are capable of detecting endogenous skeletal TnI in the blood of patients with skeletal muscle injury. *The authors marked with an asterisk equally contributed to the work.

P-04.1-106
TRPM5 and TRPM7 channels form complexes with calcium binding proteins CaM and S100A1

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Out of the transient receptor potential (TRP) channel superfamily, the melastatin (TRPM) subfamily is the largest. Its eight members (TRPM1-TRPM8) have diverse physiological functions and biophysical properties. TRPM5 belongs to a group critical for sensory physiology, specifically for taste transduction. TRPM7 represents channel significant for cation permeability and kinase activity and participates in important processes of the nervous and cardiovascular systems. TRPM channels can be activated in response to a wide range of physiochemical stimuli and are known to be highly modulated through interactions with extracellular and intracellular agents. The binding sites for these ligands are mostly located at the intracellular N- and C- termini of the TRP channels, and they can demonstrate the character of an intrinsically disordered protein, which allows such a region to bind various types of molecules. In the present study, we explored the N-termini of TRPM5 and TRPM7 and identified novel binding regions for important intracellular modulators calmodulin (CaM) and calcium-binding protein S1 (S100A1) using *in vitro* binding assay fluorescence spectroscopy, microscale thermophoresis and *in silico* approaches. Molecular modeling and molecular dynamics simulations of the discovered complexes reveal their potential binding interfaces with common interaction patterns and the uniqueness of the basic residues present in the N-terminal binding region of TRPM. Our results may help to a deeper understanding of the mechanism of TRPM interactions with intracellular regulatory molecules and may further assist in the design of new therapeutics for ion channel dysfunctions associated with severe disorders.

P-04.1-107
Screening of an organophosphate's effect on the plasma membrane organization of the mouse brain

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Organophosphates (OPs) such as nerve agents are lipophilic molecules that inhibit the enzyme acetylcholinesterase (AChE), which has an essential role in hydrolysing the neurotransmitter acetylcholine. The inhibition of AChE leads to the onset of life-threatening symptoms induced by the overstimulation of nicotinic and muscarinic receptors, which can result in death. Oxime compounds are used for reactivation of inhibited AChE, but there is no universal therapy for exposure to different OPs. Moreover, the effect of the OPs is not limited to cholinergic functions but affects neuritogenesis, cell-cell interactions, apoptosis, synaptogenesis, amyloid fibre formation, and they possess membrane disrupting potential due to their lipophilicity. The compartmentalization of the plasma

membrane into lipid rafts has an important role in cellular signalling, endocytosis, exocytosis, and other cellular processes. Lipid raft (LR) disruption can lead to apoptosis, disorders in regulation of neurotransmission and protein trafficking, and is associated with several diseases. Herein, we investigated whether an OP induces the reorganization of the membrane subdomains. Mice exposed to a sub-lethal dose of nerve agent were compared to mice treated with an oxime 30 minutes after OP and both groups compared to control mice without any treatment. Mice were sacrificed 1.5 h, 2 and 4 days after OP administration, brains were dissected and snap-frozen. LR were isolated from brain cortices by ultracentrifugation in discontinuous sucrose gradients and western blotting was performed for LR markers ganglioside GM1 and Flotillin-1, and non-lipid raft marker transferrin receptor. The results showed that there were slight disturbances in the distribution of the markers with regard to the OP exposure and therapy when compared to control mice, which makes this a promising base for further investigation. Supported by HDTRA-19-1-006-UCSD-113020, Croatian Science Foundation (IP-2018-01-7683; IP-2016-06-8636)

P-04.1-108
Modification of protein synthesis and secretory pathway to improve surface display efficiency

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Major disadvantages of protein surface display in yeasts as an alternative to classical solid-surface immobilization techniques are low production, secretion, and binding capacity, or simply said low surface display efficiency of recombinant proteins. As several problems could lead to low display efficiency, we attempted to investigate how modification of different cellular processes involved in the production and secretion of proteins can affect and possibly enhance display efficiency. For this purpose, we tested the effects of mutations of genes involved in transcription and translation (*Δgcn2, Δgcn5, Δopi1, Δrrp6*) and mutations affecting properties of the endoplasmic reticulum (*Δyop1*). We also tested the effect of mutations of genes involved in unfolded protein response (UPR) and the endoplasmic reticulum-associated degradation (ERAD) pathways that are possibly activated due to stress conditions caused by increased protein production of overexpressed recombinant protein. To test the effect of these mutations we used two reporter systems previously developed in our laboratory. Both systems contain β-lactamase fused with either Pir2 protein or with the GPI anchoring signal sequence of Ccw12. Results obtained by measuring the activity of surface-displayed β-lactamase have shown that it was possible to increase surface display efficiency significantly by modifying processes involved in protein production and secretion in yeast cells.

P-04.1-109
Altering the cell wall to increase cell surface display efficiency

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Yeast surface display is a powerful and robust method for the surface immobilization of heterologous proteins with potential application in different biomedical and biotechnological processes. In biotechnology, it could be used in various bioconversion processes as enzymes of interest can be fused with anchoring proteins. This allows secretion of such protein constructs and their binding to the cell wall. The major disadvantage of this method is the low binding capacity and low overall surface display efficiency of currently used systems. There are several possible approaches to resolve this problem, and in this work, we focused on the modification of the cell wall of host cells. Several mutant strains were developed and/or tested to see if it is possible to increase display efficiency by introducing changes in cell wall structures. Tested strains contained mutations in genes coding for cell wall proteins (the strain with deleted genes coding for Pir proteins, and *Δcwp2* strain) or in genes coding for proteins involved in the synthesis of the GPI anchor, and in the localization of GPI anchored wall proteins (*Δgpi7, Δdfg5, Δdcw1* strains). Also, the effect of deletion of the SSD1 gene coding for the translation factor involved in the translation of many cell wall proteins was tested. Effects of these mutations were investigated using reporter systems previously developed in our laboratory, composed of β-lactamase fused with either Pir2 or Ccw12. Our preliminary results show that it is possible to obtain more than two-fold higher surface display efficiency by these modifications.

P-04.1-110
Functional and stability analysis of Glucan binding protein C

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Bacterial strains forming biofilms are a major challenge in eradication of dental plaques. These extracellular, chemically heterogeneous structures include several commensal microbial populations, providing them with an isolated and protected habitat. Amongst a wide variety of microbes, the Gram-positive bacterium *Streptococcus mutans* is a dominant member of the normal flora of the human oral cavity. The polysaccharide backbone of biofilm can be produced by cell surface glycosyltransferases, which promotes cell-cell adhesion through cell surface glucan-binding proteins. Although the biofilm is a central component of defense against the oral cavity invading pathogenic microorganisms, its overgrowth contributes to the extensive plaque formation on tooth enamel and, in turn, to the development of tooth decay. Recently, a key protein component of biofilm formation, the Glucan binding protein C (GbpC) of *S.mutans*, has received increasing attention from oral biologists. Its crystal structure and interaction with its natural ligands have been described [1]. However, its thermal stability – especially in native-like milieu – has not been studied yet. Our research aimed at analysing stability properties of the GbpC protein. To implement our goal, we produced 6xHis-labeled GbpC using the *E.coli* overexpression system and purified an ample amount of protein of interest. Our ALPHA and BLI measurements demonstrated the glucan binding capacity of the purified protein confirming its functionality. Finally, the stability of GbpC was determined by using Thermal Unfolding Assay in a variety of conditions. We concluded that close-native conditions have major effect on studied properties. [1] Mieher et al. (2018) Infect. Immun., American Society for Microbiology 86(7). DOI: 10.1128/IAI.00146-18

P-04.1-111
The influence of amino acid substitutions and modifications on the functioning of human eIF3j in translation termination

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In addition to the main release factors, eRF1 and eRF3, additional proteins are involved in translation termination. eIF3j is one of the eukaryotic translation factors originally reported as the labile subunit of the eukaryotic translation initiation factor eIF3. We recently demonstrated that human eIF3j stimulates translation termination by loading release factors into the ribosome (previously published in: Egorova T. at al. (2021) Nucleic Acids Res 49(19), 11181-11196), but the details of its functioning in the termination of translation remain unclear. Using a reconstituted mammalian *in vitro* translation system, we elucidated the impact of mutations and modifications of eIF3j on its activity in translation termination. We have obtained the following recombinant mutants and phosphorylated form of eIF3j: eIF3j_E214Q, eIF3j_W52A, eIF3j_S127A, eIF3j_LQ123-124AA, and eIF3j with a phosphorylated serine residue at position 127. We determined the activity of mutant and phosphorylated forms of eIF3j in translation termination *in vitro* upon recognition of stop codons and hydrolysis of peptidyl-tRNA by release factors eRF1 and eRF3a. In peptidyl-tRNA hydrolysis experiments, it was found that eIF3j_W52A demonstrated approximately 15% of eIF3j activity. Additionally, we determined the ability of mutant and phosphorylated forms of eIF3j to interact with eRF3a; as well as their possible influence on the GTP hydrolysis, performed by eRF3. We have revealed that phosphorylated eIF3j inhibits the GTPase activity of eRF3. It was previously shown that phosphorylation of this amino acid residue by protein kinase CK2 increases the efficiency of translation. So, obtained results will be useful for revealing the mechanism of the influence of this modification on translation. This work is supported by the Grant of the President of the Russian Federation for state support of young Russian scientists - Ph.D. no. MK-6150.2021.1.4.

P-04.1-112
New RNA handling tools: engineering polymerases using high-throughput directed evolution

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The current rise of RNA biotechnologies, such as, among others, mRNA vaccines or aptamers, brought to light a lack of molecular biology tools, allowing cheap and rapid RNA amplification. Whereas many methods already exist for DNA exponential amplification (PCR, RCA, SDA...), the production of RNA strands requires the use of DNA-dependent RNA polymerase, limiting the growth to a linear function of time. Moreover, the many modifications increasing the RNA molecule stability are generally added randomly afterwards by costly organic chemistry reactions. Here, we propose to engineer a thermophilic DNA-dependent DNA polymerase to make it accept (modified-)RNA both as template and as substrate through directed evolution. Such an RNA-dependent RNA polymerase should be able to perform RNA PCR or could pave the way for innovative techniques. To do so, we use two different ultra-high-throughput and microfluidic droplets-based approaches. The first one, based on the Compartmentalized Self-Replication (CSR), links an increase in the desired activity to a higher gene replication. The variants are cloned and expressed in bacteria that are encapsulated in water-in-oil droplets containing all the necessary compounds for PCR except the enzyme and the template. The emulsion composed of up to 10^8 droplets is then subjected to thermocycling. Confined in their own droplets, the variants are to replicate their own gene, giving, after emulsion break, a gene library enriched in the best mutants. The second method is using a Fluorescence-Activated Droplet Sorting (FADS) system that can send droplets, containing each one variant of the proteins, in different channels as a function of their fluorescence. The selective pressure is then adjusted by a suitable fluorogenic activity test. We are currently making rounds of selection towards the reverse transcription activity that will then help to select for the desired RNA polymerase activity.

P-04.1-113
Revealing the allosteric coupling during the gating cycle of the prototypical KcsA potassium channel using advanced homo-FRET approaches

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Potassium channels are integral membrane proteins widely distributed in all living organisms. Due to their key role in many physiological processes, their functional properties must be finely regulated. In this work, a homo-Förster Resonance Energy Transfer (homo-FRET) approach based on steady-state and time-resolved fluorescence polarization measurements was implemented to evaluate the allosteric interplay between the activation gate (inner gate) and the selectivity filter (SF; outer gate) of KcsA, the prototypical model of the K^+ channels superfamily. The structural re-arrangements at the SF from W67 KcsA (harboring a single Trp residue located at the nearby pore-helices) were tracked by monitoring its intrinsic fluorescence properties [1], whereas the pH-induced opening of the inner gate was detected by the changes on the fluorescence anisotropy of the extrinsic dye tetramethylrhodamine attached to the C116 position [2]. We found that triggering the inner gate opening of KcsA by a pH drop allosterically decreased the affinity of the SF for the permeant cations K^+, Rb^+ and Cs^+, while the SF (and the hydrogen-bonded pore helices) displayed similar conformational changes associated with the conversion from a non-conductive to a conductive state. Inversely, the occupation of the SF by a saturating concentration of K^+, but not Rb^+ nor Na^+, shifted the activation pK_a of the pH sensor at the inner gate to lower values. These results will be discussed in terms of their possible impact on the C-type inactivation process of KcsA. [1] Renart et al (2019) Sci Reports 9:6215 [2] Díaz-García et al (2021) Int J Mol Sci 22:11954 This work was financed by grant PGC2018-093505-B-I00 from the Spanish "Ministerio de Ciencia e Innovación"/

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P-04.1-114
Exploring a novel β-glucosyltransferase from GH17 family for modifying (β1→3) linked gluco-oligosaccharides

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ß-glucans are made of β-1,3 backbone with β-1,6 branching and are available in the cell wall of bacteria, plants, fungi, and seaweeds; however, degree of polymerisation, and branching affect their bioactive properties significantly. A novel β-glucosyltransferase from GH17 family, isolated from *Bacteroidetes* bacteria, was characterized regarding kinetics, substrate specificity and product formation using linear β-1,3 linked oligosaccharides as the substrate. It was found out that the enzyme has a very high substrate specificity toward β-1,3 linked glucans with a degree of polymerisation (DP) higher than five. Product profile of the enzyme, using laminaripentaose (DP5) and laminarihexaose (DP6), as the substrate displays that the enzyme cleaves two sugar units from the reducing end and transfers the remaining part to the second molecule of the substrate functioning as an acceptor. MS and NMR analysis were performed to analyse the transferase activity in terms of DP of the product and potential branch formation.

P-04.1-115
Heterologous expression and purification of SH2 domain-containing protein 3C, protein with a predicted high content of intrinsically disordered regions

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SH2 domain-containing protein 3C (SH2D3C) is one of only three members of the family of proteins that contain both SH2 domain and a domain similar to guanine nucleotide exchange factor domains for Ras family GTPases (Ras GEF-like domain). It acts as an adapter protein and has proposed roles in cell migration and adhesion, tissue organization, and the regulation of the immune response. The data about the structure of the SH2D3C protein are scarce. Only one structure can be found in the Protein Data Bank, and it is the structure of the C-terminal, Ras GEF-like domain in the complex with the Breast cancer anti-estrogen resistance protein 1 (BCAR1). SH2D3C protein was identified as a prey in the SILAC-MS analysis of the dipeptidyl peptidase 3 (DPP3) interactome. To prepare the purified protein for the biochemical and biophysical analysis of SH2D3C and its interaction with DPP3, we have expressed isoforms 2 and 3 of SH2D3C and the C-terminal domain in *E. coli* with HIS-, GST- and MBP-tag. We were able to purify only the C-terminal domain with GST- and MBP-tag, but the proteolytic removal of the tag rendered the domain unstable, however, isoform 3 was successfully expressed and purified by baculovirus-mediated protein expression in the insect cells. Isoform 3 lacks the N-terminal SH2 domain and has only the Ras GEF-like domain. According to the AlphaFold prediction of the structure of the longest, isoform 1 which contains both SH2 and Ras GEF-like domains, a large part of isoform 3 should be disordered. This was confirmed by CD measurements of the purified isoform 3 which showed that around 43 % of the protein is disordered. SEC analysis of isoform 3 indicated that it forms dimers, however, SEC-MALS showed that it is a monomer, which also indicates that the protein is probably largely disordered. Despite having a high proportion of internally disordered regions, SH2D3C-isoform 3 is stable and will be used for further biochemical, biophysical and structural analysis.

P-04.1-116
Histone chaperone FACT reorganizes nucleosomes into a nearly linear structure

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FACT (facilitates chromatin transcription) is a histone chaperone that participates in nucleosome removal and reassembly during transcription and replication. Previously we have shown that yeast FACT dramatically alters the nucleosome structure without ATP hydrolysis, but the extent of these alterations depends on the presence of HMGB domain-containing protein Nhp6. The detailed mechanism of this process remains unknown. Here FACT, FACT:Nhp6 and FACT:Nhp6: nucleosome complexes were studied using electron microscopy. All complexes are highly flexible and adopt broad ranges of configurations. DNA-binding protein Nhp6 binds to the C-terminal tails of both FACT subunits and induces formation of more open FACT complexes, thus altering the structures of FACT and the nucleosomes and facilitating nucleosome unfolding. The data suggest that Nhp6 has multiple roles before and during nucleosome unfolding by FACT, and that the process proceeds through a series of energetically similar intermediate structures, ultimately leading to an extensively unfolded form. We proposed FACT-dependent nucleosome unfolding pathway based on a large number of potential intermediates revealed by electron microscopy. This work was supported by the Russian Science Foundation (#19-74-30003). Electron microscopy was performed on the Unique equipment setup "3D-EMC" of Moscow State University, Department of Biology. Previously published in: Sivkina AL et al. (2022) Communications biology 5(1), 1-9 *The authors marked with an asterisk equally contributed to the work.

P-04.1-117
Activity characterization of PL25 family novel ulvan lyase SH2L_Ulv3 from seaweed biomass metagenome

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The green macroalgal cell wall polysaccharide ulvan remains an underutilized biopolymer with unique chemical and physiochemical properties. Ulvan is a water-soluble sulfated irregular polysaccharide mainly consisting of rhamnose, xylose, glucuronic acid and iduronic acid. The various ulvan degradation derivatives possess potential to be utilized in food, pharma and chemical industries. Ulvan lyases degrades ulvan by a β-elimination mechanism cleaving β-glycosidic bond between sulfated rhamnose and glucuronic acid or iduronic acid while glycoside hydrolases end ulvan degradation acting on ulvan oligosaccharides. Ulvan-degrading enzyme characterization remains rather sporadic as very few ulvan lyases were ever characterized confirming activity. Activity characterization of PL25 family novel ulvan lyase SH2L_Ulv3 from green and brown seaweed biomass metagenome enriched in intertidal coastal hot spring in Skarðshver (Iceland) was performed in pursuit of novel ulvan lyases with favourable activity characteristics for ulvan degradation. Sequence of SH2L_Ulv3 was most identical (52.43% identity, 97% query coverage) to ulvan lyase PLSV_3936 (PDB 5UAM) from *Pseudoalteromonas* sp. PLSV. Heterologous overexpression of SH2L_Ulv3 via rhamnose induction in *Escherichia coli* JM109 ensured high yield production of soluble recombinant ulvan lyase. SH2L_Ulv3 purified by nickel affinity chromatography remained stable soluble. Ulvan extracted from *Ulva armoricana* was actively degraded by SH2L_Ulv3 as assessed by DNS and TLC. Characterized ulvan lyase activity optima was pH 7.4 at 25°C and 1% (w/v) sodium chloride. Ulvan degradation product profile were assessed by HPAEC-PAD. Degradation products were identified by NMR spectroscopy. Acknowledgement ERA-NET Cofund BlueBio MARIKAT (New Catalytic Enzymes and Enzymatic Processes from the Marine Microbiome for Refining Marine Seaweed Biomass) project (grant no. 127).

P-04.1-118
Development of artificial enzymes with activity towards CO_2 and NO_x based on copper enzymes

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Carbon dioxide (CO_2) and nitrogen oxides (NO_x) mitigation is an emergent world goal. These compounds are abundant and low cost making them interesting feedstock for valuable chemicals/fuels production, bringing together two important needs, the mitigation of these compounds and the production of other valuable compounds. The obstacles that hamper the conversion of CO_2 and NO_x are related to their properties. To transform them into less harmful and added-value compounds, it is necessary to use the optimal reactions, namely the ones requiring the less energy. Nature has created enzymes that can react with CO_2 and NO_x, such as RuBisCo (Ribulose-1,5-biphosphate Carboxylase), responsible for CO_2 fixation, resulting in two molecules of glyceraldehyde 3-phosphate subsequently converted to biomass (1). For NO_x, there are enzymes like nitrate reductases that are involved in the denitrification route (2). In this work, the identification of promising binuclear copper sites that may be used for CO_2 reduction, is explored, by testing enzyme models in molecular dynamics simulations combined with density functional theory, starting with tyrosinase enzymes, although, not disregarding other enzymes. The chosen enzymes are from archaeal and prokaryotic origin for the simplicity of the system. This work aims to construct efficient artificial enzymes, that can produce valuable compounds from CO_2 or NO_x, taking as basis the already known natural enzymes, and proper catalytic site mutations. GNV acknowledge FCT/MCTES for funding through grant number 2021.06216.BD. This work was supported by the Associate Laboratory for Green Chemistry—LAQV which is financed by national funds from FCT/MCTES (UIDB/50006/2020) and the Research council of Norway, grant number 301022. 1. Schwander T *et al*., Science. (2016)354(6314):900-904. doi: 0.1126/science.aah5237. PMID: 27856910; PMCID: PMC5892708. 2. Carreira C *et al*., PeerJ. (2018);6:e5603. doi: 10.7717/peerj.5603. PMID: 30258713; PMCID: PMC6152468.

P-04.1-119
Enhanced PARP-1 binding and remodeling of nucleosomes flanked by linker hairpins

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PARP1 is involved in the processes of DNA repair, replication, transcription, cell cycle regulation, and apoptosis. PARP1 is one of the first proteins recruited to a site of DNA damage, where it attracts repair enzymes and facilitates their access to the DNA damage site via polyADPribosylation of neighboring proteins and chromatin decondensation. PARP1 can bind both to DNA breaks and to intact nucleosomal DNA, producing complexes with nucleosomes of different stoichiometry (1:1, 2:1 and 3:1) [Maluchenko et al., 2021, PMID: 34830005]. Formation of the PARP1-nucleosome complex induces reversible changes in the conformation of nucleosomal DNA, significantly increasing the distance between adjacent DNA gyres and probably modulating in this way the processes of DNA replication and transcription [Sultanov et al., 2017, PMID: 28804761; Maluchenko et al., 2021, PMID: 34830005]. To discriminate the role of PARP1 binding to intact nucleosomal DNA vs. its binding to DNA breaks during the nucleosome remodeling, we studied the features of PARP1 interactions with mononucleosomes containing hairpin loops at the ends of linker DNA (DNA break-free model of mononucleosomes; H-nucleosomes) by spFRET microscopy and electrophoretic mobility shift assay. We found that PARP1 binding to H-nucleosomes causes concentration-dependent changes in conformation of nucleosomal DNA, and reorganization of H-nucleosomes occurs more readily than that of conventional mononucleosomes. It is concluded that PARP1 interaction

with nucleosomal DNA strongly contributes to PARP1-mediated nucleosome remodeling. Studies were supported by Russian Science Foundation (grant 21-64-00001).

P-04.1-120
Auto-catalytic breakdown of alkaline phosphatase from a Vibrio marine bacteria

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A consistent drop in activity was observed in a cold-active Vibrio alkaline phosphatase (VAP) when the enzyme was stored at ambient room temperature in low ion-strength buffer. Gel electrophoresis showed that the inactivation was accompanied by appearance of smaller peptide bands originating from the intact protein. The breakdown required a pre-incubation of 1 day, indicating a time-dependent change in enzyme structure. The addition of 500 mM NaCl increased structural stability greatly and prevented the appearance of the extra bands in polyacrylamide gels. The breakdown was still observed after employing a three-step purification procedure, as well as when crystals were redissolved. This makes contamination by an extraneous proteinase highly unlikely. Various inhibitors prevented the autolysis, such as metal chelators (EDTA, EGTA), activated serine reagents (PMSF, AEBSF), activated cysteine reagents (iodoacetic acid, iodoacetamide, pHMB), and inorganic ions that mimic the product (tungstate, vanadate, sulphate, molybdate, aluminium tetrafluoride). Mass spectrometry analysis of gel bands or liquid samples gave results that indicated wide specificity, again excluding the presence of a specific protease. The exact mechanism for the autolysis is still unproven, but the lag-time may indicate the slow leakage of catalytic ions from the active site. Atom absorption results showed that the enzyme becomes deficient in the magnesium ion and one of the zinc ions as the proteolytic activity developed and the phosphatase activity declined. This supports the possibility that a catalytic triad involving the nucleophilic serine in conjunction with prior metal ion ligands is forming. There is precedence for similar proteolytic activity in shrimp and rat APs (Chakraborty, S. et al., 2011, PLoS One 6, e28470; Ghosh, K. et al., 2013 J. Struct. Biol. 184, 182-192).

P-04.1-121
Identification of transient protein interactions in *Arabidopsis thaliana* protoplasts by proximity biotinylation

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Detection of transient protein-protein interactions by classic immunoprecipitation can be a challenging task. The recently published proximity labelling approaches have been developed to address this hurdle of protein interaction studies. These techniques rely on *E. coli* BirA biotin ligase mutants, such as TurboID, that promiscuously biotinylate the exposed lysine residues of proximal proteins. The expression of bait–biotin ligase fusion proteins is followed by the addition of exogenous biotin that results in biotinylation of prey proteins and allows their isolation by streptavidin coated beads. We set out to convert the system for applications in *Arabidopsis* protoplasts derived from cell suspension culture. First, we modified the compatible pRT vector to permit N- and C-terminal TurboID labelling of bait proteins and we introduced a ligation-independent cloning site for convenient insertion of bait protein coding sequences. Next, the coding sequence of AtRCE2 was cloned into the N-terminal TurboID construct and the protein was transiently expressed following PEG-based transfection. We performed time course experiments with various biotin concentrations to determine the optimal exogenous biotin concentration for efficient and specific biotinylation in protoplasts. We evaluated our preliminary mass-spectrometry results and optimized the system further by introducing a biotin removing pre-clearing step. We identified a promising prey protein by using TurboID-AtRCE2 as bait and TurboID as negative control and confirmed their direct interaction by *in vitro* pull-down assay. Our results demonstrate that the presented vectors and optimized labelling conditions are suitable for *in vivo* protein biotinylation in *Arabidopsis* protoplasts and enable purification and identification of interacting proteins by mass spectrometry. These results are expected to aid the characterization of *in vivo* transient plant protein interactions. This research was supported by NTP-NFTÖ-21-B-0075.

P-04.1-122
The impact of SARS-CoV-2 Omicron variant on the human ACE2 binding: a computational study

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the COVID-19 pandemic, which escalated into a global pandemic in early 2020, accounting for more than 400 million infections and more than 6 million confirmed deaths worldwide (as of 2022/03/10). The SARS-CoV-2 mechanism of transmission and infection involves the binding of the virus to the angiotensin-converting enzyme 2 (ACE2) host receptor through the receptor-binding domain (RBD) of the spike (S) protein. The RBD is a privileged target of our immune system and antiviral therapies. Throughout last year multiple vaccines and new therapeutics against SARS-CoV-2 have been developed. However, their effectiveness is challenged by the continuous evolution of SARS-CoV-2, accompanying the origin and spread of new variants of concern (VOC): Alpha, Beta, Gamma, Delta, and recently, Omicron. Among the reported mutations in the VOC S proteins, several are specific to the RBD, which are associated with higher transmissibility or the ability to escape the immune response of previously infected patients. (Previously published in: Greaney, A.J. et al. (2021) Cell Host Microbe 29,44-57). In late 2021, the newly SARS-CoV-2 Omicron VOC raised considerable global concern due to the presence of more than 30 mutations in the S protein, 15 of which occur in the RBD (Previously published in: Mannar D et al. (2022) Science 375,760-764). Here we investigated the impact of the VOC RBD mutations on its interaction with ACE2, with a major focus on the Omicron RBD, by performing microsecond molecular dynamics (MD) simulations of this complex. Our analysis of the binding and structural dynamics of these mutations provided a detailed characterization of the binding mode between the VOC RBDs and the receptor. This allowed us to understand the role of key residues in the VOC RBD-ACE2 interface and the effect of specific substitutions on the binding affinity via the establishment of new

inter-protein contacts. *The authors marked with an asterisk equally contributed to the work.

P-04.1-123
Targeting *Mycobacterium tuberculosis* dUTPase to fight against the TB disease

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While the new coronavirus has turned our lives upside down causing millions of deaths, the historically known tuberculosis (TB) disease caused by *Mycobacterium tuberculosis* (MTB) was responsible for the loss of approximately 1.5 million lives alone in 2021. New anti-TB drugs are in an urgent need. A promising target is dUTPase, an enzyme preventing uracil incorporation into DNA. It is present in all multicellular species and in most microbes. Abolition of its activity potentially leads to DNA double strand breaks and cell death. Therefore, species-specific inhibition of MTB dUTPase may be a successful way of TB disease treatment. Currently no species-specific dUTPase inhibitor exists, but an interaction partner, protein Stl shows significantly different ability to inhibit dUTPase homologues from various species. We use Stl as a model to understand how species-specific differences in dUTPase structure may be harnessed in future inhibitor development. A remarkable species-specific characteristic of MTB dUTPase is a small surface sequence loop playing no direct role in enzyme activity but being essential for mycobacterial survival in a yet unknown way. What is the exact structural background of MTB dUTPase–Stl interaction? For this reason, we have crystallized a complex of MTB dUTPase and a truncated Stl protein mutant. And how the loop sequence may affect the MTB dUTPase protein structure on its own? For this answer, we obtained another X-ray diffraction dataset of a loop-lacking mutant of MTB dUTPase with 1.3 Å resolution. Surprisingly, electron density of the flexible C-terminal "arm" segment of the mutant dUTPase was missing from our dataset, contrary to the already crystallized wild-type MTB dUTPase structures. We postulate that the loop sequence may restrict conformational flexibility of the dUTPase "arm", making it more inhibitable by Stl compared to the loop-lacking mutant, as we know from our comparative steady-state enzyme activity inhibition measurements. *The authors marked with an asterisk equally contributed to the work.

P-04.1-124
New three-finger protein acting on nicotinic acetylcholine receptor from starfish

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Three-finger proteins form the Ly6/uPAR family exhibit the wide spreading across different organisms. Thus, snake neurotoxins represent the main component of a venom. For different members of the human Ly6/uPAR proteins the ability of regulation of cognitive processes, anti-proliferative activity, and participation in the number of regulatory pathways, such as the Wnt/beta-catenin signalling were shown. The main target of the Ly6/uPAR proteins in the most cases is nicotinic acetylcholine receptors (nAChRs). In this work, we, for the first time, obtained recombinant analogue of the three-finger protein from the starfish and characterized it. Using bioinformatics, we found the series of proteins containing the LU-domains in the two starfish genomes. One of them, named Ly17, demonstrated the high (57%) sequence homology with human neuromodulator Lynx2, for which the correlation with anxiety-related behavior was shown earlier. We developed the expression system for Ly17 in the *E. coli* inclusion bodies with subsequent renaturation. Electrophysiology studies on the *Xenopus* oocites showed the inhibitory effect of Ly17 on nAChRs. NMR study of ^{13}C,^{15}N-labelled Ly17 confirmed the three-finger organization of the protein. The study was supported by the Russian Science Foundation (Project № 19-74-20176).

P-04.1-125
Mechanisms of protein dysfunction in rare metabolic disorders – from protein misfolding to cellular impairment

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Inborn Errors of Metabolism (IEM) comprise a large class of inherited genetic diseases which affect genes coding for enzymes involved in different metabolic pathways. Although individually rare, they are collectively quite numerous and affect millions of people worldwide. The vast majority arise from single gene mutations that lead to impaired cellular metabolism, often as a consequence of defective protein folding. The era of genomics permit to identify an increased number of cases, though a clear correlation between genotype and phenotype has been difficult to establish. To contribute to a better molecular understanding of these disorders we have investigated disease-related variants combining biochemical, biophysical and structural methods to establish the effects of point mutations on protein folding, stability and function. Here we report our latest research on two IEM, Glutaric Aciduria-type I (GA-I) and Multiple Acyl-CoA Dehydrogenase Deficiency (MADD). GA-I is a neurometabolic disorder caused by deficiency of glutaryl-CoA dehydrogenase (GCDH). Resorting to studies on two disease variants (GCDH-p.Arg227Pro and -p.Val400Met) we established genotype/phenotype correlations showing that both variants retain the overall protein fold but different conformational stabilities [1]. Additionally, compromised enzymatic activity on both proteins depend on different features [1]. We have also provided a molecular rationale for the potential benefit of riboflavin supplementation in GA-I patients [2]. MADD results from deficiencies in the alpha or beta subunit of electron transfer flavoprotein (ETF), or in the ETF:ubiquinone oxidoreductase (ETF:QO), and is associated to impaired

mitochondria beta oxidation. Protein studies on ETF:QO variants provide a molecular rational for the decreased enzyme activity observed in patients [3, 4]. Currently, we are addressing mitochondrial dysfunction on MADD patient-derived fibroblasts with ETF:QO mutation. 1. Ribeiro et al (2020) BBA Prot Proteom 1868(1): 140269 2. Ribeiro et al. (2020) IJMS 21(19) 3. Lucas et al. (2020) BBA Prot Proteom 1868(6): 140393 4. Henriques et al. (2019) Curr Mol Med 19(7): 487

P-04.1-126
The flanking polyQ regions of Huntingtin exon 1 display distinct dynamic signatures in its membrane-bound state: insights into Huntington´s disease

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Huntington's disease (HD) is a fatal inherited neurodegenerative disorder caused by an abnormal expansion of the polyglutamine (polyQ) repeat within the first exon of huntingtin protein (httex1). The membrane interaction of httex1 is critical for its pathological self-assembly. While initial studies were mainly focused on synthetic polyQ peptide-lipid interaction, recent evidence supports that both flanking polyQ regions [the N17 segment and the proline-rich domain (PRD)] strongly modulate the httex1 aggregation at membrane surfaces. However, its structural characterization is inherently challenging due to the intrinsically disordered (IDP) features of httex1 and its high aggregation-prone propensity. Here, we initially employed fluorescence correlation spectroscopy (FCS) to probe the early steps of httex1-lipid interaction; and then acrylodan/ Atto 488 fluorescence to map the structural dynamics of both adjacent polyQ regions in the httex1 membrane-bound state. Fluorescence correlation spectroscopy (FCS) results reveal a favored partitioning of httex1 towards anionic lipid vesicles. In addition, both steady- and time-resolved fluorescence measurements of httex1 site-specifically labeled with acrylodan or Atto 488 at each segment adjacent to the polyQ domain indicate distinct local polarities and conformational features. Notably, the C-terminal PRD segment retains its conformational dynamics and remains highly solvent exposed even upon membrane binding. Instead, the N-terminal N17 segment adopts a less flexible state, as it directly anchors httex1 to the lipid membranes. Our work provides unique insight into the distinct roles of each flanking polyQ region in mediating httex1-lipid interaction and further illuminates the mechanism of httex1 aggregation at lipid membranes. Work supported by FCT-Portugal (PTDC/BIA-BFS/30959/2017 grant and CEECIND/00884/2017 contract to AMM; UIDB/04565/2020 and LA/P/0140/2020 funding to iBB and i4HB).

P-04.1-127
Zeroth Law of ribosomodynamics: *in vitro* translation assay

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Mutations in genes encoding rRNAs and ribosomal proteins result in ribosomopathies including Diamond-Blackfan Anemia, Schwachman-Diamond Syndrome, Dyskeratosis Congenita and Sarcopenia. The first approach for diagnosis is sequencing ribosomal protein genes. However 30% of the patients does not reveal any known genetic variation. A second approach is northern-blot analysis of rRNA and the third major approach is polysome profiling; a labor-intensive technique that require expertise. Here we aim to establish a zeroth approach to assess ribosome function through *in vitro* translation in suspected cases ahead of labor-intensive investigations. We pursuit time-course quantification of green fluorescent protein (eGFP) translation to assess ribosomal function. Cell lysates containing ribosomes, substrates and cofactors as test material are supplemented with a translation buffer (TB) containing all required factors for protein synthesis and *in vitro* transcribed eGFP mRNA is supplemented to achieve reproducible observations. Ribosomal function is further challenged using a translation elongation inhibitor anisomycin. HeLa cell lysates are employed as standardization material and eGFP expressing OP-9 cell lysates were used as positive control for translation. Supplementation of TB buffer to the OP-9 cell lysates reveal a dramatic increase in fluorescence signal intensity after 60 min. Addition of further eGFP mRNA doubled the fluorescence in 300 min. Initial fluorescence was detected at 180 min and a linear increment was observed till 300 min in HeLa cell lysates supplemented with TB and eGFP mRNA. Anisomicin treatment inhibited signal increment drastically in both lysates. Currently we are implementing this assay comparatively in healthy controls and patient samples as a screening step to identify ribosomopathies with respect to their translation capability before further genetic testing. This study was supported by TUBITAK,319S062 under the frame:EJP-RD JTC'19 RiboEurope.

P-04.1-128
Ixr1 transcriptional factor is an intrinsically disordered protein with prion-like properties

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Ixr1 is a transcriptional regulatory factor of *Saccharomyces cerevisiae* previously identified as a player in the response to several stress conditions, such as oxidative stress or hypoxia, as well as in the resistance to cisplatin treatment (Vizoso-Vázquez et al., 2018). Our lab has previously demonstrated that the amino-terminal part of this protein is involved in the transcriptional activation (Barreiro-Alonso et al., 2018). Nevertheless, little is known about the structure and mechanism of action of this protein. Here, we show that Ixr1 is an intrinsically disorder protein with high tendency to aggregate, displaying large disorganized regions flanking the HMG boxes which conform the DNA binding domains. Indeed, Ixr1 aggregation is highly ordered and the protein is able to form amyloids. Amyloid fibrils are one of the most frequent self-templating replicative states among the prions characterized until now. The relationship between Ixr1 function and its prion capabilities is also analyzed and discussed. - Barreiro-Alonso A, Lamas-Maceiras M, Cerdán EM, Vizoso-Vázquez Á. (2018) "The HMGB protein Ixr1 interacts with Ssn8 and Tdh3 involved in transcriptional regulation" FEMS Yeast Research 18(2). - Vizoso-Vázquez Á, Lamas-Maceiras M,

González-Siso MI, Cerdán ME. (2018) "Ixr1 Regulates Ribosomal Gene Transcription and Yeast Response to Cisplatin." Scientific Reports 8(1):3090.

P-04.1-129
Exploring novel enzymes from GH-115 family acting on decorated polymeric xylan

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Biorefinery and bioenergy sectors use plant cell wall as the main substrate. Therefore, the enzymatic degradation of plant cell wall becomes of great importance. Xylan is a major component of the plant cell wall, and the xylan present in hardwoods and grass families is frequently substituted with glucuronic acid units on the xylose backbone. In this study, two novel enzymes originating from *Belliella baltica* and *Flavobacteriaceace bacterium* from family GH115 were selected, expressed, purified, characterized, and tested on their capacity to debranch glucuronic acid from highly substituted polymeric xylan. The proteins' melting temperature was determined with differential scanning fluorimetry (DSF), and the particle size through dynamic light scattering (DLS) and their structure was further visualized with cryoEM.

P-04.1-130
The specific elongation factor to selenocysteine incorporation in *Escherichia coli*: unique tRNASec recognition and its interactions

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Several molecular mechanisms are involved in the genetic code interpretation during translation, as codon degeneration for the incorporation of rare amino acids. One mechanism that stands out is selenocysteine (Sec), which requires a specific biosynthesis and incorporation pathway. In Bacteria, the Sec biosynthesis pathway has unique features compared with the eukaryote pathway as Ser to Sec conversion mechanism is accomplished by a homodecameric enzyme (selenocysteine synthase, SelA) followed by the action of an elongation factor (SelB) responsible for delivering the mature Sec-tRNASec into the ribosome by the interaction with the Selenocysteine Insertion Sequence (SECIS). Besides this mechanism being already described, the sequential events for Sec-tRNASec and SECIS specific recognition remain unclear. In this study, we determined the order of events of the interactions between the proteins and RNAs involved in Sec incorporation. Dissociation constants between SelB and the native as well as unacylated-tRNASec variants demonstrated that the acceptor stem and variable arm are essential for SelB recognition. Moreover, our data support the sequence of molecular events where GTP-activated SelB strongly interacts with SelA.tRNASec. Subsequently, SelB.GTP.tRNASec recognizes the mRNA SECIS to deliver the tRNASec to the ribosome. SelB in complex with its specific RNAs were examined using Hydrogen/Deuterium exchange mapping that allowed the determination of the molecular envelopes and its secondary structural variations during the complex assembly. Our results demonstrate the ordering of events in Sec incorporation and contribute to the full comprehension of the tRNASec role in the Sec amino acid biosynthesis, as well as extending the knowledge of synthetic biology and the expansion of the genetic code

P-04.1-131
A peptide derived from *Lonomia obliqua* bristles is able to internalize into myoblast cells

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The crude bristle extract of *Lonomia obliqua* modulated the expression of inflammatory markers in human macrophages. In addition, a pro-coagulant protein isolated from this extract, Lopap, induced expression of adhesion molecules, and inhibited the apoptosis of endothelial cells. We found that Lopap and derived peptides modulated the expression of myogenic regulatory factors (MRF) and production of inflammatory mediators (PGE2, IL-6 and other myokines) involved in differentiation processes of myoblast cells. Since one of these Lopap-derived peptides, E8, induced modulation of some MRF and mediators, we wondered to know how this peptide interact with the myoblast cell. An internalization study of E8 was performed in C2C12 mouse myoblast, using high content screening (HCS) analysis and confocal microscopy. Peptide E8 FITC-conjugated was evaluated at 100 nM, 1 μM and 10 μM at several time periods, and cells were counterstained with fluorescein-phalloidin and DAPI. Using a designed mask to quantify total granules/well for each time and concentration by HCS, we found that peptide E8 internalized at 1 μM and 10 μM at both 3 and 6 h, and these results were confirmed by confocal microscopy. Additionally, a time-lapse study at low concentrations was performed using the confocal microscope and E8 was seen internalized at 1.2 μM, from 15 min to 4:45 h. Finally, we evaluated the C-terminal modified E8 peptide (L-Photo-leucine) at lower concentrations (150 nM, 600 nM and 1.2 μM), which after photo-activation allow a covalent crosslinking of the interacting proteins. The internalization and binding with cellular proteins was observed with streptavidin-based detection using confocal microscopy at 24 h. In summary, these findings allow us to conclude that peptide E8 internalizes the myoblast cells and interacts with intracellular proteins. Moreover, this internalization occurred at low concentration, similarly to the other biological effects previously found in these cells.

P-04.1-132
SCD5, the second human stearoyl-CoA desaturase isoform, in search of transcriptional regulation

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Elevation of free fatty acid (FA) levels is a key component in the development of severe diseases. The cellular stress caused by

saturated FA overload can be reduced by local desaturation. Thus, the stearoyl-CoA desaturase-1 (SCD1) enzyme is an important member in the cellular defense mechanism against lipotoxicity. The regulation of SCD1 is well characterized, but SCD5, the other human isoform has barely been studied yet. The present work aimed to assess the tissue-specific expression of the SCD5 promoter. Due to the marked central nervous system expression of the isoenzyme, the promoter activity of SCD5 constructs was planned to measure in neuronal cells. We also aimed to investigate the effect of two naturally occurring SNPs in the 5' region of SCD5 on gene expression. The promoter activity of SCD5 constructs were measured by luciferase assays in transiently transfected HEK293T, HepG2 and SK-N-FI cells. Promoter SNPs were generated by site-directed mutagenesis and characterized by luciferase reporter system. Putative transcription factor (TF) binding sites were analyzed using JASPAR online database. Consistent with our previous results, the 1 kb long 5′ region showed the highest promoter activity in SK-N-FI cell line. While 5-fold higher luciferase activity was measured in HepG2 cells and 10-fold higher in HEK293T cells, but the increment was 30-fold in neuronal cells compared to the control. Endogenous SCD5 mRNA levels in the three cell lines and in samples from human liver, kidney and brain tissues showed the same pattern as promoter activities of different strengths measured on cell lines. rs6841081 and rs3811792 SNPs reduced luciferase activity. Based on in silico prediction of transcription factor binding sites, rs3811792 may attenuate the binding of CEBPA on the promoter. Further research is needed to elucidate the mechanism of the observed cell type specificity of SCD5 promoter, as well as the potential contribution of SNPs in human diseases.

P-04.1-133
Antipsychotic clozapine binds catalase and preserves its activity in oxidative environment

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Oxidative stress undoubtedly accompanies mental disorders, and the pleiotropic effects of atypical antipsychotics, recommended drugs in the treatment of psychosis, are not clarified at the molecular level. Catalase is one of the key enzymes of the primary antioxidant protection system. This work studied the binding of second-generation antipsychotic drug Clozapine to commercial bovine liver catalase. Using various spectroscopic methods under simulated physiological conditions, we found moderate binding affinity of clozapine for catalase ($K_a \sim 2x10^5$ M^{-1}), the binding influenced the secondary and tertiary structure of protein (according to UV-VIS and CD spectroscopy) and it managed to slightly increase its thermal stability. In AAPH induced oxidation experiments, we found that clozapine efficiently protects catalase from free-radicals oxidation and preserves its activity. Clozapine affects catalase activity in dose dependant manner, having no significant effect at lower concentrations but significantly inhibiting enzyme at saturating concentrations. In conclusion, our results indicate that the effect of direct binding of clozapine to catalase can be both beneficial and harmful and that this effect is dose dependent.

P-04.1-134
The endoplasmic reticulum (ER)- associated degradation system regulates the intracellular level of amyloid precursor protein (APP)

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Alzheimer's disease (AD) is one of the most common dementia and neurodegenerative diseases manifested by behavioral disorders and loss of short-term memory. The pathophysiology of AD is characterized by the formation of brain senile plaques from amyloid-β peptide. The accumulation of plaques destroys brain cells and blocks cell-to-cell signaling at synapses. Neurotoxic amyloid-β is formed by sequential cleavage of the amyloid precursor protein (APP) by β-secretase and γ-secretase. APP is a transmembrane glycoprotein which serves a variety of functions related to cell adhesion and migration. Like all plasma membrane proteins, APP is processed in the endoplasmic reticulum (ER) and in the Golgi complex, before being transported to the cell surface. Aberrant processing of APP in the ER may result in overproduction of amyloidogenic products. The endoplasmic reticulum (ER)- associated degradation (ERAD) proteins, such as EDEMs and Derlin-3, support transport of proteins from the ER to the cytosol for degradation by the 26S proteasome. Our research focus on studies of the intracellular level of amyloid precursor protein (APP) dependent on ERAD proteins. The results strongly suggest that the level of APP can be regulated by Derlin-3 and EDEM proteins.

P-04.1-135
Peroxidases in green chemistry

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Peroxidases are a widespread group of enzymes belonging to a few families which can be found in plants, animals and microorganisms. They have the ability to catalyze reactions in which various organic or inorganic substances are oxidized using a hydrogen peroxide (H_2O_2) as a substrate. In addition, the family of catalases-peroxidases possesses the ability to decompose hydrogen peroxide to water and oxygen. They often provide protection against reactive oxygen species (ROS) and are therefore an important part of the antioxidant defence system. In our work we focused on production different peroxidases, namely thermostable catalase AfKatG, dye-decolorizing DypB and C-domain of MagKatG2, in *E. coli*. We mutated these enzymes on selected sites and studied effects on activities and/ or stabilities (chemical or thermal). We also observed different effects of ions on activity of some enzymes. We tried several compounds as substrates. All of these studies are parts of a project which focuses on biotransformation, mainly use of different enzymes in such reactions, to prepare interesting substances, e. g. aromas as vanillin.

P-04.1-136
The development of a novel SARS-CoV-2 virus neutralizing immunotoxin

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For decades, scientific efforts were focused on the improvement of the effectiveness of the therapeutic antibodies, mainly in order reduce the dosage and thus lower the side-effects and costs. P4A1, a potent SARS-CoV-2 virus neutralizing antibody was already engineered to contain Fc fragment mutations, that dramatically increased the blood circulation time. In this work, we aimed to further enhance this neutralizing antibody efficacy by creating a next-generation virus neutralizing agent based on the P4A1 and conjugated with a highly processive *Bacillus amyloliquefaciens* RNase (barnase). Barnase itself is known to act as a mild toxin that drives the cells to apoptosis, and we propose that its RNase activity may enhance the protective effect through the hydrolysis of viral RNA in infected cells, and thereby additionally preventing pathogen replication. The main challenge in the assembly of such molecule is the intrinsic barnase toxicity in mammalian cells, what precludes the possibility to express it as a fusion protein. Further, we had shown that barnase, being a small (12.5 kDa) protein, contains very few surface reactive moieties that are available for conventional chemical crosslinking strategies. Therefore, the antibody-barnase fusion protein was obtained by enzymatic conjugation via the sortase A enzyme. The reaction conditions for bacterially expressed barnase and HEK293 derived P4A1 modified to contain heavy chain C-terminal sortase motif were thoroughly optimized and the reaction yield approached 80%. The immunotoxin RBD binding EC_{50} was not found to differ from the unconjugated P4A1 antibody and barnase activity was found to be 33% of the one for unmodified enzyme. Thus, we obtained the promising immunotoxin with a good yield, which had retained its RNase activity for the further *in vitro* virus neutralization studies. The work was supported by Project of Ministry of Science and Higher Education № 075-15-2021-1049.

P-04.1-137
SARS-CoV-2 Mpro inhibition by zinc ion: structural features and hints for drug design

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SARS-CoV-2 main protease (SARS-CoV-2 M^{pro}) is a cysteine protease that hydrolyses the viral polyproteins at several sites with a preference for the Leu-Gln(Ser, Ala, Gly) sequences[1]. The enzyme represents one of the main drug-target candidates for covid-19 syndrome because the large and deep pocket at the active site and its crucial activity for viral replication[2-5] Here, we provide X-ray structural data on SARS-CoV-2 M^{pro} in complex with the isolated Zn^{2+} ion. The comparison with the apo SARS-CoV-2 M^{pro} shows that residues involved in zinc binding are not affected by significant structural rearrangement upon zinc binding supporting the idea that the binding site is ready to accommodate the metal. The interaction of SARS-CoV-2 M^{pro} with Zn^{2+} ion was also investigated by NMR. Moreover, zinc binding is able to inhibit protein activity, demonstrating that the zinc ion is capable of an efficient binding also in solution. These findings provide a solid ground for designing potent and selective inhibitors of SARS-CoV-2 M^{pro} suggesting that a zinc ion incorporated into suitable ligands interacting with additional sites at the protein surface can modulate the binding energy. [1] Rut W et al. (2021) Nat Chem Biol 17, 222-228. [2] Anand K et al. (2003) Science 300, 1763-1767. [3] Zhang L et al. (2020) Science 368, 409-412. [4] Jin Z et al. (2020) Nature 582, 289-293. [5] Douangamath A et al. (2020) Nat Commun 11, 5047.

P-04.1-138
Design of biologicals for blood–brain barrier crossing

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The frequency of brain diseases has increased significantly in the past years. After diagnosis, therapeutic options are usually limited, which demands the development of innovative therapeutic strategies. The use of biologicals is promising but highly limited by the existence of the blood-brain barrier (BBB) [1]. To overcome the impermeability of this barrier, antibody fragments, peptides and nanoparticles can be engineered to contain a BBB peptide shuttle (BBBpS), capable of brain penetration. PepH3, a seven-residue sequence derived from the α-helical domain of the dengue virus type-2 capsid protein, and its enantiomer D-PepH3, efficiently translocate across the BBB using a receptor-free mechanism [2]. In addition, these BBBpS presents very low toxicity and high stability (D-PepH3). Here, we linked PepH3, to the IgG fragment crystallizable (Fc) domain using the streamlined expressed protein ligation (SEPL) method. With this strategy, we obtained an Fc-PepH3 scaffold that can carry different payloads. Fc-PepH3 was shown to be non-toxic to brain endothelial cells and red blood cells (RBCs). Fc-PepH3 is capable of crossing an in vitro cellular BBB model, with a percentage of translocation 2.4-fold higher than unconjugated Fc. In addition, Fc-PepH3 binds to neonatal Fc receptor (FcRn), which is responsible for antibodies' long half-life (t1/2) at pH 6.0. The determined binding affinity was 95.4 ± 9.21 nM for Fc-PepH3, which is comparable to unconjugated Fc (89.6 ± 8.78 nM). Overall, we demonstrated the potential of Fc-PepH3 as a versatile platform readily adaptable to diverse drugs to treat different brain conditions. 1. Cavaco, M., et al. Peptibodies: An elegant solution for a long-standing problem. Peptide Science 2018, 110 (1), e23095. 2. Cavaco, M., et al. DPepH3, an Improved Peptide Shuttle for Receptor-independent Transport Across the Blood-Brain Barrier. Current Pharmaceutical Design 2020, 26, 1-12.

P-04.1-139
Optimization of renaturation method of the IL7-His and its characterization

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Investigations of interleukin-7 (IL-7) and IL-7 receptors have shown that their levels vary in different diseases such as viral infections (HIV, CMV, HCV), multiple sclerosis, Type 1 diabetes, and others. IL-7R concentration monitoring appears to play a significant prognostic value and requires highly purified IL-7. The work aimed to obtain rhIL-7 in *Escherichia coli* and allow protein renaturation with further testing of peripheral blood mononuclear cells (PBMCs) responsiveness to highly purified cytokine. The DNA sequence encoding human IL-7 was subcloned into a *pET24a(+)* expression vector under control of the T7 promoter and upstream of the vector-derived 6xHis-tag. *pET24-hIL7* was transformed into *E. coli*, protein synthesis was induced with the auto-induction protocol. Immobilized metal affinity chromatography was used for the purification of solubilized protein. Subsequent refolding protocols of rhIL7-His were developed using dilution, gel filtration, on-column refolding, and dialysis strategies. The highest renaturation efficiency of rhIL7-His was obtained in the dialysis method. Gel filtration and refolding on metal affinity sorbent showed comparable lower efficiencies. The activity of rhIL7-His renatured by dialysis and gel filtration was tested on PBMCs. The response of phytohemagglutinin activated lymphocytes of human peripheral blood was measured in the MTT test. Analysis shows the comparable activity of standard rhIL7 and rhIL7-His renatured by dialysis. It can be concluded that after dialysis the rhIL7-His protein is obtained in a fully functional form. *The authors marked with an asterisk equally contributed to the work.

P-04.1-140
Together we fuse: parainfluenza virus fusion peptides oligomerize to promote membrane fusion

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Human parainfluenza viruses are responsible for a significant proportion of respiratory infections, particularly in children. The entry process of these viruses into the host cell starts with the recognition and attachment to target receptors, followed by proteolytic cleavage of the fusion glycoprotein (F) protein, exposing the fusion peptide (FP) region. The FP is responsible for binding to the target membrane, and it is believed to play a crucial role in the fusion process, but the mechanism by which the parainfluenza FP (PIFP) promotes membrane fusion is still unclear. To elucidate this matter, we performed biophysical experimentation of the PIFP in membranes, together with coarse grain (CG) and atomistic (AA) molecular dynamics (MD) simulations. The simulation results led to the pinpointing of the most important PIFP amino acid residues for membrane fusion and show that, at high concentrations, the peptide induces the formation of a water-permeable pore-like structure. This structure promotes lipid head intrusion and lipid tail protrusion, which facilitates membrane fusion. Biophysical experimental results validate these findings, showing that, depending on the peptide/lipid ratio, the PIFP can promote fusion and/or membrane leakage. Our work furthers the understanding of PIFP-induced membrane fusion process, which might help foster development in the field of viral entry inhibition.

P-04.1-141
Cooperativity in protein–protein interactions

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Protein–Protein Interactions (PPIs) lie at the heart of cellular signalling, but are virtually unexplored from a drug discovery perspective. Especially small molecule stabilization of PPIs (molecular glues) offers enormous opportunities for providing insight in the molecular regulation mechanisms of PPIs and for drug development. Our group uses chemical biology to unravel the underlying, complex, molecular interaction mechanisms of PPIs, with the overall aim to enable innovative medicinal chemistry to stabilize PPIs and synthetic biology approaches for bottom-up construction of synthetic protein systems. Especially fundamental concepts such as cooperativity and multivalency drive our chemical biology to develop selective small molecules as probes, drug starting points, and multi-component synthetic protein assemblies. Using nuclear receptors and 14-3-3 proteins as examples this presentation aims to provide insights into questions such as 1) How can chemical biology of PPIs steer medicinal chemistry? 2) What are the key biophysical characteristics of molecules that stabilize PPIs? and 3) How can we use these insights to assembly synthetic PPI complexes.

P-04.1 142
Production of the Aspergillus giganteus keratinase using *E. coli* BL21 expression system

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Enzymes with keratinolytic activity are in demand in various fields, including biomedicine and agriculture. Dozens of articles are published about new keratinase producers and optimization of the known target proteases obtaining every year. Thus, a keratinase, secreted by *Aspergillus giganteus* A16, was expressed in *E. coli* BL21 (DE3) strain to study the possibility of using a bacterial host to produce fungal keratinase. To amplify the end fragments of cDNA of the target enzyme, we used the Step-out-RACE method with randomized PCR primers to conservative regions of *Aspergillus* micromycete subtilisin proteases utilising previously obtained *A. giganteus* A16 cDNA library as a template. Based on the results of the amplified terminal cDNA fragments sequencing, gene-specific primers were designed for subsequent amplification of the full-length cDNA sequence of the target keratinase. The bioinformatic study of the obtained

sequences showed that *A. giganteus* A16 keratinase is belonged to the proteinaseK-like subtilisin proteases, and consists of signal sequence, propeptide and protease domains. A cDNA fragment encoding target enzyme without signal sequence was cloned into the pET23a vector to obtain an expression plasmid, which was subsequently transformed into *E. coli* BL21 (DE3) host strain. The crude lysate of resulting biomass after expression was applied to the Toyopearl Sulfate 650F for purification. The obtained keratinase recombinant form has a high specific activity, as well as the same properties as the native enzyme. Both forms of the protease have the same molecular weight, isoelectric point, pH- and temperature optimum of activity (pH 8 and 30-37°C, respectively), which indicates the prospects for obtaining of fungal keratinases in *E. coli* BL21 expression system. This research was supported by the Russian Science Foundation (grant #20-16-00085). Author contributions: investigation – E. Popova, S. Komarevtsev, S. Timorshina; conceptualization – A. Osmolovskiy.

Lipids

P-04.2-001
A new kid in the Bloch? The non-catalytic protein ERG28 plays a functional role in mammalian cholesterol synthesis and is co-regulated transcriptionally

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The enzymatic pathway of cholesterol biosynthesis has been well characterized. However, there remain a number of potential interacting proteins that may play ancillary roles in the regulation of cholesterol production. Here we identified ERG28 (C14orf1), a homologue of the yeast protein Erg28p, as a player in mammalian cholesterol synthesis. ERG28 is conserved from yeast to humans and while overexpressed in some cancers, the function of this protein has been largely overlooked in mammals. Using qRT-PCR, luciferase assays, and publicly available ChIP-seq data, we found that transcription of this gene is driven by the transcription factor SREBP-2, akin to other cholesterol synthesis enzymes, and also identified sterol responsive elements and co-factor binding sites in its proximal promoter. Generation of Huh7 ERG28-knockout cell lines revealed that knockout cells exhibit reduced total cholesterol levels in sterol-depleted environments. Additionally, radiolabelled metabolic flux assays showed up to a 75% reduction in the rate of cholesterol synthesis in the knockout versus wild-type cells, which could be rescued by expression of ectopic ERG28. These results indicate that ERG28 is clearly involved in cholesterol synthesis, although the precise role this non-catalytic protein plays in this complex metabolic pathway remains to be fully elucidated. A deeper understanding of ERG28, and other ancillary proteins of cholesterol synthesis, may help inform therapeutic strategies for diseases associated with aberrant cholesterol metabolism, including a variety of cancers.

P-04.2-002
Tunicamycin induced sphingolipid and polyunsaturated fatty acid changes in human kidney epithelial cells

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This study aimed to determine tunicamycin (TM)-related sphingolipid and polyunsaturated fatty acid (PUFA) changes in human kidney cells (HEK-293) and their effects on inflammatory and apoptotic pathways. Tunicamycin was employed to induce endoplasmic reticulum (ER) stress in HEK-293 cells and an ER stress inhibitor, tauroursodeoxycholic acid (TUDCA), was given to minimize cytotoxicity. Cell viability was determined by MTT assay. Sphingomyelin (SM), ceramide (CER), and PUFA levels were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Glucose-regulated protein 78-kd (GRP78), cleaved caspase-3, and cyclooxygenase-1 (COX-1) protein levels were assessed by immunofluorescence. Cytosolic phospholipase A2 (cPLA2), total COX, and prostaglandin E2 (PGE2)

were measured to evaluate changes in enzyme activity. Decreased cell viability was observed in TM-treated cells. Administration of TUDCA following TM treatment significantly increased cell viability compared to TM treatment alone. Tunicamycin-induced ER stress was confirmed by significantly increased protein levels of GRP78. A significant increase was observed in C18-C24 CERs and caspase-3 activity, while a significant decrease occurred in sphingosine-1-phosphate (S1P) and cPLA2 activity in cells treated with μg/ml versus controls. The decrease in cPLA2 activity was accompanied by significantly increased PUFA levels in TM-treated cells. Tauroursodeoxycholic acid treatment in conjunction with TM significantly decreased ER stress, C18-C24 CERs, caspase 3 activity, and increased S1P levels. The results show the build-up of long-chain CERs and PUFAs in HEK-293 cells undergoing ER stress alongside increased apoptotic activity. Tauroursodeoxycholic acid administration, along with TM treatment alleviated the build-up of CERs and TM-induced apoptotic activity in kidney epithelial cells. Keywords: Kidney, Tunicamycin, Sphingolipid, Ceramide, PUFA

P-04.2-003
The change of line tension of the pore edge in bilayer lipid membranes in the presence of anionic surfactants

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The formation of transient pores in lipid bilayer membranes (BLM) occurs in a number of cellular processes. In addition, pore formation in membrane is used in various biotechnological and biomedical applications. In the classical theory pore formation considered as spontaneous creation and diffusion of membrane defects such as lipid pores in the space of radii. The main parameter that determines the stability of the BLM is the average lifetime - the time from the moment of formation of the BLM to its rupture. The average lifetime of a BLM depends on a number of reasons, but the main one is the line tension of the pore edge. Analysis and calculations show that the presence of surfactant molecules with positive spontaneous curvature in the solution surrounding BLM leads to a dramatic decrease in the average lifetime of the BLM. This is due to the fact that surfactants from the surrounding solution of BLM are adsorbed and embedded in the BLM structure, reducing the linear tension of the pore edge, thus facilitating the formation of pores on the BLM. Anionic surfactants with different head groups (dodecylbenzenesulfonic acid, sodium dodecyl sulfate, sodium dodecanoate) and the same hydrocarbon chains were studied. These molecules have different values of the packing parameter. Taking into account the known analytical relationship between the packing parameter and the value of the spontaneous curvature of a monolayer, it is possible to determine the value of the spontaneous curvature and use it to analyze the experimental data on the dependence of the BLM stability on the structural features of surfactant molecules. With an increase in the size of the head group of surfactants, the value of spontaneous curvature increases, which leads to a decrease in the average lifetime of BLM. The surfactants mentioned above are the most commonly used ingredients for the production of household detergents and are of great interest for use in targeted drug delivery systems. *The authors marked with an asterisk equally contributed to the work.

P-04.2-004
Deciphering the lipid composition of the human nuclear envelope during healthy aging

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Aging is a major risk factor for many pathologies. At a time when we are living longer than ever before, untangling the mechanisms of this biological deterioration is absolutely critical if we aim at providing a better quality of life for aging individuals. The loss of nuclear integrity, and the consequent defects in nuclear mechanics, are already among widely considered hallmarks of aging. In mammalian cells, the structural properties of the nucleus are maintained by the nuclear lamina and its interaction with the nuclear envelope (NE). The NE is mainly composed of phosphatidylcholine and phosphatidylethanolamine phospholipids, together with a series of other minor components. However, the link between variations of the NE lipid content and altered biological functions within the nucleus is only now starting to be explored. Here, we start to elucidate the role of specific lipid classes in maintaining the proper nuclear integrity during healthy aging. We isolated nuclei from human primary fibroblasts prepared from healthy individuals of young and old age, and Hutchinson-Gilford Progeria Syndrome (HGPS) patients. Nuclei isolation was confirmed through atomic force microscopy, confocal microscopy, histology and transmission electron microscopy. Upon lipid extraction, lipidomics analysis through liquid (and gas) chromatography coupled with mass spectrometry was carried out. Our results show significant differences in NE lipid composition as age progresses. Interestingly, there seems to be a general decrease in ether lipids in aged individuals. Ether lipids, especially plasmalogens, are known antioxidants and their decrease can be directly linked to the loss of nuclear integrity/function. Moreover, other lipid classes such as sphingolipids appear to be altered, suggesting that aged cells might contain nuclei with significantly different bulk biophysical properties that could affect many biological processes, from diffusion to mechanics of the overall nuclear lamina.

P-04.2-005
How the absence of just a few water molecules affects the structure and dynamics of cell membranes

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Self-assembly of biomembranes results from the intricate interactions between water and the lipids' hydrophilic head groups. The lipid–water interplay strongly contributes to modulating membrane architecture, lipid diffusion, and chemical activity. We have recently developed a methodology that allows for precise control of the hydration of biomimetic cell membranes. Consequently we can now routinely study the structure and dynamics of supported lipid bilayers (SLBs) over a wide range of hydration states. Our recent experiments revealed that biomimetic membranes can survive even in very harsh dehydration conditions, which so far was thought to be impossible and that membranes in such conditions require chemical and mechanical modifications to maintain their properties. We learned that the lipid domain structure of phase-separated SLBs is largely insensitive to the

changes in the hydration layer. In stark contrast, lipid mobility is drastically affected by dehydration, showing a 6-fold decrease in lateral diffusion. At the same time, the diffusion activation energy increases approximately 2-fold for the dehydrated membrane. The obtained results, correlated with the hydration structure of a lipid molecule, revealed that about six to seven water molecules directly hydrating the phosphocholine moiety play a pivotal role in modulating lipid diffusion. Most importantly, the above-described experiments revealed many previously unseen effects that we are currently exploring. Amongst others these include hydration dependent migration of cholesterol between different lipid phases; hydration induced changes to the line tension and the shape of lipid domains; and asymmetric response of SLB leaflets to changing hydration conditions. These findings provide deeper insights into the fundamental cellular processes that involve local and transient dehydration, for instance during cell–cell fusion, and help us to better understand the survivability of anhydrobiotic organisms.

P-04.2-006
Hydrophobic free energy for linear alkylic primary acids, alcohols and amines, based on experimental values of Henry's law constants

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The solubility of alkylic molecules in water is unavoidable in biochemistry, as well as in areas such as environmental conservation, detergency, pharmaceutics and biomedicine. Yet, the comprehension of hydrophobic hydration is still a matter of debate from its thermodynamic grounds to protein stability and ligand biding [1]. In 1891, Traube's rule launched a qualitative perception for the solubility of aliphatic acids, alcohols and amines based on the length of methylenic chains. This work intends to quantitatively verify Traube´s rule through the definition of the hydrophobic free energy (HFE) as a simple transfer process of solutes from the gas phase to water [2], based on experimental values of Henry´s law constants for small linear alkylic primary acids, alcohols and amines (less than 10 carbons) [3]. Also, corresponding hydrocarbons are used as standards to assess the differences in HFE between apolar and amphiphilic molecules. This approach presents a twofold advantage of excluding other processes (thermodynamic cycles) from the unitary transfer and of upmost importance, when treating the initial apolar phase as ideal gas, allows to obtain the experimental values of the dimensionless Henry constants. These values, for each class of molecules, were obtained from tabulated track [3] and only experimental and consistent sources were considered. Next, the values of HFE were computed for all molecules, and plotted against the number of methylene groups. For hydrocarbons, the plot from propane to decane, is linear (R = 0.993) and provides an intercept value of 7.1 kJ/mol, which compares with 7.56 kJ/mol for ethane. For the amphiphiles the outcomes are in accordance with their respective ionic or polar features. This study received Portuguese national funds from FCT-Foundation for Science and Technology through project UIDB/04326/2020. [1] Silverstein TP (2020) ChemTexts 6, 26 [2] Baldwin RL (2013) FEBS Lett 587, 1062-1066 [3] Sander R (2015) Atmos Chem Phys 15, 4399-4981

P-04.2-007
Direct effect of biomimetic cell membrane hydration on Laurdan fluorescence

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Water hydrating biological membranes are essential for maintaining cell biochemical activity. Studies of the membrane hydration properties particularly benefited from the results obtained using environment-sensitive fluorescent probes, such as Laurdan, whose maximum fluorescence wavelength is extremely sensitive to the polarity of its local molecular environment. In this regard, changes in the polarity of the probe's immediate vicinity, produced by any stimulus (e.g. migration of cholesterol, viral entry), were ascribed to alterations in hydration level at the Laurdan location. *Ironically, no direct measure of the influence of the hydration state of lipid membrane on Laurdan spectra has been available*. To address this, *we investigated fluorescence spectra of Laurdan embedded in planar supported lipid bilayers for the first time as a function of their hydration*. We varied the membrane hydration by applying a controlled drying process with a slow and sequential reduction in relative humidity of the membrane environment. We conducted the study in both single component and phase-separated membranes. Additionally, we evaluated the effect of cholesterol, which induces the polarity decrease in fluid membranes. The measurements revealed a membrane phase-dependent change in the Laurdan's fluorescence spectrum as a function of hydration. The observed spectral changes are fully reversible upon membrane rehydration. We provide evidence that Laurdan can be a reliable indicator of water level and hydration state of individual lipids in the liquid disordered membranes. Furthermore, preliminary results indicate that cholesterol simply dehydrates lipids by displacing the water molecules from in between the lipid headgroups. The provided data is of great importance for the proper interpretation of data obtained with Laurdan, routinely used by scientists worldwide in cell membrane research.

P-04.2-008
Engineering cell membranes – the effect of pH on the formation, structure, and mobility of biomimetic cell membranes

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The lateral segregation and compartmentalization of lipids within cell membranes are strongly related to many biological processes occurring in the body such as protein sorting, ion-channel regulation, signalling, organization of the cytoskeleton, and pathogen entry. Moreover, lipid domains composed of ordered phase are associated with the binding of toxins and their penetration inside the cell. The importance of the lipid domain formation and activity triggered studies on the active manipulation of their structural properties. Here, we present systematic study on the formation, dynamics, and stability of the phase-separated, supported lipid bilayers (SLBs) under varying pH conditions. The used composition of phosphatidylcholine (14:1 PC), cholesterol and sphingomyelin (SM) leads to the formation of liquid-ordered (L_o) and liquid-disordered (L_d) phases. The effect of pH on the formation,

structure and dynamics of biomimetic membranes was investigated in a wide range of pH values: 1.7–9.0, with the use of fluorescence confocal microscopy and fluorescence recovery after photobleaching (FRAP) techniques, respectively. We discovered that lipid domain size is very sensitive to the pH conditions and increases significantly with increasing pH this allows for modulating and inducing the formation of L_o domains of a specific size solely by varying pH of the environment, without any external modification of the solid support or altering of membrane composition. Interestingly, the diffusion coefficient of lipids in both L_o and L_d phase remains unaltered at different pH conditions. Our studies demonstrate the possibility of active manipulation of the lateral organization of lipid membranes and enables further studies focused on mutual interactions between proteins and other molecules binding specifically to lipid domains in membranes subjected to varying pH conditions. Previously published in Krok E, Batura A. et al. (2022) J Mol Liq 345, 117907. *The authors marked with an asterisk equally contributed to the work.

P-04.2-009
Sodium ions support lipid mobility in dehydrated biomembranes

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Cellular membranes are surrounded by an aqueous buffer solution containing various ions, which interact with the hydration layer of the lipid head groups. At the same time, water molecules hydrating the lipids play a major role in facilitating lipid diffusion. Based on fluorescence microscopy imaging and fluorescence recovery after photobleaching (FRAP) measurements, we demonstrate that the presence of sodium ions is essential to sustain PC lipid mobility after the removal of the direct hydration layer of lipid membrane. Firstly, our study reveals that for fully hydrated phase-separated supported lipid bilayers (SLBs) an increase of Na^+ concentration leads to stronger lipid phase separation that increases liquid ordered domain size, but has little effect on lipids' mobility. A detailed picture of the effect of Na^+ ions on lipid mobility emerges when structure and mobility is investigated at lower membrane hydration conditions. After removal of SLB outer hydration layer, lipids showed nearly the same mobility as for fully hydrated SLB, but only when prepared in HEPES buffer solution containing NaCl between 50 mM to 1.5 M concentration range. In contrary, at similar hydration conditions, lipid diffusion was observed to nearly cease in absence of Na+ ions. Systematic studies of lipids mobility as a function of salt concentration and hydration state of the membrane revealed that upon membrane dehydration Na^+ ions strongly bound to the phosphate oxygen by ionic bonds help to retain the water clathrate cage around the phosphocholine group and facilitate the lateral mobility of lipids. Many biochemical processes, such as cells fusion or adsorption of macromolecules, involve both: local and transient membrane dehydration as well as local variation of Na^+ ion concentration. Our study provides new insights into the interplay between the ionic composition of membrane local environment and the hydration properties of the membrane.

P-04.2-010
Altered anti-inflammatory resolvin E1 in an *in vivo* model of age-related macular degeneration

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Resolvin E1 (RvE1) is an eicosapentaenoic acid-derived pro-resolving lipid mediator, involved in resolution of inflammation. Its alterations are documented in several chronic inflammatory diseases. Here, we investigated for the first time whether RvE1 metabolism and signaling may be involved in age-related macular degeneration (AMD), a widespread retinal degenerative disease. We used an *in vivo* model of AMD, in which light damage (LD) induces photoreceptors and retinal pigment epithelial cells dysfunction and degeneration, impairment of electroretinography (reduction of a-,b- waves and oscillatory potentials), and gliosis (microglia and Müller cells reactivity) (Previously published in: Tisi et al. (2019) Exp Eye Res 188, 107797; Tisi et al. (2020) Cells 9, 1617). Upon assessment of physiological retinal function, through electroretinography, Sprague Dawley albino rats (n = 6/group) were dark-adapted for 12 h and then exposed to 1000 lux white light for 24 h. A group of animals was euthanized immediately after LD; another one was returned to standard dim cyclic light conditions (5 lux) and euthanized seven days after LD. Eyes were enucleated and eye cup samples collected for subsequent analyses. The main RvE1 receptor (ChemR23) and metabolic enzymes (5-LOX, COX-2), and major pro-inflammatory cytokines (IL-4 and IL-1β) were analyzed through western blot on retinal lysates. ChemR23 was significantly up-regulated seven days after LD compared to unexposed animals (control) ($P = 0.035$) and to animals euthanized immediately after LD ($P = 0.011$); at the same time point, IL-4 was significantly up-regulated compared to the control ($P = 0.025$). No differences were found for IL-1 β and the metabolic enzymes (5-LOX, COX-2) analyzed for each time point. Altogether, our data indicate that RvE1 alterations are implicated in AMD pathogenesis and may be a target for the development of new therapeutic strategies, possibly promoting resolution of inflammation in AMD patients.

P-04.2-011
CFTR modulators and ganglioside GM1: a new combination for the treatment of cystic fibrosis

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Cystic fibrosis (CF) is a severe inherited disorder caused by loss of function mutations in the gene encoding for the Cystic Fibrosis Transmembrane conductance Regulator (CFTR). CFTR is a

chloride/bicarbonate channel expressed at the apical surface of epithelial cells. In particular, it is localized in specific areas of the plasma membrane (PM) where it is stabilized by the interaction with the ganglioside GM1, which is strongly reduced in CF bronchial epithelial cells. The new therapeutic approaches are based on the use of correctors that rescue the biosynthesis, as well as potentiators that enhance the function of the mutated protein. Unfortunately, the chronic treatment with the most effective potentiator (VX-770) determines a PM instability of the rescued mutated-CFTR. VX-770 is present also in the new formulation Trikafta® but importantly, we verified that in this case the side effect is strongly reduced. Considering the important role of the GM1 in the stabilization of the channel, we have focused our attention on the effect of the Trikafta® on the lipid composition of bronchial epithelial cells expressing CFTR with the most common mutation F508del. We observed an increase in the GM1 and GD1a levels even if their content is less to that observed in healthy bronchial epithelial cells. Moreover, we explore the possibility to rescue the level of the GM1 by its exogenous administration in order to further promote the pharmacological activity of the Trikafta®. Interestingly, exogenous administration of GM1, and of its pharmacological analogues LIGA20, seems to ameliorate the efficacy of Trikafta® on the rescue of F508del-CFTR expression at the cell PM level. Taken together, our data indicate that GM1 is important for the stability of the CFTR at the PM, and even if the new formulation partially compensates the GM1 reduction, acting on this ganglioside could be considered an adjuvant approach to increase the efficacy of the CFTR modulators.

Saccharides

P-04.3-001
A deeper dive into MUC1 O-glycosylation: the case of C1GalT1 and ST6GalNAc-I

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Mucin type (GalNAc-type) *O*-glycosylation is a complex mechanism regulated by the coordinated activity of specific glycosyltransferases (GTs). Misregulation of GTs is a common feature of cancer that yields tumor-associated carbohydrate antigens (TACAs). Two of the most common TACAs are the TF-(Galβ1-3GalNAcα1-*O*-Ser/Thr) and STn (Neu5Acα2-6GalNAcα1-*O*-Ser/Thr) antigens (Pinho SS, Reis C (2015) Nat Ver Cancer 15, 540-555). The TF-antigen is naturally formed by the action of the inverting C1GalT1 enzyme that catalyzes the transference of a Gal residue from UDP-α-Gal sugar donor to GalNAc-mucin peptides. However, this antigen is overexpressed in cancer (González-Ramírez A, Grosso AS et al (2022) Nature Comm (accepted)). The STn antigen is generated by the action of the inverting ST6GalNAc-I enzyme, which adds a Neu5Ac residue from the CMP-β-Neu5Ac sugar donor to GalNAc-mucin peptides and is only found in cancer cells (Marcos NT et al (2004) Cancer Res 64, 7050–7057). Understanding the molecular mechanism of glycosylation and recognition of TACAs by these enzymes is of extreme importance to develop strategies targeting C1GalT1 and ST6GalNAc-I. On this basis, here we report new insights on the mechanism of mucin-1 *O*-glycosylation by C1GalT1 and ST6GalNAc-I, through the concerted application of advanced NMR methodologies and molecular modelling techniques. Acknowledgements: Authors thank the FCT-Portugal for funding: GLYCANTIGENS project PTDC/BIA-MIB/31028/2017; UCIBIO project UIDB/04378/2020 and UIDB/04378/2020, i4HB project LA/P/0140/2020 and the Infrastructure project 22161. ASG thanks to FCT-Portugal for the PhD grant SFRH/BD/140394/2018 and FM and HC thank the CEEC contracts 2020.00233.CEECIND and 2020.03261.CEECIND, respectively.

P-04.3-002
Structural characterization of infertility-associated seminal plasma N-glycans

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Seminal plasma is a rich source of proteins, including glycoproteins that could serve as additional fertility biomarkers. Therefore, this study aims to characterize and determine the association of total protein seminal plasma N-glycans in men with male infertility. The study included 82 normozoospermic men and 84 men with pathological sperm parameters (count, motility, morphology, and vitality) separated into asthenozoospermic (A, N = 29), oligozoospermic (O, N = 21), and oligoasthenozoospermic (OA, N = 34) sub-groups. Basic sperm parameters were determined by computer-aided sperm analysis (CASA). Seminal plasma was then separated from the cellular components by centrifugation, while N-glycans were isolated from proteins and fluorescently labeled. Glycans were detected and measured by ultra-performance liquid chromatography based on hydrophilic interactions. The structures of 5 statistically significant N-glycans between the normozoospermic men and infertile sub-groups were determined by liquid chromatography-mass spectrometry. The total protein seminal plasma N-glycans separated into 37 individual peaks (SPGP1 – SPGP37). Statistically significant N-glycans between at least one pathological sub-group and the normozoospermic group were SPGP5, SPGP14, SPGP17, SPGP26, and SPGP32. However, after Bonferroni's correction, only SPGP14 remained statistically significant and showed a declining trend from A, O to OA groups. All N-glycans were of the complex type with, mono- (SPGP5), bi- (SPGP14, SPGP17) tri- (SPGP26, SPGP32), or tetra- (SPGP32) galactosylated and sialylated antennas. The SPGP5 also contained monogalactosylated bisected N-acetylglucosamine. We also confirmed the Lewis y structures in the SPGP32. Hence, we showed the diversity of significant N-glycans from total protein seminal plasma, and SPGP14 as the most prominent biomarker candidate with a declining trend in men with pathological semen parameters. *The authors marked with an asterisk equally contributed to the work.

DNA and RNA

P-04.4-001
Studies on DNA dynamics relevance to neurodegeneration

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DNA topology plays a critical role in maintaining the integrity of the genome and cellular functions. Although, changes in DNA conformation and structural dynamics in the brain have been associated with various neurological disorders, its precise role in the pathogenesis is still unclear. Previous studies from our lab have shown that there is a conformational change in the genomic DNA of Parkinson's disease (PD) (B to altered B-DNA) and Alzheimer's disease (AD) brain (B to Z-DNA). However, there is limited information on the mechanism on DNA dynamics changes in brain. In the present study, we have investigated the DNA conformation and sequence specific binding ability of a-Synuclein and Tau with reference to B-DNA and Z-DNA using oligonucleotide (CGCGCGCG)2 as novel model DNA system. This sequence is predominantly present in the promoter region of the genes of of biological relevance. Natively, (CGCGCGCG)2 sequence exists in B-DNA conformation, but in the presence of high sodium concentration (4 M NaCl), the oligo converted into Z-DNA form. We used Circular Dichroism, melting temperature and fluorescence studies to understand protein-DNA interactions. CD studies indicated that both a-Synuclein and Tau bind to B-DNA conformation of (CGCGCGCG)2 and induce altered B-form. Further, these proteins increased the melting temperature and decreased the number of EtBr molecules bound per base pair of DNA in B-form indicating that DNA stability is favored to alter B-DNA conformation, which could an intermediate form favoring Z-DNA conformation. Furthermore, both a-Synuclein and Tau also bound to disease-linked Z-DNA conformation of (CGCGCGCG)2 and further stabilized the Z-conformation. The present study provides vital mechanistic information on synuclein and tau binding to DNA in a conformation-specific manner causing conformational transition.

P-04.4-002
microRNA as predictive molecule for the *in vitro* fertilization process

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Infertility currently affects a significant part of the population. Success of *in vitro* fertilization (IVF) derives from implantation window and the endometrial receptivity. In the context of infertility, specific miRNAs appear as potential predictive marker. Our research aimed to differentiate miRNAs using sequenation (NGS) and bioinformatic analyzes, which would predict the patient's readiness in the IVF process for embryo implantation. 112 patients were selected for the study (59 unsuccessful IVF, 53 with successful IVF). The outcome of the IVF process was determined on serum hCG concentration (>30 IU/l defined as successful). Plasma samples were used for miRNAs isolation (miRNeasy Micro Kit, Qiagen). Subsequently, a sequencing library (QIAseq miRNA Library Kit, Qiagen) was prepared. Sequencing was performed using a NextSeq 500 (Illumina). Final results of the study were obtained by combination of ncRNA sequencing and a bioinformatics data analysis. The evaluation of the selection of biomarker molecules was based on at least one of the methods with the determination of the correlation of the molecules and the machine learning methods (e.g. Random Forest Classifier, SVC, SVM, SGD Classifier). Of the total number of miRNAs identified were selected 6 specific molecules (miR-26a-2-3p, miR-874-5p, miR-6734-5p, miR-4767, miR-4775, miR-18b-3p), which showed a high predictive specificity of the patient´s successful outcome in the IVF process. Taking into account the relationship between specificity and sensitivity, the Area Under Curve (AUC) in the Receiver Operating Characteristic (ROC) model was over 0.7 (ROC/AUC >70%). The use of sequenation defined biomarkers in the form of 6 miRNA selected by analysis in the presented study seem to be promising prognostic-predictive molecules. This could play a key role in predicting a woman's readiness for the IVF process and its results concurrently. This work was supported by grants VEGA 1/0540/21 and VEGA 1/0620/19. *The authors marked with an asterisk equally contributed to the work.

P-04.4-003
Urinary levels of active demethylation products of 5-methylcytosine in patients with non-cancerous bowel diseases and colorectal cancer

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The mechanisms of active DNA demethylation include involvement of Ten Eleven Translocation enzymes (TETs) in oxidation of 5-methylcytosine (5-mCyt) to form 5-hydroxymethylcytosine (5-hmCyt) which can be further oxidized to 5-formylcytosine (5-fCyt) and 5-carboxylcytosine (5-caCyt). This process, in turn, may also be involved in the aberrant DNA methylation pattern, what may play significant role in carcinogenesis. We used two-dimensional ultra-high performance liquid chromatography with tandem mass spectrometry (2D-UPLC/MS/MS) and gas chromatography with mass spectrometry detection (GC/MS) to analyze products of 5-metylcytosine demethylation in human urine. The study included four groups of subjects: healthy controls, patients with inflammatory bowel disease (IBD), adenomatous polyps and colorectal cancer. Significant differences in the excretion of 5-fCyt and 5-hydroxymethyluracil (5-hmUra) in urine were found between healthy volunteers and the group of patients with carcinoma. Strong positive correlation was observed between 5-methyl-2'-deoxycytidine (5-mdC) and 5-(hydroxymethyl)-2'-deoxycytidine (5-hmdC). This work was supported by Polish National Science Centre [2013/09/B/NZ5/00767].

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Abstract withdrawn

P-04.4-005

Distinct activity of individual TET proteins generates characteristic profile of epigenetic DNA modifications and may be modulated by vitamin C

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Active DNA demethylation, an epigenetic process carried out by Ten-Eleven Translocation proteins (TET) plays a crucial role in regulating gene expression. Abnormalities in TET activity are one of key factors of carcinogenesis. TET proteins (TET1, TET2 and TET3) oxidize 5-methylcytosine (5-mCyt) to 5-hydroxymethylcytosine (5-hmCyt), which is subsequently oxidized to 5-formylcytosine (5-fCyt) and 5-carboxycytosine (5-caCyt). They are also capable to generate 5-hydroxymethyluracil (5-hmCyt) from thymine. Activity of TET proteins may be modulated by vitamin C, as it is a necessary co-factor of those enzymes. However, very little is known about the enzymatic activity of individual members of the TET protein family and their participation in the formation of epigenetic derivatives of 5-mCyt. Therefore, we have cultured HAP1 cells with functional knockouts of TET genes – a cellular model in which only one member of TET protein family is active and expose them to vitamin C for 24 h. We have analyzed the levels of TET proteins products in DNA isolated from cultured cells by 2D-UPLC-MS/MS. In each knockout cell line, as well as in parental cell line, we have observed significant increase in the level of 5-hmCyt, 5-fCyt, 5-caCyt and 5-hmUra in DNA after exposure to vitamin C. However, in each knockout cell line distinct pattern of epigenetic DNA modifications was observed, and was only to some extent susceptible to modulation by vitamin C. Moreover, TET2 seems to play a dominant role in generation of 5-hmCyt, 5-fCyt and 5-caCyt as loss of its function had the greatest impact on reducing the level of these modifications. Our findings suggest that each member of TET protein family have distinct enzymatic activity, that may be modulated by vitamin C to some extent, but loss of function of one protein cannot be fully compensated by enhancing activity of other family members. This work was supported by Polish National Science Centre grant no: 2018/29/N/NZ1/00497

P-04.4-006

Preliminary studies on the interactions between antitumor unsymmetrical bisacridines, UAs, and DNA using monomer acridine derivatives as model compounds

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New unsymmetrical bisacridines, UAs, developed by our group, demonstrated high antitumor activity against a set of tumor cell lines and human tumor xenografts (European Patent No EP 3 070 078 B1). UAs were constructed from two acridine monomers: imidazoacridinone (IA) and 1-nitroacridine (NA), linked by diaminoalkyl chain. In order to investigate the interactions of IA, NA and UAs with nucleic acids, a set of dsDNA and G4 sequences (G4s) were selected. Basing on our previous results, 5'-TA-3' (TA) step in dsDNA helices was suspected to be the target binding cavity for acridine derivatives (Laskowski T et al. (2020) Sci Rep 10, 11697). Hence, two palindromic octamers were selected for structural studies. For IA, which was found to bind at the centre of the studied helices, NMR-derived structures were obtained and improved by NOE restrained MD analysis. Also, binding constants of this compound to all palindromic 4-nucleotide steps containing TA at centre were established. In contrast, NA monomer and UAs dimer neither have interacted with these sequences, nor with any other longer dsDNA palindromes containing TA cavity. Since the recent studies have suggested that G4s might constitute molecular targets for acridine derivatives, Pu22, 22RT and human telomeric 22-mer were chosen to investigate them. IA monomer and UA dimer with hydroxyl group, C-2045, were found to form a well-defined DNA/drug 1:2 complex with Pu22 and 22RT, whereas NA formed a DNA/drug 1:1 adduct with solely Pu22 G4. The latter complex exhibited different geometry in comparison to the one formed by IA. Non-hydroxy UA, C-2028, interacted only with the 22RT sequence, whereas human telomeric 22-mer interacted solely with IA. In conclusion, we showed that IA monomer represented a good dsDNA intercalating agent, as well as G-quadruplex ligand, whereas UAs were suggested to target G4s with pronounced sequence-specificity. This study was supported by National Science Centre under grant No 2019/33/B/NZ7/02534. *The authors marked with an asterisk equally contributed to the work.

P-04.4-007

Novel circular RNAs (circRNAs) of the human apoptosis-related BCL2L12 gene, discovered using targeted 3rd generation (nanopore-based) sequencing, disclose novel aspects of circRNA biology

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Circular RNAs (circRNAs) constitute a type of RNA molecules, produced via head-to-tail splicing. Initially, they were considered by-products of splicing, but recently, their broad functions have emerged. However, our knowledge regarding circRNAs deriving from apoptosis-related genes is still limited. BCL2L12 is a member of the BCL2 family, which exerts an anti-apoptotic function and which is widely expressed in colorectal cancer. In the current study, we aimed at developing a high-throughput approach to identify novel BCL2L12 circRNAs. For this purpose, total RNA extracts from seven colorectal cancer cell lines (Caco-2, COLO 205, DLD-1, RKO, HT-29, HCT 116, and SW 620) were reversely transcribed using random hexamers. Next, multiple nested PCR assays using divergent primers, specific for each BCL2L12 exon, were performed. After library construction, 3rd generation (nanopore-based) sequencing was conducted using the MinION Mk1C platform and the Flongle adapter. Sequencing data analysis was performed using publicly available tools and our Perl-based algorithms. In this way, 62 novel BCL2L12 circRNAs were identified, hence indicating that numerous circRNAs can be transcribed from the human BCL2L12 gene. Interestingly, almost the

entire genomic sequence of BCL2L12 is covered by sequencing reads. Thus, several circRNAs comprised novel exons and/or extensions of the currently annotated exons. Additionally, we noticed that short sequence identity is shared between cryptic exons and micro-exons that form the back-splice junction. Last, poly(A) stretches were also detected in BCL2L12 circRNAs, supporting the notion that polyadenylation of primary transcripts starts prior to the formation of the back-splice junction. Concluding, besides leading to the identification of novel circRNAs spanning intronic regions from the apoptosis-related BCL2L12 gene, this study elucidated unexplored aspects of circRNA biology, providing evidence about an unknown mechanism of circRNA biogenesis.

P-04.4-008
Novel RNA molecules interacting with the bacterial transcription machinery

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Regulatory 6S RNA molecules that interact with the RNA polymerase are widespread among bacteria. We discovered a new type of regulatory RNA in mycobacteria, named it Ms1 and showed that Ms1 regulates the amount of RNA polymerase in nonpathogenic *Mycobacterium smegmatis*. In addition, we found Ms1 homologs among other *Actinobacteria* using bioinformatic search. This phylum includes severe human pathogens (for example *Mycobacterium tuberculosis, Mycobacterium leprae, Corynebacterium diphtheria*), industrially important producers of amino acids (*Corynebacterium glutamicum*) and antibiotics (*Streptomycetes*) or probiotic bacteria (*Bifidobacterium*). We performed RIP-seq (RNA immunoprecipitation coupled with next-generation sequencing) and identified a complete set of regulatory RNAs interacting with the transcription machinery in several bacterial species - *Mycobacterium smegmatis, Streptomycetes celicolor*, and well-established model organism Bacillus subtilis. Our data show that in addition to 6S and Ms1 RNA, other RNAs associate with the different forms of bacterial RNA polymerase. These novel RNAs expand the portfolio of possible mechanisms of bacterial transcription regulation. We propose that 6S RNA and Ms1 were the first RNAs to be identified due to their high abundance; however, other; less abundant regulatory RNAs are waiting to be discovered. *The authors marked with an asterisk equally contributed to the work.

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Abstract withdrawn

P-04.4-010
Identification of the mechanisms by which exoribonucleases affect biofilm formation and acidic stress response

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In their natural habitats, bacteria constantly deal with different stress exposure, possessing pathways that regulate gene expression and enable rapid responses. RNA degradation mechanisms play an important role in these processes since they control mRNA levels in the cell. Ribonucleases (RNases) are the enzymes responsible for RNA degradation, being their action sometimes interconnected with small-RNAs (sRNAs). These sRNAs can bind to target mRNAs and stimulate their degradation by RNases. Although RNases are involved in the metabolism of several sRNAs, the link between them and stress adaptation is still not very well understood. In our work, we have focused on *Escherichia coli* three main degradative exoribonucleases (RNase II, RNase R and PNPase) and their relevance in stress responses such as biofilm formation and acidic stress. Previously it was demonstrated that these three RNases play a key role in biofilm formation in *E. coli*. The RNase II and RNase R deletion mutants (Δ*rnb* and Δ*rnr*) form more biofilm than the wild type, while the PNPase deletion mutant (Δ*pnp*) forms less biofilm than the wild type. Through the analysis of RNA-Seq data, we found that Δ*rnb* and Δ*rnr* present upregulated transcripts related to biofilm formation. Additionally, RNA-Seq results indicate that intracellular levels of c-di-GMP, a metabolite that regulates biofilm, may be altered in the mutant strains. Together these results may help to explain the phenotype observed in the mutants. Simultaneously, we shown that RNase II and PNPase mutants have their growth affected after acidic shock, indicating these RNases participate in acidic stress response. It should be noted that in Δ*rnb* we found that ArrS, an acidic stress response sRNA is downregulated, which may explain the growth arrest. In summary, our results indicate that exoribonucleases play a key role in biofilm formation and response to acidic shock in *E. coli*, revealing one more role of these enzymes in stress adaptation.

P-04.4-011
Tau pathological aggregation and liquid-liquid phase separation are precluded by the S100B chaperone

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Liquid–liquid phase separation (LLPS) of tau is increasingly recognized as a process implicated in the initiation of tau aggregation and in the formation of pathogenic conformations in Alzheimer's Disease (AD) (Kanaan NM et al. 2020 Nat Commun). Tau pathology is accompanied by neuroinflammation, and while alarmin released by astrocytes in late AD stages is aggravating, early inflammatory responses encompass protective functions. This is the case of the Ca-protein S100B, which we recently implicated as a proteostasis regulator inhibiting amyloid β (Cristovão et al. 2018 Sci Adv) and tau aggregation and seeding (Moreira et al. 2021 Nat Commun), suggesting its widespread

action as an holdase-type chaperone in the prevention of misfit protein conformations. Here we report that S100B is also a Ca-dependent inhibitor of tau LLPS, suggesting that this chaperone also controls phase separation processes. Phase diagrams of PEG-induced tau LLPS denote a clear effect of Ca-bound but not of apo S100B. The addition of Ca^{2+} to PEG-induced tau droplets containing apo S100B leads to an immediate decrease in the levels of tau droplets, revealing the ability of Ca-S100B to interfere with highly dynamic LLPS processes. Likewise, S100B can completely inhibit PEG-free Zn-promoted tau LLPS, in virtue of its combined Zn-buffering ability and interactions with tau. Altogether these data implicate this S100 chaperone in the regulation of the formation of multiple pathological conformers and phase-separated systems, uncovering its central role as a proteostasis regulator relevant in neurodegeneration. Acknowledgments: FCT/MCTES (Portugal) is acknowledged for funding (UID/MULTI/04046/2013) and for PhD grant 2020.06443.BD (to GGM). Kanaan NM et al. (2020) Nat Commun 11(1), 2809; Cristovao et al. (2018) Sci Adv 4(6), 1-14; Moreira et al. (2021) Nat Commun 12(1), 6292

P-04.4-012
The RNA-binding protein PNPase regulates biofilm formation and virulence in *Listeria monocytogenes*

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Biofilm-related diseases are typically persistent infections, and a challenge for medical treatment. Biofilms are communities of bacteria that attach to surfaces and are enclosed in an extracellular matrix. These sessile microorganisms can endure external stresses like nutrient deprivation, antibiotic treatments, and immune defences. Therefore, biofilms create conditions favourable for bacterial pathogenesis. The knowledge of novel biofilm regulators may contribute to develop new strategies to fight microbial infections. In this work we study the role of the RNA-binding protein and RNA-degradative enzyme polynucleotide phosphorylase (PNPase) from the human pathogen *Listeria monocytogenes*. We show that inactivation of *Listeria* PNPase not only leads to strong defects in biofilm production, but also affects biofilm morphology. We demonstrate that PNPase is a previously unrecognized regulator of the composition of the biofilm extracellular matrix, greatly affecting the levels of proteins, sugars, and extracellular DNA. This reduction correlates with a thinner biofilm found in PNPase-deficient bacteria, as observed by confocal laser scanning microscopy and scanning electron microscopy. RNA-seq analysis of the RNA extracted from biofilms of the wild-type and the PNPase mutant strains revealed major changes in the expression of genes affecting the metabolism of carbon. We also show that the less stable and less resistant biofilm produced in the PNPase mutant is more susceptible to antibiotic treatment. Lastly, infection assays in eukaryotic cell lines confirmed that PNPase deletion leads to the severe attenuation of *Listeria monocytogenes* pathogenicity. Overall, our results show that PNPase is a novel regulator of biofilm formation and human cellular invasion of a bacterial pathogen. This work presents PNPase as a new and attractive target for the control of bacterial infection and highlights the expanding role of RNA-binding proteins as critical players in pathogenicity.

P-04.4-013
Control of mRNA poly(A) tail synthesis in *Saccharomyces cerevisiae* by the nuclear polyadenylated RNA binding protein Nab2p

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Eukaryotic mRNA 3′ends carry polyadenosine (poly(A)) tails whose exact lengths impact when and how mRNAs are transported, translated and degraded. Poly(A) tails are not encoded in the genomic DNA, instead, they are synthesized by a dedicated protein machinery called the Cleavage and Polyadenylation Factor (CPF) complex. Poly(A) tails on newly synthesized mRNAs have uniform lengths which arise through a cooperation between the CPF complex and regulating protein factors. To study how polyadenylation is regulated in the budding yeast, *S. cerevisiae*, we utilized an *in vivo* assay capable of assessing nuclear poly(A) tail synthesis, analyzed tail length distributions by direct RNA sequencing, and reconstituted polyadenylation reactions with purified components. This work revealed that the nuclear polyadenylated RNA-binding protein 2 (Nab2p) is the primary factor that controls mRNA poly(A) tail synthesis, and that this primary pathway can be backed up by two failsafe mechanisms (Previously published in: Turtola *et al.* (2021) *Genes & Development* 35: 1290-1303). Here, I discuss our progress in investigating the mechanism how Nab2p controls polyadenylation and "measures" the lengths of poly(A) tails. Biochemical reconstitution experiments suggest that the association of Nab2p with the growing poly(A) RNA inhibits the polyadenylation activity of the CPF complex. *In vivo*, several structural domains of Nab2p collaborate to distinguish the full-length poly(A) tail from shorter polyadenylation intermediates. Furthermore, an interplay of structured and unstructured regions of Nab2p facilitates termination of poly(A) tail synthesis. Our work reveals unanticipated complexity in the essential last steps of eukaryotic mRNA biogenesis and provides a starting point to study how completion of polyadenylation is coupled to the subsequent nuclear export of mRNA.

P-04.4-014
nido-Carborane-containing purine derivatives as precursors of boronated nucleosides

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Chemo-enzymatic glycosylation of purine derivatives makes it possible to obtain modified nucleosides under mild conditions and with high stereoselectivity. Previously, we showed the

possibility of obtaining purine nucleosides with fragments of amino acids and chiral heterocyclic amines at position 6 of purine using recombinant *E. coli* purine nucleoside phosphorylase (PNP) (Eletskaya BZ et al. (2019) Chem Biol Drug Des 93, 605-616; Krasnov VP et al. (2020) Pure Appl Chem 92, 1277-1295). The presence of bulky heterocyclic substituents in the structure of modified nucleobases, as a rule, did not affect the enzyme substrate specificity, and conversion to ribosides, deoxyribosides, and arabinosides was high. Synthesis of new derivatives of polyhedral carboranes is of interest taking into account the possibility of obtaining selective boron delivery agents for boron neutron capture therapy of tumors. Moreover, carborane-containing derivatives of adenosine and other nucleosides are lipophilic analogs of natural compounds, among which receptor modulators, enzyme inhibitors, and compounds capable of binding to DNA can be found. To study the effect of the bulky carborane fragment on the *E. coli* PNP substrate specificity, we synthesized a number of new purine bases containing *nido*-carborane residues in position 6 by the reaction of 6-chloropurines with *nido*-carboranyl ethylenediamine and 6-aminohexanoic acid. In preliminary experiments, (purin-6-yl) and (2-aminopurin-6-yl) *nido*-carboranes were not subjected to ribosylation under conditions where the corresponding derivatives of 3-methyldihydrobenzoxazine and 2-methyltetrahydroquinoline easily reacted (*E. coli* PNP, inosine, phosphate buffer pH 7.0). At present, the works are underway to modify the structure of carboranyl derivatives of purine and optimize the glycosylation reaction conditions. The work was financially supported by the Russian Science Foundation (project 21-73-10073).

P-04.4-015
MicroRNA of the bovine milk lipid fraction

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Exosomes are extracellular vesicles with a diameter of 40–100 nm. Exosomes play an essential role in intercellular communication, as they deliver proteins and nucleic acids to cells. Due to their small size and high biocompatibility, milk exosomes are promising delivery vehicles for therapeutic molecules. Hundreds of different microRNA (and even mRNA) were described in bovine milk exosomes. These high numbers of nucleic acid molecules cannot fit in a 40–100 nm diameter vesicle. According to data obtained earlier in our laboratory, the number of proteins and nucleic acids attributed to milk exosomes is greatly overestimated. Here we tested the hypothesis that the data on the content of hundreds of molecules of different microRNAs in milk exosomes is a consequence of impurities from the lipid fraction of milk co-isolating with the exosomes. We analyzed the lipid fraction obtained from two samples of bovine milk volume of one liter. Small RNA fraction was isolated using the TRIzol analog on spin columns with silica membrane. MicroRNA analysis was performed by reverse transcription using stem-loop primers and subsequent qPCR. This method makes it possible to specifically generate cDNA corresponding to short RNAs using a unique SL primer for each microRNA and then quantitatively analyze it by PCR. A standard curve was constructed using synthetic microRNA let-7f-5p to estimate the number of microRNA in samples. We determined the content of several miRNAs in eluates from the spin column. Some microRNAs specific for milk were not found in the bovine milk lipid fraction samples. *The study was supported by the Russian Science Foundation project No. 18-74-10055* *The authors marked with an asterisk equally contributed to the work.

P-04.4-016
Formation of a G-quadruplex-like structure may trigger the ribosomal protein S1-promoted termination of the RNA synthesis by Qβ replicase

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Qβ replicase, the RNA-depended RNA polymerase of bacteriophage Qβ, exponentially amplifies RNA *in vitro* at an unprecedented rate. Synthesis of GGG, complementary to the 3'-terminal CCC of the template, drives the replicase into a closed conformation, from which the growing RNA strand cannot dissociate. Termination (release of the completed RNA strand) is promoted by the ribosomal protein S1, one of the four Qβ replicase subunits, as well as by its N-terminal fragment OB_{1-2}. We discovered that with GTP as the only substrate, Qβ replicase produces long polyG strands, which on denaturing gel electrophoresis produce a ladder with at least three clusters of bolder bands of about 15, 25 and 35 nt. Varying the GTP concentration or incubation time changes the distribution of material among the clusters, but the positions of the clusters in the gel remain preserved. This synthesis is template-directed, it only occurs in the closed replicase conformation, and is prevented by incorporation of the next template-encoded nucleotide; the latter indicates that it results from transcript slippage. This is the first time that slippage is demonstrated for the replicase of a positive strand RNA phage, and the first time ever that transcript slippage is found to generate products whose amount periodically changes with their size. The most intriguing observation is that protein S1 and its fragment OB_{1-2} promote release of the G_{15} product suggesting that they recognize it as a termination signal. In view of the known propensity of G-rich sequences to form quadruplexes, this indicates that a G quadruplex-like structure may be formed by the replicative complex at the termination step.

P-04.4-017
Association of GH (c.2141C>>G) polymorphism with amino acid composition of muscle tissue

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Different allelic variants of growth hormone gene are associated with growth and differentiation of body tissues in cattle. GH (c.2141C>G) polymorphism in exon 5 of GH gene is associated with the substitution of leucine for valine (L127V, rs 4192384). The aim of the work was to study the association of GH (c.2141C>G) polymorphism and amino acid composition in

cattle. Hereford bulls were genotyped using PCR-RFLP technology. System of capillary electrophoresis was used to study the amino acid composition of proteins. The content of essential amino acids varied within 38.94-39.37%. The maximum level (by 0.32-0.43%) of essential amino acids was found in animals with the GHGG genotype. In particular, the superiority of GHGG carriers was in the content of arginine by 0.06-0.26%, lysine – by 0.04-0.10% and leucine – by 0.13%. The heterozygous group had leadership in the presence of methionine and valine by 0.06-0.16% and 0.14%, respectively. Bulls with GHCC genotype were distinguished by an increased level of phenylalanine by 0.07-0.20% relative to their peers. The concentrations of histidine and threonine in the muscle protein of animals with GHCG and GHGG genotypes were equal and exceeded the content of amino acids of their peers by 0.30 and 0.07%, respectively. Muscle tissue from GHGG carriers was characterized by an increased content of non-essential amino acids, which provided them with a superiority of 0.03-0.18%. The greatest contribution to the structure was made by tyrosine, proline and glycine, the content of which exceeded by 0.07-0.14%, 0.04-0.07 and 0.03% the corresponding amino acids in GHCC and GHCG bulls. The maximum level of serine and cystine was found in the protein of animals with heterozygous genotype, which exceeded the corresponding amino acids in GHCC and GHGG bulls by 0.07 and 0.07-0.10%. Thus, the amino acid composition of the muscle tissue is determined by the GH (c.2141C>G) polymorphism in Hereford bulls.

P-04.4-018
Effect of LEP (p. 73 C>>T) polymorphism on the chemical composition of longissimus dorsi muscle in Hereford bulls

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The leptin gene codes for the hormone leptin, which is synthesized by adipose tissue cells. Leptin plays an important role in energy and fat metabolism, feeding behavior, and feed efficiency in cattle. The aim of the research was to study the effect of LEP (p. 73 C>T) polymorphism on the chemical composition of the longissimus dorsi muscle in Hereford bulls. Tissue samples were obtained at the level of 12-13 ribs from 21-month-old bulls. The chemical composition was determined as per methods of AOAC (2000). Genetic variants of the LEP (p. 73 C>T) polymorphism (GenBank AF120500) were studied using PCR-RFLP technology. The maximum amount of dry matter was noted in the muscle of bulls with LEPTT genotype (25.62%) with superiority relative to their peers with LEPCC and LEPCT genotypes of 0.11-0.38% (P>0.05). Moisture occupied the largest share in the muscles of LEPCC carriers, surpassing analogues by 0.27-0.38% (P>0.05). The intramuscular fat content in LEPTT variant was 0.23-0.64% (P>0.05) higher compared to peers. The maximum protein content in the longissimus dorsi muscle was observed in the heterozygous group with an advantage of 0.13-0.27% (P>0.05) relative to homozygous bulls. The protein of the longissimus dorsi muscle from LEPTT carriers contained more tryptophan by 5.00-12.22 mg% and hydroxyproline by 0.65-0.88 relative to peers with LEPCC and LEPCT genotype, respectively. The muscles from bulls with LEPCC genotype were characterized by the minimum content of oxyproline. The pH level of the longissimus dorsi muscle ranged from 5.18 to 5.44 units. The muscle solution of LEPCC group was somewhat more acidic than peers by 0.25-0.26 units. Thus, LEP (p. 73 C>T) polymorphism was associated with variability in the chemical composition of the longissimus dorsi muscle in Hereford bulls.

P-04.4-019
Abundance of sno-derived RNAs in patient-derived primary glioblastoma tissues, their potential biogenesis and role in cancer progression

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In the last few decades, knowledge about the role of small nucleolar RNAs (snoRNAs) in tumor development as both suppressors and oncogenes in different cancers is still rising, peculiarly their involvements in gliomagenesis. Mature snoRNAs undergo processing into stable, shorter 20-30 bp fragments, so called sno-derived RNAs (sdRNAs). Research work involving sdRNAs changes in expression provides a range of new potential biomarkers and/or therapeutic targets, although the importance of sdRNAs expression pattern in glioblastoma (GBM) is still rarely known. The major aim of our study was to analyze the expression pattern of sdRNAs and their snoRNAs precursors in patients-derived primary GBM tissues seeking for potential suppressors and oncogenes. The list of differentially expressed sdRNAs subjected to further analyses were obtained based on RNA-seq data. Moreover, we analyzed the secondary structures of snoRNAs according to their potential processing into smaller fragments. To further investigate sdRNAs mechanism of action we identified the sdRNAs potential mRNA targets using databases and finally performed the preliminary functional analysis. In conclusion, the negative correlation between abundance of sdRNAs and snoRNAs expression pattern was observed, which might confirm the processing of the snoRNAs into shorter fragments. Also, secondary structures analyses allowed to remark structural elements most favored by enzymes tasking to generate sdRNAs. The functional studies upon knockdown or overexpression of selected sdRNAs confirmed their involvements in cell migration and the additional computational analyses identified also sdRNAs putative mRNA targets suggesting their miRNA-like mechanism of action.

P-04.4-020
Enzyme tethering protein – proximity labeling for everything

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Proximity labeling enables the characterization of biomolecules of interest near a specific target. A tag is deposited on adjacent biomolecules, later to be used for purification. Here, we combine the use of antibodies to bind the target of interest, with a protein for optimized labeling-enzyme delivery. For example, to characterization of DNA binding sites for transcription factors, we have developed a Chromatin Antibody-mediated Methylating Protein (ChAMP) composed of a GpC methyltransferase fused to protein G. By tethering ChAMP to a primary antibody directed against the DNA-binding protein of interest, and selectively switching on its enzymatic activity *in situ,* we generated distinct and identifiable methylation patterns adjacent to the protein binding sites. This method is compatible with methods of single-cell methylation-detection and single molecule methylation identification. Indeed, as every binding event generates multiple nearby methylations, we were able to confidently detect protein binding sites in single cells and from single molecules. Similarly, a RNA-DNA ligase is used for the identification of RNA molecules adjacent to a target of interest. Thus, our Enzyme Tethering Protein (ETP) can be used to target different biomolecules with different enzymes.

P-04.4-021
Ultrahigh-resolution X-ray structures of Z-DNA in complex with biogenic polyamine and potassium cations

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A Z-DNA duplex with the self-complementary d(CGCGCG) sequence was crystallized in complexes with the polyamines putrescine^{2+} and cadaverine^{2+}, and the crystal structures were determined at sub-atomic resolution (0.60 Å and 0.69 Å, respectively). In addition, in both structures the electron density maps clearly revealed novel metal coordination sites, interpreted as potassium. The positions of the potassium cations relative to the Z-DNA duplex are similar in both structures and indicate that K^+ has preference for binding between two phosphate groups. The putrescine^{2+} dications interact with the Z-DNA bases, while the longer cadaverine^{2+} dications are hydrogen-bonded, directly or via water bridges, to the O atoms of the phosphodiester groups from different Z-DNA molecules. The water-mediated N-H...O interactions are reinforced by C-H...O bonds. The entire putrescine^{2+} and cadaverine^{2+} dications are clearly visible in the electron-density maps, despite their fractional occupancy, 0.38 and 0.54, respectively. The presented structures are the first examples of the interactions of putrescinium/cadaverinium and potassium cations with DNA duplexes. The study provides insight into the efficiency and competition of polyamine and metal cations for interactions with Z-DNA, and confirms that even a simple biogenic diamine can adopt different conformations and consequently enter into a variety of interactions with their biomacromolecular partners. The partial occupancy of the K^+ ions confirms previous findings that most monovalent cation sites in DNA crystals are partially occupied and that they are mobile and easily exchanged with water molecules.

P-04.4-022
Expression of HuINFalpha-2b gene in transgenic tomato plants of the cv. Shedevr long shelf-life variety and maintenance of physiologically active human interferon alpha-2b during fruit storage

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The aim of our investigation was to validate the expression of the heterologous *HuINFalpha-2b* gene in transgenic tomato lines. The tomatoes harbouring HuINFalpha-2b and *nptII* genes were previously obtained by *Agrobacterium*-mediated genetic transformation. Total RNA was extracted from the leaves and fruits of several transgenic tomato lines to investigate *HuINFalpha-2b* gene expression. Primers specific to mRNA enabled the synthesis of the total cDNA pool in the reaction of reverse transcription. Further PCR amplification of cDNA with primers specific to the *HuINFalpha-2b* gene confirmed the presence of the appropriate mRNA in specimens isolated from fresh leaves, just harvested, and 2 week-stored tomato fruits. We failed to synthesize cDNA in specimens from tomatoes stored for 2 months. These data showed the efficient transcription of mRNA from the heterologous *HuINFalpha-2b* gene and active degradation of it after 2-week storage of fruits. We conducted RT-qPCR to quantify gene expression levels during the prolonged storage of fruits. RT-qPCR was done with the same samples used for RT-PCR. The quantity of HuINFalpha-2b gene in fruits stored for 2 weeks was three times lower compared with those which were analysed immediately after ripening. In the fruits stored for 2 months, the presence of *HuINFalpha-2b* transcripts was not detectable at all, also revealing the degradation of mRNA during prolonged storage of fruits. Although the gene expression stopped and levels of mRNA decreased after 2-week storage, biological interferon activity was detected even after 5 months of storage at moderate room temperature. We suggest that the protective influence of crude extracts from transgenic tomato lines against cytopathic effects of the Vesicular stomatitis virus was maintained for such a long period because the interferon was driven by the calreticulin apoplast targeting signal. The protein was sent to cell walls, where it was accumulated and protected from cell proteases. *The authors marked with an asterisk equally contributed to the work.

P-04.4-023
Myoinhibitory peptide (MIP) signaling in the hard tick *Ixodes ricinus*

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Multicellular organisms such as ticks developed plastic and adaptable nervous system that helps them to survive and thrive in the ecosystem. Neuropeptides represents the key players in this system. They can regulate wide range of biological processes including feeding activity, reproduction, and metabolism. Several neurotransmitters

and neuropeptides have already been described in ticks, however there is still a lack of information about their identity, function, and expression level in pathogen-carrying vectors like the hard ticks Ixodidae. In this study we focused on MIP signaling in *Ixodes ricinus*. We cloned the gene coding MIPs (MIP1, MIP2 and MIP3 transcribed under one promoter) in plasmids, and we used the identified nucleotide and peptide sequences for development of the specific hybridization probe and peptide-specific antiserum. We successfully disclosed nerve cells producing MIPs in central nerve system, in the innervation of hindgut, salivary glands, and reproductive organs, and endocrine cells producing MIPs in anterior lobes of the gut via *in situ* hybridization and fluorescent immunohistochemistry. Then we performed homology-based search for MIP receptors, and we identified two putative G protein-coupled receptors (MIPR1 and MIPR2) in the *I. ricinus* genome. In the aequorin functional assay we confirmed that both receptors interacted specifically with MIPs. Interestingly, as we have tested more G-proteins (stimulating or inhibiting), we observed luminescence responses in both types, suggesting MIP′s pleiotropic effect in different tissues in ticks. We detected higher expression levels of MIP receptors mostly in the salivary glands, gut, and reproductive organs using qRT-PCR. Based on our experiments, we expect that MIP, and its receptors may be involved in early stages of blood feeding, and in the regulation of reproduction in *I. ricinus*.
Keywords: neuropeptides, MIP signaling, *I. ricinus*, aequorin functional assay, qRT-PCR

P-04.4-024
Mechanisms of topoisomerase 1 DNA-protein crosslink repair

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DNA-protein crosslinks (DPCs) consist of proteins that are irreversibly covalently bound to DNA and can be resolved by different repair pathways. Repair of DPCs is mainly studied at the cellular and biochemical level, while almost nothing is known at the level of organism. One of the most abundant DPCs in cells are Topoisomerase 1-DPCs (TOP1-DPCs). Trapped TOP1 interferes with all DNA transactions (replication, transcription, repair) and can cause double-strand breaks. In cancer therapy, many drugs have been developed that induce TOP1-DPCs and cause cell death. In humans, a mutation in the active site of TDP1 causes SCAN1 syndrome, a neurological disorder characterized by slowly progressive cerebellar ataxia in late childhood. Main repair proteins involved in TOP1-DPC repair are Tyrosyl DNA phosphodiesterase 1 (TDP1) and the protease SPRTN. Upstream SPRTN proteolysis of TOP1-DPC is considered to be a prerequisite for the action of TDP1, which hydrolyzes the covalent bond between tyrosine in the TOP1 peptide residue and the 3' end of DNA via its phosphodiesterase activity. To investigate whether SPRTN and TDP1 act within the same pathway at the organismal level, as suggested by previous in vitro studies, and to determine the dominant pathway for TOP1 DPC repair, we created a TDP1-deficient zebrafish line. Although the mutant embryos did not accumulate total DPCs, they had significantly higher amounts of TOP1 DPCs compared with wild-type embryos. In addition, even higher accumulation of TOP1 DPCs was observed after embryos were treated with camptothecin (CPT), a model TOP1- DPC inducer. We are currently investigating the interplay with SPRTN in TOP1-DPC repair by silencing SPRTN in TDP1-deficient embryos. This study and a novel TDP1 animal model will reveal the role of TDP1 in DPC repair at the level of organism and help us better understand diseases and cancer treatments related to the DPC repair pathway.

P-04.4-025
3rd-generation (nanopore) sequencing unveils novel circular RNAs (circRNAs) of the human apoptosis-related BAX gene in leukemic cells, providing evidence for an alternative back-splicing mechanism

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Circular RNAs (circRNAs) constitute an emerging research field, mainly due to their pivotal roles in human physiology and pathology. Some major gaps in this field include poor knowledge about alternative splicing of circRNAs, the existence of apoptosis-related circRNAs and their role in hematological malignancies. Therefore, we sought to determine the identity of circRNAs deriving from the apoptosis-related BAX gene in acute myeloid leukemia (AML), due to its key role in this malignancy. Total RNA from 4 human AML cell lines (NB-4, HL-60, KASUMI-1, and MOLM-13) was extracted and reversely transcribed. in-house–developed nested PCR assays were conducted for the specific amplification of BAX circRNAs, followed by targeted 3rd-generation (nanopore) sequencing, thus revealing the full-length sequence of circRNAs. Bioinformatic analysis using our custom and publicly available algorithms, followed by *in-silico* functional analysis, was conducted. This process revealed 28 novel BAX circRNAs in these AML cell lines, with distinct expression patterns. One of the most interesting findings was the discovery of a general pattern in the formation of the back-splice junction through the splicing of microexons and cryptic exons. Surprisingly, besides known exons, intronic regions were also frequently included in BAX circRNAs, augmenting the repertoire of microRNAs predicted to be bound by BAX circRNAs. Interestingly, one of the most abundant circRNAs comprises a large intronic region, while it is predicted to interact with splicing factors and potentially affect the expression of its linear counterparts. Finally, one circRNA was shown to be composed of exonic regions of both BAX and its neighbor gene, DHDH, adding so far unknown perspectives regarding BAX transcription. Overall, these findings provide novel insights regarding the structure of BAX circRNAs and back-splicing mechanism, paving the way for further investigation of the localization and function of these novel molecules.

P-04.4-026
The analysis of oxyphilic metagenomic communities of Lake Baikal

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The study aimed to access the metagenomic communities of Lake Baikal water in zones with increased concentrations of oxygen.

The sampling was carried out from 4 zones with different oxygen concentrations of 9.97 mg/L, 10.78 mg/L, 11.91 mg/L, and 12.19 mg/L. The samplings were performed in villages B. Goloustnoe and Buguldeyka (South Baikal). Water samples were filtered through bacterial filters for metagenome sequencing. According to the primary analysis, the phyla *Armatimonadetes* (OTU, % - 0.04%), *Epsilonbacteraeota* (OTU, % - 0.1%), *Spirochaetes* (*Treponema* sp.) (OTU, % - 0.02%) were found in samples collected from the zone with the highest oxygen content (12.19 mg/L). Also, we found the *Cyanobacteria* phylum (class *Oxyphotobacteria*) in all zones. However, the content of mentioned bacteria was significantly higher (OTU, % - 38%) in samples collected from the zone with a high level of oxygen. In the water sampled in areas with lower oxygen content, *Oxyphotobacteria* were a minor class of bacteria (OTU, % - 1-5%). Such phyla as *Firmicutes*, *Bacteroidetes* and *Actinobacteria* were found in all zones. However, the individual representatives of such phyla as the genera *Brachybacterium* (OTU, % - 0.04%), *Porphyromonas* (OTU, % - 0.02%), *Murdochiella* (OTU, % - 0.06%), *Bacillus* (OTU, % - 0.04%), *Abiotrophia* (OTU, % - 0.04%), *Peptoniphilus* (OTU, % - 0.02%) were found in the zone with the highest oxygen content. Thus, it was shown for the first time that oxygen influences on the diversity of Lake Baikal microorganisms, and these organisms can be named oxyphiles. The study is carried out with the primary financial support of the Ministry of Science and Higher Education of the Russian Federation (State registration 121111100025-5), Grant of the President of the Russian Federation No MK-1245.2021.1.4., Grants of ISU № 091-21-317, № 091-21-318 and RSF grant № 20-76-00001. *The authors marked with an asterisk equally contributed to the work.

P-04.4-027
Using UBF silencing as a tool to investigate the regulation of rRNA transcription

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RNA polymerase I (PolI)-driven rRNA transcription is central to ribosome biogenesis. The upstream binding factor UBF is established as a master transcriptional regulator because of its role in the formation of the pre-initiation complex PIC, in maintaining an open chromatin state at rDNA repeats, and in enhancing elongation rate. Here we generated and characterized a stable UBF-silenced mouse cell line to further investigate mechanisms regulating rRNA transcription. Consistent with the known role of UBF, we observed reduced transcription of the precursor 47S rRNA, as well as repressed mTOR growth signalling in UBF-silenced cells, relative to wild-type (WT). In silenced cells, although the absolute amount of phospho-UBF (pUBF), the chromatin-binding form of UBF, is reduced, there is a higher proportion of pUBF as a fraction of the total UBF protein. This indicates mobilisation of the pUBF to counteract UBF paucity and maintain life-sustaining levels of rRNA transcription. Additionally, the pUBF fraction is in proportion to growth-stimulating extracellular cues such as serum concentration in WT and silenced cells, whereas total UBF levels remain relatively stable but different in the two cell types. Importantly, we found that PolI concentration is also reduced upon UBF silencing, both at mRNA and protein levels, suggesting that PIC formation may be impacted both through UBF silencing but also through direct downregulation of PolI. Furthermore, we found modulation of PolI concentration not to be solely linked to UBF silencing, but likely a more generalised mode of rRNA transcriptional regulation under normal circumstances. Specifically, we observed that in WT-cells, PolI levels also fluctuate proportionally to serum concentration in different cell lines and mammalian species. Collectively, our findings consolidate UBF as a main regulator, but also add novel insight to further mechanisms by which rRNA transcription maybe regulated, through expression of PolI itself.

P-04.4-028
Exploration of DNA processing features of a MOBT relaxase unravels novel properties for ICE conjugation in Gram-positive bacteria

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Bacterial conjugation is one of the main mechanisms of horizontal gene transfer, playing a crucial role in the rapid evolution of bacterial genome. It also contributes to the spread of antibiotic resistance and virulence genes. Conjugation is mediated by conjugative plasmids or by integrative and conjugative elements (ICEs). The analysis of streptococcal genomes showed the high prevalence of ICEs belonging to the ICE*St3*/Tn*916* superfamily. The initiation of conjugation requires a key protein named relaxase, which nicks the DNA to be transferred at the origin of transfer (*oriT*). ICEs of the ICE*St3*/Tn*916* superfamily encode uncanonical relaxases belonging to a unique family called MOB_T, which is related to the rolling circle replication initiators of the *Rep_trans* family. The *nic* site of these MOB_T relaxases is conserved with *Rep-trans* proteins, however, the DNA binding site is still unknown. In this study we determined the binding site of the relaxase RelSt3 from ICE*St3* (*Streptococcus thermophilus*) on its *oriT* using electrophoretic mobility shift experiments (EMSA). Unexpectedly, we found this *bind* site distantly located from the *nic* site. We showed that this binding is mediated by the N-terminal HTH domain of RelSt3, and that this domain is required (i) for efficient nicking activity *in vitro* and (ii) for conjugation *in vivo*. We also characterized the enzymatic activities of RelSt3 using labeled oligonucleotides containing both binding and nicking sites. We demonstrated that RelSt3 harbors a strand transfer activity, and that RelSt3 nicking leads to the formation of a covalent DNA-relaxase intermediate. Thus, we deciphered the implication of RelSt3 in the initial and final stages of DNA processing during conjugation, for the first time for a MOB_T relaxase. *The authors marked with an asterisk equally contributed to the work.

P-04.4-029
RT-qPCR standardization and novel normalization method for profiling of circulating microRNAs in Alzheimer's and other aging-related diseases

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Circulating miRNAs in blood plasma present significant potential for use as presently unavailable minimally-invasive biomarkers for diagnosis of aging-related diseases, such as cancer or

Alzheimer's disease (AD). The most sensitive and inexpensive analysis method of circulating miRNA is RT-qPCR. However, lack of RT-qPCR standardization is one of major causes of data inconsistencies across different studies. Moreover, current methods of identifying optimal normalisers are lacking in their evaluation of the stability of normalizers and their combinations in aging populations. To address these needs, we created a novel, transparent, method for selecting optimal normalisers in aging population. Our method for identifying normalisers was written in the Python programming language (v. 3.7.3), using pandas (v. 1.2.3), numpy (v. 1.17.1), scipy (v. 1.3.1). The comparators are two distinct biological groups of interest (such as Alzheimer's disease and cognitively normal controls). The miRNA data were collected from Polish subjects including 44 AD and 50 blood donors. Circulating miRNA molecules were isolated from whole-blood plasma, followed by RT-qPCR with a selection of 7 miRNAs tested as potential normalizers, along with suitable controls for mRNA isolation and reverse transcription. We provide a novel method for identification of optimal normalisers with the advantages of assessments of a greater number of potential normalisers (7 in this study) and transparency of decision making. Moreover, we recommend the standard protocol for assessment of plasma miRNA levels in an aging population employing a novel set of normalizers. This work has been supported by the Polish National Science Centre grant OPUS 2018/29/B/NZ7/02757and by the EU Horizon2020 FETOPEN grant no 737390 (ArrestAD).

P-04.4-030
Drosophila zinc finger protein CG9609 interacts with DUB-module of SAGA complex and involved in transcription regulation

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In previous studies, we showed that zinc finger proteins Su(Hw) and CG9890 interact with ENY2-containing transcriptional complexes, including DUB module of SAGA, and play role in regulation of genetic processes. In this project, we decided to identify new zinc proteins that interact with the DUB module and study their effect on transcriptional regulation. The extract from the nuclei of Drosophila embryos was fractionated step by step using ion exchange chromatography and gel filtration to segregate the DUB-module of the SAGA complex. The Sgf11-contaning fraction were incubated with anti-Sgf11 antibodies linked to Protein-A-Sepharose. The bound material was analyzed by MALDI-TOF MS. As a result, proteins of the DUB module were identified: Sgf11, ENY2, and Nonstop. More than 100 proteins involved in transcription and formation of an mRNP particle were also coprecipitated with the DUB module. Among them were several zinc finger proteins, one of which was CG9609. The interaction of CG9609 and Sgf11 was confirmed by coimmunoprecipitation. We raised antibody against CG9609 and showed the nuclear localization of this protein. We performed ChIP-Seq analysis of CG9609 protein and found that it is localized predominantly on the promoters of Pol II-dependent genes, including promoters of bnl, cv-2 and pnt genes. To investigate the role of CG9609 in the regulation of transcription we have performed analysis of mRNA level of bnl, cv-2 and pnt genes in cell upon RNA interference of CG9609 protein compared to the control cells. After knockdown of the CG9609 protein, the transcription of this genes was higher more than 2-fold. Thus, the CG9609 protein is a new member of the cell transcriptional network which is localized on active promoters and is involved in the regulation of transcription. This work was supported by the Russian Science Foundation (Grant No. 20-14-00269).

P-04.4-031
New innovative methods for fast identification of MALAT1 long non-coding RNA small molecule ligands

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MALAT1 (metastasis-associated lung adenocarcinoma transcript 1) is an abundantly and ubiquitously expressed long non-coding RNA of 8kb that plays a pivotal role in biological processes of cell proliferation, migration and invasion in many cancer types [1]. Upon folding, the 3'-terminal end engaged a genomically encoded poly(A)-rich tract in a highly stable triple helical conformation that promotes the nuclear accumulation and persistent functions of MALAT1 [2]. Using a new screening assay based on time-resolved fluorescence energy transfer (TR-FRET) technology we facilitated the fast and robust discovery of potential MALAT1 triple helix binders [3]. A pilot screening of an RNA-focused library validated the high-throughput screening suitability and allowed to evaluate larger libraries of 10,000 compounds. The mechanism of action at the molecular level of the most promising interactions is currently under study by using biochemical and biophysical assays as well as cellular studies. Our study promotes the use of an HTS method to identify small molecules ligands MALAT1 triple helix and explore the druggability of highly complex RNA tertiary structures. [1] Zhang X et al. (2017) RNA Biol. 14, 1705-1714 [2] Brown JA, (2014) Molecular Biology, 21, 10 [3] Abulwerdi FA et al. (2019) ACS Chem Biol. 14, 223-235

P-04.4-032
A primate conserved element of intron 7 of caspase-1 with promoter activity governed by GATA switch regulates erythropoiesis

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Hematopoietic stem and progenitor cells (HSPC) differentiate into completely functional erythrocytes in an extremely complex process, called erythropoiesis, which is regulated at multiple levels by still many unknown factors. Recently, our group has shown that the inflammasome plays an evolutionarily conserved

role in erythropoiesis, since caspase-1 (CASP1), the effector of the canonical inflammasome, regulates the level of the master erythroid transcription factor GATA1 by its direct cleavage and inactivation (Previously published in: Tyrkalska SD. et al. (2019), Immunity 51: 50-63). Fine tuning of GATA1 protein level by the inflammasome determines both erythroid/myeloid decision and terminal erythroid differentiation. Here, we report an additional level of regulation within this crosstalk, involving a GATA switch (GATA1 displaces GATA2) that occurs at a primate conserved sequence located at the intron 7 of CASP1 gene upon induction of erythroid differentiation. Deletion of the binding sites of GATA1/2 present in this sequence by CRISPR-Cas9 technology in human erythroleukemic K562 cells facilitates terminal erythroid differentiation. Luciferase reporter assays reveal that this sequence shows TATA box-dependent promoter activity, which is negatively regulated by GATA1, and drives transcription of a short transcript in both K562 and CD34+ HSPCs encoding a 6 kDa polypeptide, which is stabilized upon erythroid differentiation, assayed by RT-qPCR and western blot. Current studies are in progress to illuminate this GATA switch-mediated control of inflammasome activity, that potentially controls a novel erythroid gene-regulatory network.

P-04.4-033
Antisense non-coding transcription modulates PHO5 gene regulation

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RNA-sequencing studies discovered pervasive transcription across eukaryotic genomes leading to the transcription of numerous non-coding RNAs (ncRNAs). ncRNAs have been shown to be important regulators of gene expression, acting via modulation of chromatin structure, promoter interference and/or overlapping sense/antisense pairs. The *PHO5* gene encodes secreted nonspecific acid phosphatase, which is located in the periplasmic space of the cell and plays a role in phosphate metabolism. Accordingly, *PHO5* expression is regulated in response to phosphate concentration in the cell via the PHO signalling pathway, so that it is suppressed when intracellular concentration is abundant and induced under conditions of phosphate deficiency. This regulation is primarily achieved through phosphorylation of the major activator Pho4. At high phosphate concentrations, Pho4 is phosphorylated by the cyclin-dependent-kinase (Pho80-Pho85), accumulates in the nucleus, and activates transcription of the *PHO5* gene. Another level of complexity of *PHO5* promoter regulation was revealed by mapping the *PHO5* antisense transcript, CUT025. This transcript initiates from the 3' region of *PHO5* ORF and extends across the promoter region with a size of $\sim$2.4 kb. It is produced only in cells growing under repressive (phosphate-rich) conditions and is more abundant in rrp6D mutant than in wild-type cells, indicating its degradation by the nuclear RNA exosome. In this work, we examined the effects of noncoding antisense transcription on *PHO5* gene expression by enhancing or impairing elongation of the *PHO5* antisense transcript. In both cases, our results demonstrate that antisense transcription has a negative effect on *PHO5* gene transcription. Furthermore, we provide evidence that this negative effect occurs through a chromatin based mechanism mediated by antisense transcription that reduces the accessibility of chromatin structure at the *PHO5* gene promoter.

P-04.4-034
The impact of cLTP induction on mRNA polyadenylation in cultured neurons

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Almost all mRNAs are modified by polyadenylation, an addition of a poly(A) tail to their 3'-end. Polyadenylation stabilizes transcripts and increases the probability of translation. At the neuronal synapses, translation occurs upon stimulation and is strictly regulated. The elongation of poly(A) tail length induced by synaptic stimulation was shown for several mRNAs. We used nanopore sequencing for measuring mRNA poly(A) tails in cultured neurons. We analyzed changes in mRNA polyadenylation upon induction of synaptic plasticity by chemical long-term potentiation (LTP), which is a process that mimics *in vitro* LTP induced in the brain. Chemical LTP was evoked in cultured hippocampal neurons by application of agents that raise intracellular cAMP concentration. RNA from stimulated and unstimulated cultures was extracted and prepared for sequencing. To sequence RNA directly and precisely measure the length of poly(A) tails nanopore sequencing was performed. We observed significant changes in the length of poly (A) tails upon stimulation. Further analyzes will give an insight into molecular events happening locally at the synapses.

P-04.4-035
HnRNPA1 and G-quadruplexes regulatory role in KRAS expression

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KRAS oncogene transcription is controlled by a G-rich motif (called 32R), located immediately upstream of the transcription starting site (TSS). 32R is known for being polymorphic, with two principal G-quadruplex conformers in thermodynamic equilibrium, G9T (TM = 61.2°C) and G25T (TM = 54.7°C). Both conformers have the ability to recruit hnRNPA1 regulatory protein and promote several contacts with the RRM protein domains. A truncated version containing only the RRM motifs (UP1) display similar binding properties. Following the DNA imino protons by 1D NMR spectroscopy, shows that, upon binding to UP1, G25T is readily unfolded at both 5' and 3' tetrads, while G9T is more resistant to the protein action. The impact of hnRNPA1 on KRAS expression was followed by comparing Panc-1 cells with two Panc-1 knockout cell lines in which hnRNPA1 was deleted by the CRISPR/Cas9 technology. The results showed that the expression of KRAS is inhibited in the knockout cell lines, indicating that hnRNPA1 is essential for the transcription of KRAS. In addition, the knockout cell lines, compared to normal Panc-1 cells, show a dramatic decrease in cell growth and capacity of colony formation. Pull-down and western blot experiments indicate that conformer G25T is a better platform than conformer G9T for the assembly of the transcription preinitiation complex with PARP1, Ku70, MAZ and hnRNPA1.

Together, our data prove that hnRNPA1, being a key transcription factor for the activation of KRAS, can be a new therapeutic target for the rational design of anticancer strategies. *The authors marked with an asterisk equally contributed to the work.

P-04.4-036
Dynamics of the possible dTet product 5-hydroxymethyluracil during the fruit fly (*Drosophila melanogaster*) life cycle

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Drosophila melanogaster is a well-known model organism. For a long time it was hypothesized, that *Drosophila* lost ability to methylate DNA. However, more sensitive methods enabled the determination of low levels of 5-methylcytosine in all developmental stages of *Drosophila*. In 2015, the presence of another methylated DNA modification, N6-methyladenine, was found in *Drosophila* embryos and in adult flies. Additionally, a very low content of 5-hydroxymethylcytosine was also found. In mammals, 5-hydroxymethylcytosine is considered as an important epigenetic modification and product of 5-methylcytosine oxidation by TET enzymes. *Drosophila melanogaster* has a single ortholog of TET proteins (dTet) which can oxidase 5-methylcytosine. Some evidence from experimental studies suggests that mammalian TET may be also involved in synthesis of 5-hydroxymethyluracil, a compound with potential epigenetic functions. We analyzed the content of DNA modifications on the whole-genome level using isotope-dilution automated online two-dimensional ultra-performance liquid chromatography with tandem mass spectrometry and stable isotope-labeled internal standards. The developed methodology enabled quantification of the 5-methyl-2′-deoxycytidine, 5-(hydroxymethyl)-2′-deoxycytidine, 5-(hydroxymethyl)-2′-deoxyuridine, 2′-deoxyuridine, 8-oxo-7,8-dihydro -2′-deoxyguanosine and N6-methyl-2'-deoxyadenosine in DNA of *D. melanogaster* larvae, pupae and imago. Each stage was characterized by a specific DNA modification pattern and the level of these compounds fluctuates throughout the *D. melanogaster* life cycle. Moreover, it was possible to observe significant differences between groups of insects bred under different temperature conditions (different metabolic rate). The obtained results suggest that each of the analyzed modifications may play a role as a potential epigenetic DNA marker in *D. melanogaster*. The work was supported by Polish National Science Centre grant No. 2016/21/N/NZ1/00563.

P-04.4-037
Conditional Grsf1 knockout mice develop normally but show distinct transcriptomic and proteomic alterations in their testes

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The guanine-rich RNA sequence binding factor 1 (GRSF1) is an RNA-binding protein of the hnRNP H/F family and has been implicated in a variety of different cellular functions such as embryogenesis, mitochondrial RNA metabolism and redox homeostasis. Only recently, the first *in vivo* study described the muscular phenotype of skeletal muscle specific Grsf1 knockout mice. To further gain insights into the role of Grsf1 *in vivo* we created a mouse strain that carries a compromised Grsf1 gene lacking exons 4 and 5 (Grsf1-/-) and compared the basic functional properties of these animals with those of wildtype mice. We found that Grsf1-deficient mice are viable and reproduce normally. Interestingly, we found an elevated monocyte count of Grsf1-deficient animals. However, other basic hematological parameters did not differ significantly. Female body weight kinetics of Grsf1-deficient mice were almost identical to wildtype control animals. Meanwhile, after week 15 Grsf1-deficient male mice consistently gained less weight than the corresponding control animals. We subsequently profiled mRNA and protein expression of Grsf1 in different tissues and found highest expression in testes. We thus performed transcriptomic and deep proteomic analysis of testes from wildtype and Grsf1-deficient mice and GO term analysis of the differentially regulated transcripts revealed steroid biosynthesis to be most significantly upregulated. In conclusion, for the first time, we could show that conditional Grsf1 knockout mice show no major phenotypic alterations but distinct transcriptomic and proteomic alterations in testicular tissue, hinting at a role for Grsf1 in steroid biosynthesis.

P-04.4-038
Relationship between vitamin C transporters expression and active DNA demethylation pathway in acute leukemia

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Acute myeloid leukemia (AML) is a haematopoietic malignancy that frequently exhibits various genetic and epigenetic alterations, including aberrant DNA methylation, which in turn may be linked with active demethylation process. Active demethylation involves ten-eleven translocation (TET) enzymes to catalyze oxidation of 5-methylcytosine (5-mCyt) to 5-hydroxymethylcytosine (5-hmCyt) and further oxidation products. TETs are α-ketoglutarate and Fe^{2+}-dependent dioxygenases requiring vitamin C as an additional cofactor. It has been found that vitamin C may increase TET activity thus it can play a crucial role in the demethylation processes. Vitamin C action may depend on the efficacy of vitamin C transport into the cell. It is transferred to the cells with Na^{+}-dependent transporters (SVCT1 and SVCT2) which are products of a different gene, *SLC23A1* and *SLC23A2*, respectively. SVCTs are capable to accumulate vitamin C against a concentration gradient. We have examined 3 groups: patients with AML, myelodysplastic syndrome (MDS), which may evolve into AML, as well as the healthy controls. In all groups we have examined the expression of *SLC23A1* and *SLC23A2* genes using qRT-PCR. We have also measured concentration of vitamin C inside cells and the level of epigenetic DNA modifications in leukocytes using 2D-UPLC-MS/MS. The expression SLC23A2 gene in leukocytes from AML was reduced compared to control group as well as MDS patients. We also observed differences between expression of genes encoding vitamin C transporters. Additionally, differences in epigenetic modifications level as well as intracellular vitamin C concentration between groups were

also observed. Our findings suggest that alterations in expression of genes encoding vitamin C transporters may affect vitamin C level inside cell, which may induce changes in TET proteins activity and then in DNA methylation pattern. The work was supported by the Polish National Science Center 2020/04/X/NZ4/00745 and 2015/19/B/NZ5/02208. *The authors marked with an asterisk equally contributed to the work.

P-04.4-039
Alternative splicing of hag mRNA coding for Flagellin from a thermophilic eubacterium *Geobacillus* sp. Kps3 *in vivo* and *in vitro*

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Recently, in our laboratory, thermophilic eubacterium has been isolated and are called *Geobacillus* sp. Kps3. As a result of previous studies in our laboratory, it was found that two intron-like sequences exist in the hag gene encoding Flagellin of *Geobacillus* sp. Kps3. Both of these two introns are due to the secondary structure characteristic of Group I introns, self-splicing *in vitro*, and the fact that they show the requirement for Mg^{2+} ions and GTP during splicing. Was proved to be Group I introns(1). Flagellin is one of the proteins constituting the flagellum of eubacteria, and a flagellar filament structure is formed by stacking several thousand molecules of Flagellin. These two Group I introns can produce two kinds of Flagellin by alternative splicing. Alternative splicing has never been reported with eubacteria so far, if it can be confirmed that selective splicing actually occurs, it will be the first discovery in eubacteria. We produced *hag* deficient strains and produced complementary strains expressing two types of Flagellin, respectively. Both types of Flagellin also worked, but differences in motility were seen due to differences in structure after splicing. We discovered that under normal growth conditions, this strain uses Flagellin which is expressed when both introns are spliced together. Furthermore, it was also found that another type of Flagellin is used when the second intron is not spliced by changing the salt concentration of the medium. *In vitro*, it was found that intron splicing was inhibited in the presence of high concentrations of NaCl, but intron splicing was not inhibited in the presence of high concentrations of KCl. In this paper, we report on the relationship between the change in salt concentration and the mechanism of splicing of hag mRNA *in vivo* and *in vitro*, together with the above results. (1) M. Ishizuka, et al., FEBS 2019, Symposia Topics: RNA processing, Krakow, Poland, July 6-11, 2019

P-04.4-040
Analysis of invasive aquatic plant *Elodea canadensis* (Michx) polymorphism by microsatellite SSRs DNA loci in Northern Europe

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The goal of this study was to reveal the ability to apply polymerase chain reaction (PCR) as a highly efficient and reliable approach for analysis of non-native invasive plant *Elodea canadensis* (Michx.) SSRs polymorphism in natural ecosystems populations in Northern Europe, Latvia (*the Basin of Daugava River, and nearby lakes*) [1-2]. Defined the homogeneity of Canadian waterweed populations in Latvia and estimated the distribution level of this aquatic plant species. Carried out the PCR optimization and testing of different conditions and identified suitable parameters for succeeded primers amplification: quantity of the cycles, concentration, denaturation temperature, annealing, and elongation for selected primers. Ten pairs of polymorphic microsatellite primers were selected: Ecan5b2, Ecan5c, Ecan16, Ecan45b, Ecan46Lb, Ecan55, Ecan58, Ecan60b, Ecan105 and Ecan103. Seven of the ten examined microsatellite loci can be considered as successful markers and can be perspective for future analysis: Ecan45b, Ecan105, Ecan16, Ecan55, Ecan46Lb, Ecan103, and Ecan60b. Successful primer amplification allows identification of stress-responsive genes and also compares the structure and distribution intensity of genetic variation in *E. canadensis* populations based on neutral and adaptive available markers. References: [1] Petjukevičs, A. & Škute, N. (2017). Application of Raman scattering in the analysis of the Elodea canadensis genomic dsDNA at different stages of the plant development. Biologia, 72(9),1017-1022 [2] Skute N, Savicka M, Petjukevics A, Harlamova N (2020) Application of the Liminometric Methylation Assay for plant ecological researches: the study of global DNA methylation in leaves of *Elodea canadensis* under laboratory conditions and in leaves of fen orchid from wild populations. Plant Omics 13(1):30–36 The research was funded by the ESF Project No.8.2.2.0/20/I/003 "Strengthening of Professional Competence of DU Academic Personnel of Strategic Specialization Branches 3rd Call"

P-04.4-041
The interplay between long non-coding RNAs expression and DNA methylation in hypoxia

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The influence of hypoxia on the epigenome represents a major topic in cancer research and it is well documented. Nevertheless, data regarding the interplay between lncRNAs profiles and DNA methylation under hypoxic conditions is scarce. The objective of this study was to investigate the expression levels of some lncRNAs associated with factors involved DNA methylation process in a hypoxia model. Thus, SiHa cell line was used and hypoxia was chemical induced by treatment with $CoCl_2$. From treated/untreated cells nucleic acids as well as proteins were extracted using commercials kits and subsequently used in qRT-PCR, DNA methylation and western blot experiments. LncRNA Profiler qPCR Array Kit (System Biosciences) and ELISA was used for lncRNA profiles and global methylation (5-mC and 5-hmC quantification). All determinations were performed in triplicate and the statistical analysis was calculated using GraphPad Prism 6. The obtained data indicates a significantly increase in HIF-1α expression both at mRNA and protein levels, the best result being noted for SiHa cells treated with 200 μM $CoCl_2$ and for 72 hours. Next, under these experimental hypoxic conditions, DNMT1 and DNMT3B reduced expression levels were observed along with an increase in TET enzymes levels, especially for TET1 and TET2. On the other hand, following hypoxia an increase in the global percentage of 5-hmC was noted alongside

with a slight decrease in 5-mC levels. Moreover, 36 lncRNAs displayed a significantly modified pattern of expression in hypoxic cells (fold change >3), including several lncRNAs that affects DNA methylation. The results showed a significantly overexpression for WT1-AS ($P = 0.021$) and KCNQ1OT1 ($P = 0.039$), while lincRNA-p21 was found to be down-regulated without statistical significance. Our findings illustrate an important cooperation between different epigenetic mechanisms in hypoxia having a great potential to be explored. Acknowledgements: TE 39/2020. *The authors marked with an asterisk equally contributed to the work.

P-04.4-042
Comparison of *in silico* methods to increase quality of ancient DNA authentication in next generation sequencing reads

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One of the main methods for ancient DNA (aDNA) authentication in Next Generation sequencing (NGS) reads is the detection of post-mortem modifications at the ends of DNA molecules which appear as cytosine to thymine substitutions. Often, for this purpose, Perl script mapDamage is used which computes nucleotide misincorporation and fragmentation patterns using NGS reads mapped against a reference genome. When working with highly degraded human archeological samples from 8th to 11th century AD burials, we observed large amounts of softclipped sequencing reads aligned to the human reference sequence GRCh38, which indicate a high probability that not all of these reads actualy belong to human DNA, but rather represent burial microbiome. In order to address this issue and to improve the downstream aDNA authentication quality, we explored two methods of *in silico* filtering of NGS reads from Illumina shotgun libraries: a) detection of NGS reads aligning to bacterial genomes using the MALT software with subsequent removal of these reads prior the alignment to GRCh38; b) removal of the softclipped reads after alignment to GRCh38 followed by the repeated mapDamage analysis. The results showed that the removal of bacterial reads had negligible effect, as the mapDamage still showed high amounts of the softclipping. On the other hand, the removal of softclipped reads after alignment to GRCh38 resulted in a complete loss of softclipping while maintaining characterisic aDNA damage patterns. In addition, in several cases, when lower quality NGS reads were obtained from severely degraded archeological samples, this strategy enabled the detection of higher post-mortem damage percentage that characterizes aDNA. Funding: This research was funded by the Latvian Council of Science, project No. lzp-2018/1-0395.

P-04.4-043
Maintenance of genomic integrity in the malaria parasite *Plasmodium falciparum*

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Our research focuses on the maintenance of genomic integrity and the characterization of the molecular background that underlies the quick adaptability of the malaria parasite, Plasmodium falciparum. Our group previously showed the elevated presence of uracil, a non-canonical base in the genomic DNA of *P. falciparum* throughout its intraerythrocytic life cycle (Molnár et al. (2018) FEBS Open Bio 8(11): 1763–72). This observation may have relevance with regard to the AT-rich genome of P. falciparum (80% in coding and 90% in non-coding regions). Results obtained so far raised the question of whether and how genomic uracil may be involved in the accumulation of AT in the parasite genome. We focused on the building blocks of DNA using an EvaGreen-based dNTP incorporation assay to measure the relative ratio of dNTPs in every intraerythrocytic stage. Our findings show that the dNTP pool of the early-stage parasites (ring) differs from the more mature trophozoite and schizont stages. Based on the high AT-content of the genome we expected a high dATP and dTTP ratio throughout the life cycle. In contrast, a high dATP ratio is predominant in the ring stages, which becomes less abundant in later stages. Meanwhile, the dTTP ratio rises with the maturation from ring to later stages. Furthermore, we found that the relative ratio of dCTP is comparable to dATP and dTTP during the different developmental stages. With this method, we were also able to detect dGTP levels in P. falciparum for the first time in literature – it was the least abundant in all stages. We also investigated the effect of widespread antimalarials (WR99210, DSM265) that inhibit the pyrimidine biosynthetic pathway. Our measurements show that the parasite can compensate the effect of the drug at IC_{50} concentrations and no change can be detected in the dNTP pool ratio. Interestingly at higher concentrations both drugs prominently perturb dGTP levels, indicating a potentially more complex mechanism of action. *The authors marked with an asterisk equally contributed to the work.

P-04.4-044
AmnSINE1 transcripts are depleted in the cortex of autism spectrum disorder patients

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Repetitive sequences (RS) represent about half of the human genome, but studies on their transcription are sporadic at best, because they are very difficult to map by conventional approaches. However, when studied, RS showed to possess a role in almost every aspect of human biology. Of particular interest for the topic of this study is their role in the development of human brain. Autism Spectrum Disorder (ASD) is a heterogeneous condition, and many studies suggest a genetic etiology or

predisposition, however the molecular basis of ASD is still poorly understood. We investigated published transcriptomic data obtained from postmortem dorsolateral prefrontal cortex of 13 ASD patients and 39 matched controls. We applied a custom bioinformatic pipeline of analysis in order to retrieve specifically the transcriptomic data of RS form the general datasets. We analyzed the differential expression of both canonical genes and RS (that represent about 5% of ribo-depleted reads) by adapting DESeq2 analysis, and only 37 genes result differentially expressed with a p-adj< 0.05: among them only one RS was identified as differentially expressed: AmnSINE1, of the Small Interspersed Nuclear Elements (SINE). AmnSINE1 is expressed in all the samples investigated (base mean from DESeq2 = 1268 reads), but it is strongly downregulated in ASD specimens (log2 Fold Change = -0.82 ± 0.15; P-value = 4,73E-08; P.adj = 0.0002). Many data in literature strongly suggest a role of this and other SINE in mammalian-specific brain formation. Second level analyses showed that many genes where AmnSINE1 sequence resides are associated with ASD (from SFARI database) and with neuron differentiation and activities by PANTHER overrepresentation test. At least, analyzing the potential perturbation of AmnSINE1 expression on microRNA network we retrieved a panel of microRNAs and targets reported to play a role in ASD. Overall, AmnSINE1 is a candidate gene for the development of ASD worth to be further investigated.

P-04.4-045
Characterization of two novel long non-coding RNAs associated with brain cancer

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Brain cancer, mainly glioblastoma multiforme (GBM), is a relatively rare malignancy with an extremely high mortality rate in patients. Localization of these tumors often excludes the possibility of surgery, while the lack of specific biomarkers disables detection of the disease before its manifestation. Long non-coding RNA (lncRNA) are known to be largely affected upon carcinogenesis, thus changes in their expression levels might be used for future cancer diagnostics. Recently we characterized a new liver-specific lncRNA HELIS and demonstrated its diagnostical potential in discriminating different liver tumors [Burenina OY et al. (2021) J Cancer Res Clin Oncol, 147, 49-59]. We investigated genome locus of HELIS and revealed a number of uncharacterized transcripts with preferential expression in brain. After preliminary bioinformatic analysis we selected two brain-specific candidates named CABR1 and CABR2 with probable diagnostic and prognostic potential for GBM according to available TCGA data. We demonstrated that both lncRNAs are miserably expressed in conventional brain cancer cell line SK-N-MC, but not in U-87 MG and U-251 MG. In contrast, active expression of CABR1 and CABR2 was observed in a set of 13 non-immortalized primary glioma cell lines, in particular their maximum levels were attributed to rather low-proliferative but chemotherapy-resistant gliomas. Considering a large number of transcripts annotated for each lncRNA in different databases we determined their exact isoforms by 3'-/5'-RACE method. In case of CABR1 we identified a single abundant isoform, as well as a number of minor alternative transcripts. Notably, we discovered a couple of tiny CABR1 exons which were not annotated previously. We also determined several major isoforms of CABR2 with partially overlapping exons. All of them represent chimeric versions sharing features of 35 transcripts annotated for CABR2 in the Ensembl database. This work is supported by RFBR grant No. 21-34-70042.

P-04.4-046
The nsp15 nuclease as a good target to combat SARS-CoV-2: mechanism of action and its inactivation with FDA-approved drugs

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The pandemic caused by SARS-CoV-2 is not over yet, despite all the efforts from the scientific community. Vaccination is a crucial weapon to fight this virus; however, we still urge the development of antivirals to reduce the severity and progression of the COVID-19 disease. For that, a deep understanding of the mechanisms involved in viral replication is necessary. Nsp15 is an endoribonuclease critical for the degradation of viral polyuridine sequences that activate host immune sensors. This enzyme is known as one of the major interferon antagonists from SARS-CoV-2. In this work, a biochemical characterization of SARS-CoV-2 nsp15 was performed. We saw that nsp15 is active as a hexamer, and zinc can block its activity. The role of conserved residues from SARS-CoV-2 nsp15 was investigated, and N164 was found to be important for protein hexamerization and to contribute to the specificity to degrade uridines. Several chemical groups that impact the activity of this ribonuclease were also identified. Additionally, FDA-approved drugs with the capacity to inhibit the in vitro activity of nsp15 are reported in this work. This study is of utmost importance by adding highly valuable information that can be used for the development and rational design of therapeutic strategies.

P-04.4-047
Fighting COVID-19: potential targets for drug design

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Coronaviruses have emerged as important agents of human infection. SARS-CoV-2, the causative agent of COVID-19, has triggered a global pandemic with devastating consequences. The understanding of fundamental aspects of these viruses is of extreme importance. Fast vaccine development has been a crucial factor in preventing serious disease, but the fast-paced emergence of new variants raises many problems. Viral non-structural proteins are fundamental for viral replication. SARS-CoV-2 nsp16 is a 2′-O-methyltransferase with a pivotal role in Interferon antagonism. Nsp16 methylates viral RNA to mimic the host mRNA and then the cell stops recognizing it as foreign. This activity is stimulated by the interaction with nsp10. This protein also acts as a co-factor for the exoribonucleolytic activity of nsp14. Nsp14 also has significant anti-interferon importance that stems from its

2 distinct activities: the N-terminal 3'-to-5' exoribonuclease (ExoN) and a C-terminal N7-methyltransferase (N7-MTase). Unlike Spike proteins, these nsp10, nsp14, and nsp16 are highly conserved among viral variants. In this work, we are studying them and finding inhibitors in order to develop new therapies. Nsp10 is the prime target of our focus since it is the central player in the regulation of both nsp14 and nsp16.

P-04.4-048
Biochemical characterization of SARS-CoV-2 nsp14/nsp10 complex: a promising target for drug design

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The global pandemic prompted by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has already caused more than 6 million deaths worldwide, calling for urgent effective therapeutic measures. A deep understanding of the mechanisms involved in viral replication is required. Among the nonstructural proteins (nsps) encoded by SARS-CoV-2 genome, there is the nsp14 ribonuclease, the main object of study in this work. Ribonucleases are key factors in the control of all biological processes, ensuring maturation, degradation, and quality control of all types of RNAs. Nsp14 is a bifunctional protein, holding a 3'-5' exoribonucleolytic activity (ExoN) in the N-terminal domain, stimulated through the interaction with nsp10, and a C-terminal N7-methyltransferase activity (MTase). Both are critical for the coronavirus life cycle. In this work, we provide a complete biochemical characterization of SARS-CoV-2 nsp14-nsp10, addressing several aspects of the complex for the first time. Moreover, using a homology model, we have identified residues involved in the nsp14-nsp10 interaction that were extensively studied. We have confirmed the SARS-CoV-2 nsp14 dual function and we have shown that both ExoN and MTase activities are functionally independent. We demonstrate that the nsp14 MTase activity is independent of nsp10, contrarily to nsp14 ExoN that is upregulated in the presence of the cofactor. Additionally, our results show that the ExoN motif I has a prominent role on the ribonucleolytic activity of SARS-CoV-2 nsp14, contrasting to what was previously observed in other coronaviruses, which can be related to the pathogenesis of SARS-CoV-2. The knowledge provided in this work can serve as a basis to design effective drugs that target the pinpointed residues in order to disturb the complex assembly and affect the viral replication, ultimately, treating COVID-19 and other CoV infections. *The authors marked with an asterisk equally contributed to the work.

Metabolism and metabolic regulation

P-04.5-001
Control of Rrp6 stability in yeast sporulation

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The conserved 3'-5' exoribonuclease Rrp6 (EXOSC10 in mammals) is a catalytic subunit of the nuclear RNA exosome, which degrades and processes RNAs in eukaryotic cells. Rrp6 and EXOSC10 are important for efficient meiotic development in yeast and male and female gametogenesis in mammals, respectively. We have shown earlier that yeast Rrp6 protein levels decrease during meiotic M-phase and yeast spore formation although the mRNA continues to be expressed. Given that Rrp6/EXOSC10 undergoes a variety of post-translational modifications, including ubiquitination, we hypothesized that Rrp6 is degraded by targeted proteolysis in differentiating cells. We first confirm that Rrp6 becomes unstable when cells progress from fermentation to respiration and sporulation and then show that the protein rapidly accumulates during initial rounds of cell division after spore germination. Furthermore, we show that Rrp6 degradation requires an active Anaphase Promoting Complex/Cyclosome (APC/C). Finally, we demonstrate that an Rrp6 allele lacking the sole APC/C target motif (destruction box) conserved from yeast to humans remains detectable during sporulation. We conclude that Rrp6 protein stability is under nutritional control via targeted proteolysis by the APC/C-proteasome pathway. These results are relevant for mammals because EXOSC10 is also ubiquitinated, diminishes during spermatogenesis and becomes unstable after cold-shock via SUMOylation.

P-04.5-002
NCLX modulates astrocytic metabolism and function

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Ca^{2+} is an ion of utmost importance for the maintenance of numerous processes in the central nervous system (CNS). In the mitochondrial matrix, Ca^{2+} regulates energy metabolism, coupling ATP production to energetic demands under different stimuli. Hence, mitochondrial Ca^{2+} transport – including efflux through the mitochondrial Na^{+}/Ca^{2+} exchanger (NCLX) –

comprises a critical metabolic regulation point. Cell types across the CNS, such as neurons and astrocytes, have distinct metabolic profiles, although these profiles are tightly coupled. However, the impact of NCLX-mediated Ca^{2+} homeostasis on astrocytic energy metabolism and neuronal function is unknown. Here, we aimed to evaluate this issue using both pharmacological and genetic NCLX inhibition. Through public RNAseq database mining, we found that NCLX mRNA is over-enriched in astrocytes in comparison to whole tissue (hippocampus, cortex, and striatum, Srinivasan R et al. (2016) Neuron 92, 1181-1195) and neurons (Zhang Y et al. (2014) J Neurosci 34, 11929-11947). *In vitro* pharmacological inhibition of NCLX induced a decrease in total ATP production rate, while increasing relative glycolytic activity. Increased lactate secretion was observed both in pharmacologically inhibited and NCLX KO astrocytes, indicating that NCLX activity can modulate glycolytic flux. *In vivo* hippocampal NCLX genetic deletion in astrocytes improved cognitive performance of mice in novel object recognition and Y-maze tasks. Interestingly, animals with neuronal hippocampal NCLX deletion showed an opposite trend, showing poorer performance in these tasks. In summary, our data suggest that modulation of astrocytic NCLX activity may be an important connecting hub between Ca^{2+} signaling, mitochondrial metabolism, and glycolysis. *The authors marked with an asterisk equally contributed to the work.

P-04.5-003
Role of peroxiredoxin 6 in biosynthesis of anti-diabetic and anti-inflammatory FAHFAs

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Fatty acid esters of hydroxy fatty acids (FAHFAs) represent a diverse group of recently discovered lipids with distinct biological activities – mainly anti-diabetic and anti-inflammatory. However, their metabolism has not been fully elucidated yet. Peroxiredoxin 6 (Prdx6), which belongs to a family of antioxidant enzymes, was shown to be involved in biosynthesis of FAHFA. Prdx6 is a multifunctional enzyme with ability to repair peroxidized membrane phospholids through phospholipid hydroperoxide GSH peroxidase (PHGPx), phospholipase A_2 (PLA_2) and lysophosphatidylcholine acyl transferase (LPCAT) activities. During this process, it generates precursors that could serve as a source of hydroxy fatty acids needed for FAHFA synthesis. In this study, we used three different mouse models with genetically altered Prdx6 to a certain extend and a wild-type group as a control. Collected tissues were extracted and subjected to lipidomic profiling together with targeted analysis of FAHFA using LC-MS platforms. Supported by the Ministry of Education, Youth and Sports of the Czech Republic (LTAUSA18104).

P-04.5-004
Simulated marine heatwaves alters green-lipped mussel metabolic regulation: A near-natural experimental approach

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Marine heatwaves and elevated sea surface temperatures are expected to increase in frequency and intensity, with catastrophic effects on New Zealand aquaculture species, such as the green-lipped mussel (*Perna canaliculus*). Thermal stress affects mussel health, metabolic regulation and physiological functions, illustrating the kind of adaptive capacity that allows organisms to survive heatwaves. In this study, mussels were exposed to 3 near-natural seawater temperature regimes: 1) low (18°C), 2) medium (18-24°C ramped +1°C/week) and 3) high (18-24°C ramped +2°C/week). Sampling was conducted over time to determine the effect of simulated marine heatwaves on survival, condition index, immune system and the metabolome of *P. canaliculus*. Temperature increases resulted in the greatest mortalities in the high temperature group in the middle of the experiment, with the surviving animals experiencing a metabolic shift aimed at aerobic energy production for the activation of molecular defence mechanisms. Additional evidence for immune functions was seen by cytology results where temperature affected haemocyte counts, superoxide positive haemocytes and levels of total antioxidants. Increased antioxidant metabolites were seen at high temperatures, possibly as a means to counteract the production of oxidative stress. Mussels utilised anaerobic metabolism via the succinate pathway for energy production likely to sustain biological functions and survival. The effect of time was largely seen on long chain fatty acids, where increases were seen at weeks 7 and 8, linked to membrane storage functions, whereafter decreases at week 11 can be attributed to energy metabolism. This study demonstrates that integrated physiological assessments are required to investigate how mussels respond to heatwaves, with specific metabolic pathways and cellular markers now highlighted for future investigations, with the potential to identify resilient mussel traits and support industry management.

P-04.5-005
Riboregulation of human serine hydroxymethyltransferase activity in cellular metabolism

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Serine hydroxymethyltransferase (SHMT) is an enzyme that catalyses the reversible conversion of serine and tetrahydrofolate into glycine and 5,10-methylenetetrahydrofolate. SHMT plays a pivotal role in the one-carbon metabolism (OCM), a complex network fuelling the biosynthesis of nucleotide precursors, NADPH fundamental for highly proliferating cells. As expected SHMT isoforms are overexpressed in tumours: indeed, SHMT is considered as a good chemotherapeutic target candidate [Paone et al. (2014) Cell Death Dis. 5(11)e1525]. Unfortunately, although several molecules have been proposed and tested in the past years, now there aren't inhibitors that can be successfully used in vivo [Ducker, J. et al. (2017) PNAS 114, 11404-11409], for this reason a completely new approach is needed. We have previously demonstrated that SHMT1 is a moonlighting protein [Guiducci G. et al (2019) NAR, 47, 4240–4254]: beyond its enzymatic function it can bind RNA. In detail, we revealed that SHMT1 has the capability to bind 5' UTR of SHMT2 isoform regulating its concentration. We also observed that this RNA binding selectively inhibits the SHMT1 enzymatic activity: the conversion of serine to glycine is significantly affected in presence of inhibitory RNA sequences. These results suggest that the RNA-mediated inhibition may contribute to the control of serine

consumption by SHMT1. To better understand what happens when SHMT binds RNA now we are using plasmids controlled by the Tetracycline-inducible system to finely regulate the expression of the shmt2-derived UTR sequences and studying energy metabolism, survival and proliferation rate of cancer cells. Our data suggest that the moonlighting function of SHMT1 could be used as a Trojan horse to control cancer cell OCM and inhibit cancer growth. Due to the low efficiency in vivo of the small molecule inhibitors tested in past years, we are focusing on this alternative approach based on inhibitory RNA that it seems to be a promising strategy.

P-04.5-006
O-GlcNAc characterization during *Tribolium castaneum* development

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O-GlcNAc (O-linked β-N-acetylglucosamine) is a dynamic intracellular post-translational modification (PTM) that regulates several cellular and metabolic processes. This PTM is catalyzed by the covalent attachment of GlcNAc on residues of serine/threonine in proteins by the enzyme O-GlcNAc transferase (OGT), while its removal is performed by O-GlcNAcase (OGA). This PTM is limited by the concentration of UDP-GlcNAc, the end-product from the hexosamine biosynthetic pathway (HBP) that, together with OGT and OGA levels and activity, will regulate protein O-GlcNAcylation. Although O-GlcNAc has been reported in all studied metazoans, functional analysis during development in Hexapoda is restricted to the model organism *Drosophila melanogaster*. In this study, the role of O-GlcNAc during development was investigated in an emerging model system, the beetle *Tribolium castaneum*. Comparison of the transcriptional profile of the HBP rate-limiting step enzyme glutamine-6-phosphate amidotransferase (GFAT) during developmental stages showed higher levels during larval stages. Interestingly, OGT is modulated during embryonic development with an increase of mRNA levels during gastrulation while OGA remained unaltered in all stages. *In situ* hybridization analysis revealed an embryonic limited ubiquitous pattern of OGA during egg development, while GFAT is expressed in both embryonic and extra-embryonic cells. Finally, parental knock down of OGT by RNA interference (RNAi) shows physiological impairment of oocyte maturation and a decrease in egg laying and embryonic survival, while OGA knockdown did not lead to physiologically and phenotypic significant changes. Altogether, these results provide an important characterization of the O-GlcNAc machinery in the most diverse order of insects, and suggest a critical role of O-GlcNAcylation in a stage-specific manner during *Tribolium castaneum* development.

P-04.5-007
Sex and age-related effects of insulin therapy on the subcellular activity of arginase and nitric oxide synthase in peripheral blood leukocytes of patients with type 1 diabetes mellitus

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Arginase and nitric oxide synthase (NOS) are reciprocally regulated and involved in immune cell function and autoimmune diseases, including type 1 diabetes mellitus (T1DM). We aimed to study the sex and age-related changes in the subcellular activity of arginase and NOS these enzymes in peripheral blood leukocytes (PBL) of patients with T1DM, depending on glycemic status and duration of insulin therapy. Armenian girls and boys with recent-onset T1DM (RO-T1DM) and long-term T1DM (LT-T1DM) were enrolled in the study. HbA1c test used to control glycemic status. Arginase activity increased in the cytoplasm and mitochondria of PBL in all patients with RO-T1DM, compared to healthy volunteers. In pre-adolescent girls, long-term insulin therapy normalized arginase subcellular activity with good glycemic control, while in boys with LT-T1DM, it increased, regardless of glysemic status. In pre-adolescent patients the production of bioavailable NO in cell compartments significantly decreased, regardless of glycemic control and duration of T1DM. In boys with LT-T1DM with good glycemic status, it returned to normal in the cytoplasm. With RO-T1DM in adolescent girls, NO production increased above normal in cell compartments, while in boys it decreased by almost 5 times, although it improved with good glycemic control. With LT-T1DM in girls NO production returned to normal in the cytoplasm, but dropped in mitochondria, and in boys it kept reduced in both cell compartments, regardless of glycemic status. In PBL of young patients with T1DM arginase activity and the production of bioavailable NO/nitrite in the cytoplasm and mitochondria of circulating leukocytes change differentially up to opposite depending on sex, puberty, glycemic status and the duration of T1DM. The results obtained can be useful for understanding the heterogeneity of T1DM and developing new therapeutic strategies for treating T1DM.

P-04.5-008
Butyrate treatment of DSS-induced ulcerative colitis affects the hepatic drug metabolism in mice: role of microbiome

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Gut microbiome significantly contributes to regulation of diverse host physiological functions and when corrupted it is implicated

with various pathologies. One of such diseases where the intestinal microbiome plays a crucial role are inflammatory bowel diseases (IBD). Moreover, gut microbiome influences pharmacotherapy outcome by affecting the biotransformation and pharmacokinetics of a wide range of clinically used drugs. For this reason, it is very important to deepen our knowledge of the effect of gut inflammation and followed therapeutic interventions on the ability of the organism to metabolize drugs. Butyrate with its well-known anti-inflammatory effect is auspicious candidate as alternative for pharmacotherapy of IBD. However, the effect of butyrate on the hepatic drug metabolism is unknown. The aim of presented study was to clarify not only the protective effects of the butyrate on colonic inflammation induced by dextran sulfate sodium but also to evaluate the effects of butyrate on hepatic drug metabolism in inflammation murine model. To assess the role of microbiome, the both germ-free (GF) mice lacking the intestinal flora, and control, specific-pathogen-free (SPF) mice were used. Besides the ability of butyrate to alleviate gut inflammation in our model, it was observed that administration of butyrate (as a therapeutic intervention) alongside with gut inflammation influences the hepatic drug metabolism through modulation of expression and enzyme activity of cytochromes P450 (CYPs). Especially, enzyme activities of hepatic CYP2C and CYP3A was significantly affected in mice with colitis and/or by butyrate administration. Differences between SPF and GF mice have shown the importance of gut microbiome presence in the regulation of selected CYPs expression during inflammation. In summary, these data clearly highlight that butyrate affects inflammation process and hepatic drug metabolism by modulation of expression and enzyme activity of CYP enzymes.

P-04.5-009
Serine hydroxymethyltransferase: more than a metabolic enzyme

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Serine hydroxymethyltransferase (SHMT) is a metabolic enzyme involved in the reversible conversion of serine and tetrahydrofolate, into glycine and N5,N10- methylenetetrahydrofolate. This protein is a pyridoxal 5' phosphate dependent enzyme, whose aggregation state is tetrameric, working as a dimer of obligate dimers. SHMTs is a part of a complex network of metabolic pathways, the one carbon metabolism (OCM) that fuels the synthesis of nucleotides, NADPH homeostasis, exploitable by cancer cells to grow and proliferate without control. Given the central role of this protein in sustaining anabolic reactions, SHMTs are overexpressed in several types of tumors, being an interesting target for cancer therapy. In humans, there are two genes encoding SHMT, encoding a cytosolic (SHMT1) and a mitochondrial (SHMT2) isoform. In addition to having a catalytic activity, it has shown SHMT1 also has nucleic acids binding affinity. In particular, in cytosol SHMT1 binds SHMT2 transcript 5'UTR, through which negatively regulates SHMT2 expression; in parallel mRNA ribo-regulates catalytic activity of SHMT1, by inhibiting the conversion of serine to glycine without significantly affecting the opposite reaction (Guiducci et al. (2019) NAR 47, 4240-4254). Furthermore, SHMT1 also translocates into the nucleus (during S and G2/M phase of cell cycle) or in response to DNA damage (after UV exposure), where it makes a protein complex with dihydrofolate reductase and thymidylate synthase for de novo thymidylate biosynthesis in situ (Amanda J. MacFarlane et al. (2011) Cancer Res. 71, 2098-107). Therefore, the aim of my project is to characterize the molecular basis of SHMT1-DNA interaction into the nucleus and the eventual role of this interaction also in the cytoplasm, in response to those events promoting cytoplasmic DNA accumulation (infectious agents, genomic instability, genotoxic xenobiotics, genetic diseases).

P-04.5-010
The metabolic and physiological responses of daphniids upon chronic exposures to pharmaceuticals

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In recent years pharmaceuticals have been recognised as emerging contaminants of significant concern as via several pathways of entry, pharmaceuticals eventuate into freshwater systems where they pose sub-lethal effects to aquatic organisms. Exacerbated by their increased consumption and poor removal rates from waste-water treatment plants, pharmaceutical compounds have been classed as pseudo-persistent in the aquatic environment and their monitoring is imperative. In some cases, their environmental levels could be undetectable, thus, highlighting the need for sensitive tools to monitor the aquatic environment. In this study, molecular and phenotypic endpoints of *Daphnia magna* were measured following chronic exposure to five frequently detected pharmaceuticals in surface waters. Markers of enzyme activities were combined with metabolic perturbations to assess the impact of diclofenac, metformin, gabapentin, carbamazepine, gemfibrozil and their mixtures on daphniids. Physiological markers of enzyme activities included phosphatases, β-galactosidase, peptidase, lipase, lactate dehydrogenase, glutathione-S-transferase and glutathione reductase activities and revealed significant trends upon different exposure. These observations were also accompanied by metabolic perturbations in glycolysis, the pentose pathway and the TCA cycle which revealed distinct groups and metabolic fingerprints for each pharmaceutical and their mixture. Keywords: *Daphnia magna*, pharmaceuticals, chronic, metabolomics

P-04.5-011
Biochemical studies on the effect of non-enzymatic succinylation of electron transfer flavoprotein

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Non-enzymatic post-translational modifications, such as glutarylation and succinylation, constitute major players in the control of metabolism via sirtuin-mediated regulatory pathways. Despite

of the current available knowledge on these acylations, the structural and functional consequences of these modifications on mitochondrial proteins remain to be elucidated. Recent studies showed that increasing amounts of glutaryl-CoA lead to glutarylation of glutaryl-CoA dehydrogenase (GCDH), a flavoprotein involved in the metabolism of tryptophan, lysine and hydroxylysine, affecting its enzymatic activity (Ribeiro JV et al. (2020) BBA-Proteins and Proteomics 1868, 140269, Bhatt DP et al. (2022) J Biol Chem 101723). Currently, we are evaluating the effect of succinylation on electron transfer flavoprotein (ETF), a vital mitochondrial protein that participates in fatty acid, amino acid and choline metabolism (Henriques BJ et al. (2021) Gene 776, 145407). Using a biochemical multidisciplinary approach, that combines *in vitro* and *in silico* methods, we showed that ETF succinylation abolishes the enzymatic activity of the protein, without causing major structural changes on the native structure. Also, the activity impairment can be reverted by sirtuin 5, a NAD^+-dependent protein deacylase. Tryptic digestion of the *in vitro* modified ETF allowed the identification by LC-MS/MS of 8 succinylated lysines, two of which are in the region surrounding the flavin binding pocket. Carrying out a mutagenesis analysis, we confirmed that negative charges in this region alter the flavin cofactor's properties and impair ETF function. Indeed, *in silico* studies demonstrated that electron transfer in the succinylated protein is less efficient, a likely explanation for the compromised enzymatic activity. Altogether, our results reveal possible molecular mechanisms for the observed effects of acylations on metabolic proteins.

P-04.5-012
Cellular studies to evaluate mitochondria function on MADD, a rare metabolic disorder

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Multiple acyl-CoA dehydrogenase deficiency (MADD) is an inborn disorder of fatty acid, amino acid and choline metabolism triggered by mutations on the genes encoding for electron transfer flavoprotein (ETF) or ETF:ubiquinone oxidoreductase (ETF:QO) proteins (Henriques et al (2021) Gene, 776, 145407). This rare metabolic disorder presents broad clinical phenotypes and variable evolution, and treatment is based on a restricted diet, fasting avoidance and supplementation with riboflavin and/or carnitine. In the last decade we have made several contributions to understand MADD molecular mechanism such as: provide a molecular rationale for impaired protein function in a ETF:QO variant (Lucas et al (2020) BBA-Proteins and Proteomics, 1868 (6), 140393), or gathered genotypic, clinical and biochemical data for a set of MADD patients to correlate biochemical and clinical outcomes with the effects on protein structure and stability (Henriques et al (2019) Current Molecular Medicine, 1868 (1), 140269). Nevertheless, the tie between MADD variants and mitochondria dysfunction, that could explain the unpredictable disease outcomes, remains to be fully explored. Here we report a comprehensive study of mitochondrial health on mild-MADD patient-derived fibroblasts, by characterizing mitochondria morphology and bioenergetics. Our results indicate that MADD fibroblasts have altered electron transport chain (ETC) complexes content, decreased membrane potential, lower ATP content, and raised ROS production. We were also able to analyze mitochondria network and observed that patient-derived cells display heterogeneous organelle morphology. Under riboflavin supplementation these cells did not improve the analyzed parameters. Overall, our data indicates that in MADD patients fibroblasts mitochondria is compromised, supporting our hypothesis that ETF:QO missense mutations lead to cellular and mitochondrial impairment.

P-04.5-013
3D liver organoids as an *in-vitro* model to explore the metabolic requirements of liver progenitor cells

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Liver progenitor cells (LPCs) derive from bile ducts and are facultative stem cells that contribute to liver regeneration upon severe injury. Following chronic hepatocyte damage, cholangiocytes de-differentiate into LPCs, invade the surrounding parenchyma and expand to repopulate the liver, a process referred to as ductular reaction (DR). LPCs can be clonally expanded *ex-vivo* as self-assembling 3D liver organoids, that serve as an experimental model of DR. Liver organoids show stem cell features including high proliferative potential and differentiation capacity into both hepatocytes and ductal cells or cholangiocytes. However, the molecular mechanisms by which cholangiocytes acquire cellular plasticity, initiate organoids and proliferate to regenerate the damaged tissue remain poorly understood. In particular, the role of cell metabolism in controlling this process is unknown. To explore the role of cellular metabolism in controlling LPCs expansion, we designed a custom screening of both inhibitors and activators of key metabolic pathways using liver organoids. Using this platform, we were able to identify several molecules able to either increase or limit LPCs expansion and their capacity to generate organoids. In particular, we report that, during regeneration, LPCs undergo a metabolic remodelling characterized by up-regulation of anabolic signals required for lipid biosynthesis. Inhibition of these metabolic pathways, using the FDA-approved drugs, was sufficient to blunt the viability of LPCs, while having no effect on terminally-differentiated hepatic cells. Conversely, we found that molecules that fine-tune the cellular redox state were able to potentiate the proliferative capacity of LPCs in our experimental setup. Our results provide new insight into the mechanisms of liver regeneration and unveil metabolic addictions of LPCs, during DR, that can be therapeutically exploitable in patients with chronic liver diseases.

P-04.5-014
Toxicity responses of 1-butyl-3-methylimidazolium ionic liquids on daphniids – metabolic implications and responses

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Ionic liquids have been described as green solvents developed with the intention of replacing some of the more unsavory

volatile organic compounds used as solvents in industry due to the number of hazards the latter pose to organisms and the environment. Ionic liquids are organic salts made of bulky organic, asymmetric cations and small organic or inorganic anions. In this study, five ionic liquids containing the 1-butyl-3-methylimidazolium (BMIM) as cation were compared for their toxicity on daphniids. Daphniids were exposed for 24 hours to each BMIM ionic liquids (hexafluorophosphate, chloride, methanosulfonate, tetrafluoroborate and hexafluoroantimonate) in the absence of food. Lethality, assessed as immobilization of animals, showed similar levels for all ionic liquids and an EC5 concentration was selected for acute exposures. Enzyme activities were used as markers to assess the impact on physiology of daphniids on acute and chronic exposures. Specifically, activities of key enzymes for phosphate metabolism (acid and alkaline phosphatases), sugar, lipid and protein catabolism (galactosidase, lipase and peptidase, respectively), as well as enzyme responsive to xenobiotics (glutathione-S-transferase) were assessed with kinetics to illustrate the effects of ionic liquids on daphniids. Phosphatase activities were significantly decreased in all exposure scenarios to ionic liquids. Different patterns of responses were documented among acute and chronic exposures and the different ionic liquids, indicating distinct toxicity patterns. Further investigation to the level of metabolic networks was performed by targeted liquid chromatography coupled with mass spectrometry and revealed significant changes in metabolic fingerprints.

P-04.5-015
Biochemical parameters of quail blood in experimental gastrointestinal tract candidiasis

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The aim of the work is to investigate changes in the biochemical profile of Quails blood experimental infection with *Candida albicans* ATCC 10231. Female Texas white broiler quails 21 days old, 30 heads, 350 g, were used. The birds were divided into experimental and control groups, 15 in each. The experimental group was infected with *C. albicans* 1 mL (concentration 0.5 McFarland) through a digestive probe once a day for five days. The experiment lasted for 20 days: five days for infection and 15 days for the course of the experiment. Blood hematology was carried out using a CELL-DYN 3700 analyzer, within 45 min after blood collection, for the total red-blood-cell count (RBC), hemoglobin (HGB), and differential white-blood-cell count/mean leukocytes (LEU) including pseudoeosinophils (PSEUs), eosinophils (EOSs), monocytes (MONOs), basophils (BASs), and lymphocytes (LYMs). The biochemical analysis of blood was performed on the serum within 24 h. Creatinine (CRE), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), glucose (GLU), mass concentration of cholesterol (CH) and triglycerides (TG), and total bilirubin (TBIL) estimations were carried out using Vetscan VS 2 analyzers. In experiment: RBC,1012-1.86; HGB,g/L-85.4; LEU,109-40.5; PSEU,%-22.2; EOS,%-3.3; MONO,%-3.4; BAS,%-0.30; LYM,%-71.5; CRE,mmol/L-20.6; AST,ME/L-2.1; ALT,ME/L-0.11; ALP,ME/L-3.7; GLU,mmol/L-13.7; CH,mmol/L-4.3; TG, g/L-1.11; TBIL,mmol/L-1.6. Control: RBC-1.42; HGB-77.2; LEU-24.8; PSEU-14.1; EOS-2.6; MONO-1.9; BAS-0.24; LYM-60.2; CRE-14.3; AST -0.9; ALT-0.08; ALP-2.4; GLU-11.4; CH-3.2; TG-0.70; TBIL-0.9. Blood samples indicators were higher in the experimental group compared to control the group. This paper has been supported by the RUDN University Strategic Academic Leadership Program and Research project № 031622-0-000 of the Department of Microbiology and Virology RUDN Medical Institute (Moscow, Russia) under the direction of Podoprigora I.V.

P-04.5-016
Unraveling diterpenoid alkaloid biosynthesis in *Aconitum*

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Diterpenoid alkaloids (DA) are specialized metabolites found mainly within the *Aconitum* plant genus which has a long history of use in Chinese traditional medicine. They are highly toxic upon ingestion and even topical applications, leading to heart failure or — in worst cases — death. The infamous reputation is owed to the presence of C_{19} diterpenoid ester alkaloids, best seen in the most famous DA representative, aconitine. In addition, various medicinal roles (e.g. anticancer, antiarrhythmic, antimicrobial, anti-inflammatory) and structural complexity of DAs have long captivated scientists. Out of over 1,000 reported DAs, only a handful can be produced by total synthesis, which limits their usage in the pharmaceutical industry. Furthermore, although some of the initial enzymes involved in their biosynthesis have been characterized, the complete biosynthetic network remains to be elucidated. We analyzed the chemical space of five *Aconitum* species by means of LC-MS/MS, and based on the uniqueness of their DA profiles, we chose two species (*Aconitum lycoctonum* and *Aconitum plicatum*) for *de novo* transcriptome assembly with the goal of elucidating the biosynthetic pathway of aconitine-like diterpenoid alkaloids. Metabolomic analysis of *A. plicatum* and *A. lycoctonum* tissues (root, leaf, stem, flower, fruit) showed the highest presence of unique DAs in the roots, with all the tissues showing distinct metabolic fingerprints in relation to the amount of DAs. According to the structure of DAs, we hypothesized the enzymatic steps needed for the biosynthesis of aconitine which, together with metabolomic and transcriptomic data, will be used for the prioritization of candidate genes. Upon completion, the obtained results will open an avenue for the biosynthetic production of medicinally valuable DAs using metabolic engineering.

P-04.5-017
Metabolic rewiring of epithelial cells and myofibroblasts during kidney fibrosis

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Chronic kidney disease (CKD) affects 10% of the population and fibrosis driven by excessive accumulation of extracellular matrix (ECM) is the hallmark of CKD. Myofibroblasts are the

key ECM producing cells and are activated by cross-talk with injured proximal tubule and immune cells. Although a metabolic derangement is identified in CKD pathophysiology, the metabolic requirements of kidney cells during this response remain elusive. We characterized the metabolic phenotype of proximal tubular epithelial cells (CD10+) and myofibroblasts (PDGFRβ+) by using immortalized human cells, kidney organoids and the aristolochic acid nephropathy (AAN) mouse model by combining Seahorse, SCENITH and gene expression technologies. Basally, CD10+ cells relied on fatty acid oxidation (FAO), while PDGFRβ+ cells mainly depended on glycolysis. Under an AA-induced profibrotic stimuli, both FAO and glutaminolysis were impaired in CD10+ cells, whereas both glycolysis and glutaminolysis were increased in PDGFRβ+ cells. In kidney organoids, AA treatment reduced mitochondrial respiration and enhanced glycolysis. In this system, KIM1+ damaged epithelial cells exhibited impaired mitochondrial function, while PDGFRβ+ cells exhibited higher dependence on glycolysis and glutaminolysis. In AAN renal tissue, FAO dependence was reduced in KIM1+ cells, whereas PDGFRβ+ cells exhibited enhanced dependence on mitochondrial function, glycolysis and glutaminolysis. This metabolic reprogramming likely contributes to development of kidney fibrosis since FAO gain-of-function in CD10+ cells reduced the expression of markers associated with epithelial cell dedifferentiation. In turn, FAO gain-of-function and both glutaminolysis and glycolysis inhibition reduced the expression of ECM genes in PDGFRβ+ cells. In kidney organoids, glycolysis inhibition counteracted the AA-induced ECM production. Strategies based on the modulation of these metabolic shifts may prove useful in therapies against renal fibrosis and CKD.

P-04.5-018
Branched chain amino acids are an important energy source in cancer cells

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It is known that degradation of branched chain amino acids (BCAAs) — leucine, isoleucine and valine — is one of the common pathways in different cancer cells. The degradation of BCAAs could support the growth of cancer cells, though the mechanisms of this process are still unclear. We performed ^{13}C experiments in order to clarify the role of BCAA degradation in breast cancer cell lines MCF-7 and BCC. Our results have shown that BCAA degradation supports up to 36% of the energy production via respiration in MCF-7 cells, and ~14% in BCC cells. Our findings propose that inhibition of leucine degradation could affect cell proliferation, when the cell capability for the *de novo* synthesis of cholesterol decreases. About 67% of the synthesis of cholesterol precursor mevalonate in MCF-7 and 30% in BCC was originated from leucine degradation pathway. Labelling pattern of mevalonate was used for Metabolic Flux Analysis, the obtained results showed that leucine degradation determine production of an abundant amount of acetoacetate. We suggest that acetoacetate could be used for lipid biosynthesis after transfer from the mitochondria to cytosol. The fraction of labelled mitochondrial acetyl-CoA was estimated. Interestingly, there was no difference between fractions for the experiment with labelled leucine, and the three BCAAs labelled in BCC cells. So, we predict that only leucine is responsible for the supply of mitochondrial acetyl-CoA.

P-04.5-019
The role of seminal plasma polyamines in the biological effects of microwave irradiation on human semen *in vitro*

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The aim of the present study was to investigate the influence of millimeter-wave electromagnetic (MW) irradiation on normal and pathological human sperm *in vitro*, and to evaluate a possible role of polyamines (PA) in this process. The stability of sperm membranes, the number of apoptotic gametes, and the content of seminal plasma PA in the ejaculates of fertile and subfertile men were compared before and after short-term MW electromagnetic exposure *in vitro*. The ejaculate samples were collected from healthy donors [n = 25, age 22-38 years old (y.o.), average age 30.6 ± 1.1 y.o. (mean ± SEM)] and from subfertile men (n = 78, age 25-48 y.o., average age 34.1 ± 0.8 y.o.) and exposed to MW radiation. The electromagnetic field had a wavelength of 7.1 mm, a frequency of 42.194 GHz and an exposure time of 20 min. The fragility of sperm membranes was evaluated by their resistance to sodium chloride solution (Milovanov test) and to acetic acid (Joel test). Acrosin activity was assayed spectrophotometrically. Apoptosis was determined by the externalization of phosphatidylserine on the outer side of the sperm membrane and propidium iodide staining. The PA levels were determined by agar gel electrophoretic fractionation. An increase in the resistance of sperm membranes, a decrease in acrosin activity, a decrease in the number of apoptotic gametes and a decrease in the seminal plasma PA concentrations were found after exposure of the native human sperm to low-intensity MW irradiation. Two types of reactions were revealed for the subfertile samples. The results revealed positive bio-effects of specific microwaves on the human semen and the participation of PA in the realization of these effects.

P-04.5-020
Assessing biological oxidant ligand affinity of peroxiredoxins by computer assisted molecular simulations and biochemical approaches

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Peroxiredoxins (Prx) are ubiquitous enzymes that use redox-active cysteines to reduce hydroperoxide. Prx can be divided into two groups, the 1-Cys and 2-Cys Prxs, based on the number of

cysteine residues involved in catalysis. In some organisms these enzymes can scavenge up to 90% of all hydrogen peroxide produced in the cells. Additionally, Prx are able to decompose organic hydroperoxides (OHPs). The challenge with these compounds is able to enhance their expression and the deletion of Prx confers high sensitivity to OHPs. To date, most of the investigations use synthetic OHPs as substrates, and few studies have tested biologically important substrates to evaluate the activity of these enzymes. In this work we evaluated the affinity of biological OHPs, derived from lipids and nitrogen bases, by molecular docking and assess the enzymatic activity over OHPs for distinct Prx from four different organisms: Prx 2-Cys from bacteria (AhpC from *Escherichia coli* and *Pseudomonas aeruginosa*) and humans (Prx2) and the Prx 1-Cys from the pathogenic fungus *Paracoccidioides brasiliensis*. The docking results indicated high affinity for elongated organic hyperoxides such as monounsaturated and polyunsaturate fatty acids (MUFAs and PUFAs) presenting free Gibbs energy (ΔG) ranging from -6,8 to -4.5 kcal/mol. The peroxide function is very close to the catalytic Cys (3.70–3.30 Å), the hydrophobic carbon moieties are stabilized by several hydrophobic interactions and the carboxylic acid by hydrogen bonds from several residues very conserved among the different enzymes. Accordingly, the biochemical approaches revealed that both classes of Prx are able to decompose MUFAs derived hydroperoxides more efficiently than hydrogen peroxide or synthetic OHPs (10-20 ×). Since MUFAs and PUFAs are important molecules in oxidative stress and signaling events, our results suggest that the Prx may play important roles modulating the OHPs levels in the living cells. Financial Support: FAPESP and CNPq

P-04.5-021
Stem cell factor modulates endothelial glycolysis at hypoxia

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Angiogenesis, the formation of new blood vessels, occurs in response to hypoxia. Endothelial cells greatly rely on glycolysis to generate the energy required for this process. Here, we explored the previously unrecognized role of stem cell factor (SCF) in endothelial metabolism during hypoxia-driven angiogenesis. At hypoxia, SCF significantly upregulated key glycolysis-related genes and increased glycolysis in human endothelial cells. Mechanistically, SCF-induced increase in endothelial glycolysis occurred through promoting the translation of hypoxia-inducible factor (HIF) 1*a* and HIF 2*a* via Akt and Erk pathways. Moreover, inhibition of glycolysis using chemical inhibitors blocked the SCF-induced *in vitro* angiogenesis in human endothelial cells at hypoxia. In mice with oxygen-induced retinopathy, a hypoxia-driven angiogenesis model, blockade of SCF signaling using c-KIT mutant mice substantially reduced the expression of glycolysis-related genes and suppressed pathological angiogenesis in the retina. These findings demonstrate that SCF is a novel regulator of endothelial glycolysis in hypoxia-driven angiogenesis process and suggest anti-SCF therapy as a potential approach to inhibit endothelial angiogenic switch in hypoxic tissues and treat diseases dependent on pathological angiogenesis.

P-04.5-022
Hispidin biosynthesis catalyzed by plant type III polyketide synthases in mammalian cells

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Bioluminescent systems are found among different phyla. Various techniques in molecular biology are based on bioluminescent processes, and new applications are constantly emerging. Our research group has characterized a bioluminescent system from the fungus *Neonothopanus nambi*. Bioluminescence results from a chain of reactions catalyzed by different fungal enzymes with the final steps of fungal luciferin (3-hydroxyhispidin) biosynthesis and oxidation. The fungal luciferin derives from caffeic acid through a couple of reactions catalyzed by two enzymes. The first enzyme in the chain is a multidomain hispidin synthase protein. Literature analysis suggests that hispidin might alternatively be produced from caffeic acid or caffeoyl-CoA by the plant type III polyketide synthases (PKSs), and we have proven that indeed PKSs can catalyze hispidin production from caffeoyl-CoA. PKSs might be advantageous over the fungal hispidin synthase since PKS is a smaller protein molecule. We compared activity of eleven PKSs to hispidin synthase on mammalian cells, and seven PKSs efficiently catalyzed caffeoyl-CoA conversion into hispidin. In this work we also identified the most effective of these enzymes for heterologous expression in mammalian cell cultures. The reported study was funded by Russian Science Foundation, grant 21-74-00075, https://rscf.ru/project/21-74-00075/.

P-04.5-023
Tissue transglutaminase knock-out preadipocytes and beige cells of epididymal fat origin possess decreased mitochondrial functions required for thermogenesis

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During cold exposure, beige adipocytes with thermogenic function are activated in white adipose tissue through the process of browning. These cells, similarly to brown adipocytes, dissipate stored chemical energy in the form of heat with the help of uncoupling protein-1 (UCP1). Recently, we have presented that tissue transglutaminase (TG2) knock-out mice have decreased cold tolerance because of reduced browning and lower utilization of their epididymal adipose tissue (Mádi A et al. (2017) Biochim. Biophys. Acta. Mol. Cell Biol. Lipids. 1862 (12), 1575- 1586) To explore the underlying molecular mechanism, we have isolated preadipocytes from epididymal adipose tissue of wild-type ($TG2^{+/+}$) and TG2 knock-out ($TG2^{-/-}$) mice and differentiated them to beige direction. Although $TG2^{+/+}$ and $TG2^{-/-}$

preadipocytes differentiate similarly, the mitochondria of the knock-out beige cells have multiple impairments including an altered electron transport system generating lower electrochemical potential difference, reduced oxygen consumption and UCP1 expression, and a higher portion of fragmented mitochondria. These differences are detectable in the precursor preadipocytes and the differentiation process cannot overcome the functional disadvantages completely. $TG2^{-/-}$ beige adipocytes produce more DIO3 which may decrease thyroid hormone levels required for establishing optimal thermogenic potential. Both $TG2^{-/-}$ preadipocytes and beige cells are hypometabolic compared to the $TG2^{+/+}$ controls which can be explained by the lower expression of solute carrier proteins SLC25A42, SLC25A47, and SLC25A42 responsible for the transportation of acylcarnitine, Co-A, and amino acids, respectively, into the mitochondrial matrix. As a result, the mitochondria in $TG2^{-/-}$ beige adipocytes cannot reach the energy-producing threshold required for normal thermogenic functions leading to decreased cold tolerance of $TG2^{-/-}$ mice.

P-04.5-024
The role of mitochondrial phospholipase A2γ in the regulation of cellular redox homeostasis and oxidative stress signalling

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Redox-dependent regulations play an essential role in a wide range of biological activities. Mitochondria in numerous tissues represent a primary source of superoxide and subsequent downstream oxidants, notably H_2O_2 and lipid hydroperoxides. However, the understanding of the role of mitochondrial oxidant production in pathology and normal physiology is limited. Mitochondrial calcium-independent phospholipase $A_{2\gamma}$ ($iPLA_{2\gamma}$) belongs to a family of enzymes that participate in cellular signalling by simultaneously producing free fatty acids (FAs) and lysophospholipids. Here we hypothesize that redox activation of $iPLA_{2\gamma}$ has an antioxidant effect on the brain, as it contributes to FA-dependent H^+ transport, which leads to subsequent partial attenuation of mitochondrial oxidant production. We demonstrate the oxidant-induced activation of $iPLA_{2\gamma}$ by increasing respiration in brain mitochondria isolated from wild-type mice. The oxidant-induced increase in respiration was prevented by (1) R-bromoenol lactone (R-BEL), a selective inhibitor of $iPLA_{2\gamma}$, (2) carboxyatractyloside, an inhibitor of adenine nucleotide translocase (ANT), and (3) bovine serum albumin, the carrier of FAs. Oxidant-induced changes in respiration were absent in mitochondria isolated from $iPLA_{2\gamma}$-KO mice. Employing detailed lipidomic analysis, we also established a typical cleavage pattern for activated $iPLA_{2\gamma}$. The data show an increase in relative concentrations mainly of docosahexaenoic, arachidonic, and stearic acid which was prevented by R-BEL but not by ANT inhibitor. The acute antioxidant role of $iPLA_{2\gamma}$-released FAs is supported by monitoring both intramitochondrial superoxide and extramitochondrial H_2O_2 release. These results suggest that the redox-activated $iPLA_{2\gamma}$ releases free fatty acids, which promote ANT-dependent H^+ transport, leading to a decrease in the mitochondrial protonmotive force and subsequent attenuation of mitochondrial superoxide production.

P-04.5-025
Drug contamination in Baikal endemic amphipods

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This study was aimed to detect trace amounts of drugs in littoral crustaceans of ancient Lake Baikal. For the first step, these drugs were presented by ibuprofen and azithromycin. Ibuprofen is widely used to reduce fever and pain. Azithromycin is an antimalarial drug, that was recently reported to be active against acute respiratory coronavirus syndrome. Endemic crustaceans related to species *Eulimnogammarus verrucosus* were collected near village B. Goloustnoe. Animals were homogenized with acetonitrile and shaken intensively. Then precipitation of proteins was performed with a solution of TCA, and centrifuged. The supernatant was removed and filtered in chromatographic vials. The analysis was performed using an HPLC Agilent 1290 Infinity coupled with Agilent 6470 Triple Quadrupole. Mobile phase A consisted of 0.1% formic acid in the water, and mobile phase B was presented by 100% acetonitrile. The registration of ions was performed in different modes, such as: Full scan, MRM, Product Ion. For reliable identification of drug contaminants, we used the pharmaceutical substances of azithromycin and ibuprofen. Thus, here we first time reliably detected drugs in the amphipods of Lake Baikal and demonstrated the contamination of the Baikal ecosystem by azithromycin and ibuprofen. It indicates the ability to accumulate the drug substances from wastewater by amphipods, and increasing anthropogenic load on the ancient lake. The study is carried out with the primary financial support of the Grant of the President of the Russian Federation No MK-1245.2021.1.4. The research was funded by the Ministry of Science and Higher Education of the Russian Federation (State registration 121111100025-5 at November 11 2021). The study was performed in the ISU, and supported by GreenTechBaikal LLC, and Project of RSF 20-76-00001.

P-04.5-026
Effect of Zn excess under two temperatures regimes on activity of antioxidant enzymes in two species of mustard

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Zinc (Zn) in high concentration negatively affects metabolism. It is well known that temperature affects metal availability. However, effect of chilling on reactions of plants, growing under Zn excess still unknown. The activation of antioxidant system plays an important role in plant's adaptation. According to this, the aim of our study was to investigate an effect of Zn excess under two temperatures regimes (22 and 4°C) on activity of antioxidant enzymes in two species of mustard (*Brassica juncea* and *Sinapis alba*) with different strategy to metal accumulation. Plants grown on sand substrate with different Zn concentrations: 5 mg/kg (optimum) and 15, 30 and 45 mg/kg (excess) in climatic chambers until bud stage. Further, all plants separated into 2 groups, one of them placed under 4°C (Zn+4°C) another one leaft under 22°C (Zn+22°C). The MDA content and antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT) and guaiacol-

dependent peroxidase (G-POD) activity were analyzed after 4 days. The MDA content rose from 15 mg/kg to 45 mg/kg of Zn in *S. alba* but not in *B. juncea*. In this case, the SOD, CAT and G-POD activity rose under all studied Zn concentrations in *S. alba*, whereas in *B. juncea* SOD activated at 30 and 45 mg/kg of Zn and CAT, G-POD at 45mg/kg. The chilling and 5 mg/kg of Zn leads to increase in MDA content in both *S. alba* and *B. juncea*, while the antioxidant enzymes activity increased only in *S. alba*. The impact of Zn+4°C resulted in decrease in MDA content in both species compare to Zn+22°C. The activity of SOD, CAT and G-POD in *S. alba* was lower under Zn+4°C compare with Zn+22°C. According to data, the response of *S. alba* to Zn excess, chilling and their combination links to antioxidant enzymes activation in contrast with *B. juncea*, perhaps *B. juncea* uses other protective mechanisms for adaptation. The study was carried out under financial support of RSF (project No.22-24-00668)

P-04.5-027
Serine phosphorylated pathway in Alzheimer's disease

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During aging, the brain undergoes metabolic changes that primarily involve glucose (metabolism and transport) and insulin signaling. Indeed, a correlation between cerebral glucose metabolism and synaptic activity has been described in Alzheimer's disease (AD) patients. Glycolysis in astrocytes supports the production of L-serine (L-Ser), which is needed for the synthesis of a plethora of relevant biomolecules, among them glycine and D-serine, two co-agonists of the NMDA receptor. In the brain, de novo L-Ser synthesis proceeds via the phosphorylated pathway (PP) consisting of three reactions involving 3-phosphoglycerate dehydrogenase (PHGDH), phosphoserine aminotransferase (PSAT) and phosphoserine phosphatase (PSP). Here, we have evaluated the alterations observed in hippocampal regions of AD patients compared to healthy individuals by using a multi-omics approach. The transcriptomic profile of AD patients did not show any variation in the expression levels of the three PP enzymes as well as in levels of the transcripts identified for each gene. Western blot and proteomic analyses highlighted that PHGDH and PSAT levels were significantly increased in AD: these changes were associated with a significant decrease of L-Ser levels and an increase of D-/(D+L)-serine ratio in AD patients. The mechanism by which NMDA receptors are activated is different between the sexes in AD: in females it is mainly due to serine metabolism downregulation, while in males it is linked to the upregulation of proline/arginine metabolism. Our data indicate that altered L-Ser level may contribute to damaged neurotransmission and synaptic plasticity in AD patients (triggering an increase in brain D-serine availability), highlight how different pathophysiological mechanisms are active in males and females, and suggest novel therapeutic approaches. This work was supported by PRIN 2017 2017H4J3AS "Dissecting serine metabolism in the brain".

P-04.5-028
D-3-phosphoglycerate dehydrogenase: a key enzyme, not for amino acid metabolism only

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Human D-3-phosphoglycerate dehydrogenase (hPHGDH, EC 1.1.1.95) is responsible for the first and rate-limiting step in the "phosphorylated pathway" for the *de novo* biosynthesis of L-serine. PHGDH catalyzes the reversible transformation of D-3-phosphoglycerate (generated by glycolysis) into 3-phosphohydroxypyruvate. The overexpression of the gene encoding PHGDH has been reported in human cancers and PHGDH inhibition was reported to reduce cell proliferation: PHGDH is involved in nucleotide synthesis by supporting central one-carbon metabolism. Furthermore, several mutations have been correlated to PHGDH deficiency and neurodegenerative diseases. hPHGDH belongs to the structurally most complex type I class of PHGDH, which contains two common domains (the substrate-binding domain and the cofactor-binding domain) and two additional regulatory domains at the C-terminus: the structure of the full-length hPHGDH has not been solved yet. Here, we performed a biochemical characterization of recombinant hPHGDH. We clarified the substrate specificity by establishing the kinetic parameters in the forward and in the reverse reaction. We highlighted the presence in solution of different conformations and/or oligomeric states of hPHGDH. Moreover, the effect of different ligands related to several metabolic pathways (such as glucose-6-phosphate, L-serine, acetyl-CoA, ATP, L-lactate) and ions on hPHGDH activity was investigated to clarify a putative allosteric regulation of its functionality. Furthermore, selected hPHGDH variants (namely V261M, V425M and V490M) corresponding to known SNPs related to pathologies were also characterized. Taken together, biochemical properties of hPHGDH allow a better understanding of the molecular mechanisms related to L-serine synthesis under physiological and pathological conditions. This work was supported by PRIN 2017 2017H4J3AS "Dissecting serine metabolism in the brain".

P-04.5-029
Dysfunction in mitochondrial metabolism in cerebral cortex of mice with mucopolysaccharidosis type II

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Hunter syndrome or mucopolysaccharidosis type II (MPS II) is a lysosomal storage disorder caused by the deficiency of iduronate 2-sulfatase (IDS), which leads to the accumulation of

glycosaminoglycans in a variety of tissues. Patients affected by the severe form present with a multisystemic disease which include brain abnormalities, but the mechanisms of such alterations are unknown. Since recent studies suggest that mitochondrial alterations may participate in the pathogenesis of lysosomal disorders, in this work we evaluated mitochondrial bioenergetics and redox homeostasis in the brain cortex of MPS II mice. Cerebral cortex from 6-month-old mice (MPS II: IDS knockout; or controls: Wild Type) were used for the determination of the parameters. Our results showed a significant increase in mitochondrial respiration (measured by oxygen consumption using an OROBOROS Oxygraph-2) in MPS II animals, compared to controls. While state 3 (ADP-stimulated) was increased either when stimulated by the substrates pyruvate, malate and glutamate (PMG; NADH-linked) or by PMG plus succinate (FADH 2 - linked), uncoupled respiration (CCCP-stimulated) was augmented only in the presence of succinate. State 4 (non-phosphorylating) was also significantly increased. Furthermore, a reduction in citrate synthase and malate dehydrogenase was verified in MPS II mice. In contrast, the activities of alfa-ketoglutarate dehydrogenase, isocitrate dehydrogenase and respiratory chain complexes, as well as the redox homeostasis parameters were not significantly altered. Our data suggest that disturbances in mitochondrial energy metabolism may contribute to the pathogenesis of neurological dysfunction observed in MPS II.

P-04.5-030
L-Serine biosynthesis in the brain: functional and structural characterization of human phosphoserine aminotransferase

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L-serine plays a crucial role in the central nervous system, being involved in several processes including the biosynthesis of nucleotides, lipids and of the neuromodulators glycine and D-serine. L-serine is mainly synthetized in astrocytes through the phosphorylated pathway from the glycolytic precursor 3-phosphoglycerate. The enzyme phosphoserine aminotransferase (PSAT) is a PLP-dependent enzyme (belonging to the fold-type I structural group) that catalyzes the second step of serine biosynthesis, the conversion of 3-phosphohydroxypyruvate to L-O-phosphoserine (L-OPS), using L-glutamate as the amino group donor. Here, we present the first functional and structural characterization of human PSAT. The determination of the kinetic parameters for both the forward and reverse reactions of PSAT showed that the former exhibits higher catalytic efficiency in comparison to the latter. The pH dependences of the activity and the absorption UV-Vis spectra of the cofactor showed that at pH 6.9, the value corresponding to maximal activity, the cofactor is predominantly in the protonated form. The thermal unfolding of PSAT was monitored by circular dichroism spectroscopy and revealed a two-step process, with formation of an intermediate that is more stable in the PLP-PSAT with respect to the PMP enzyme form. Crystal structures of PSAT were solved both in the absence and in the presence of L-OPS at 2.5 and 2.8 Å resolution, respectively. The electron density maps suggest the presence in the active site of a sulfate ion, which interacts with the same, positively charged residues involved in the binding of the phosphate moiety of L-OPS. In keeping with this finding, preliminary results showed that phosphate and sulfate inhibit the activity of PSAT.

P-04.5-031
Pyruvate dehydrogenase phosphatase 1 (PDP1) is a regulator of HIF activity via an Acetyl-CoA dependent mechanism

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Cancer cells exposed to the hypoxic tumor microenvironment, respond by activating the Hypoxia-inducible factors (HIF). HIF-1 mediates extensive metabolic re-programming and its expression is associated with poor prognosis in cancer. To understand the role of reversible phosphorylation in the regulation of HIF-1 pathway, we have previously silenced expression of the catalytic subunits of human phosphatases using a siRNA library in HeLa cells. Pyruvate dehydrogenase phosphatase 1 (PDP1) was one of the most prominent regulators of HIF activity among the phosphatases tested. PDP1 is a key metabolic enzyme that dephosphorylates and activates pyruvate dehydrogenase (PDH), leading to increased conversion of pyruvate into Acetyl-CoA. Phosphorylation dependent inactivation of PDH is catalyzed by PDK1, expression of which is regulated by HIF-1. Silencing of PDP1 down-regulated HIF mediated transcription and the expression of HIF-1-dependent genes, including PDK1. Accordingly, PDP1 overexpression or its hormonal stimulation by insulin enhanced HIF activity under hypoxia. Furthermore, we showed that in tumor cells silencing of PDP1 under hypoxia decreased PDH activity and affected Acetyl-CoA production. Consequently, acetylation levels of histones and HIF binding on hypoxia regulated gene promoters was markedly reduced. Treatment of PDP1-silenced cells with acetate or with the histone deacetylases (HDACs) inhibitor trichostatin A led to recovery of HIF-1 activity. Overall, our data suggest the operation of a homeostatic metabolic circuit between the mitochondria and the nucleus involving PDP1, HIF1 and PDK1. These data could provide valuable insights into the use of HIFs as anticancer therapeutics targets.

P-04.5-032
The effect of p21 and TXNIP interaction on autophagy in HepG2

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Autophagy is an intracellular recycling mechanism that degrades damaged cellular components under various stimuli, such as ROS, starvation, hypoxia. Autophagy is also observed to be increased on individuals with heart disease, diabetes, and obesity showed increased autophagy, implicating the importance of autophagy in the regulation of body homeostasis. And by various studies, the impaired autophagy flux was observed in both

the murine model of NAFLD and human, indicating the importance of this process in hepatocellular function. p21 is also one of autophagy activator that is known as downstream effector of p53, which induces apoptosis and cell cycle arrest. According to the previous study, p21 is directly regulated by TXNIP (Thioredoxin Interacting Protein), a redox regulator that inactivates antioxidant TRX (Thioredoxin) in normal condition, suggesting a link between the two regulators of autophagy. So this experiment was performed to investigate the effect of p21 and TXNIP interaction to autophagy in HepG2. As a result, both endogenous p21 and TXNIP level upregulation were identified in starvation conditions. And p21 showed direct binding with LC3, the general experimental marker for detecting and quantifying autophagosome in cells, which implies the association between p21 and autophagy. In addition, p21 increases LC3-I to LC3-II conversion while it doesn't affect the p62 significantly, suggesting that p21 increases early-stage autophagy. And in starvation conditions, the binding between p21 and TXNIP are strengthened, and this interaction decreased autophagy, which is different from when they were expressed independently. Also, immunostaining results showed increased LC3 puncta when p21 or TXNIP expressed alone, but it decreased when both are expressed together. Overall, this study shows that TXNIP binding on p21 inhibits p21's positive regulation on autophagy, and this shows tht p21 and TXNIP could be used as the therapeutic target for NAFLD. *The authors marked with an asterisk equally contributed to the work.

P-04.5-033
Investigation of time-course and dose-dependent regulation of circadian clock genes with aryl hydrocarbon receptor-induced human bone marrow mesenchymal stem cells

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Long-term dioxin exposure may induce dysregulation of the circadian clock through Aryl hydrocarbon receptors (AhR). It has been revealed that is associated with many diseases such as metabolic syndrome, leukemia, and circadian-related. AhR reciprocally interacts with the circadian clock involved in cellular homeostasis and metabolism. 6-formylindolo[3,2-b]carbazole (FICZ) is an agonist of AhR. Tumor necrosis factor (TNF-α) is a pro-inflammatory cytokine and has a role in cancer development by suppressing the expression of circadian genes. On the other hand, there is a reciprocal interaction between inflammatory cytokines such as TNF-α and AhR. To determine whether FICZ and TNF-α interfere with circadian rhythm and to investigate physiological turnover, we used human bone marrow mesenchymal stem cells (hBM-MSCs) synchronized by Dexamethasone and cultured with two different doses of FICZ and TNF-α for 24h and replaced cell culture medium thereafter up to 48h. RNA was extracted every 6h up to Zeitgeber time (ZT) ZT48 and analyzed by RT-qPCR. We demonstrated that treatment altered the circadian expression patterns of selected AhR and clock genes (AHR, AHRR, CYP1A1, PPARG, BMAL1, CRY1, REVERBA, ADRB2) in a dose-dependent manner. Cells exposed to high dose FICZ and TNF-a showed similar gene expression patterns in both AHR and circadian clock gene regulation. Our study provides data for a better understanding of the potential interactions between inflammation and AhR mediated circadian clock gene regulations and provides novel insight into exploration of the connection between physiological FICZ response and high dose FICZ compared with TNF-α-induced anti-inflammation pathway through increased pro-inflammatory clock gene response. These preliminary results provide new perspectives for the development of therapeutic drugs for the regulation of rhythmic biological processes.

P-04.5-034
Exploring the prevalence of weak effectors in non-allosteric metabolic enzymes

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Protein allostery is an intriguing regulatory phenomenon that has been given various definitions in the literature over the decades, with the latest suggesting that all dynamic proteins have the potential to behave allosterically under certain circumstances. The interactions involved in allostery can range from weak (K_d = μM-mM) to strong (K_d = nM-pM). In this study, we intended to examine the prevalence of (weak) allosteric interactions in enzymes that have been classically defined as non-allosteric. As proof of principle, we performed binding assays on several non-allosteric enzymes in the glycolytic pathway with natural metabolites from a customized compound library. Here we used the Microscale Thermophoresis (MST) as a screening method, which is capable of detecting binding events in the mM range. Having identified potential binders ($K_d \leq 15$ mM), we next proceeded to assess the effect of these binders on the enzymes' activity using enzyme assays. A few potential hits appeared to have weak inhibitory effects on the enzymes, leading to a partial decrease in their activity, and two of the metabolites exhibited non-competitive mode of inhibition, possibly due to binding to an allosteric site. Furthermore, the effect of the ligands on glycolytic and mitochondrial activities was tested using an *in vitro* cell assay (SeaHorse XFe96). In order to examine how these ligands affect protein stability, we monitored their interaction with the enzymes using thermal shift assays. Our next task will be to reveal their binding sites using X-ray crystallography. All the hits we have identified so far are relatively weak binders, with K_d in the range of 6-15 mM, thus we are hoping to expand the compound library to evaluate how extensive weak metabolite regulation is in metabolic pathways and reveal its significance in maintaining cell homeostasis. *The authors marked with an asterisk equally contributed to the work.

P-04.5-035
PAF level regulation are crucial for embryonic development of zebrafish

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Cytosolic PLA2 (cPLA2) was found in the zebrafish embryo and mobilization of arachidonic acid (AA) was relevant to the developing from mammalian to fish embryos. AA production by cPLA2 also generates platelet activating factor, which is a mediator in the pathogenesis of several disorders with an inflammatory component. Malathion (MTON, 50g/Liter) used commercially for the control of *Aedes aegypti* and *Musca domestica* at concentrations above 10mg/L inhibited zebrafish embryonic development and at doses of 2.5mg/L embryonic development was significantly affected with teratogenic effects mainly evidenced by decreased body development and smaller eye diameter. In *in silico* molecular docking studies using (Chimera, Autodock Vina, Discovery Studio, LIGPLOT and Pymol) showed that malathion binds strongly to the active site of PAF AH but cannot interact with the active site of cPLA2 and experimental data showed that it dose-dependently decreased the enzymatic activity using commercial colorimetric kit for Platelet Activating Factor-Acetyl Hydrolase (PAF-AH) showing an IC50% of 5 μM/ L, being less effective than MAPF 1.0 μM/ L. Malathion at high concentrations can be a potentially teratogenic agent and our data suggest among several factors that AA generation and PAF control are two crucial factors for zebrafish embryo development. Thus, long exposures of malathion and at high concentrations can strongly contribute to impairing zebrafish embryonic development by inhibiting the PAF AH enzyme, which cannot control PAF levels. Financiamento: FAPESP 2017/20291-0/ CNPQ 304153/ 2019-2. *The authors marked with an asterisk equally contributed to the work.

P-04.5-036
Chronic treatment with sertindole, but not clozapine and ziprasidone, affects redox homeostasis in brain of male rats

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Atypical antipsychotic drugs (APDs) are being used to treat acute psychotic episodes in schizophrenia and schizophrenia-related diseases as well as a variety of nonpsychotic disorders. Despite their effectiveness, we showed that APDs clozapine (CLO), ziprasidone (ZIP) and sertindole (SER) increased oxidative stress and reduced antioxidative defence capacity in rat kidneys and heart. Given the scarcity of data about CLO, ZIP and SER actions on the brain redox homeostasis, the goal of current study was to investigate their impact on antioxidant enzymes: total superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) in brain of 3 month old male Wistar rats treated daily via intubation with water (control group), CLO (45 mg/kg/day), ZIP (20 mg/kg/day) or SER (2.5 mg/kg/day) for 6 weeks. There were no significant changes in investigated parameters in CLO and ZIP groups. However, in SER group the total SOD activity was decreased while CAT and GPx activities were increased compared to control group (One-way ANOVA with Tukey's HSD test, $P < 0.05$). Since both CAT and GPx catalyze decomposition of H_2O_2, their increased activity in SER group suggests an elevated peroxide pressure. This conclusion can be strengthened since the activity of SOD can be decreased by elevated concentration of H_2O_2. Our results suggest that SER could cause a redox imbalance in brain with possible negative effects on mitochondrial respiration and neural tissue metabolism. *The authors marked with an asterisk equally contributed to the work.

P-04.5-037
Characterization of the human bronchial epithelial cells with deletion of the α subunit of mitoBKCa channels

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Air pollution has a negative impact on the functioning of the respiratory system. Urban dust (particulate matter, PM), which is one of the main components of smog, contributes to the development of respiratory diseases and death. Recent studies indicate that PM cytotoxicity might be related to the mitochondrial dysfunction in bronchial epithelial cells. There are indications that the survival of cells exposed to PM increases as a result of the activation of potassium channels located in the inner membrane of mitochondria (IMM). The transport of potassium ions across the IMM is essential for the optimal course of oxidative phosphorylation, regulation of reactive oxygen species synthesis, mitochondrial volume and membrane potential. Pharmacological activation of potassium channels alleviates the effects of hypoxia/reperfusion injury in heart or brain tissue. Mitochondrial potassium channels in the bronchial epithelium may be a new pharmacological target. Our recent study has revealed the presence of large conductance calcium-activated potassium (mitoBK$_{Ca}$) channel in the IMM of the human bronchial epithelial (16HBE14o-) cells. In this project, we focus on the role the mitoBK$_{Ca}$ channel in the mitochondrial physiology and it is the potential cytoprotective role against PM-induced cytotoxicity. We used the CRISPR/Cas9 technique to develop 16HBE14o- cell line with BK$_{Ca}$ α subunit knockout. In the newly developed cell lines we did not observe the mitoBK$_{Ca}$ channel activity. However, we did not observe significant changes in the expression of BK$_{Ca}$ auxiliary subunits in knockout cells. We also checked the effect of deletion on the expression of selected mitochondrial genes and mitochondrial physiology. In the near future, we plan to check how the lack of the channel affects the cytotoxicity induced by PM. This study was supported by a grant (2019/35/ B/NZ1/02546) from the National Science Centre, Poland. *The authors marked with an asterisk equally contributed to the work.

P-04.5-038
DJ-1 proteoforms in breast cancer: proteomic strategies to relate metabolic rewiring and epigenetic disregulation

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In cancer cells, the high glycolytic flux induces carbonyl stress, a damaging condition that increases reactive carbonyl species making DNA, proteins and lipids more susceptible to glycation. One of the main glycating agent is methylglyoxal, a highly diffusible molecule capable of entering the nucleus where it reacts with lysine-arginine-rich tails of histones leading to the formation of advanced glycation end-products (AGEs). This modification leads to a fatal deconstruction of the histone code and has been associated with cancer and several age-related diseases. In this scenario, the activity of DJ1, a protein that protects cells from oxidative stress and that has been described as a deglycase enzyme, appears to be crucial for ensuring the survival of cancer cells. Indeed, in several human tumours its expression, localization, oxidation, and phosphorylation were found to be altered. This work aims to explore the molecular mechanism that triggers the peculiar cellular compartmentalization and the specific post translational modifications (PTM) that influences DJ-1's dual role in breast cancer cells. Using a proteomic approach, we identified on DJ-1 a novel threonine phosphorylation, part of a putative Akt consensus. Interestingly we found that pharmacological modulation of Akt pathway induces a functional tuning of DJ-1 proteoforms revealing that the pathway is critical for DJ-1 protumorigenic abilities. In breast cancer cells, the overactivation of Akt signalling enhances DJ1-phosphorylation. Phosphorylated DJ-1 increases its glyoxalase activity, therefore, preventing glycation-induced histones misregulation. The novel proteoform of DJ-1 was used as a molecular template for docking simulation, to identify a parterre of DJ-1 inhibitors able to selectively impair the ability of cancer cells to preserve their malignant proliferative potential. Our results demonstrated that targeting the novel DJ-1 proteoforms might be a promising therapeutic strategy in fighting cancer.

P-04.5-039
MUC2 expression as a trigger of intestinal epithelium dysregulation and microbiome dysbiosis in the context of ulcerative colitis

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Chronic inflammation of the intestinal epithelium is the main characteristic of ulcerative colitis (UC). Numerous factors have been identified as a triggering cause for pathogenesis, among which an impaired mucosa layer. The abnormal mucosa causes an aggravated immune response that acts upon the commensal microbiota in the intestine, causing dysbiosis. Our aim was to evaluate the gene and protein expression of mucin 2 (MUC2), in order to correlate the level of expression with the bacterial phyla found in the colon. For this study, we used colon tissue samples isolated from 4 mice conditions: BALB/C, 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced UC BALB/C, STAT6 knockout and TNBS-induced UC STAT6 knockout. Quantitative Real-Time PCR was performed for gene expression evaluation of MUC2, whereas immunohistochemistry for protein expression evaluation was possible by using specific antibody for MUC2. Assessment of MUC2 gene and protein expression indicated increased values in BALB/C models when compared to BALB/C TNBS-induced mice. Moreover, MUC2 expression was slightly decreased in STAT6 knockout mice when compared to BALB/C, whereas the expression in STAT6-TNBS induced mice was very low. In addition, metagenomics sequencing of 16S RNA from cecum samples of BALB/C mice revealed a positive ratio between the two dominant phyla, *Firmicutes* and *Bacteroidetes*, when compared to *Proteobacteria*. Moreover, although bacterial diversity was at a lower extent in STAT6 knockout mice, *Firmicutes* and *Bacteroidetes* were the most present in the samples. However, STAT6 knockout mice had a 50% *Proteobacteria*, confirming the inflamed state of the colon, with an exacerbated immune response. The results showed MUC2's modified expression in the intestinal epithelium of UC-induced mice and suggested its abnormal expression affects the epithelial barrier integrity, leading to microbiome dysbiosis, and further on promoting colon inflammation.

P-04.5-040
The role of NAD biosynthesis in maintaining pluripotency and differentiation of mouse embryonic stem cells

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Nicotinamide adenine dinucleotide (NAD) is an essential intracellular metabolite controlling key metabolic and signaling pathways in mammalian cells. It has recently been shown that NAD-dependent processes play a crucial role in stem cells regulation. The proper control of NAD-dependent metabolic and signaling processes depends on how efficiently stem cells can maintain a certain level of the dinucleotide. The major means to maintain NAD^+ levels is through its biosynthesis from various forms of vitamin B3. In this study we examined the input of different NAD^+ biosynthetic pathways into pluripotent state maintenance and differentiation of mouse embryonic stem cells E14. Pluripotency status of E14 cells was assessed by immunocytochemical and immunoblotting analysis using antibody to the transcription factor Nanog, the marker of pluripotency in mammals. Using NMR spectroscopy, we established that E14 cells in pluripotent and differentiated state have the same level of intracellular NAD^+. Moreover, neither activation nor suppression of NAD^+ biosynthesis affects the maintenance of pluripotency and differentiation of E14 cells. When cells were treated with deamidated NAD^+ precursor nicotinic acid riboside (NAR), considerable accumulation of nicotinic acid adenine dinucleotide (NAAD) was observed in pluripotent, but not in differentiated cells. NAAD is a direct NAD^+ precursor, which is involved in all deamidated pathways of NAD^+ biosynthesis. The amidation reaction of NAAD to NAD^+ is catalyzed by the NAD synthetase (NADS). Our results suggest that NADS is suppressed when cells are in pluripotent state, whereas in the process of differentiation its

activity can be stimulated. In support of this assumption, we also demonstrated that in pluripotent E14 cells NAD^+ biosynthesis from NAR (via deamidated pathway) is much less efficient than in differentiated cells. This work was supported by the Russian Science Foundation (grant no. 21-14-00319).

P-04.5-041
Modulation of NAD biosynthesis does not significantly affect the efficiency of DNA double-strand break repair in human dermal fibroblasts

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Nicotinamide adenine dinucleotide (NAD) serves as a substrate for a number of regulatory proteins such as protein deacetylases (sirtuins) and poly(ADP-ribose) polymerases which play an important role in cellular response to genotoxic stress. There is increasing evidence that NAD-dependent protein deacetylation and ADP-ribosylation are involved in DNA double-strand break (DSB) repair. In this study, we have examined how modulation of NAD biosynthesis affects the efficiency of DSB repair in primary human dermal fibroblasts (HDF) after exposure to ionizing radiation (IR). DSB repair capacity was estimated by immunocytochemical analysis using an antibody to histone H2AX phosphorylated at Ser139 (gH2AX), the marker of DSBs. We have demonstrated that pharmacological stimulation of NAD biosynthesis by nicotinamide riboside (NR) significantly increases the level of intracellular NAD, but does not affect the efficiency of DSB repair in HDF after exposure to ionizing radiation at a dose of 1 Gy. Moreover, we did not observe any depletion of NAD pool during the DNA damage response induced by IR at a dose of 1 or 5 Gy. To elucidate if NAD depletion can affect the efficiency of DSB repair in HDF, we optimized the conditions for the suppression of NAD biosynthesis by FK866, an inhibitor of nicotinamide phosphoribosyltransferase. We have shown that moderate decrease of intracellular NAD (by 50%) after 1 day of treatment with FK866 does not affect the DSB repair capacity. Surprisingly, even after 4 days of FK866 treatment when intracellular NAD was no longer detectable, HDF effectively eliminated IR-induced DNA DSBs. Critical depletion of NAD only moderately decreased DSB repair capacity comparing to control cells. Our data suggest that NAD and NAD-dependent processes, such as protein poly-ADP-ribosylation, may be important but not crucial factors controlling the repair of DNA DSBs induced by IR. This work was supported by the Russian Science Foundation (grant no. 20-74-00145).

P-04.5-042
Cold plasma-induced stimulation of natural sweeteners biosynthesis in *Stevia rebaudiana* Bertoni

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Seed treatment with cold plasma (CP) stimulates seed germination, plant morphometric parameters, biomass production, and disease resistance in different plant species by inducing changes in plant biochemical phenotype, however, the underlying mechanisms of CP-induced changes in secondary metabolite biosynthesis are not elucidated. *Stevia rebaudiana* Bertoni is an economically valuable plant due to its secondary metabolites steviol glycosides (SGs) that are responsible for the sweetness of stevia and are widely used as natural sweeteners. Stevioside (Stev) and rebaudioside A (RebA) are the most abundant SGs in stevia. The aim of this study was to determine the effect of *Stevia rebaudiana* Bertoni seed treatment (2-7 min) with different types of CP (dielectric barrier discharge (DBD) and capacitively coupled (CC) CP) on the amount and ratio of Stev and RebA in the leaves of stevia, the kinetics of stimulated biosynthesis and the possibility to transfer the CP-induced stimulating effect to the vegetatively propagated plants. We have demonstrated for the first time, that seed treatment with CP of both types can increase Stev and RebA concentrations several times. CC CP increased the RebA concentration 1.5-fold and the concentration of Stev 7-11-fold depending on treatment duration. The optimal 2-min pre-sowing seed treatment with DBD CP increased the RebA concentration 2-fold, Stev - 14%, RebA/Stev ratio - 1.7-fold. Stimulating effect persisted 14 weeks; however, the CP-induced stimulating effect was lost in vegetatively propagated plants. The concentrations of other bioactive compounds as phenolics and flavonoids were decreased or unchanged by both types of CP treatment leading to lower or unchanged antioxidant activity of stevia leaf extracts rich in SGs. It can be concluded that a short time pre-sowing treatment of seeds with CP can be a powerful tool for the enhancement of biosynthesis/accumulation of SGs in stevia plants.

P-04.5-043
Targeting unstable regions of the most common variant medium-chain acyl-CoA dehydrogenase to develop strong candidates for pharmacological therapies

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The human medium-chain acyl-CoA dehydrogenase (hMCAD) is a homotetrameric flavoprotein, involved in the first step of mitochondrial fatty acid β-oxidation (mFAO), catalyzing the dehydrogenation of medium-chain acyl-CoA (C6-C12)[1]. Each hMCAD monomer (44 kDa) has three structural domains: two α-helical domains in both N- and C-terminus and a central β-sheet domain. MCAD deficiency (MCADD), the most common inborn error of mFAO, without an available pharmacological treatment. The therapeutic approach for MCADD relies mainly on dietary control, not always effective as many patients still present acute life-threatening metabolic crises[2]. MCADD is a loss-of-function conformational disorder, with p.K329E MCAD variant representing ≈80% of the disease-causing variants. Using *in silico* approaches our group showed that the central β-domain was the most affected region of p.K329E, with severe disruption in the pockets' architecture and in the FAD and octanoyl-CoA binding affinities[3]. These studies were extended, allowing the identification of additional unstable regions. Through MD simulations, several molecules were designed to interact with those regions. After evaluation, molecules with the best scores were selected and experimentally tested for their effect on p.K329E

stability and functionality. These molecules were able to stabilize the p.K329E structure, by increasing the protein's melting temperature or decreasing its denaturation rate, without compromising the enzymatic activity. In fact, some molecules had the capacity to improve the variant's activity after protein-molecule incubations at 37 °C for 10 and 30 minutes. The results showed that stabilizing the p.K329E structure is essential for rescuing the protein's enzymatic activity, thus supporting that MCADD is a strong candidate for pharmacological therapies. 1- Satoh, A et al. (2003) J Biochem 134, 297-304 2- Gregersen, N et al. (2008) J Inherited Metab Dis 31, 643-657 3- Bonito, CA et al. (2016) Biochem 88, 7086-7098

Food and nutrition in biochemistry

P-05.1-001
Cooking oil-derived 'hydroxynonenal' may cause diverse lifestyle-related diseases

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Although excessive consumption of deep-fried foods is regarded as an important epidemiological factor of lifestyle diseases such as Alzheimer's disease, type 2 diabetes, nonalcoholic steatohepatitis etc., the underlying mechanism remains unknown. Deep-fried foods cooked by ω-6 polyunsaturated fatty acid (PUFA)-rich vegetable oils contain lipid peroxidation product 'hydroxynonenal' which induces carbonylation of Hsp70.1 with dual functions as chaperone protein and lysosomal stabilizer. This impairs ability of cells to recycle damaged proteins and stabilize the lysosomal membrane. It was not reported that daily consumption of ω-6 PUFA-rich vegetable oil causes lysosomal/autophagy failure with the resultant cell degeneration/death in diverse organs. Using monkeys after the consecutive injections of the synthetic 'hydroxynonenal' to be relevant to the serum concentration of human 60', calpain activation, Hsp70.1 cleavage, lysosomal integrity, and autophagy deficiency were studied in the brain, liver, and pancreas by immunofluorescence histochemistry, electron microscopy, and western blotting. Light microscopy showed abundant vacuole formation in the hydroxynonenal-treated cells compared to the controls, which were identified as enlarged rough ER by electron microscopy. In addition, lysosomal membrane permeabilization/rupture and accumulation of autophagosomes were confirmed in all cells after hydroxynonenal treatment. Immunofluorescence histochemical analysis showed an increased co-localization of activated μ-calpain and Hsp70.1, extralysosomal release of cathepsin B, and overexpression of p62. Western blot showed upregulation of fatty acid receptor GPR40 (brain), GPR120 (liver), or GPR109A (pancreas). Such GPCR activation caused calpain activation and calpain-mediated Hsp70.1 cleavage. Based on the 'Calpain-Cathepsin Hypothesis' formulated in 1998, the molecular implication of hydroxynonenal for lysosomal destabilization/rupture and autophagy deficiency was discussed. Conclusion: By covalently modifying Hsp70.1, both the dietary PUFA- (exogenous) and the biomembrane phospholipid- (intrinsic) peroxidation product 'hydroxynonenal', combined together, plays crucial roles in the occurrence of diverse cell death, leading to lifestyle-related diseases.

P-05.1-002
Influence of seed coat color on germination, morphometry, and the amount of phytohormones in red clover seeds after their treatment with cold plasma and electromagnetic field

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This study aimed to assess the effect of seed coat color on seed germination of red clover (*Trifolium pratense L.*), seedling growth, and the amount of phytohormones in seeds induced by an electromagnetic field (EMF) and cold plasma (CP). In this study, two varieties of red clover seeds were used – 'Vychiai' and 'Arimaichiai'. Before sowing, seeds of different coat colors (yellow, brown, and black) were treated using these physical stressors. The effect of treatments on seed germination was assessed using an *in vitro* germination test. The following germination kinetic parameters were determined: final germination percentage, mean germination time, and quartile of deviation. Morphometric analysis of seedlings was carried out 7 days after sowing. Seed extracts for the analysis of phytohormones were prepared on the day of sowing and analyzed by HPLC. The amounts of phytohormones such as abscisic acid, gibberellin A7, salicylic acid, indole-3-acetic acid, indole-3-butyric acid, and zeatin were analyzed. Five weeks after sowing, the number of root nodules was measured in the 'Arimaichiai' plants. It was found that the *in vitro* germination kinetics of both varieties of red clover do not depend on the color of the seed coat. And the pre-sowing treatment of seeds improved the kinetic parameters of seed germination of both varieties. However, the effect of treatment on the early growth of clover seedlings depended on the color of the seed coat. The treatments increased the length of seedlings from yellow and black seeds of the 'Arimaichiai' variety and the weight of seedlings from brown seeds. The amount of seed phytohormones involved in germination control also depended on the color of the seed coat of both varieties. Cold plasma treatment significantly increased the amount of abscisic acid in yellow seeds and decreased gibberellins in brown seeds. Likewise, the treatment of seeds with CP affects nodulation, especially for seedlings from black seeds.

P-05.1-003
Covalent modification of bovine serum albumin with phycocyanobilin using Traut's reagent

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Phycocyanobilin (PCB) is an open-chain tetrapyrrole chromophore of phycocyanin (PC), chromoprotein derived from the cyanobacterium *Arthrospira platensis*. Our group has previously

demonstrated the potential of PCB to covalently modify free cysteine residue of proteins using food protein β-lactoglobulin as a model protein. Relying on the proven ability of PCB to be attached to sulfhydryl groups of proteins, we propose a new method for covalent attachment of PCB to potentially any protein. We used Traut's reagent (TR, 2-iminothiolane) to introduce free sulfhydryl groups in the model protein, bovine serum albumin (BSA), by modifying its lysine residues. All tested molar ratios of TR per mole of protein successfully modified BSA. A higher degree of modification by TR induced more profound alterations of BSA structure, as evidenced by near-UV and far-UV circular dichroism spectroscopy. At the same time, minor changes in BSA oligomerization and aggregation profile occurred with increasing TR molar ratio. PCB was covalently attached to introduced sulfhydryl groups at pH 9 at 20–fold molar ratio of PCB per mole of protein. An increase in the molar ratio of TR per mole of BSA leads to amplification of fluorescent signal of PCB-modified BSA, most significantly observed starting from 50-fold and higher TR ratios. Using BSA as a model protein, a 50-fold molar excess of TR seems to be the optimal choice for balancing a satisfactory signal amplification level and the negative effect on protein structure. BSA covalently modified with PCB has higher antioxidative activity than free BSA. The proposed method thus serves as a proof of concept for labeling virtually any protein with PCB as means of either functionalization through covalent attachment of bioactive PCB or obtaining fluorescent probes for application in fluorescence-based techniques.

P-05.1-004
Cheeses with modified alpha-s-casein to beta-casein ratio

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Use of microfiltration (MF) enables fractionation of milk proteins: casein micelles and serum proteins (SP), when the process is carried out at cold temperatures beta-casein (CN) migrates from casein micelles and passes through the membrane. The cheeses were produced from milk with normal (control) (C), higher (H) and lower (L) content of beta-CN (Iwaniak et al. 2021, doi.org/10.3390/ijms22062949). The proportions of alpha-s to beta-CN were 1.52, 2.58 and 1.28 for C, H and L milk. The produced cheeses were characterized by a protein content of 25.77%, 26.88% and 26.88% for C, H and L cheeses, respectively. Significant differences ($P > 0.05$) in protein retention were found between the cheeses. For the control, increased and reduced beta-CN cheeses, protein retention was respectively: 80.25%, 87.20% and 89.99%. The proteolysis was studied by determining the amount of water soluble nitrogen (WSN), 12% TCA and 5% PTA. During ripening, WSN content increased in all cheeses. Higher values were recorded for L cheeses (27.86% WSN%TN after 60 days of ripening) compared to C cheese (11.88%) and H cheese (23.77%). Similar trends were observed for the content of nitrogenous compounds soluble in 12% TCA and 5% PTA. After 60 days of ripening, the content of these compounds was 8.06% TCA%TN and 3.41% PTA%TN, for control cheese, 12.17% and 3.11% for H cheese, and 16.13% and 4.07% for L cheese. During ripening of the cheeses, the content of free amino-acids (FAA) increased in all samples. In L cheeses, FAA content was lower than in the control samples. Cheeses with reduced β-CN content showed the highest level of FAA. Project financially supported by Minister of Education and Science in the range of the program entitled "Regional Initiative of Excellence" for the years 2019-2022, Project No. 010/RID/2018/19, amount of funding 12.000.000 PLN

P-05.1-005
Transforming Okara into a high value by-product: enzymatic production and molecular characterization of antifungal bioactive peptides

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Conversion of by-products and reduction of food wastage for the full use of natural resources is one of the main challenges the industrialized world must face nowadays. Okara is a by-product generated in huge quantities during soymilk or tofu production, thus posing a significant disposal problem since, up to now, it has been mainly used as such in animal feeds or as industrial waste. Okara's high protein content (about 25–40% on a dry weight basis), makes this by-product interesting and exploitable from a biotechnological point of view [1]. Production of antifungal peptides has been described in plant seeds and Okara as well, showing its potential as a valuable source of such compounds, exploitable for integrated pest management [2]. This work aims to describe a rapid and economic procedure to isolate proteins from Okara, to produce and characterize an enzymatic proteolyzed product, active against fungal plant pathogens. A dose-response inhibitory activity was established against fungi belonging to the *Fusarium* genus. Enrichment of the active fraction was obtained through the combination of different chromatographic techniques. Mass spectrometry analyses allowed the identification of potential candidate bioactive peptides. The exploitation of okara to produce antifungal bioactive peptides has the potential to turn this by-product into a paradigmatic example of circular economy, since field-derived food waste is transformed into a source of valuable compounds, to be used in field protection of economically valuable crops for human and animal nutrition. [1] Jiménez-Escrig, A et al. (2010) Eur. Food Res. Technol 230, 655–663 [2] De Benedetti S et al. (2021) Molecules 26, 4858.

P-05.1-006
Biotransformation of flavonoids by combination of acid treatment and fermentation to improve bioavailability

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Most natural occuring flavonoids such as naringin or hesperidin exhibit neohesperidoside or rutinoside residues that prevent their efficient absorption in the small intestine, while free aglycones such as naringenin or hesperetin can cross the cell membrane enabling potential health beneficial effects. Thus, deglycosylation is a promising approach to convert less biologically active compounds into more active ones. In this work, we used an innovative combination of acid treatment and fermentation to increase

aglycone contents in citrus extracts. In a first step, citrus extracts rich in naringin and hesperidin were treated with citric acid to remove terminal rhamnose groups. This was followed by incubation with lactic acid bacteria to cleave the remaining glycosidic bonds and to release the aglycones naringenin and hesperetin. The composition of flavonoids was analyzed before and after biotransformation by high performance liquid chromatography (HPLC). As expected, hydrolysis with citric acid resulted in a significantly higher content of naringenin-7-O-glucoside and hesperetin-7-O-glucoside. Subsequent biotransformation of these so-called monoglucosides by bacteria significantly increased the yield of aglycones. In conclusion, we introduced an innovative method to enrich the aglycone levels in flavonoid-rich extracts. Molecular mechanisms affected by the relevant bioactive compounds will be investigated in future experiments.

P-05.1-007
Ghee butter from bovine colostrum reduces inflammation in dextran sulfate sodium-induced colitis in mice

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Pharmacological treatment and/or remission maintenance in inflammatory bowel disease (IBD) is currently one of the most difficult challenges in the field of gastroenterology. The available therapies are mostly aimed at alleviating symptoms rather than addressing the underlying cause of the disease. Ghee butter from bovine colostrum (GBBC) is a clarified butter produced by heating milk fat to 40°C and separating the precipitating protein. As colostrum mainly contains fatty acids, immunoglobulins, maternal immune cells, we hypothesized that it may exert anti-inflammatory effects. We characterized the effect of GBBC on intestinal barrier function in dextran sulfate sodium (DSS) mouse model of colitis. 100% GBBC (per os, 100 µL/mouse) significantly reduced colon-damage score, MPO activity, stool score, and concentration of FITC dextran in serum in comparison with DSS mice. GBBC notably reduced the level of TNF-α, IL-17, and IL-23 while compared to the DSS mice group. Additionally, administration of the FFAR4 antagonist followed by treatment with 100% GBBC significantly increased the anti-inflammatory effect of GBBC by decreasing production of IL-17 and 23 and the IL-6 level in comparison with DSS. We also assessed tight junctions (TJs) mRNA expression using distal colon samples collected from control, DSS-treated, DDS+GBBC-treated mice, and mice-treated DSS with the combination of GBBC+FFAR1 or 4 antagonists. Administration of GBBC alone not only restored the expression of OCLD 1 and CLDN1 to the level of control but also notably increased expression of these TJs mRNA above the basal level. FFAR1 antagonist in combination with GBBC significantly potentiated this effect. Of note, attenuation of FFAR4 expression reversed the effect of GBBC, thus indicating that FFAR4 receptor may affect the expression of OCLD1 and CLDN 1 in the colon. This is the first study to show the anti-inflammatory potential of a nutritional supplement derived from GBBC in the colitis animal model.

P-05.1-008
Interaction between alpha-2-macroglobulin and phycocyanobilin – structural and physiological implications

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The interaction between phycocyanobilin (PCB), a bioactive chromophore of blue-green cyanobacteria Spirulina's phycobiliproteins, and human alpha-2-macroglobulin (α2M), a universal anti-proteinase, was investigated in this study under simulated physiological conditions using spectroscopic techniques and α2M activity assay. Protein α2M was found to bind PCB with a moderate affinity, as assessed by spectrofluorimetric titration. The binding constant was calculated to be 6.3×10^5 M^{-1} at 25°C. The binding of PCB to α2M did not cause significant change in the secondary structure of the protein, as determined by circular dichroism. PCB protected α2M from oxidative damage in the presence of AAPH-induced free radical overproduction. PCB binding also effectively preserved α2M anti-proteinase activity. Since α2M is involved in controlling the action of enzymes during the inflammatory process, the protection that PCB expresses could indirectly influence the intensity and direction of the body response to impaired homeostasis, especially under oxidative stress.

P-05.1-009
Stability of oligosaccharides and rutin after *in vitro* digestibility of enzymatically hydrolyzed common buckwheat (*Fagopyrum esculentum* M.)

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Enzymes-assisted extraction of common buckwheat (*Fagopyrum esculentum* M.) is an exciting tool for developing food with higher-added value and characteristics because of their mild extraction conditions. During extraction, the permeability increases of the plant cell wall, releasing functional compounds such as rutin and forming new derivatives and properties. The research aims to determine rutin and oligosaccharides' stability after enzymes-assisted extraction and INFOGEST *in vitro* digestibility afterward. For analysis, two different batches were performed. Milled common buckwheat flours (> 0.5 mm) were homogenized with distilled water in a ratio of 1:5. Continuously, for the first batch, 0.15% of amylase (AL) and 0.15% non-starch polysaccharides enzymes (NSP), and for the second batch, 0.45% AL+ glucoamylase (AG) and 0.15% NSP was incorporated. After 2.5 hours at 68°C enzymatic extraction, a liquid fraction of buckwheats was collected. Samples were lyophilized, and rutin content was determined using HPLC. Furthermore, the HPLC SEC – NSP methodology was implemented for sugars and

oligosaccharides molecular mass profile comparison. The results indicate that rutin stability after *in vitro* digestion was not significantly changed. The quantity of rutin before and after digestion was 2.34 ± 0.02 and 2.37 ± 0.03 mg/100g. It shows rutin's stability with digestive fluids and enzymes. However, sugars and oligosaccharides alteration after enzymes-assisted extraction showed the highest variation in samples without glucoamylase. Moreover, after *in vitro* digestion analysis, different oligosaccharides' stability and continuous releases were identified. The alteration from 1 to 4 sugar chains (DP1 – DP4) showed the impact of digestive fluids and enzymes. In general, the results indicate that specific enzymes incorporation could lead to stable oligosaccharide formation, which potent have the prebiotic function, and rutin, which is involved in many health-promoting properties.

P-05.1-010
A methylotroph to produce L-malic acid in a techno-economically feasible way

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Worldwide methanol production has climbed from 32 million metric tons (MT) in 2006 to 62 million MT in 2012, and then to 85 million MT in 2016. Increasing numbers have forced strong legislations in favor of environmental benefits for sustainable processes for both methanol production and consumption. While there are still challenges in realizing sustainable production, assessment of methanol as a feedstock paves the way for circular carbon economy. Advantage of using native/synthetic methylotrophs for production relies on their ability to consume a reduced C1 compound instead of food-competitive carbon sources. This study evaluates the economic feasibility of l-malic acid production from methanol with *Bacillus methanolicus* MGA3 (Müller JEN et al. (2015) Appl Microbiol Biotechnol 99, 535–551; Wendisch VF et al. (2021) Advances in Biochemical Engineering/Biotechnology Springer 1-44). The target product has been recognized by The US Department of Energy as one of the most important platform chemicals that can be produced from biomass. First, a process flow diagram has been constructed with methanol, oxygen, ammonia, and a salt solution as inputs, biomass and l-malic acid as outputs, and fumaric, succinic and glutamic acids and CO_2 as by-products. Data for uptake/secretion rates and yield coefficients have been retrieved from relevant publications to make a mass balance (Delépine B (2020) mSystems 5, e00745-20). By-products have been selected based on literature data on l-malic acid producers. Finally, the bioprocess was simulated with SuperPro Designer® (Intelligen, Inc.) software for 20 g/L l-malic acid production using a batch mode bioreactor. Downstream processing comprised capture of the extracellular product, followed by separation from by-products and a formulation step. Different scenarios for the bioprocess simulations enabled evaluation of l-malic acid overproduction in terms of the feasibility of the operation. Support by TUBITAK (220N291) is acknowledged. *The authors marked with an asterisk equally contributed to the work.

P-05.1-011
Molecular aspects of nutritionally improved sorghum and buckwheat, two sustainable crops

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Drought and global warming are the most prominent abiotic stresses limiting crop production and productivity. Buckwheat is a short-season crop that grows well in low-fertility or acidic soils, while sorghum does best in semi-arid and arid tropics. Both crops are a major staple for roughly a billion people, mostly in developing countries. Buckwheat and sorghum are gluten-free grains, since their proteins do not contain coeliac-toxic sequences. In spite of their high protein and fiber content, the use of these crops in human nutrition may be limited by the presence of several anti-nutritional factors that limit nutrient bioavailability. Processes based on the use of eso- or endo-enzymes represent a suitable method for improving the bioavailability of both micro- and macro-nutrients, and are a sustainable way to obtain high quality foods from sustainable species. The biochemical changes ensuing from the action of endogenous seed proteases after short-time sprouting (48-72h) of buckwheat and sorghum grains included a less compact protein structure and a high release of peptides. The overall features of proteins in the sprouted grains made them more suitable for formation of the inter-protein network fundamental for appropriate texturing of specific foods. Sprouting-associated enzymes also decreased the levels of anti-nutritional factors such as enzymatic inhibitors, phytates and chelating compounds, increasing the bioavailability of metals, fatty acids, and free polyphenols. All together these results suggest that flour from germinated buckwheat and sorghum may be regarded as a nutritionally and technologically improved food ingredient. This research is part of the project "MIND FoodS HUB (Milano Innovation District Food System Hub) cofunded by POR FESR 2014-2020_BANDO Call HUB Regione Lombardia.

P-05.1-012
The effect of fermentation for leaves of *Aralia cordata* Thunb on the content of polyphenols and antioxidant activity

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Different traditional fermentation cultures, such as symbiotic culture *Meducomyces gisevii* (MG) and Tibetian kefir grains (TKG), lead to various bioactive compounds upswing for the same plant material. In most cases, fermentation of plant material increases phenolic content and antioxidant activities, which has a positive outcome on health. *Aralia cordata* Thunb (*A. cordata*) is a medicinal plant of the Araliaceae family with anti-nociceptive, antioxidant, and anti-inflammatory properties. This study aims to compare lactic and acetic acid fermentation impact, using TKG and a symbiotic culture of MG with *A. cordata* leaves, respectively. Fresh leaves were mixed with symbiotic cultures in ratio 1:10 and fermented at 25°C for 48 hours. Furthermore, the total phenolic content was performed using the Folin Ciocalteu method, and antioxidant activity was tested using $DPPH^{\bullet}$ scavenging capacity, $ABTS^{\bullet+}$ radical

cation assays for fermented samples. In addition, the color of fermented *A. cordata* fresh leaves using CIEL*a*b coordinates was measured. Results indicated that both symbiotic cultures significantly modified the color of fermented *A. cordata* leaves. Also, results show that different strains fermentation with *A. cordata* leaves contributed to enhanced total phenolic content, 58.7 mg/100g in TKG fermented extract and 69.8 mg/100g of MG fermented extract. Also, antioxidant activity in $ABTS^{\bullet+}$ increases by 96.8% in TKG fermented extract and 79.3% in MG fermented extract and for $DPPH^{\bullet}$ 98% in TKG fermented extract and 89% in MG fermented extract. A significant increase in bioactivity contributes to fermented *A. cordata* leaves being valuable for producing value-added functional food.

P-05.1-013
The effect of the real food matrix interaction on the cytotoxicity of digested SiO2 nanoparticles

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Silicon dioxide (SiO_2) nanoparticles (NP) are widely found in foods and there is a limited number of studies regarding the changes in the properties during digestion when consumed with foods and the effects of these changes on human health. It was known that some characteristic properties of NP, such as aggregation behaviour and corona structure, alter the cytotoxicity based on the reactivity of the NP. In this study, it was investigated the effects of digested 30 nm SiO_2 NP (Si30) and digested 100 nm SiO_2 NP (Si100) on cytotoxicity at two distinct doses (4 μg/mL (the daily consumption limit) and 400 μg/mL) in a real food matrix, milk. Firstly, the aggregation state of bare and milk-interacted NP was analyzed via SEM and DLS analysis, and these findings demonstrated that the aggregate size of Si30 was considerably enhanced after food interaction at the intestinal phase, while Si100 showed no significant change. SDS-PAGE was used to identify the protein composition of corona on NP and TEM images revealed the corona formation for both NP at the end of the digestion. After the characterization analysis, the cell viability of the human Caco-2 cell lines was measured using MTT assay and ROS experiments were used to determine reactive oxygen species activity within cells. According to MTT analysis, the viability of Caco-2 cells was higher for interacted Si30. These analyses revealed that the toxicity of Si30 was dramatically reduced at interacted NP due to the change of aggregate size rather than corona formation. Since any significant change was not observed in aggregate size following food interaction with Si100, it was thought the cytotoxicity would not alter, either. As a consequence of this research, it has been revealed that food interactions of NP could change the particle toxicity and these interactions should be considered for determining the potential toxicity of NP used as food additives.

P-05.1-014
Extraction of polyunsaturated fatty acids-rich oils from alternative sources from the food industry

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Food industry aims to reduce the waste of food products, including by-products resulting from casual manufacturing processes, like making jams, syrups, juices, and other drinks. By-products left after the first processing are still rich in valuable nutritional components: oil, fiber, proteins, etc. Another potential source for valuable compounds is secondary plant parts, for example, leaves. In this study, we aimed to process food industry by-products for the second time, extracting the oil and identifying the profile of polyunsaturated fatty acids to determine the further application possibilities. Three different by-products were collected from food industry companies in Babtai, Lithuania: processed sea buckthorn (*Hippophae rhamnoides* L.) fruits cake, sea buckthorn leaves and raspberry (*Rubus idaeus* L.) seeds. All by-products were dried until moisture content was less than 8% and extracted using high pressure CO_2 extraction. Collected oils were analysed by gas chromatography-mass spectrometry, and major saturated, monounsaturated, and polyunsaturated fatty acids identified and compared. Additionally, the content of carotenoids and tocopherol in oils were analyzed. Determined fatty acid composition revealed that sea-buckthorn cake oil had higher content of polyunsaturated acids (more than 51%) than oil from leaves, which was about 30%. Dominating polyunsaturated acids was linoleic 31.13% in cake oil and alpha-linolenic 16.06% in oil from leaves. Raspberry seed oil had the highest content of polyunsaturated acids among all extracted oils with more than 82% detected. The extraction of oils from sea-buckthorn and raspberry by-products revealed that nutritional oils could be obtained and used in the further development of polyunsaturated acid supplements. Secondary processing of by-products is proved to create high-value products and should be encouraged in the whole food industry.

P-05.1-015
Memory improvement resulting from the medium-chain triglyceride treatment is accompanied by altered glutamatergic gene expression in the rat brain: a pilot study in rats

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Medium-chain triglyceride (MCT) supplementation improves cognition in young and aged healthy subjects, and in neurodegenerative conditions. Although the benefits of MCT are linked to ketogenesis, which provides alternative fuel for the brain, the exact mechanisms remain poorly understood. Changes in number and subunit composition of NMDA- and AMPA- ionotropic glutamatergic receptors (iGR) underly long-term potentiation, one of the key mechanisms in learning. We studied the effects of MCT feeding on learning and mRNA expression of the iGR subunit and EAAT2 (glutamate transporter) genes in medial prefrontal cortex (mPFC), dorsal (DH) and ventral hippocampus (VH), of adult male Wistar rats. The animals were tested in Y-maze (YM), Open Field (OF) tests. After 2 weeks of daily o/g treatment with 2 ml/kg MCT (water as control), the animals were tested in YM, OF, and Morris water maze (MWM) tests. Gene expression was assessed with RT-PCR. After the last MCT treatment, animals were sacrificed, blood was collected for biochemical tests. β-hydroxybutyrate level was elevated, while pyruvate

and cholesterol levels decreased after MCT treatment compared to control. In YM, the rate of spontaneous alternation decreased in control but not MCT-fed animals, indicating that MCT improved working memory. In OF, MCT animals demonstrated higher extinguishing in exploratory activity, indicating better memory of the surroundings. In MWM, MCT animals spent more time in the target sector, indicating improved spatial memory. GluN2a- and GluN2b- NMDA-iGR subunit and GluA1- and GluA2 AMPA-iGR subunit expression was upregulated in mPFC of MCT animals, suggesting enhanced memory consolidation. MCT feeding increased EAAT2 expression in DH and decreased it in VH. Therefore, memory-enhancing effects of the intermittent ketosis established by MCT feeding are at least partially mediated via the effect on glutamatergic transmission. Funded by the Russian Science Foundation, grant 19-75-10076.

P-05.1-016
Assessment of *in vitro* biological activities of a natural product made from fermented bee pollen

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The immune system must be targeted to avoid reacting to harmless molecules and must be balanced in its reaction, responding at the appropriate level and for an appropriate period of time. Fortifying immunity with functional nutrition is one of the most effective public health techniques for disease prevention. Our aim was to investigate a complex product, which includes various health-related compounds by fermentation of the bee collected pollen with a Kombucha consortium and adding a mixture of kefir, in different concentrations and at various stages of the fermentation process. The monocyte/macrophage cell line (ATCC 9855) was used to evaluate *in vitro* cell viability (MTS test), cytotoxicity (LDH test) and anti-inflammatory capacity (xMAP array) of our natural product. Using multiplexing technology, the immune response to natural product treatment was investigated by analyzing a 10-plex inflammatory cytokine panel. Natural compounds based on pollen and Kombucha, in different concentrations stimulate monocyte proliferation, inducing a doubling of the values obtained in the MTS tests, compared to the control cells, at 48 hours for treatment. At 72 hours of treatment this value decreases to values similar to those obtained for untreated (control) and treated cells with a consortium of kefir and colostrum. Subsequently, the anti-inflammatory potential of the natural product, at different concentrations, was evaluated by multiplexing, using as positive control LPS (lipopolysaccharide) stimulation. The supplement inhibited the expression of main inflammatory cytokines in activated monocytes/macrophages cells, indicating that it has anti-inflammatory properties. Partially supported by the grants PNIII.P2-2.1-PED-2019-3141(contract no 382/2020), PN 19.29.01.04 (Core Programm), COP A 1.2.3., grant ID: P_40_197/2016, and 31PFE/30.12.2021. *The authors marked with an asterisk equally contributed to the work.

P-05.1-017
Production and characterization of a novel GH5_34 subfamily arabinoxylanase for exploitation in cereal fiber valorization

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Carbohydrate active enzymes are valuable tools in cereal processing in order to valorize under-utilized side streams. By solubilizing fibers and modifying the fiber polysaccharide structure, novel food products with increased nutritional value can be created. In our current work, we have identified, produced and characterized a novel GH5_34 subfamily arabinoxylanase, *Hh*Xyl, for the intended exploitation in oat processing to solubilize potential prebiotic fibers; arabinoxylooligosaccharides. Using the 3,5-dinitrosalicylic acid assay for activity measurement and commercially available homologue *Ct*Xyl5A (nzytech) as a reference enzyme, the *Hh*Xyl enzyme was characterized by evaluating substrate utilization and reaction products, optimal reaction conditions as well as investigating domain organization influence on activity and stability. The two-domain *Hh*Xyl demonstrated activity on various commercial cereal arabinoxylans as well as on alkali extracted oat fibers, while the single catalytic domain of *Hh*Xyl alone was inactive. By applying experimental design, optimal temperature and pH conditions for *Hh*Xyl on wheat arabinoxylan (Megazyme) was determined to 50°C and pH 6.5, respectively. The corresponding maximal reaction rate was 0.21 mM/min at 20 mg/L enzyme concentration and *Hh*Xyl was stable at these reaction conditions for at least 36 h. Our results indicate the benefits of exploiting *Hh*Xyl for fiber solubilization and modification and in addition provide a deeper insight into the importance of domain organization when establishing novel enzymes for biorefinery processes. This study was supported by funding from the Swedish Foundation for Strategic Research (the Industrial Research Center ScanOats).

P-05.1-018
Bioavailable polyphenol metabolites impact renal cancer cells migration

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Plants have developed different strategies to fight biological stress, triggering metabolic responses with production of secondary metabolites, including polyphenols. These compounds are largely included in human diet and its consumption has been related to a range of health benefits, including renal health. Most studies addressing the role of polyphenols in health use *in vitro* strategies relying on the direct incubation of specific polyphenols with target cells. Nevertheless, polyphenols undergo a series of biological transformations along the human digestive tract, leading to the formation of different metabolites that reach circulation in higher concentrations than their parent compounds, hence prone to exert higher biological activity at target tissues. We are interested in exploring the potential role of polyphenol metabolites on cell features related with renal cell carcinoma

(RCC) progression, under physiological relevant conditions. Our initial strategy involved a survey on the polyphenol metabolites that have been previously identified and quantified in human urine, through an extensive data collection from available literature and databases – Phyto Hub and Phenol Explorer. This approach provided a selection of 34 polyphenol metabolites, previously identified in human urine in maximum concentrations >1 μM. Physiologically relevant concentrations of 15 selected metabolites were then used for the assessment of potential effects on cell viability using the crystal violet staining, on two cell lines representing clear cell RCC (786-O) and non-tumor-like renal cells (Vero-E6). Cell viability was not affected on both cell lines. Collective cell migration was assessed in 786-O cells through the wound healing assay, and some polyphenol metabolites have shown anti-migratory properties. Overall, our results demonstrate that polyphenol metabolites can impact cancer cells phenotype, contributing to understand how polyphenols may influence RCC progression.

P-05.1-019
Natural functional food based on fermented bee pollen with chemopreventive potential – *in vitro* studies

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Bee pollen is a natural functional food containing valuable bioactive compounds with impact on human health. Due to the limited bioavailability of bee pollen nutraceuticals, different fermentation processes have been developed to increase their absorption. This study presents the results of *in vitro* investigations of biological effects induced by products obtained through a two-step bee pollen fermentation process. Fermented bee pollen with Kombucha was mixed with bovine colostrum co-cultivated with yeast. The freeze-dried day 1 and day 6 fermentation products were tested on normal and tumorigenic human hepatic cell lines. Cytotoxicity of samples at concentrations (0.05-0.55 mg/ml) were measured by MTS and LDH tests. The proliferation rate was assessed by real-time cellular impedance monitoring. Cellular antioxidant activity was performed using OxiSelect™ CAA kit on treated and control-treated tumorigenic cells and was confirmed by CellROX™Green staining. No cytotoxicity and an increased cellular proliferation rate were observed for normal cells treated with day 6 fermentation products in comparison with control- and day 1 fermented products-treated cells. At the same concentration, a significant anti-proliferative effect and cytotoxicity was induced on carcinoma cells. The cellular antioxidant activity of 6 days fermentation-products was comparable with quercetin at 250 μM, used as positive control. Our study demonstrated that bee pollen fermentation products can induce chemopreventive effects by reducing the cellular proliferation rate and the viability of hepatic tumour cells. Moreover, the 6 days fermentation products presented higher cytoprotective activity against ROS generation in tumour cells, compared with day 1 samples. The two-step fermentation process enhanced biological activities of bee pollen, providing in vitro chemopreventive activity. Acknowledgement: partially supported by grants 31PFE/30.12.2021; COP A 1.2.3., ID: P_40_197/2016 and PN 192901.03.

P-05.1-020
Change in phenolic profile, neuroprotective and antihypertensive potentials of wine and grape juice during *in vitro* digestion and cell metabolism

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Moderate wine and grape intake and lower incidence of chronic diseases are the basis of French paradox and Mediterranean diet concept. Most health benefits attributed to wine and grape intake are associated with phenolic compounds, that are known to undergo an extensive metabolism in the digestive tract. Since phenolic metabolites are the main carriers of wine/grape bioactivity, there is an increased interest in their metabolic pathways and stability under physiological conditions. Thus, the aim of this study was to compare the phenolic profile of undigested, *in vitro* digested and *in vitro* Caco-2 cell metabolised samples of Cabernet Sauvignon wine and grape juice. In total, 39 phenolic compounds were determined by LC-MS/MS, HPLC-UV/VIS and UHPLC-DAD-MS in undigested and digested wine and juice samples, as well as in samples obtained from the cell lysate and apical and basolateral compartments (after a 5-h incubation). Higher amounts of most compounds were detected in undigested samples compared to digested and metabolised samples, with a few exceptions. High levels of catechin and epicatechin were found in cell lysates, suggesting their accumulation in cells, along with some flavonol-glucosides and phenolic acids. Only 3 phenolic acids passed unchanged to the basolateral compartment in low concentrations, where p-coumaric acid displayed a dose-dependent flux. Furthermore, neuroprotective and antihypertensive properties of digested and undigested samples were assessed by inhibiting acetylcholinesterase (AChE) and angiotensin-I converting enzyme (ACE), respectively. Undigested samples inhibited only AChE, while digested samples inhibited only ACE, indicating that digested metabolites are responsible for the antihypertensive effect. In both assays, grape juice stood out as a more potent sample than wine. This study gives information on the fate of wine and grape-related phenols in the gastrointestinal tract and the change in biological activity due to their metabolism.

P-05.1-021
Antioxidant content of basil leaves (*Ocimum basilicum* L.) different colors

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Common basil belongs to the subfamily Nepetoidea of the Lamiaceae family. The diversity of types of this species and the presence of essential oils and aromatic compounds determine the

commercial interest in this green crop. In our study, 4 green colored and 5 purple colored basil samples were studied for ascorbic acid content, total water-soluble antioxidant content, content of polyphenols and total antioxidants in the alcohol extract, and total anthocyanin content for the purple colored samples. According to the amount of ascorbic acid, cv. Caramel and selected sample from VIR k-143 (France) (9.68 mg%) stood out from green-colored samples, and from purple-colored – cv. Free Gift (9.68 mg%), while the values of the sum of water-soluble antioxidants were comparable in all samples. Our calculated correlation coefficient between these two parameters was - 0.73. For the content of polyphenols and the sum of antioxidants in the alcohol extract the highest values were observed for the purple-colored cv. Free Gift (19.80 mg/g and 21.97 mg/g in EGA), while the lowest values were observed for the selected sample VIR k-13 (Armenia) (14.11 mg/g and 15.65 mg/g in EGA). Whereas among the green-colored varieties the content of both parameters was the same. The correlation coefficient was also high - 0.94. The total anthocyanin content was also the highest in the leaves of the cv. Free Gift - 0.74 mg/g in terms of cyanide-3,5-diglycoside. Other purple-colored basil samples contained 2-3 times less anthocyanins than the cv. Free Gift. The correlation coefficient between the content of anthocyanins and the sum of water-soluble antioxidants was 0.95, whereas the correlation between the content of anthocyanins and the sum of antioxidants in the alcohol extract was lower at 0.81, and the correlation between the sum of anthocyanins and the content of polyphenols was even lower at 0.76. The study of correlational interactions between biochemical parameters in the leaves of the model plant of common basil, both green- and violet-colored, will probably reveal the relationship of antioxidant parameters.

Sensors and nanotechnology

P-05.2-001
Antibacterial activity of silver nanoparticles biosynthesized from *Stevia rebaudiana* extract

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Nowadays, silver nanoparticles (Ag NPs) attracted much attention as alternate antibacterial agents, which are widely used in biomedicine. *Stevia rebaudiana* is a medicinal plant which contains proteins, carbohydrates, glycosides and antioxidant compounds, and its extract can be used as a valuable platform for the Ag NPs biosynthesis. The present work focuses on the biosynthesis of Ag NPs using hydroponic *S. rebaudiana* extracts and evaluation of antibacterial activity of extracts. Biosynthesized NPs demonstrate antibacterial activity against Gram-negative (*Escherichia coli* BW25113) and Gram-positive (*Enterococcus hirae* ATCC9790) bacteria. Ag NPs demonstrate concentration dependent effect on the growth rate of these bacteria. In the presence of 10 μg/mL Ag NPs ~9-fold inhibition of *E. coli* growth was detected, whereas in case of *E. hirae*, it was suppressed ~2-fold. Thus, biosynthesized NPs showed more obvious antibacterial effect on Gram-negative *E. coli*, in comparison with Gram-positive *E. hirae*, which is coupled with the differences in the bacterial cell wall structure. Ag NPs not only suppressed the growth of bacteria, but also changed the energy-dependent H^+-fluxes across the bacterial membrane. Ag NPs increased energy-dependent H^+-fluxes in *E. coli* and *E. hirae* ~1.3-fold, in comparison in the assays without NPs. Moreover, NPs affect the *N,N'*-dicyclohexylcarbodiimide (DCCD)-dependent H^+-fluxes. The obtained data indicate that biosynthesized Ag NPs affected the structure and permeability of the bacterial membranes. In addition, Ag NPs biosynthesized from hydropnic *S. rebaudiana* extract demonstrate an antibacterial activity against bacteria and can be applied as an alternative to antibiotics. *The authors marked with an asterisk equally contributed to the work.

P-05.2-002
Folic acid-unsymmetrical bisacridine conjugated quantum dots for improving tumor-targeted drug delivery

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Targeted delivery of drugs to the tumor cells by nanoparticles (NPs) can increase the efficiency of cancer treatment. Chemotherapeutics can be delivered with NPs targeting surface proteins, overexpressed on the membrane of cancer cells. One of them is the folic acid receptors, which have a high affinity for folic acid (FA). C–2028 is the agent of patented [EP 3070078 B1 and US 10,202,349, B2], active antitumor drugs – unsymmetrical bisacridines. Here, we investigated the effect of the presence of navigating molecule (FA) in conjugates of quantum dots (QDs) with C–2028 (QDs–CD–FA–C–2028) on cytotoxicity, cellular uptake, and the mechanism of internalization to cancer (H460, Du-145, and LNCaP) as well as normal (MRC-5 and PNT1A) cells. The QDs–CD–FA–C–2028 conjugate was characterized using DLS (dynamic light scattering), ZP (zeta potential), QCM-D (quartz crystal microbalance with dissipation), and UV-vis spectroscopy. The obtained results showed that the conjugate is stable in pH 7.4 (ZP = -29.1 mV) in the form of small aggregates (size *ca.* 200 nm). The QCM-D measurements showed that the intensity of drug release from the conjugate is more effective in pH 5.2. Conjugation of C–2028 with non-toxic QDs or QDs–CD–FA did not change the cytotoxic activity of this compound. Confocal microscopy images showed that the use of FA in conjugates significantly increased the amount of delivered compound to the cells, mostly in cancer cells. The internalization of QD_{green}–CD–FA–C–2028 to the cells was taken up by three endocytosis pathways: clathrin- and caveolae-mediated endocytosis, as well as macropinocytosis in different levels, depending on the cell line. To conclude, QD_{green}–CD–FA–C–2028, compared to C–2028 alone and QD_{green}–C–2028, showed much higher cell uptaking ability due to the multiple pathways of endocytosis and overexpressed receptors on the membrane of cancer cells. These studies were supported by the National Science Center, Poland, Grant No. 2020/37/N/NZ7/01979.

P-05.2-003
Abstract withdrawn

P-05.2-003
Properties of electrode-supported lipid cubic mesophase films with embedded ROMK2 channel

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One of the biological membranes models is a lipid cubic phase (LCP), which is a nanomaterial composed of water channels surrounded by lipid bilayers. LCPs have many features that give them an advantage over other membrane bilayer models. This is a lyotropic liquid crystal, an inexpensive membrane mimetic system that allows for the immobilization of membrane proteins. Due to the structure, its internal surface area is huge, meaning it can host and orient a large number of membrane protein molecules in a favorable way, providing the lipid environment for the hydrophobic part of the protein while the extra and intracellular domains are exposed to the aqueous channels, similarly to their contact with the aqueous phase on both sides of the biological membrane. The properties of the LCP resemble those of biological bilayers, allowing the incorporated proteins to potentially maintain their activity. In this study, we focus on the changes of cubic phase film properties upon embedding mitochondrial renal outer medullary potassium channel (ROMK2). We chose it due to its pivotal role in inducing cytoprotective mechanisms. For this purpose, we obtained a protein by in vitro transcription-translation and prepared a stable LCP with and without the ROMK2. The K^+ transport efficiency was studied by electroanalytical methods, while the structural changes of the LCP were investigated using small-angle X-ray scattering (SAXS). The presented results prove that the LCP preserves native properties of ROMK2 and allows more efficient movement of K^+ through the lipid film than in the absence of the ROMK2, and thus provides a suitable platform for electrochemical studies of other membrane ion channels. This matrix allows us to employ membrane proteins in sensing biodevices. LCP in the form of a film supported on the electrode surfaces can be viewed as a convenient model membrane for the studies of membrane transport phenomena, as well as for screening therapeutically relevant drugs.

P-05.2-005
A yeast-based biosensor to monitor tebuconazole in environmental samples

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Agriculture is a human activity with high impact in the environment. Due to a constant demand for a high and stable crop production, pesticides and other chemicals have been used extensively, however once introduced in the environment these substances can have a negative impact in ecosystems and in humans. Environmental monitoring of these agrochemicals is therefore of utmost importance, because of their collateral effects on ecosystem and human health; however, most current-use analytical methods require procedures that are too complex and costly for routine monitoring. In recent years, there has been an increased interest in biosensors as alternative or complementary tools for detection and quantification of environmental contaminants. In this sense, we aimed to develop a biosensor for environmental monitoring of tebuconazole, a fungicide commonly used as agrochemical and frequently detected in aquatic ecosystems, which has been reported to be hazardous to non-target fungi. We engineered cells of the yeast *S. cerevisiae* with a reporter gene downstream of specific gene promoters that are expressed after exposure to tebuconazole and characterized the sensitivity and specificity of this model system. After optimization, we found that this easy-to-use reporter strain is specific to tebuconazole at realistic environmental concentrations. This biosensor can be used as a complementary tool in environmental monitoring programs, namely in high throughput scenarios requiring triage of numerous samples.

P-05.2-006
In vivo imaging of cytokinins activity in plants with autonomous bioluminescence

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Cytokinins are a major group of plant hormones responsible for the growth and development of plants. However, studies of their localization and activity are typically invasive, or limited to the use of either fluorescence-based reporters that require light for excitation, or luminescence-based reporters that require external addition of substrate. Herein we investigated the possibility of using a fully encoded bioluminescence system of fungi *Neonothopanus nambi* for imaging of cytokinins via *Agrobacterium*-mediated transient transformation of *Nicotiana benthamiana* leaves and BY-2 cell culture line. We placed the luciferase gene under the control of cytokinin-induced promoters: pARR6 promoter from *Arabidopsis thaliana* (Zürcher et al. 2013) and synthetic pTCSv2 promoter. Other genes of the bioluminescence system of fungi (hispidin synthase, 4-phosphopantetheinyl transferase, hispidin-3-hydroxylase, caffeoyl pyruvate hydrolase) were placed under the control of constitutive promoters. We detected induction of pARR6 and pTCSv2 promoters by different cytokinins proving their suitability for the development of genetically encoded luminescence-based tools for imaging of phytohormones. The reported study was funded by RFBR and GACR, project number 20-54-26009.

P-05.2-007
Chaetopterus variopedatus bioluminescence system low-molecular-weight components

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Chaetopterus variopedatus, a marine polychaete worm inhabiting U-shaped tubes burrowed into the sediment, is capable of secreting luminescent mucus in response to mechanical stimulation[1]. A long-lasting study of its bioluminescence system by various scientific groups yielded contradictory results and the mechanism of *Chaetopterus* luminescence remains unclear. Thus, early investigations have suggested that *Chaetopterus* bioluminescence system consists of a photoprotein and five auxiliary components: O_2, Fe^{2+}, H_2O_2, and two cofactors – lipid-like substance and additional protein[2]. Further kinetic studies confirmed the luciferin-luciferase mechanism of the reaction[3], but luciferin has not yet been isolated due to its low stability. Three compounds demonstrating bioluminescent activity with the luciferase fraction of *Chaetopterus* in the presence of Fe^{2+} ions were isolated from the algae *Chaetomorpha linum*. Their structures were established using NMR spectroscopy and mass spectrometry. We also developed methods for the synthesis of these substances and their functional analogs, which was necessary for the determination of their role in the *Chaetopterus* bioluminescence system and elucidation of the light emission reaction mechanism. We believe that our study of *Chaetopterus* bioluminescentce system may lead to the development of new analytical tools for the analysis of reactive oxygen species in living cells. The work was supported by the Russian Science Foundation grant no. 18-74-10102, https://rscf.ru/project/18-74-10102/. (1) Mirza J.D. et al. (2020) Photochem. Photobiol., 96(4), 768-778. (2) Shimomura O., Johnson F.H. (1966) Bioluminescence in Progress, 495–521. (3) Purtov K.V. et al. (2019) Dokl Biochem Biophys., 486(1), 209-212.

P-05.2-008
Calcium sensor development based on bioluminescence system from higher fungi

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Calcium is a secondary messenger involved in signal perception and transduction during plant responses to environmental changes. However, imaging of calcium signaling in plants is mostly limited to fluorescence-based calcium indicators. Fluorescent imaging is hampered by autofluorescence in heavily pigmented plant tissues. In contrast, luminescence-based sensors do not require light excitation, have almost no background signal and impose no light-induced physiological effects. Nevertheless, almost all available luminescent tools to date require exogenous addition of substrate, thus not allowing non-invasive imaging. Recently we characterized a fully genetically encoded bioluminescent system from higher fungi (Kotlobay et al., 2018, Mitiouchkina et al., 2020). Herein we describe our ongoing effort to engineer an autoluminescent calcium indicator. We implemented a bioluminescence resonance energy transfer-based approach by coupling fungal luciferase with a red fluorescent protein through a calcium-binding domain of troponin C and assessed its functionality via transient expression in plant cells. The reported study was funded by RFBR and MOST, project number 21-54-52004.

P-05.2-009
Doxorubicin loaded albumin modified nanoparticles for targeted chemo-photothermal therapy in breast cancer cells

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An encouraging strategy to perform targeted diagnosis and therapy in tumors is nanotheranostic which uses, as carriers for contrast agents (CAs) and drugs, particles with nanoscale size (nanoparticles). Recent studies have combined photoacoustic imaging with chemotherapeutic drugs (e.g. Doxorubicin, DOX) and/or Phototermal Therapy (PTT). In previous studies, PA properties of hybrid NPs, namely MelaSil_Ag NPs, composed of a metallic silver cluster surrounded by a silicon and a melanin like compound shell and functionalized with Human Serum Albumin (HSA), were tested. Here, HSA-NPs were loaded with DOX (MelaSil_Ag-HSA@DOX NPs) and then their chemo- and photo-thermal efficiency was analyzed. Our results showed that DOX delivered by NPs is more toxic compared to the free drug under the same experimental conditions, allowing a better effect by using lower drug concentrations and time exposure, probably due to the combined effect of targeted delivery and overcoming multidrug resistance. Subsequently, the effect of the DOX-loaded NPs administration followed by photothermal laser irradiation was investigated. After 6 h of treatment at the lowest concentration, a relatively higher cytotoxicity compared to dark conditions was observed, due to a synergistic effect of temperature increase and doxorubicin toxicity, associated to the improved drug release after photothermal heating at 808 nm. All together, these data confirm that PTT is a good strategy to improve the cytoxicity of MelaSil_Ag-HSA@DOX NPs. In conclusion, the use of MelaSil_Ag-HSA@DOX permit to reduce the timing of treatment, combining chemo- and photothermal approaches, reducing the efficacy concentration of the DOX and the time exposure.

P-05.2-010
A novel wound healing agent with anti-microbial properties: BSA-inorganic hybrid nanoflowers

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Bovine serum albumin (BSA), which has been used for a long time in medias for mammalian cell cultures, stimulates DNA synthesis and has a growth-stimulating effect. It leads to a consideration of the extracellular and intracellular actions of the molecule, and importantly the role of its interactions with numerous ligands or bioactive factors that influence the growth of cells in culture: these include hormones, growth factors, lipids, amino acids, metal ions, reactive oxygen and nitrogen species. However, free proteins have some disadvantages, such as short lifetime in solution, inconvenient recovery and reusability, which strictly hinder their aforementioned applications. The study has been focused on the synthesis and characterization of BSA-inorganic hybrid nanoflowers (BSA-Nfs), then evaluating wound healing with anti-microbial properties. CCD-1072Sk fibroblast cell line was studied to observe effect of BSA-Nfs on wound healing. Firstly, cell proliferation was tested with 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium to determine the best concentration of BSA-Nfs. Then, the spreading and migration capabilities of CCD-1072Sk fibroblasts were assessed using a scratch wound assay which measures the expansion of a cell population on surfaces. Fibroblasts were treated with BSA free (2 ng/ml), BSA-Nfs (100 ng/ml), CuSO4(100 ng/ml) and Cu-Nfs(100 ng/ml). There was a non-significant, but marked increase in the number of cells present at 24 h in BSA-Nfs versus control and BSA free. Besides, the *in vitro* antimicrobial activities of the BSA nfs against Gram-positive and Gram-negative bacteria and *C. albicans* were determined by the microbroth dilutions technique using the Clinical Laboratory Standards Institute recommendations. BSA Nfs showed antimicrobial activity against S. epidermidis, E. faecalis, E. coli and K. pneumoniae with the MIC = 1250 μg/ml. However, BSA-Nfs did not exhibit any activity on the yeast *C. albicans* in the study.

P-05.2-011
High-throughput droplet microfluidics for protein engineering

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Improving the properties of biological molecules is vital for many industrial and medical applications. Droplet-based microfluidic systems present high-throughput capabilities to screen and sort peptide and protein libraries with different features [1]. Here, we present three different examples of how droplet-based approaches could be used to upgrade three different classes proteogenic species: (a) Computationally designed tetratricopeptide repeat (TPR) rational libraries that coordinate with porphyrins with enhanced catalytic properties, (b) The fatty-acid-binding protein UnaG with improved fluorescence when it binds to a bilirubin molecule and, (c) Epitope library screenings against different antibodies using fluorescence anisotropy. Overall, our results show that droplet-microfluidic techniques are a powerful tool to overcome the limiting screening steps and could allow the analysis of variants within increasingly complex libraries. [1] Manteca, Aitor, et al. "Directed Evolution in Drops: Molecular Aspects and Applications." ACS Synthetic Biology 10.11 (2021): 2772-2783.

P-05.2-012
Potential biotechnological applications of low molecular weight poly-γ-glutamic acid fractions

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Polyglutamic acid (PGA) is a non-immunogenic and biodegradable anionic homopolyamide that is made of D- and L-glutamic acid units and that can be differentiated into two isoforms depending on the attachment of the carboxyl group. γ-PGA is extensively synthesized using bacteria, especially those of *Bacillus* species, in a ribosome-independent manner, since glutamate is polymerized inside the cell via γ-amide linkages. The potentialities of applications of low Mw γ-PGA chains (< 100-200 kDa) have been so far only partially explored and, thus, different molecular families of them were separated and characterized for their potential bioactivity and ability to form biodegradable films. In order to obtain size-specific γ-PGA fractions, ultra and nano-filtration membrane-based separation methods were investigated by starting from an inexpensive commercial γ-PGA source. The results obtained by size exclusion chromatography equipped with a triple detector array showed that the membrane-based separation led to separate two fractions with homogenous MW of 57 kDa and 18 kDa, respectively. The samples were then tested as bioactive polymer sources to produce films by either casting or hot compression techniques both in the absence and presence of different concentrations of glycerol added as plasticizer. The mechanical and thermal properties, as well as the hydrophilicity of the produced materials indicated a significant increase of the elongation at break as a function of glycerol amount present in all the film samples examined. Moreover, the materials obtained by hot compression were less water-soluble than the ones prepared by casting and revealed an increased stability as they decomposed at 330°C. All these findings, together with the observed ability to counteract the desiccation and the oxidative stress of keratinocyte monolayers, suggest potential applications of the low molecular weight γ-PGA fractions to vehicle bio-active molecules for pharmaceutical and food purposes.

P-05.2-013
The contribution of phospholipase A2 and metalloproteinases to the synergistic action of viper venom on the bioenergetic profile of VERO cells

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The increasing concern about the use of animal models has stimulated the development of *in vitro* cell culture models for the analysis of the biological effects of snake venoms. However, the complexity of animal venoms and the extreme synergy of the venom components during the intoxication calls for critical review and analysis. The epithelium is a primary target for injected viper venom toxic substances, and therefore a focus in modern toxicology. We used Vero epithelial cell line as a model to compare the actions of a crude *Macrovipera lebetina obtusa* venom with the actions of the same venom with inhibited two key enzymatic components (specifically, phospholipase A2, PLA2; and metalloproteinases) on the bioenergetic cellular response, i.e. oxygen uptake and reactive oxygen species generation. In addition to the rate of free radical oxidation and lipid peroxidation, we measured real-time mitochondrial respiration (based on the oxygen consumption rate) and glycolysis (based on the extracellular acidification rate) using a Seahorse analyzer. Our data have shown that Levantine viper venom drives an increase in both glycolysis and respiration in Vero cells, while blockage of PLA2 or/and metalloproteinases affects only the rates of oxidative phosphorylation. PLA2 blocking in venom also increases the cytotoxic activity and overproduction of the reactive oxygen species.

P-05.2-014
The role of self-assembled Congo red complexed with albumin or carbon nanotubes as a carrier of drugs (dasatinib or doxorubicin) in anticancer therapy

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Nowadays, targeted drug delivery in cancer therapy is of great interest in nanotechnology. Carriers that bind a drug can reduce its toxicity and allow targeted transport into the cell, and provide controlled dosing. Examples of such carriers are ribbon-like supramolecular structures formed by self-association which can attach compounds by intercalation, interact with carbon nanotubes or albumin. An example of this type of structure is Congo red (CR). One of the special features of supramolecular structures formed by CR is the ability to target binding to antigen-bound antibodies, while not binding to free antibodies. There is also a phenomenon of strong enhancement of the interaction of antibodies with the antigen which ensures their use in immunotargeting and may be particularly promising for the recognition of neoplastic cells. The proposed structures are also easily removed from the body. The project aimed to analyze the complexes between dasatinib (BMS) or doxorubicin (Dox) and the carrier (CR). The analysis was performed using the following methods: electrophoresis, DLS, UV-VIS spectroscopy, and CD, as well as tests on bladder cancer cell lines (results are presented in the abstract of Lasota et al.). We have chosen the CR-drug optimal molar ratios. The results showed that the presence of CR increases the solubility of the BMS, giving the CR-BMS complex. CR-Dox complex, in contrast to CR-BMS complex, has optical properties (CD results). Earlier results also showed CR binding to carbon nanotubes and albumin. The combination of these elements forms the basis for the creation of carrier systems and their use for the transport of anti-cancer drugs. *We acknowledge the financial support from the National Science Centre, Poland (no. 2016/21/D/NZ1/02763) and the international project (no. U1C/P03/NO/03.23) in the strategic program Initiative of Excellence at the Jagiellonian University.*

P-05.2-015
Nanobiotechnology based research in the last decade: evaluation of the related publications and patents

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Since the concept of 'nanotechnology' emerged in the 1990s, the design and engineering of functional nanostructured materials at the molecular scale are fast-growing. The development of biosensors for diagnosis, drug targeting, controlled release applications, medical implants, imaging techniques, and vaccine developments are the top research topics in nanobiotechnology. Here, we provide a critical evaluation of the tremendous progress of nanobiotechnology over the last decade (2011-2021) in regard to related papers and patents using Statnano, Web of Science (WoS), European Patent Office (EPO), and The United States Patent and Trademark Office (USPTO) databases. The evaluation indicates that many developed countries give their priority to nanotechnology research and supported the field with various funds. The top players in the global market are the USA (estimated share of $13.2 billion in 2021, China (estimated share of $5.1 billion by 2026), Japan, Canada, and Germany. WoS search indicates that China, the USA, India, Iran, and South Korea are the top five countries with published ISI-indexed articles in 2011–2021. The number of ISI publications exceeded 220,000 in 2020. The evaluation of granted patents indicates that the USA, Germany, and South Korea are the top countries of patents in the field of nanotechnology. Despite this rapid growth and investments made by start-ups, governments, universities, etc. nanobiotechnology is faced with similar challenges to other applied sciences. There is an increasing number of good 'applied' papers however the development of successful technologies is not always parallel. Academia and industry should strengthen their cooperation, but governments and funding agencies need to play a key role. Thus, the authorities should give focus and support to this area taking the fact that it has great potential, especially in the health sector.
Keywords: nanobiotechnology, biomedical, medicine, nanomaterials

P-05.2-016
Nanocristalline graphite is an optimum substrate for antibodies functionalization for capture of circulating tumor cells

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Capture and quantification of circulating tumor cells is a novel research target for early diagnostic. An optimum substrate should combine low non-specific binding to high efficiency in capturing only targeted cells. Recently, it was shown that nanostructuring of substrates increased cell binding when compared to planar substrates. Our aim was to test different functionalized graphene-based substrates and select one optimum combination for capture of breast tumor cells. Simple graphene, vertical graphene and nanocrystalline graphene substrates were functionalized with monoclonal Epcam antibodies and tested for cell-binding with normal monocytes (ATCC-9855) and three different breast tumor cells (ATCC-MCF-7, MDA-MB-231, MDA-MB-361). 100 cells were incubated for 1h at room temperature, fixed in 4% formaldehyde for 10 min and washed 3 times in PBS. Cell binding was assessed by DAPI staining of attached cells. Of all tested combinations, nanocristalline graphite decorated with covalently bound gold nanoparticles and functionalized with 11-mercaptoundecanoic acid showed the highest efficiency for functionalization with anti-Epcam antibodies. Non-specific binding of cells was assessed by monocytes attachment (EpCam negative suspension cells) and it was less than 10% for the selected substrate. For tested breast cancer cells, the selected substrate captured with highest efficiency MDA-MB-231 cells. Nanocristalline graphite functionalized with anti-EpCam antibodies is a useful substrate for capture of triple negative metastatic breast tumor cells. This work has been supported by a grant of the Ministry of Research, Innovation and Digitization, CNCS/CCCDI – UEFISCDI, project number PNIII.P2-2.1-PED-2019-3141, contract no 382/2020, within PNCDI III and grant no. PN 19.29.01.04 (Core Programm) and Contract No. 31PFE/30.12.2021 *The authors marked with an asterisk equally contributed to the work.

P-05.2-017
Drug delivery systems in targeted cancer therapy using supramolecular compounds of Congo red or Evans blue, complexed with tyrosine kinases inhibitors (dasatinib or imatinib)

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The incidence of many diseases has increased in recent decades. Among all the diseases there is a huge problem with the incidence of neoplastic diseases. It is estimated that in 2022 alone, almost two million new cases of cancer and over half a million deaths as a result of cancer will be diagnosed in the United States. Every year, there is a growing demand for new drugs, methods of diagnosis, treatment and drug administration. One of the solutions to deal with the problem is using a supramolecular ribbon-like structures such as Congo red or Evans blue. Targeted therapy seems to be a requirement for cancer pharmacotherapy progress. The research is aimed at optimizing targeted therapy related to target drug delivery with the use of a supramolecular carrier and heat-aggregated immunoglobulins (HAI). The main goal is to deliver the drug to the cell while maintaining an optimal therapeutic dose. An analysis of the complex formation between the supramolecular carrier such as Congo red or Evans blue and selected c-KIT receptor tyrosine kinases inhibitors (imatinib, dasatinib) was performed using electrophoresis, chromatography, DLS and UV-VIS spectroscopy. TEM and agglutination method was used to evaluate the structures of selected carriers with HAI. The optimal carrier-drug molar ratio was determined and a preliminary evaluation of complex formation was made. Drug intercalation into ribbon-like carrier structures was confirmed. The supramolecular complexes with drugs may be used in targeted anti-cancer therapy. The results of the research on the effect of the compounds tested by us on bladder cancer cells are presented in the abstract by Lasota M. et al. We acknowledge the financial support from the National Science Centre, Poland (grant no. K/MNT/000232) and the international project (no. U1C/P03/NO/03.23) in the strategic program Initiative of Excellence at the Jagiellonian University.

P-05.2-018
Comparison of electrochemical immunosensor platforms for indiscriminate SARS-CoV-2 variant detection

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Since the first days of the pandemic, diagnosis of patients infected with the SARS-CoV-2 has been one of the most important parameters to control the virus. For this reason, many studies have been carried out to develop various methods for rapid and accurate diagnosis, but mutations and the occurrence of successive variants have made accurate diagnosis difficult. In this study, a screen-printed carbon electrode was used to develop magnetic nanoparticle (MNP)-based electrochemical biosensing systems that selectively detect SARS-CoV-2 virus and its variants (original, alpha, beta, and delta) in nasopharyngeal swabs. These electrodes were modified with MNPs conjugated to SARS-CoV-2 S1, S2 proteins and swab samples. Then, commercially available SARS-CoV-2-specific anti-S1 and anti-S2 antibodies and antibody cocktails purified from serum samples were applied to the surface and the performance of the platforms was compared.

Analytical parameters and electrode surface characterizations were performed by electrochemical measurements after each modification step. After optimization studies of the developed biosensor platforms, the detection of limit for the antibody cocktail-based sensors were determined to be 0.53-0.75 ng/mL, while it was calculated to be 0.93-0.99 ng/mL for the anti-S1 and anti-S2-based sensors. The performance of the platforms in real nasopharyngeal swab samples (negative, original, alpha, beta, and delta variants) was evaluated and it was found that the polyclonal antibody cocktail outperformed the commercial anti-S1 and anti-S2 antibodies. As a result, polyclonal antibody cocktail, with an overall sensitivity, specificity, and accuracy of 100%, is a versatile electrochemical biosensor system for the detection of the different variants of SARS-CoV-2. We hope that the biosensor platform modified with polyclonal antibodies can be used as a potential diagnostic tool that can be applied to such epidemics in the future.

Synthetic biology

P-05.3-001
Expression genes encoding cholesterol carrier proteins to increase the efficiency of P450scc system in *E. coli* cells

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Cytochrome P450scc (CYP11A1) with its redox partners adrenodoxin reductase (AdR) and adrenodoxin (Adx) forms a complex of the P450scc system. This system provides the initial reactions of mammalian steroidogenesis, resulting in the formation of pregnenolone. Earlier it has been shown that the P450scc system can function in *E. coli* cells. However, transport of P450scc substrate – cholesterol – into cells is inefficient (Previously published in Efimova et al. (2018) Mol Biotechnol, 61 (4), 261-273), thereby placing a limit on the yield of the target product. To solve this problem, we used a fundamentally new approach – the incorporation of the human cholesterol transporter into the bacterial membrane. We were examining the potential of the mitochondrial protein StARD1 (steroidogenic acute regulatory protein 1), a native partner of cytochrome P450scc, as well as StARD3, delivering cholesterol to endosomes. Obtained DNA constructions provide synthesis in *E. coli* of StARD1 or StARD3. Both of them are predominantly localized in the membrane fraction. For the first time, human cholesterol-carrying proteins were shown to manifest their activity in bacterial cells. According to the results of HPLC-MS and fluorescence spectroscopy, the carrier proteins expressed in bacteria increase the intracellular level of both cholesterol and 22-NBD-hydroxycholesterol by ~2-fold compared to the control strain. Most notably, this is the first study demonstrating the functional coupling of recombinant StARD1 and StARD3 with the P450scc system. Also, it was shown that StARD1 and StARD3 increase the whole cell cholesterol biotransformation activity of recombinant strain of *E. coli* with reconstructed P450scc system by ~2.5 and ~6 times. Pregnenolon is excreted in the culture medium and is almost absent in cells. The data obtained demonstrates that the approach used, may be a useful tool for the creation of artificial whole-cell biocatalysts. The research was supported by RFBR (20-08-00467A)

P-05.3-002
Preparation and characterization of argan-amylose-based novel bioplastics

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This work aimed to investigate the possibility to produce novel bioplastics, made up of argan proteins extracted from oilcakes, and amylose obtained from barley by RNA interference technique. Argan, *Argania spinosa*, is a plant typically widespread in arid and semiarid regions of Northern Africa useful for protecting soil from desertification and erosion. Until now, argan was mainly used to obtain a biologically active oil extracted from its seeds producing oilcake, a by-product rich in proteins, generally used as animal feed. Recently argan oilcakes have been attracting attention as a waste to be recovered to obtain high-added value products for different applications. Amylose is an optimal raw material for bioplastic purposes because of its linear molecular structure and its high thermal stability. It was already demonstrated that amylose-based films provided better properties compared to normal starch-based films. We investigated the performance of these novel blended bioplastics in terms of mechanical and barrier properties, and we also studied the effect of the enzyme microbial transglutaminase (mTGase) as reticulating agent for the argan protein component. Finally, we verified that both films, prepared respectively with unmodified and mTGase-modified argan proteins, are completely digested during oral and gastric digestion. In fact, amylose is digested by amylase in the simulated oral digestion process and the remaining proteins are digested by simulated gastric digestion because of the presence of pepsin. Our results confirmed the possibility to valorize a by-product, as argan proteins, using it as new raw material possibly destined to different applications in several industrial sectors, thus contributing to the development of new sustainable processes of production.

P-05.3-003
Mining and prioritization of GH43_29 subfamily novel xylan-debranching arabinofuranosidases

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Xylan is a highly abundant biopolymer with potential to be modified obtaining outstanding chemical and physiochemical properties. Utilization of xylan remain limited as processing of the biopolymer remain not efficient due to shortage of relevant enzymes needed for modification. Efficient enzymatic xylan-debranching is desirable to reduce water solubility of the biopolymer for successful introduction to biobased consumer products. GH43_29 subfamily α-L-arabinofuranosidases liberating L-arabinose directly from branched xylan are required for effective

conversion of the raw biopolymer to a high-value product component. Manual as well as programmatical sequence-curated mining was implemented for candidate arabinofuranosidase prioritization for production and characterization aiming subsequent exploitation in xylan debranching. Candidate sequences (1375) indexed in CAZy database as attributed to GH43_29 subfamily were analyzed in respect of domain organisation, conservative motif patterns and sequence identity to characterized xylan-debranching enzymes resulting in prioritized sequences (6) of manual mining. SINTEF data mining pipeline based on the HMMER suite and the dbCAN2 meta server with the profile Hidden Markov Model (HMM) of GH43_29 subfamily was used for programmatical sequence mining. Candidate sequences indexed in Marine Metagenomics databases of University of Tromsø (Norway) and proprietary databases of SINTEF Industry (Norway) were analyzed resulting in prioritized sequences (6) of programmatical mining. Prioritized sequences were selected considering soluble expression predictions from a shortlist of sequences (30) curated by Multiple Sequence Alignment and Sequence Similarity Network from HMM hits (~ 4200). Acknowledgement EU H2020 EnXylaScope (Mining Microbes and Developing Advanced Production Platforms for Novel Enzymes To Rapidly Unleash Xylans' Potential In a Scope Of Products For the Consumer Market) project (grant no. 101000831).

P-05.3-004
Biodegradation of the polyesters by the engineered yeast *Yarrowia lipolytica*

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Cold-adapted filamentous fungal strain *Geomyces* sp. B10I has been reported to decompose polyesters such as poly(e-caprolactone) (PCL), poly(butylene succinate) (PBS) and poly(butylene succinate-co-butylene adipate) (PBSA). Newly identified hydrolases (named hydrGB10I and chitGB10I) from *Geomyces* sp. B10I (Urbanek et al.2022) have been overexpressed in yeast *Yarrowia lipolytica*. Both enzymes, showed the activity toward the polymers at 14°C and 20°C, what makes the biodegradation process economically beneficial. The unconventional yeast *Y. lipolytica* is known for its capacity to assimilate atypical carbon sources, production of extracellular lipases and biosurfactants. The functional overexpression was tested by RT-PCR and the strains with the highest gene expression was tested toward polyesters biodegradation. Moreover, the influence of ion supplementation on biodegradation was tested. This work was financially supported by the National Science Centre, Poland, project UMO-2017/27/B/NZ9/02218. Urbanek AK, Arroyo M, de la Mata I, Mirończuk AM. Identification of novel extracellular putative chitinase and hydrolase from Geomyces sp. B10I with the biodegradation activity towards polyesters.AMB Express. 2022 Feb 5;12(1):12. *The authors marked with an asterisk equally contributed to the work.

Genomics

P-06.1-001
Pseudoautosomal region at the long arm of human Y chromosome is a hot spot of inter-chromosomal contacts of nucleoli in different cell lines

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rDNA units shape dynamic inter-chromosomal contacts with different chromosomal regions including the contacts with the regions where genes controlling development and differentiation reside (Tchurikov et al., 2015, doi: 10.1093/jmcb/mju038; 2019, doi: 10.3390/cells8111393). However, there are also the regions with very frequent contacts spanning tens of kb, which are stable in different cell types. One of such 63-kb region is located in human Y chromosome with coordinates 56820-56883 kb (hg38) corresponding to its subtelomeric pseudoautosomal euchromatic region at the long arm. The same pattern of frequent contacts were mapped in 4C-rDNA experiments in the region in both HEK293T and K562 cells that have different origin. Interestingly, in UCSC Browser this rDNA-contacting region exactly corresponds to a TAD and is decorated by the prominent H3K27ac marks in 7 cell lines from ENCODE. At present, only AF527552 gene was localized in the middle part of this rDNA-containing region. The gene was generated by reshuffling of the histone-Lysine N-methyltransferase (KMT2C/MLL3) gene and is associated with melanomas. We suppose that nucleoli form stable contacts with this region in different cell types to ensure the proper regulation of the gene. The study was supported by the grant from Russian Science Foundation No. 21-14-00035.

P-06.1-002
Identification of PRE DNA-binding factors interactomes: purification of the Combgap, Zeste, Psq, and Adf1 protein complexes

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The Polycomb group (PcG) and Trithorax group (TrxG) proteins are key epigenetic regulators controlling the silenced and active states of genes in multicellular organisms, respectively. In Drosophila PcG/TrxG proteins are recruited to the chromatin via binding to specific DNA sequences termed Polycomb Response Elements (PREs). While precise mechanisms of the PcG/TrxG protein recruitment remain unknown, the important role is suggested to belong to sequence-specific DNA-binding factors. At the same time, it was demonstrated that the PRE DNA-binding proteins are not exclusively localized to PREs but can bind other DNA-regulatory elements, including enhancers, promoters, and boundaries. In the present study, to gain an insight into the PRE DNA-binding protein regulatory network, we, using immunoaffinity purification coupled to the high throughput mass

spectrometry, isolated factors associated with the Combgap, Psq, Zeste, and Adf1 PRE DNA-binding proteins. We show that Combgap and Zeste are more tightly associated with the Polycomb repressive complex 1 (PRC1), while Psq interacts strongly with the TrxG proteins, including the BAP SWI/SNF complex. The Adf1 interactome contained Mediator subunits as the top interactors. In addition, Combgap efficiently interacted with AGO2, NELF, and TFIID. Combgap, Psq, and Adf1 have architectural proteins in their networks. We further investigated the existence of direct interactions between different PRE DNA-binding proteins and demonstrated that Combgap-Adf1, Psq-Dsp1, and Pho-Spps can interact in the yeast two-hybrid assay. Overall, our data suggest that Combgap, Psq, Zeste, and Adf1 are associated with the protein complexes implicated in different regulatory activities and indicate their potential multifunctional role in the regulation of transcription. The reported study was funded by the Russian Science Foundation (RSF) № 18-74-10091.

P-06.1-003
Impact of vitamin C on the levels of TET products in model of cell lines with a single knockout of TET genes

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In recent years, the effects of vitamin C supplementation of cell cultures and the following changes in the levels of chemically modified nucleotides have been increasingly investigated. This is related to the role of vitamin C as a cofactor for enzymes from the TET protein family. There are 3 TET proteins, which are dioxygenases responsible for the oxidation of 5-methylcytosine (5-mC) and to 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5-fC) and 5-carboxycytosine (5-caC), as well as thymine to 5-hydroxymethyluracil (5-hmU). Due to the influence of modified nucleotides on gene expression, supplementation with vitamin C may lead to changes in cell function. In our experiment, we used 4 models of cell lines: HAP1 wild type and HAP1 with a knockout of one of the 3 genes coding for TET proteins. Cells were grown under dedicated conditions in Dulbecco's Modified Eagle's Medium with the addition of filtrated bovine serum in sterile conditions and the presence of antibiotics. 100 mM vitamin C solution in phosphate-buffered saline was added to the culture and cells were harvested after 0, 3, 6, 9, 12, 18, 24, 36 and 48 h of exposure. DNA was isolated and hydrolyzed to single nucleosides and their content was determined using ultra performance liquid chromatography and mass spectrometry. We observed continuous accumulation of 5-hmC, 5-fC, 5-caC and 5-hmU until approximately 18-24 hours after start of exposure to vitamin C which was delayed by TETs knockouts. The highest levels of modified bases were observed in the wild type HAP1 line. This indicates the diversified involvement of individual forms of TET proteins in the oxidation process of 5-mC derivatives and its strong dependence on the availability of vitamin C. This work was supported by the Polish National Science Centre (grant number UMO-2018/29/N/NZ1/00497)

P-06.1-004
Inter-chromosomal contacts of nucleoli with CADM2 gene are important for its expression in HEK293T cells

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Gene ontology data indicate that CADM2 gene is involved in synapse organization, providing regulated trans-synaptic adhesion. It was demonstrated that the gene is associated with a range of behavioural and metabolic traits, including physical activity, risk-taking, educational attainment and some others (Morris et al., 2019, https://doi.org/10.1038/s41598-019-43861-9). 4C-rDNA approach was used for detection of inter-chromosomal contacts of rDNA clusters that are involved in epigenetic regulation of developmental genes in human cells (Tchurikov et al., 2019, doi: 10.3390/cells8111393). We detected that CADM2 gene shapes very frequent contacts with nucleoli in HEK293T cells that have neuronal origin. After heat shock treatment many genes change their contacts with rDNA clusters, but CADM2 gene increased the association with them. Our RNA-Seq data indicate that after heat shock treatment the expression of CADM2 gene is not affected. As many other rDNA-contacting genes CADM2 gene is jointly regulated by several transcription factors including POU6F2, FOXP2, XNF804B, ESRRG and MYT1L. We suppose that nucleoli form stable contacts with the region of chr3 where the gene resides to ensure the proper regulation of the gene. The study was supported by the grant from Russian Science Foundation No. 21-14-00035.

P-06.1-005
The population genetic study of the Kazakh national dog breed Tazy: the first steps

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The Tazy or Kazakh Borzoi is one of the oldest dog breeds officially recognized as a national cultural asset of Kazakhstan. Since ancient times, hares, wild boars, foxes, badgers, deer, wolves and saigas were hunted by the Tazy. Currently, the breed is in a very difficult situation, its population is only a few thousand dogs. Currently, there is almost no information about the breed research in international databases. Providing information about this unique dog to the international scientific community will help to preserve the Tazy breed and achieve its international recognition. Information on the genetic structure of populations is the basis for assessing the genetic differentiation of animals within a breed. Here are the results of studies on the genetic structure and genetic differentiation of the Tazy population in the southern regions of Kazakhstan. DNA from 101 Tazy dogs was used for molecular testing of 19 STR loci recommended by ISAG. The percentage of polymorphic loci in the tested population was 100% (4 to 13 alleles per locus). The effective number of alleles (4.405) indicates sufficient variability in the studied breed. The values of observed heterozygosity were lower than expected (Ho = 0.745, He = 0.756). This was also evidenced by the obtained positive values of inbreeding coefficient (0.016),

indicating inbreeding and genetic homogeneity. The obtained positive values of F_{IS} (0.016) and F_{IT} (0.041) indicate the related mating. The F_{ST} coefficient 0.026 shows a slight but remarkable divergence between subpopulations. For the first time in the world, we initiate a large-scale genetic study of the Tazy breed. The data obtained suggest a low level of crossbreeding and genetic homogeneity in the breed, which is an important indicator for assessing purebredness. Further studies will help confirm the isolation of the Tazy breed and take a step towards its international recognition.

P-06.1-006
NF-YA and SP1 factors regulate the PNPT1 promoter, a mitochondrial RNA translocase

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PNPT1 gene encodes for polynucleotide phosphorylase (PNPase), a highly conserved protein present in both prokaryotes and eukaryotes. PNPase is predominantly located in the mitochondrial intermembrane space. It is a 3'-5'exoribonuclease involved in RNA processing and degradation by phosphorolysis. In addition, plays a key role in maintaining mitochondrial homeostasis by regulating adenine nucleotide levels. PNPase promotes the import into mitochondria of small RNA components, necessary for mitochondrial DNA replication and RNA processing. The mechanisms regulating the import of proteins into mitochondria are well known, but the mechanisms regulating the transport of RNA into the mitochondria are unclear. Several studies have described that RNA import into mitochondria is a highly selective process whose failure causes metabolic, morphological, and physiological alterations. Examples are the Leigh syndrome, a hereditary hearing loss disease, and pathologies caused by deficiencies in the respiratory chain. Recently, the overexpression PNPase has been related with a poor prognosis in some types of cancer. For this reason, we have characterized the activity of the *PNPT1* promoter using the pGL4-basic vector with the Luciferase reporter gene system. We have also performed gel retardation (EMSA) and gene silencing assays. Our results provide evidence that the *PNPT1* promoter contains putative binding sites for ISRE, SP1 and NF-Y transcription factors. In addition, NF-Y and SP1 seem to have a predominant role in the regulation of PNPase expression. NF-Y and PNPase has been previously found to be overexpressed in liver, breast and lung cancer, and to be a negative prognostic factor in several oncologic disorders. In conclusion, our results suggest an important role for NF-Y and Sp1 transcription factors in the regulation of *PNPT1* expression.

P-06.1-007
The first assessment of metagenomic communities of Russian black truffle *Tuber aestivum*

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This study aimed to assess the diversity of symbiotic communities of black summer truffle *Tuber aestivum*. Fragments of the fruiting bodies of truffle were sampled to analyze the diversity of prokaryotic and eukaryotic organisms (according to the 16S rRNA and ITS genes) using the metagenome sequencing approach. The black summer truffle was collected near Sochi city in Russia. According to the obtained data, the fruiting bodies of *T. aestivum* contained representatives of the *Enterobacteriaceae* family (OTU, % - 95%) in the bacterial metagenome. Moreover, from this microorganism the genera *Enterobacter* sp. and *Serratia* sp. also were isolated. Such microorganisms as *Stenotrophomonas* sp., *Pseudomonas* sp., *Delftia* sp., *Achromobacter* sp., *Pedobacter* sp., *Nocardioides* sp. and others did not exceed 0.1%. Thus, the bacterial community of the Russian black truffle *T. aestivum* was presented by the phylum *Proteobacteria* (99.6%), and minor phyla, the number of which did not exceed 0.2% (*Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Cyanobacteria*). Besides *T. aestivum* (OTU, % - 52%), fruiting bodies of *T. aestivum* contained representatives of the class *Oligohymenophorea* (OTU,% - 11%) and the *Sordariales* family (OTU,% - 9%), as well as organisms of the genera *Ascobolus* sp. (OTU,% - 9%), *Tylospora* sp. (OTU,% - 4%), *Papulaspora* sp. (OTU,% - 3%), *Penicillium* sp. (OTU,% - 3%), *Alternaria* sp. (OTU,% - 3%), *Rhodotorula* sp. (OTU,% - 2%) and unidentified microorganisms (OTU,% - 16%). Thus, eukaryotic organisms of the black truffle communities were presented by fungi (74%), protists (11%) and not identified species (15%). Finally, the microbiome of the Russian black truffle *T. aestivum* was presented by soil fungi and bacteria. Most of the obtained microorganisms can grow on plant residues and they are parasites or symbionts of plants, and Truffles. The study is carried out with the main financial support of the Russian Science Foundation, project 20-76-00001.

P-06.1-008
The assessment of first-line anti-tuberculosis drug exposure, inflammatory biomarkers and pulmonary tuberculosis severity association with treatment response

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Pharmacokinetic variability in rifampicin (RIF), one of the first-line anti-tuberculosis (TB) drugs, can lead to insufficient drug exposure,

and together with patient-dependent factors and disease severity can significantly affect treatment response. In this study, we evaluated the connection between TB treatment response and acute-phase inflammatory biomarker C-reactive protein (CRP), RIF plasma concentration, as well as patient-and disease-related factors. Plasma samples were obtained from TB patients (n = 43) admitted to the Centre of Tuberculosis and Lung Diseases. Plasma levels of anti-TB drugs were measured using Liquid Chromatography-Tandem Mass Spectrometry at 2h post-dose. The chest radiography and computer tomography findings were used to assess the extent and severity of the disease. Time to sputum culture conversion (tSCC) was used as a treatment response marker. All clinical data were obtained from the medical records. Logistic regression models were constructed to assess the association of RIF exposure, CRP level before treatment initiation, and extent of lung lesions with treatment response within 60 days. Univariate models demonstrate significant association between tSCC within 60 days and CRP (OR = 0.96 (0.92, 0.99), $P = 0.019$), and severity of lung lesions (OR = 0.28 (0.08, 0.99), $P = 0.048$). Multivariate logistic regression model was adjusted for age and sex; only the association between tSCC within 60 days and CRP remain statistically significant (CRP: OR = 0.96 (0.93, 1), $P = 0.037$; severe lung damage: OR = 0.33 (0.07, 1.62), $P = 0.172$; RIF exposure: OR = 1.15 (0.87, 1.51), $P = 0.34$). In conclusion, this study shows that the observed tendency of higher CRP levels, severe lung lesions before treatment initiation appeared to be a promising prognostic marker of subsequently delayed treatment response in TB patients, and corresponds to findings reported globally. Acknowledgments. This study was supported by the Latvian Council of Science, project No. lzp-2020/1-0050.

P-06.1-009
The role of the chromatin-remodeling complex PBAF–BAF200 in the activation of NF-κB dependent genes

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SWI/SNF regulates the expression of inflammation genes through interaction with transcription factors, including factors of the NF-kB signaling pathway. The variability of the subunits of this complex determines the specificity of its binding to the chromatin and various transcriptional activators. In this work, we studied the interactions of the remodeling chromatin complex PBAF and the MEDIATOR complex, as well as their effect on the induced transcription of pro-inflammatory genes. We found tha BAF200, the subunit of PBAF specific module, co-precipitates with the subunits of the MEDIATOR kinase module: CDK8, MED12, and the core MEDIATOR subunit - MED1. Also, antibodies against the core subunit of PBAF - BAF155 precipitated MED1 and MED12. In reciprocal precipitations, anti-MED12 antibodies precipitated BAF155 and other subunits of the PBAF specific module, BRD7. BAF250, a specific subunit of BAF complex BAF250 did not precipitate any MEDIATOR subunits. This demonstrates that these interactions occur for the PBAF, but not for the BAF complex, and reveal the functional implementation of the PBAF complex in the regulation of inducible NF-kB transcription. We found that knockdown of the BAF200 subunit leads to degradation of the PBAF module. Reduced expression of BAF200 negatively affects the activation of NF-kB luciferase reporter. At the same time endogenous CDK8-dependent genes expression was increased, decreased, or not changed after the BAF200 knockdown during TNF activation in HEK293T cells, demonstrating complex regulation of transcription by these complexes. In the HEK293-8KD cell line, in which the kinase function of CDK8 is impaired, we found that the kinase function of CDK8 doesn't change the effect of BAF200 knockdown. In other words, the kinase function does not affect the properties of the PBAF module to regulate the activation of the NF-kB signaling pathway. This study was supported by the Russian Science Foundation [grant number 21-14-00258 to NS]. *The authors marked with an asterisk equally contributed to the work.

P-06.1-010
Analysis of the mechanism of interaction of the Su(Hw)-dependent complex with the Bonus, ADD1, and XNP proteins in *Drosophila melanogaster*

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The Su(Hw)-dependent complex is a convenient model for studying the functions of insulators and the mechanism of their binding to DNA. The Su(Hw) insulator includes three main proteins - Su(Hw), Mod(mdg4)67.2, and CP190. We found that Bonus, ADD1, and XNP proteins interact with the Su(Hw) complex. We created constructs expressing the full-length Bonus, ADD1, XNP proteins and their individual domains. Interactions between these proteins and components of the Su(Hw) insulator were tested in a yeast two-hybrid system, by protein coimmunoprecipitation (IP) and protein coprecipitation on glutathione sepharose (GPD). We have demonstrated interactions between proteins and identified the domains which involved in interactions. The ADD1 protein directly interacts with the Bonus and Mod(mdg4)67.2 proteins. The Bonus directly interacts with the Su(Hw) protein, but not with the Mod(mdg4)67.2 or CP190 proteins. The XNP interacts with the Mod(mdg4)67.2 protein and the C terminal region of the Su(Hw) protein in GST and IP. It has been proven that *in vivo* the Bonus and ADD1 proteins form a common complex with each other, the components of the Su(Hw) insulator and the HP1a repressive protein. We propose a new model for the tissue-specific formation of Su(Hw) complexes at different genome sites. At the sites where both the Su(Hw) and Mod(mdg4)67.2 proteins are present, Mod(mdg4)67.2 directly interacts with ADD1. Then, through ADD1, the Bonus protein is attracted to the complex and stabilized there by a weaker interaction with the Su(Hw). At the sites where Mod(mdg4)67.2 is absent, firstly Bonus is recruited into the complex through interaction with Su(Hw) and then attracts ADD1 protein. It is possible that the XNP protein is recruited into the complex via the ADD1 protein only in those tissues where Su(Hw) represses nearby genes. At the next stage, the ADD1 and XNP proteins recruit the HP1a protein. This work was supported by Russian Science Foundation grant No. 21-14-00205.

P-06.1-011
Differentially expressed genes associated with tumor cells differentiation in localized prostate cancer

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Most cases of localized prostate cancer (LPC) have a Gleason score (GS) of 7. The GS reflects the degree of differentiation of tumor cells on a 5-point scale, where 1 point is a highly differentiated tumor, 5 points is a poorly differentiated. Recently, a classification based on the sum of points on the GS was introduced, but there is no division into primary and secondary points, these are the prognostic groups of ISUP, where the scale varies from 1 to 5. According to that, the GS of 7 was divided into two ISUP scores: 2 (3 + 4) and 3 (4 + 3). Our research is aimed at identification of differentially expressed genes associated with ISUP scores in LPC within GS of 7. The study included RNA-Seq data of 88 LPC samples within GS of 7 based on TCGA. Patients included in the cohort belonged to the Caucasian population, had not neoadjuvant therapy. The cohort was divided into two groups: tumors with ISUP group 2 (n = 64), and ISUP group 3 (n = 24). Differential expression analysis (DEA) performed in statistical environment R using the edgeR package. Gene set enrichment analysis (GSEA) performed using clusterProfiler R package in order to find involved pathways. During the DEA 209 genes were identified between ISUP 2 and ISUP 3 in LPC, 9 genes of which had the most considerable change: *FREM2*, *HRAS*, *ITGA1*, *ITGA2*, *ITGA4*, *ITGB8*, *MYL5*, *PDGFRA*, and *VCL*, and that were involved in three significantly enriched KEGG pathways: Focal adhesion (hsa04510), ECM-receptor interaction (hsa04512), and Regulation of actin cytoskeleton (hsa04810). Based on the research between ISUP groups 2 and 3 significant differences have been shown on biological pathways. Decreased activation of the following pathways: focal adhesion, ECM-receptor interaction, and regulation of actin cytoskeleton was demonstrated. This work was founded by the Russian Science Foundation, grant 18-75-10127. This work was performed using the equipment of EIMB RAS "Genome" center (http://www.eimb.ru/ru1/ckp/ccu_genome_c.php).

P-06.1-012
Sequences related to Rhizobium phage 16-3 in genomes of geographically distant *Sinorhizobium meliloti* strains, root nodule symbionts of alfalfa

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Bacteriophages can have a significant impact on the total microbial population density by reducing bacteria titer by lysis. Alternatively bacteriophages have an ability to integrate into genome of the host as a prophages, and that could increase the host's resistance to homoimmune infections, but also could results in bacterial lysis in the event of its exposure to stress factors. Root nodule bacteria forming nitrogen fixing symbiosis with legumes most often attacks by phages from Caudovirales order. Temperate Rhizobium phage 16-3 firstly described in 1970s is assumed as a model bacteriophage, a member of *Siphoviridae* family. Its genome size is 60.2 kb and 110 ORFs were identified in it. Our focus of interest was to assess the abundance of sequences related to Rhizobium phage 16-3 in full sequenced genomes of *Sinorhizobium meliloti* strains native to geographically distant regions. Analysis of 48 genomes from the NCBI database revealed prophage sequences of interest in 8 strains from hill mountains of Caucasus, Aral Sea and Siberian regions, as well as from regions of Morocco and Spain. The 6 out of 8 prophages were intact, and other two were incomplete and defective. The sizes of last two were correspondingly 33.1 and 21.7 kb, while the size of intact prophages reached 63 kb that is similar to that of the 16-3 phage. The number of ORFs encoding protein sequences reached 144 in intact, and 29 and 27 in defective and incomplete prophages, respectively. ORFs encoding tail proteins are present in all prophages, while ORF encoding various structural elements of the virion were not in all studied sequences. ORFs encoding bacterial proteins were not identified at all. Summarizing, sequences related to Rhizobium phage 16-3 are abundant in *S. meliloti* strains since nearly each fifth strain native to geographically distant regions harboured above sequences participating in recombination processes. The work supported by the RSF 20-16-00105. *The authors marked with an asterisk equally contributed to the work.

P-06.1-013
Methods for the identification of key polymorphisms of FAD3A and FAD3B genes responsible for the content of linolenic and linoleic fatty acids in flax oil

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Flax varieties significantly differ in the oil composition (predominantly in the content of linolenic, linoleic, and oleic fatty acids (FA)) and this characteristic determines the application of seeds – for industry, food, or pharmaceutics. Stearoyl-ACP desaturases (SAD) and fatty acid desaturases 2 and 3 (FAD2 and FAD3) play a pivotal role in the fatty acid synthesis, and several key polymorphisms of FAD3A and FAD3B genes are responsible for the content of linolenic and linoleic FA, previously published in: Thambugala D et al. (2013) Theor Appl Genet 126, 2627-2641 and Dmitriev AD et al. (2020) BMC Plant Biol 20, 301. However, intravarietal heterogeneity and the presence of genotypes with different FA composition in one variety can affect the results of studies concerning the determination of flax oil composition. Thus, choosing plants which are typical for a particular variety is extremely important for the experiment to be reliable. We provide two approaches for the identification of three key polymorphisms of FAD3A and FAD3B genes. The first approach is the amplification and Illumina sequencing of partial FAD3 gene fragments containing key polymorphisms that is convenient for the analysis of a pool of plants to detect heterogeneity within a variety. The second approach is based on the Cleaved Amplified Polymorphic Sequence (CAPS) analysis,

which is preferable for the examination of individual plants and allows one to identify key polymorphisms of FAD3A and FAD3B and detect heterozygous plants. We used these two approaches to select plants for transcriptomic and mass spectrometry analysis to study the contribution of genotype and environment to the flax oil composition in varieties with different FA content. The proposed methods will be a useful resource for the marker-assisted selection and DNA-based certification of flax cultivars. This work was funded by the Russian Science Foundation according to the research project 21-16-00111.

Proteomics

P-06.2-001
Charged sequence motifs increase propensity towards liquid–liquid phase separation

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Protein phase separation has been shown to be a major governing factor in multiple cellular processes, especially ones concerning RNA and RNA-binding proteins. We have previously observed that the single alpha helix (SAH) motif, characterized by a regularly alternating pattern of charged residues, is enriched in RNA-binding proteins. This prompted us to investigate whether SAHs or charged motifs in general are preferentially associated with LLPS. Our hypothesis was that charged regions can effectively function in both aqueous environment and within the condensed phase. We have defined and analysed the presence of SAHs, charged residue repeats (CRRs) and charge-dense regions (CDRs) in proteins of the human proteome and found a strong association between such charged motifs and LLPS. Importantly, our results show that proteins involved in LLPS preferentially contain repeating or simply charge-dense motifs, but these motifs are also highly abundant in other proteins. Thus, while the presence of such motifs does not indicate the participation of the given protein in LLPS, the absence of such motifs renders this unlikely but does not preclude it. *The authors marked with an asterisk equally contributed to the work.

P-06.2-002
A comprehensive analysis and comparison of proteome profiles of EVs secreted by murine hippocampal astrocytes and neurons in mono- and co-culture

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It is well accepted that metabolic crosstalk between different types of cells in the central nervous system, especially between neurons and astrocytes, plays a crucial role in shaping neuronal function. Extracellular vesicles (EVs) are in the limelight of the recent research as a mode of such a crosstalk, both in physiological and in pathological conditions. Here, we present results of analysis of proteins secreted to the culture medium in EVs by hippocampal neurons and astrocytes, cultured alone and together in the co-culture. Comparison of these proteomes revealed a set of protein signals used by neurons and astrocytes to reciprocally modulate their physiology. We found quantitative and qualitative changes in proteins secreted to the medium in EVs in different culture conditions, and involved in a number of different processes: response to cellular stress, regulation of the cell cycle, energy metabolism, ionic homeostasis, gene expression and synaptic transmission. We also found a pool of proteins involved in energy metabolism that were unique to EVs from neuronal-astrocytic co-culture, what suggests that interactions between these two types of cells stimulated secretion of these proteins. On the other hand, we identified proteins specific to EVs from monocultures of neurons or astrocytes. Significance of the observed changes for the cells' survival, energy metabolism and neuronal plasticity is discussed.

P-06.2-003
Bottom-up proteomics approach revealed significant variations of the cystatin D interactome in saliva from patients affected by different forms of mastocytosis

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Mastocytosis is a rare myeloproliferative neoplasm caused by abnormal infiltration and accumulation of clonal mast cells in different tissues. Depending on mast cell localization, it is possible to distinguish cutaneous from systemic (SM) variants, which can manifest with (SM+C) or without (SM-C) cutaneous symptoms and make disease classification often arduous. Cystatin D is a cathepsin inhibitor detectable in human saliva and in a previous study we demonstrated that its salivary level could help to discriminate the different forms of mastocytosis. In this study, we investigated the capacity of cystatin D to interact with other proteins in a salivary complex (SC-D), to discoverer potential salivary markers useful to classify the different forms of systemic mastocytosis. Whole saliva pools from SM (SM-C and SM+C) patients and from sex/age matched healthy controls were submitted to immunoprecipitation assay using cystatin D antibody followed by SDS-PAGE in reducing conditions, tryptic digestion, and nano-HPLC-high-resolution-MS/MS analysis. MS spectra were analyzed by Proteome Discoverer 2.5 software, while Perseus 2.0.3 tool was used for statistical analyses. SC-D was composed by 36 proteins and peptides involved in innate immune system and in the modulation of the inflammation, such as histatin 1, cystatin B, annexin A1, lactotransferrin, azurocidin and others. Annexin A1 was more abundant in SC-D of SM, leukocyte elastase inhibitor in both protein complexes of SM and SM+C compared to controls, while increased levels of cystatin D and cystatin S were found in SC-D of controls with respect to

these patients' groups. SM-C showed higher level of histatin 1 compared to controls and to SM and SM+C, and of cystatin S and lactoperoxidase when compared to SM+C. Finally, SM group exhibited higher levels of cystatin B and azurocidin compared to SM-C and SM+C, respectively.

P-06.2-004
Phosphoproteomic analysis of legumain deficient mice

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Legumain, a member of cysteine proteinase family, is an asparaginyl specific protease. Typically, it is located in endolysosomal system, however, under specific physiological conditions, it can also be found in the cytoplasm, cell nucleus in the extracellular milieu. Legumain's amino acid sequence is highly conserved among many different species, pointing to its significance for the normal physiological function of organisms. Interestingly, legumain knock out mice show a very mild phenotype. They are viable and fertile with no behavioural abnormalities. Compared to their wild type counterparts, they have reduced body mass, irregular kidney function and hyperinflammation. However, since no system-wide studies of these animals have been carried out, the molecular basis for the observed phenotype is largely unknown. It has been shown that levels of EGF receptor are significantly increased in legumain null mice. An effect that may cause global changes in cellular signalling. Apart from that, legumain could also be able to influence the function of other receptors and kinases. To reveal possible molecular interactions, explaining phenotype observed in legumain knock out mice, we employed phosphoproteomics to study changes in protein phosphorylation combined with immunological methods to validate selected target proteins while also measuring expression levels of certain proteins. Obtained results will enable us to explain legumain's role in the physiology of the organism and its possible involvement in the immune response.

P-06.2-005
Bottom-up analysis of salivary proteome from autoimmune hepatitis and primary biliary cholangitis patients

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Autoimmune hepatitis (AIH) and primary biliary cholangitis (PBC) are liver pathologies characterized by autoimmune etiology. The immune system attacks directly the hepatocytes in AIH, while in PBC it destroys the cells of biliary ducts. Despite the different origin district, both pathologies cause liver fibrosis, chronic inflammation, and metabolic changes. There are still critical diagnostic issues due to the sharing of symptoms with many other liver diseases and to the invasiveness of diagnostic methods. Saliva represents a promising and less invasive biofluid candidate for diagnostic and prognostic purposes since it contains both proteins from salivary glands and from other organs and tissues. To highlight any qualitative/quantitative change of the salivary proteome associated to AIH and PBC, the acid insoluble fraction of saliva from patients and age/sex matched healthy controls were submitted to SDS-PAGE under reducing conditions, tryptic digestion, and nano-HPLC-high-resolution-MS/MS analysis combined to label free quantitation. The software Proteome Discoverer 2.2 was used for proteins identification and preliminary statistical evaluation, meanwhile the functional and pathways enrichment analyses were performed using AmiGO 2 and Reactome pathway Databases. 1449 proteins were identified, 484 of them with high confidence. Only proteins statistically varied between AIH and controls (46), PBC and controls (30), and between AIH and PBC (51) have been used for enrichment analyses. Results from pathways analysis highlights that these proteins are mainly involved in processes related to immune system and inflammation. In addition, the functional analysis suggests a variation in protease activity, showing dysregulation of proteases inhibitor like Histidine-rich glycoprotein, Cystatin S and SA, Alpha-2-HS-glycoprotein, and Kininogen-1. These preliminary results denote that salivary proteome reflects the pathological condition of AIH and PBC patients.

P-06.2-006
HDX-MS as a tool for small molecule binding characterization: monitoring modulation of Cyclophilin D and Autotaxin

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Hydrogen deuterium exchange mass spectrometry (HDX-MS) is emerging as a powerful biophysical technique for probing protein interactions, structure, and conformational dynamics. In our work, we use HDX-MS to gain a deeper understanding on the binding-mode and the target modulation of a set of compounds on two target proteins: Cyclophilin D (CypD) and Autotaxin (ATX). CypD is the mitochondrial isoform of Cyclophilins, which plays an important role in the execution of cell death by regulating the mitochondrial permeability transition pore, is related to several diseases as multiple sclerosis and cardiovascular disease, making CypD an interesting target for therapeutic intervention. Merck Healthcare KGaA synthesized different classes of compounds targeting the two main pockets of CypD. We tested several of these compounds by HDX-MS and the data obtained corroborated the information given by the crystal structure, but also provided new insights on how these compounds target CypD and how they could be further optimized. ATX, or lysophospholipase D, primarily function is converting lysophosphatidylcholine into the lipid mediator lysophosphatidic acid (LPA). The ATX-LPA signaling pathway is essential for vascular and neural development. LPA acts via specific G protein-coupled receptors (GPCRs) on the cell surface, activates a variety of signaling pathways involved in wound healing, neurogenesis, tumor progression, and metastasis. Merck researchers developed compounds targeting ATX that, interestingly, display an unusual

and unique behavior on Surface Plasmon Resonance (SPR) and it is envisaged that HDX-MS studies may help decipher the mechanism at the basis of these unexpected SPR results. Successful modulation of CypD and ATX has important therapeutic potential and the elucidation of the interactions of these proteins with their candidate therapeutic compounds is expected to provide valuable and additional insights for the design and improvement of CypD and ATX inhibitors.

P-06.2-007
Environmental impacts on barley grain composition and seed viability

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To counter projected reductions in yields of the major crop barley it is essential to elucidate mechanisms of its resilience. To assist such efforts, we collected grains from plants grown in fields at 12 breeding stations, with suitable temperature and precipitation gradients for identifying environmentally induced changes in their protein and metabolite contents. We then subjected the grains to detailed molecular analysis. The results showed that numerous metabolites and at least a quarter of the grain protein content were modulated by temperature or drought, and provided insights into barley seed production under abiotic stress. Potential positive and negative markers of yield (grain mass per unit area) were also identified, including the phenolic compound catechin and storage protein levels, respectively. Complementary analyses of barley seedlings and Arabidopsis seeds respectively confirmed the role of the identified proteins in abiotic stress responses and highlighted evolutionarily conserved mechanisms. In addition, accelerated aging experiments revealed that variations in the maternal environment had stronger effects on seed longevity than the genotype. Finally, seeds with the highest longevity differed from the others in gibberellin contents and levels of 92 proteins, providing novel targets for improving resilience.

P-06.2-008
BABA pre-treatment before drought stress revealed tolerance related proteomic alterations in rice

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Rice (*Oryza sativa* L.) is a staple food for more than half of the world's population. In order to adapt to the rapid increase in the world population, rice production should be increased by approximately 60% until 2025. However, increasing environmental stress, declining arable land and scarcity of irrigation water pose serious threats to rice production on a global scale. Drought is the most important problem affecting yield in rice production. It is estimated that more than one third of the total cultivated land in the world is affected by drought stress. In order to increase productivity in the development of drought-resistant varieties, it is of great importance to identify tolerance related genes and proteins. In this study, it was aimed to determine the proteomic responses of rice plants pre-treated with beta-aminobutyric acid (BABA) molecule, which is known to increase plant resistance and tolerance by stimulating the defense mechanism in plant cells, before drought stress. Different hours and concentrations of BABA applied to Osmancık-97 and Halilbey varieties before germination and drought stress was initiated in 2-weeks-old plantlets. Protein were isolated by TCA-acetone method and separated by using two-dimensional gel electrophoresis. Between emerging or disappearing spots that showed significant expression alterations between groups, proteins thought to be drought-related were digested from the gel and analyzed by MALDI-TOF (Matrix assisted laser desorption/ionization time-of-flight mass spectrometry). Protein characterization was carried out by searching the obtained peptide sequences in protein databases. As a result, it was determined that BABA application in rice plants, depending on different genotypes and varying doses, contributed to the formation of different protein profiles and different tolerance levels. *The authors marked with an asterisk equally contributed to the work.

P-06.2-009
Use of endophytic fungus in mitigating of cadmium toxicity

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Cadmium is a widespread toxic pollutant, a nonessential element without any known benefits for organisms. It has been estimated that human activity releases approximately 13,000 tonnes of cadmium per year, and this pollutant may remain in the environment for up to 18 years. Unlike humans and animals, plants are sessile organisms that cannot escape the stress and actively compete above and below ground for the habitat. The presence of cadmium in the environment inhibits root and stem elongation by reducing mitotic division that limits root length, its dry weight, and increases root diameter. Cadmium can be rapidly taken up by the roots, transported, and accumulated in the stems. For these reasons, the extensive presence of cadmium in the soil causes deterioration symptoms in the aboveground part of plants, including chlorosis, necrotic lesions, wilting, and leaf deformation. Endophytic microorganisms are low-cost and environmentally friendly technology to improve food production and reduce abiotic stress, including cadmium toxicity. The endophytes may be bacteria, fungi, algae, and oomycetes. These microbes show a mutualistic relationship with their host plants and colonize both the intercellular and intracellular plant compartments without significant morphological change. The endophytes can significantly promote plant growth and induce tolerance to both biotic and abiotic stressors. Furthermore, they have beneficial effects on the host plant by improving nutrient uptake and modulating the level of phytohormones, siderophores, and enzymes. Here, we employed flax and analyzed its response to the presence of endophytic fungus under different cadmium ion concentrations. We found that the presence of fungi mitigated cadmium toxicity and improved plant growth. Our proteomics and metabolomics profiling provided the first molecular insights into this interaction and highlighted pathways underlying higher resilience in flax plants cultivated in the presence of fungi.

P-06.2-010
Use of peptides derived from *Lonomia obliqua* venom for searching novel molecular targets on inflammatory chondrocytes cell model

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Osteoarthritis (OA) is an inflammatory disease of synovial joints involving the loss and degeneration of articular. Several studies have shown that chondrocyte aging is associated with the degeneration of articular cartilage in OA. Lopap is the most studied Lipocalin isolated from *L. obliqua*, and the activity of its derived peptides has been the objective of several studies by our group. Peptides derived from Lopap, such as B8, may have the potential to prevent inflammation, cell aging and induced death in pro-inflammatory chondrocytes. This study aims to elucidate the molecular pathways modulated by B8 peptide on inflamed chondrocytes through mass spectrometry based-proteomic analysis. Chondrocytes were induced with IL-1β for 1 h. After this period, cells were treated with B8 peptide for 24 h. Tryptic peptides from each sample were submitted to nLC-MS/MS analysis followed by relative label-free quantification (LFQ). The relative quantitative analysis was performed for identification of differentially abundant (DA) proteins between the B8-treated sample versus control by comparing the LFQ intensities of each identified proteins. A total of 70 proteins were found to be DA, including 26 that were detected in higher abundance and 44 in lower abundance, in B8 treatment samples. Gene Ontology annotation analysis of the DA proteins showed that the most enriched terms for biological process were metabolism process, biological regulation, and response to stimulus; cytosol, nucleus and membrane were the most enriched terms for cellular component; and for molecular function annotation the most enriched terms were structural molecular activity and nucleotide binding. In conclusion, our results demonstrated that B8 peptide can modulate proteins that are important for inflammatory process in chondrocytes. Moreover, investigation to further elucidate the role of the B8 peptide in this model may provide new insights into the pathological mechanisms and therapeutic targets for OA.

Systems biology

P-06.3-001
The analysis of *Staphylococcus aureus*' NDH-2 Coarse-Grain simulations might be the beginning to unveil a future therapeutical target

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Some strains of *Staphylococcus aureus* have been gaining resistance to several different antibiotics, raising the possibility of a future pandemic. Therefore, ways of diminishing their virulence are of the utmost importance. Studies have shown a connection between Type II NADH: menaquinone oxidoreductase (NDH-2) and *S. aureus*' virulence. NDH-2 has thus promising therapeutical potential, highlighting the importance of studies of its role in respiration in general and of its binding site mechanisms in particular. In this work, we have employed Coarse-Grain (CG) simulations to represent *in silico* the substrate approximation that occurs in the binding site *in vivo*. All the compounds related to this study, such as FAD (NDH-2 prosthetic group), $NADH/NAD^+$, menaquinone/menaquinol and derivatives, were all parameterised using the most recent Martini 3 force field parameters. To perform the CG simulations, an *S. aureus* membrane of representative lipid composition was modelled together with NDH-2. Preliminary results show the preference of quinones for one of the NDH-2 binding sites (there is a separate site for NADH that the quinones do not visit). In addition, the reduction of quinones to quinols lowers their affinity for the binding site, thus promoting product egress. This hints that the binding site environment may be specifically tuned to discriminate the quinone from the quinol as a means to increase turnover. Our promising results show that CG Molecular Dynamics (MD) is well suited to studying NDH-2 substrate/product dynamics and could possibly be extended to other *S. aureus* oxidoreductases. Ultimately, we hope that with these approaches, we will be able to identify new and vital targets in the fight against bacterial resistance.

P-06.3-002
Miniaturizing nanotoxicity in daphniids – the impact of crowding and vessels

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Nanomaterial pollution is a subject of increasing severity. Gaining more insight on the mechanisms of nanoparticle toxicity is imperative given the scarcity of information. Silver nanoparticles are very toxic to aquatic organisms especially, with their yearly production increasing. Miniaturization of nanotoxicity assays can reduce costs and nanomaterial waste, while focusing on the effects at individual animal level and increase reproducibility of testing. In this study, daphniids were exposed to silver nanoparticle ink in nine plastic and two glass vessels for 24 and 48 hours. The effect of surface to volume ratio and animal density was investigated. Toxicity curves were obtained and the activity of key enzymes in the metabolism of phosphate, sugar, protein and lipids was determined to closely evaluate the impact of exposure conditions. Crowding of daphniids reduced the toxicity of silver nano-inks while miniaturization is possible as no significant differences were observed among vessels. However, biochemical markers of physiology showed differences in enzyme activity.

P-06.3-003
Estimation of diagnostic value of serum tTg antibody levels in Celiac disease confirmation consistent with histological discrepancies

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The aim of study was to show the correlation between tTG levels and degrees of duodenal damage. B, by reviewing files of patients registered in GI clinic in Children's Hospital and adult outpatients from private clinics. Files included tTg IgA titres, pathology reports of duodenal biopsy according to Marsh classification. Patients group (adults and children) n = 100 Control group n = 30 healthy adults and healthy children. We have found that the Mean tTg levels in patients with celiac disease were more than 11 times the upper limit range of normal range. Our study concluded that severity of elevation serum tTg antibodies concomitant with diagnostic symptoms as confirmation for celiac disease incidence. Therefore, tTg test can be applied for diagnosis of celiac disease in children and adults, without duodenal biopsy when tTg titres are more than 10 fold of upper limit of normal range associated with clinical symptoms. Funding: The work was performed in the framework of the Russian Federation fundamental research program for the long-term period from 2021-2030.

P-06.3-004
Sex steroid hormones status influence on antidepressant pharmacotherapy effect in male and female patients

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Depression causes immense burden on health care systems worldwide with two time s higher prevalence in women. However, both male and female patients are treated with antidepressants under same protocols. As it was demonstrated that estrogen has a prodepressant and testosterone an antidepressant affect, it is reasonable to assume that pharmacotherapeutic effect might depend also on sex hormones status. The aim of this pilot study was to explore hormonal status of female and male patients upon hospitalization on occurrence of depressive episode and to correlate it with pharmacotherapy effect after four weeks of therapy. Subjects were 42 patients, 14 males, 14 females in the first (follicular) phase of menstrual cycle and 14 females in the second (luteal) phase of menstrual cycle upon hospitalization. The Hamilton scale was used to determine degree of depressive state upon hospitalization an after 28 days. At both time points, blood was sampled and level of testosterone and estrogen for male and estrogen, progesterone and testosterone for female patients was analysed. Results of the study showed that antidepressant effect calculated as a difference in Hamilton scale was highest in male group of patients and significantly higher than in women in the second phase of the cycle (10.4 vs 8.1). This correlated with increase of testosterone in male patients during four weeks treatment (12.08 vs. 9.46), while there was no significant change in the level of testosterone in both female groups of patients. Furthermore, in female patients in the luteal phase of the cycle, with lowest response to antidepressants, both estrogen and progesterone were significantly reduced during four weeks of treatment. In conclusion, results of our pilot study suggest sex differences in response to antidepressant therapy and level of hormonal status should be evaluated for better personalized pharmacotherapy.

P-06.3-005
Comparative characteristics of the treatment of acute pancreatitis in rats using native and preconditioned human umbilical cord MSCs with hydrogen peroxide

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Acute pancreatitis (AP) is serious disorder requiring emergency hospitalization. There are currently no effective therapies for AP. MSCs can be a potential candidate for the treatment of this disease due to their immunomodulatory properties. The aim of this study was to compare the therapeutic effect of the transplantation of native MSCs and MSCs preconditioned with H_2O_2 in the rat model of AP. Wistar rats were randomized into four groups: a negative control group received normal saline; a positive control group obtained the intraperitoneal injection of L-arginine at a dose (350 mg/100 g of body weight) within 1 h interval in between; and two of experimental groups were injected intraperitoneally with native MSCs and preconditioned MSCs with 30 μmol H_2O_2 second passage at a dose (6–7x10^6 cells/kg of rat weight) 72 h after AP induction. Rats were sacrificed after 7 days, and the pancreatic tissues and blood were collected. Biochemical, histological and histochemical methods were used to determine pathological changes in the rat pancreas. Three day after the AP induction, the amylase level increased by 5 times to compare negative group. The size of fibrotic area was 5 times larger than the negative group. Schiff's reaction shows that the pancreas of positive group has a low level of insulin synthesis which leads to acute inflammation. 7 days after transplantation of native and preconditioned MSCs into the rats with induced AP, the amylase level decreased 3 times after MSC transplantation and decreased to normal after preconditioned MSCs. The size of fibrotic area was almost returned to norm after the injection of preconditioned MSCs. Other morphometric and histochemical parameters of pancreas almost return to a normal level. The results of this study show that the transplantation of native MSCs to the rats with AP led to the recovery of pancreas, but the injection of preconditioned MSCs with H_2O_2 significantly improved the therapeutic efficacy of MSC therapy. *The authors marked with an asterisk equally contributed to the work.

Atomic and molecular imaging

P-07.1-001
Total synthesis of *Odontosyllis undecimdonta* luciferin and its structural elucidation

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Odontosyllis undecimdonta is a marine polychaete that has an ability to emit visible green light. During the previous studies we isolated several low-molecular weight compounds, which were then proved to be substrates in the light-emitting transformation process. NMR, mass-spectrometry and X-ray analysis data allowed us to propose the structures of the luciferin oxidation products. We then used this information to suggest a possible structure of *Odontosyllis* luciferin, belonging to a class of 2,3-dicarboxythiochromenes. The main goal of this work was to confirm it by total synthesis. We had to develop new synthetic methods, as literature search showed that there were no reliable approaches to the synthesis of the target compound. The approach employing the use of the thia-Michael/Horner-Wadsworth-Emmons cascade transformations using quinone and diester derivative of a dicarboxylic acid as starting compounds allowed us to obtain *Odontosyllis* luciferin via convergent synthetic scheme from 4-bromo derivative of benzothiophene and dimethylacetylenedicarboxylate. The work was supported by Russian Science Foundation grant № 21-74-10152, https://rscf.ru/en/project/21-74-10152/.

P-07.1-002
Highly-fluorescent benzothiphene-based dye exhibiting a large Stokes shift

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Fluorescent dyes play a significant role in modern science and technology, where they are used as tools in a variety of fields, including nanoscience, biomedicine, and solar energy conversion. The creation of new fluorescent dyes emitting in red and near-infrared regions of the spectrum is a crucial task of modern organic synthesis. Conjugated aromatic compounds with a benzothiophene-based core have fluorescent properties, but mostly possess small Stokes shift, and require conjugation with an additional chromophore group to increase it[1]. A number of conjugated dyes that absorb visible light and emit in the near-infrared spectral region were obtained for different applications[2,3]. Meanwhile, a dimerization of the original dye may become an alternative strategy for the increase of the Stokes shift. Here we report a novel highly fluorescent benzothiophene-based dye comprising five fused rings and exhibiting a large Stokes shift (from $\Delta\lambda$ = 100 nm or $\Delta\nu$ = 3633 cm^{-1} in water to $\Delta\lambda$ = 152 nm or $\Delta\nu$ = 5482 cm^{-1} in ethanol). The compound is obtained via a simple two-step procedure from commonly available aromatic benzenethiol. Due to the presence of a protected carboxyl group, the obtained compound could be easily modified to allow a series of new fluorescent dyes. This work was supported by the Russian Science Foundation grant № 21-74-10152, https://rscf.ru/en/project/21-74-10152/. (1) Jiao C et al. (2011) Org Lett 13, 632. (2) Ni Y et al. (2014) Org Biomol Chem 12, 3774. (3) Lim S. H. et al. (2010) J Med Chem 53, 2865.

P-07.1-003
Synthesis of plant and fungal secondary metabolites – substrates for the biosynthesis of fungal luciferin analogues *in vivo*

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Bioluminescence-based bioimaging systems are widely used in biomedical research. The bioluminescent system of higher fungi was deciphered and successfully reconstructed in heterologous hosts in our laboratory. New fungal luciferin analogs will expand the area of application of fungal bioluminescent system. Wide substrate tolerance of the enzymes of fungal bioluminescent system allows the use of caffeic acid and its synthetic analogs for the biosynthesis of fungal luciferin analogs *in vivo*. We have developed approaches to the biosynthesis of plant and fungal secondary metabolites from caffeic acid analogs in the yeast cell model. This work is an important step in understanding of the process of fungal bioluminescence towards new artificial bioimaging systems. The work was supported by Russian Science Foundation grant № 22-44-02024, https://rscf.ru/en/project/22-44-02024/. *The authors marked with an asterisk equally contributed to the work.

P-07.1-004
A crystallographic snapshot of SARS-CoV-2 main protease maturation process

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SARS-CoV-2 is the causative agent of COVID-19. The dimeric form of the viral M^{pro} is responsible for the cleavage of the viral polyprotein in 11 sites, including its own N- and C-terminus. The lack of structural information for intermediary forms of M^{pro} is a setback for the understanding its self-maturation process. Herein, we used X-ray crystallography combined with biochemical data to characterize multiple forms of SARS-CoV-2 Mpro. For the immature form, we show that extra N-terminal residues caused conformational changes in the positioning of domain-three over the active site, hampering the dimerization and diminishing its activity. We propose that this form preludes the cis and trans-cleavage of N-terminal residues. Using fragment screening,

we probe new cavities in this form which can be used to guide therapeutic development. Furthermore, we characterized a serine site-directed mutant of the Mpro bound to its endogenous N- and C-terminal residues during dimeric association stage of the maturation process. We suggest this form is a transitional state during the C-terminal trans-cleavage. This data sheds light in the structural modifications of the SARS-CoV-2 main protease during its self-maturation process. *The authors marked with an asterisk equally contributed to the work.

Cellular imaging

P-07.2-001
NIR-emitting pentamethine cyanine dyes for mitochondria visualization with high cytotoxicity to cancer cells

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Mitochondria play crucial roles in cellular metabolism. Therefore, compounds that stain mitochondria selectively are essential for tracking their different functional states. Moreover, dyes with high selectivity for mitochondria in cancer cells that also exhibit toxicity can be used for cancer treatment. In this regard, we have studied the series of pentamethine cyanine dyes based on benzothiazole heterocycles with different substituents emitted in the near-infrared region (NIR) as fluorescent probes for mitochondria visualization. Spectral-luminescent properties of the dyes in a free state and in the presence of different biomolecules such as nucleic acids and serum albumins were characterized. Dyes possess low to moderate fluorescence intensity in a free state, but it increases while binding to serum albumins up to 27 times with quantum yield up to 100 times higher than in a free state. The fluorescence emission maxima of dyes in a bound state lie between 680 and 711 nm at the NIR region. We have studied the ability of the dyes to stain live mesenchymal stem cells from human bone marrow (MSC) and the human breast cancer cells MCF-7. The dyes penetrate the cell and stain the cytoplasm components in a shorter incubation time compared to the commercially available dye MitoTracker Green (less than 5 min). Performing co-localization of the studied dyes with MitoTracker Green shows that cyanine dyes colocalize with standard dye, and therefore stain mitochondria in cancer MCF-7 cells. We have also performed SYTOX/Propidium Iodide-based staining assay to investigate the cytotoxicity effect of the dyes. The higher cytotoxicity of studied dyes on MCF-7 cells was shown compared to MSC. Taken together, the combined features of good imaging, NIR emission, and the higher anticancer activity make pentamethine benzothiazole cyanine dyes powerful fluorescent probes applicable for mitochondria visualization in cancer cells and suitable candidates for further theranostic studies.

P-07.2-002
Live imaging with subcellular detail in optical coherence microscopy: is it applicable for quality assessment of mammalian zygotes?

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Confocal and fluorescence microscopy allow for detailed structural studies on intracellular level, but require fluorescent markers to visualize cellular architecture. Bright-field microscopy, although non-destructive, does not provide such data. Optical coherence microscopy (OCM) is a promising alternative, as it does not require sample preprocessing and provides 3D images of intracellular structures, including pronuclei in zygotes, which can be analyzed quantitatively. There were numerous reports indicating that the pronuclear size provides an effective indicator of the embryo's quality, with embryos containing evenly sized pronuclei exhibiting higher developmental potential. We applied OCM to correlate the observed morphological parameters of zygotes, such as number, volume, and distance between pronuclei, with their developmental capabilities, such as the ability to reach the blastocyst stage, morphokinetic parameters, and numbers of cells in the first embryonic cell lineages. We showed that OCM allows for quantitative measurement of the pronucleus volume and distance between the pronuclei in a mouse zygote. As we expected, the size and the distance between the pronuclei correlates with the time of NEBD and the interval between the NEBD and the first mitotic division, but surprisingly, pronuclei volume also correlates with the time required for the 3rd embryonic cell cycle. None of the examined parameters, however, correlated with the total number of cells, or the number of cells in the first embryonic cell lineages in 5-day old embryos. We verified that both scanned and control zygotes have the same ability to complete preimplantation development. In summary, although our results indicate that OCM indeed allows for non-invasive 3D imaging of mammalian zygotes, providing data on pronuclei architecture, it seems the analysis of the volume and distance between the pronuclei, contrary to the earlier reports, might be insufficient for embryo quality assessment.

P-07.2-003
Altered ultrastructure of synaptic mitochondria in Fragile X syndrome linked with metabolic changes

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In the synapse, an important pool of mitochondrial proteins is translated locally on the bases of mRNAs transported from cell soma. Moreover, the local synthesis of proteins constituting mitochondrial respiratory chain complexes is increased by synaptic stimulation. In Fmr1 KO mice, a mouse model of fragile X syndrome, proteomic analysis shows dysregulated levels of mitochondrial proteins. Mitochondrial functions are fundamentally linked to their morphology and inner membrane ultrastructure. We used Serial Block Face-Scanning Electron Microscopy (SBF-SEM) to analyze mitochondrial ultrastructure in the hippocampi of Fmr1 KO and WT mice. Mitochondria shapes and volumes were reconstructed with the use of RECONSTRUCT software. We compared the morphology and the number of synaptic

mitochondria in Fmr1 KO and WT mice and found significant differences between genotypes. To understand this genetic mutation's physiological consequences for mitochondrial function in the synapse, we measured the level of reactive oxygen species (ROS) and ATP using mice of three different ages. We found a significant decrease in mitochondrial ROS levels in 13 weeks old Fmr1 KO mice compared to WT mice.

P-07.2-004
FRET-based cAMP imaging in AKAP6-organized compartment

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cAMP signaling is known as crucial regulator of diverse cellular processes including neuronal outgrowth and survival following injury. Signal transduction by cAMP is highly compartmentalized because of the formation of discrete, localized multimolecular signaling complexes by A-kinase anchoring proteins (AKAPs). The subcellular compartmentation of cAMP signaling has been widely studied, but outside of dendritic synaptic regulation, only few cAMP compartments have been defined in terms of molecular composition or function in neuronal survival and regeneration. Although the concept of cAMP compartmentation is well established, the function and identity of these compartments remain poorly understood in neurons. We demonstrate that cAMP signaling within a perinuclear neuronal compartment organized by the large multivalent scaffold protein AKAP6 promotes neuronal survival and axon outgrowth. AKAP6 signalosome function has been investigated using new molecular tools designed to specifically affect local cAMP levels as studied by live-cell FRET imaging. Moreover, enhancement of cAMP signaling at AKAP6 by isoform-specific displacement of phosphodiesterase 4D3 increased retinal ganglion cell survival in vivo in mice following optic nerve crush injury. Our findings define a novel neuronal compartment that confers cAMP regulation of neuroprotection and axon growth and that may be therapeutically targeted in optic nerve degeneration. Supported by National Science Centre (Narodowe Centrum Nauki) grant number 2019/33/B/NZ4/00587

Quantitative analysis of bioimages

P-07.3-001
CRKL, AIFM3 and UBASH3A expression during human kidney development

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We aimed to investigate the spatio-temporal expression of possible CAKUT candidate genes CRKL, AIFM3, and UBASH3A, as well as AIF, CRK, and BCL2 during human kidney development. Human fetal kidney tissue was stained with antibodies and analyzed by fluorescence microscopy and RT-PCR. Quantification of positive cells was assessed by calculation of area percentage and counting cells in nephron structures. ImageJ software was utilized for cell quantitative evaluation of immunoreactivity. Figures were prepared for analysis using subtraction of the median filter and color thresholding to measure the section percentage area covered by the positive signal. Co-localization of AIFM3 and AIF was calculated by dividing the area of overlap with the combined area of the two using Adobe Photoshop. Results showed statistically significant differences in the temporal expression patterns of the examined markers, depending on the investigated developmental stage. Limited but strong expression of CRKL was seen in developing kidneys, with increasing expression up to the period where the majority of nephrons are formed. Results also lead us to conclude that AIFM3 and AIF are important for promoting cell survival, but only AIFM3 is considered a CAKUT candidate gene due to the lack of AIF in nephron developmental structures. Our findings imply the great importance of AIFM3 in energy production in nephrogenesis and tubular maturation. UBASH3A raw scores showed greater immunoreactivity in developing structures than mature ones which would point to a meaningful role in nephrogenesis. Also, its spatial relationship with CRK (an important paralog of CRKL) was examined due to a possible interaction between them but yielded minimal results. The fact that mRNA and proteins of CRKL, UBASH3A and AIFM3 were detected in all phases of kidney development implies their role as renal development control genes.

P-07.3-002
The possibility of using the fractal approach in the image analysis of the microstructure of biological samples

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When studying the structure of objects of natural or biological origin, especially under the microscope, irregularly shaped objects are very common. They are difficult to describe and compare and in this case fractals or fractal geometry can be useful. This paper presents a possible method of image analysis that allows the determination of the fractal dimension on the basis of SEM micrographs. In this study, yoghurt gels were used as a biological material and the changes in their microstructure were compared during storage for 28 days. The determined fractal dimensions characterized the porosity of yoghurt gels. The obtained results show that the fractal dimension of yoghurts increased during their storage. This indicates that the porosity of the yoghurts also increased during storage. While the increase in pore volume in the yogurt gel can be observed in micrographs, the increase in irregularities in the pore contours can only be compared using the calculated fractal dimensions. The presented method of microstructure analysis enables the determination of fractal dimensions and a direct quantitative comparison of irregular structures using a mathematical tool. This method can also be used to analyse the morphology of particles or the surface of preparations with distinct structural features that are difficult to verbally describe and compare. Project financially supported by Minister of Education and Science in the range of the program entitled "Regional Initiative of Excellence" for the years 2019-2022, Project No. 010/RID/2018/19, amount of funding 12.000.000 PLN.

POSTERS - EDUCATION

Undergraduate teaching/learning

P-E-01-01
Lessons learned from 3D printing macromolecular models for teaching and demonstration

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Advancement in three dimensional (3D) printing hardware and software makes this additive manufacturing technique rather cheap and easy to implement in schools and universities. 3D printing can be used to fabricate various teaching materials for numerous disciplines, and it has a great application in teaching life sciences. Starting with the atomic coordinates downloaded from RCSB PDB, one can create a physical model of any biomolecular structure that would help better understand/demonstrate key aspects such as molecular size, symmetry, or shape. Besides that, 3D printing of molecular models can also be used to engage students in designing their own custom printable molecule of choice, offering a great opportunity to study molecular structure first-hand. After designing and printing of more than 30 different models of molecules and macromolecular complexes (including the T7 replisome, the DNA sequencing protein nanopore, the 20S Yeast proteasome or an alpha-amylase with amylopectin), several lessons have been learned. First, surface renderings of macromolecules are extremely easy to create and print and can be tackled by any user. Surface representation of subunits of the same multimeric protein can be printed separately in different colors with a high visual impact. Unfortunately, the subunits cannot always be assembled back into the complex due to frequent overhangs and tight channels. Cartoon representations can be used only for rather small proteins as are harder to print. A higher rate of success in printing complex cartoon models can be obtained with a multilateral capable 3D printer and using soluble plastic as support. Balls and sticks models are suitable for small molecules. Printing balls and sticks or a surface model of DNA in flexible TPU has a great visual impact when demonstrating the assembly of the nucleosome. In the end, a set of models with printing instructions are provided which could serve as a starting point for anyone wishing to print its own macromolecular models on the cheap.

P-E-01-02
The Biochemist's Uncle: reading is the best medicine

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The last two years in education were like no other ones. What would we expect from 2022? The COVID-19 pandemic and subsequent health crisis has profoundly impacted our lives. Furthermore, it influences dramatically education whose implications are yet to be determined. However, even before 2020, it was verified that teaching or studying biochemistry no longer involves the reading of the biochemistry book. Moreover, recent studies referred that about 60% of Portuguese do not read any book. Thus, it was decided at the biochemistry classes, to adopt the strategy of discovering a new friend, "The Biochemist's Uncle", for the teaching and the learning of biochemistry. Some chapters of the biochemistry book were read more than 3 times during the 14 weeks of the semester. Each time the teacher traveled through a chapter, stopped at a different page where it could be found a feature about a concept or/and a topic. Students are invited to participate in this process. Certainly, students acquire better knowledge in the subjects and topics of biochemistry, if they are involved in the discovery of the topics. Furthermore, the participation of students in learning, rather than simply teaching them, leads to an increase interest in the study of biochemistry as well as in their various interfaces and applications. One problem with active learning, however, is that it is very time consuming. Herein, we described some examples arise from this methodology of teaching and learning biochemistry by purely reading the book. Students were encouraged to report the positive and negative aspects of this approach. Regarding the positive aspects, the feeling of having learned to study and the importance of biochemistry was mention. Further analysis and concerns will be given in the present communication. Still, perhaps the pandemic effects in education are yet to come. In any case, in education, the act of discovering and innovating is always an exciting adventure.

P-E-01-03
Microbe hunters – an educational program on soil microbiology and biochemistry for schoolchildren and their teachers

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The problem of education of highly qualified specialists who can conduct high-level scientific research and develop new technologies is steadily growing in most developed countries. Various programs attract young people to universities that educate specialists in natural sciences; new teaching methods are being developed to include motivated schoolchildren in scientific work. Citizen science and mass experiments involving thousands of schoolchildren are of particular interest. Danish National Center for Education in the Field of Natural Sciences and Novozymes, made a collection of lactobacilli using the help of schoolchildren. Science Education Alliance Phage Hunters Advancing Genomics and Evolutionary Science, a student search program for new bacteriophages, led by the Howard Hughes Institute, obtained more than 15000 samples of new phages. Investigation of the nitrogen-fixing bacteria distribution is of particular interest to analyze soil quality; such bacteria may produce biofertilizers. In 2019, the ICBFM SB RAS and NSU researchers organized the "Microbe Hunters" project. Schoolchildren from different regions of Russia, under the guidance of schoolteachers, collect samples of nitrogen-fixing soil bacteria. Schoolchildren carry out the primary characterization of samples of nitrogen-fixing bacteria and analyze phosphate solubilizing, proteolytic and some other activities. Metagenomes of isolated microorganisms and their consortiums are analyzed at the ICBFM SB RAS; new antibiotics, bacteriophages, genome editing instruments, nickases, DNA polymerases etc., are searched. We designed a particular educational program for

project mentors. An annual scientific conference is organized for schoolchildren participants. By 2022, more than 2,000 schoolchildren and their mentors have already implemented the educational program, and the program is ready to go to the international level. The study was supported by 2021-1930-FP5-8365-8981 (075-15-2021-1085) *The authors marked with an asterisk equally contributed to the work.

P-E-01-04
Digital technologies (DT): an important tool for training undergraduate students in the development of future competences and skills

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DT has brought significant changes in today's society, being considered one of the competencies foreseen in basic education in the 21st century. Thus, it is important that undergraduates master the ways of using DT, aiming at its use as a tool in their pedagogical practice. The Interactive Science Space (ISS) promotes, every year, a Science Club (SC) where graduates of the Exact Sciences teacher training course (USP-São Carlos) act as tutors and are encouraged to apply science teaching practices investigation with the objective of contributing to the scientific literacy of high school students from public schools. Since 2019, we have been introducing the use of active methodologies combined with DTs in the weekly meetings of the SC with the purpose of investigating whether: 1) in the tutors' conception, these methods contributed to the use of DT in their own teaching practice; 2) Club students act more actively when encouraged to use DTs to solve proposed problems. The tutors made records in a reflective diary, with (1) notes about the expectations and challenges of their pedagogical practices in 3 meetings on the topics "Bicycle Physics", "Scratch" and "Fake News", and with (2) responses to questions about the relationship between teacher training/practice and the use of DT. The results showed: a) the tutors' planning was challenging, as it was necessary to organize investigative sequences with unknown methodologies and DTs; b) identified that there are some disciplines and activities in their curricula aimed at the use of DTs, but most with a theoretical approach; c) SC students were more active when encouraged to use TDs to solve problem situations. Tutors pointed out that it was a very good opportunity to expand their knowledge and teaching practices, but in Brazil the educational institutions are not prepared for these new paradigms. *The authors marked with an asterisk equally contributed to the work.

P-E-01-05
Reaching to aliens: teaching biochemistry in a "Food Science" context

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Biochemistry is a mandatory topic for MS students in Food Science and in Human Nutrition at UNIMI. Students with a BS in Food Science at UNIMI have already taken basic biochemistry courses, so that new stimuli have to be provided while addressing the complexity inherent to food systems. All of this calls for: i) presenting topics that are seldom considered in courses offered in bio-medical settings; ii) a close integration with other area disciplines; iii) a complementary array of elective courses. The mandatory MS courses focus on methodological and safety issues (such as intolerances and allergies), discussed in terms of structural features of macromolecules in the raw materials and in changes occurring upon processing. Particular attention is placed on assessing the determinants of intermolecular interactions involving various types of macromolecules (protein/protein; protein/carbohydrates; protein/lipids) and on quantitating or predicting their relevance. Additional focus is placed on the interaction of macromolecules with small food molecules (from inorganic ions to vitamins, from food additives to food-borne xenobiotics). Links to external websites are provided as an integral part of the teaching material, and additional credits may be offered for attending seminars or short courses from visiting scientists. Lab classes are an integral component of each course – covering about one fourth of total credits – and aim at providing fundamental-type information by analyzing common practical situations. For example, temperature-dependent protein unfolding is monitored by measuring the accessibility of cysteine side-chains to inexpensive colorimetric probes. In another lab activity, the role of pH and of redox events in the metabolism of micronutrients is addressed by evaluating - with simple colored chelators - the release of iron from the storage protein ferritin at various pH values and in the presence/absence of reducing agents in food, such as ascorbic acid.

P-E-01-06
How to teach male infertility diagnostic laboratories during a pandemic: a novel approach

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In Laboratories in Biomedicine 5, a 3rd year class of the Biomedical Sciences bachelor (University of Aveiro), the cellular, molecular, and biochemical basis of male infertility diagnosis is addressed (Module 3; M3), usually involving 4 practical classes in the laboratory. In October 2020, due to the COVID-19 constrains, the practical classes needed to be restructured to avoid virus spread among students and staff. Thus, our main objective was to design and implement a new teaching strategy applied to M3 and to clarify if this methodology was valuable to students' learning process without compromising their safety. The 75 students attending to M3 were divided into 8 groups to reduce students' number in class. M3 was organized in 2 Wet labs (WL; laboratory training) and 2 Dry labs (DL; theoretical-practical classes). At the end of M3, students completed a satisfaction survey. Results showed that most students were satisfied with topics addressed, time dedicated outside the class, evaluation, and overall organization of WL and DL. Regarding WL, the protocols provided, and general conditions were satisfactory. Only positive classifications were attributed to the learning goals, the relevance of knowledge acquired, and opportunities to ask about evaluation. The qualitative data suggested that the dynamics, innovation, and organization of classes, allied to the knowledge and motivation of the professors, provided the appropriate learning environment. As a limitation of this study, there is a lack of data from previous years. To summarize, the methodology implemented in M3 represents a valid strategy to encourage and motivate students' learning, stimulate teamwork, communication, and autonomy in the laboratory. Preparing dynamic and attractive

classes increases students' satisfaction which contributes to the efficiency of the learning/teaching process.

P-E-01-07
Improving the learning environment and students' satisfaction through the combination of spaced learning and team-based learning – the impact on students' perception and engagement

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With the aim of motivating and engaging the students during the Integrative Biochemistry class, lectured in the bachelor's degree in Biomedical Sciences at the University of Aveiro, a combination of two different methodologies were implemented: Team-based Learning (TBL) and Spaced Learning (SL). This pedagogical practice emerged as an alternative to a purely theoretical 3-hour class, in which keeping the students focused and interested can be challenging. The main objectives of this pedagogical innovation were to promote the collaboration between students and, at the same time, keep them motivated throughout the semester. A typical Biochemistry class involves various steps which combine different flavors of TBL and SL: a bibliography is provided to the students for them to get familiarized with it one week in advance; in class, the students answer an individual quiz, followed by a group quiz; the next step is solving a problem. Meanwhile a SL break occurs in which students do distracting activities (usually physical activities). Lastly, a short seminar in the form of Q&A occurs, where doubts are clarified. The assessment of this class involves two written tests, the quizzes, the solved problems in class, a class project, and the attendance. Data has been collected through different means: focus group interviews, questionnaires, and observation techniques. The students feedback obtained was highly positive (results obtained in the university quality system and in the class final questionnaires). In particular, when they were asked what they liked the most the answer was – SL. The communication will present the main results of the implementation of the pedagogical innovation, with specific examples of SL activities.

P-E-01-08
Building our future, a conference by and for undergraduates

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Motivation is a key aspect of a learner's performance. The type of motivation we are interested in "fostering" is intrinsic motivation, closely linked to the concept of learning-centered goals and objectives. Two of the aspects that should be taken into account when motivating university students are the promotion of student participation and the search for practical applications. Thus, we are committed to the implementation of participatory techniques for the performance of important tasks in their professional field. The action took place in the Biology course of the first year of the Chemistry Degree. The students were divided into teams, in which they were asked about different aspects related to their professional future (Research, Teaching and Bio-Entrepreneurship). The study presented is based on the work of the teams that were dedicated to the organization of the Scientific Conference in which the students presented their projects. The themes of the teams that carried out the organizational tasks were: a) Activities before the conference. Logistics; b) Activities before the conference. Budget; c) Activities during and after the conference. The dynamics of the action were based on: i) an initial session, in which the objectives and guidelines to be followed were clarified; ii) several meetings with the teachers who guided them during the search for information, interviews with experts, organization of the information and preparation of communications; iii) a final session, in which the students presented their work in a public event at the Building our Future Conference. Based on the results obtained, we can affirm that the proposed action has had an impact on teaching practice insofar as the "research" is motivated by the reality of their professional practice, contributing to the improvement of the quality of face-to-face teaching. Teaching Innovation Group InDoBio 5.0, Universidad de Alcalá (UAH)
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P-E-01-09
Biotechnology and biochemistry laboratorial education through the SARS-CoV2 pandemic: challenges, truths and takeaways

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In early 2020, the COVID-19 pandemic forced the higher education institutions to adapt to a new form of teaching and learning, from presential to full remote, blended and hybrid environment. This challenge had a particular impact in the life sciences field where the courses have a high content of laboratorial classes. The main issue was how to transition from hands-on practical classes to remote instruction, assuring that our students continued engaged and acquiring the necessary skills, in a short amount of time. To address this, we took advantage of already available digital tools that facilitated the interface with the students such as Moodle, Skype, Teams and ZOOM and explored online resources such as virtual labs, simulations and video demonstrations. Additionally, several classes and laboratorial experiments were recorded by the teachers in the school labs and the research labs where we develop our research activity. The creation of teachers' work groups to share experiences and tools was key in the success of this process. To ensure the students evaluation we used preferentially Moodle platform which allowed the used of digital tools to control and prevent fraud by copying, plagiarism or false identity. Despite all efforts from teachers and students, crucial elements of the high education experience, particularly for undergraduates, were disrupted. Namely the student–teacher and student–student contact and interaction and the integration in the academic setting, resulting in stress and feeling of isolation

and overwhelm. Even with the difficulties faced by all the academic community, we observed no major changes regarding the academic success reflected in the final grades, comparing with previous years. In our understanding the core skills proposed for these courses were acquired successfully and the digital tools used with exception for the student's evaluation, are now considered an added value and bring flexibility to the teaching-learning process. *The authors marked with an asterisk equally contributed to the work.

P-E-01-10
The use of tactile models for the teaching of biomolecules

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Tactile models that represent molecular structures have been used in science teaching and, as demonstrated by research in the area, facilitate the learning of topics that demand molecular abstraction. Our scientific education group developed strategies and teaching materials as 3-D models representing structures of biomolecules (nucleic acids, amino acids and proteins) to teach this subject to biology teachers. The course offered in 2019 was attended by 38 high school teachers, among whom a survey was carried out regarding their impressions about the use of tactile models for teaching and learning biomolecules. The models were used within a problematizing didactic sequence, which was also applied by the teachers with their students. At the end, teachers were asked about the use of tactile models in their own learning and in that of their students. Seventeen teachers stated that the use of tactile models for assembling molecules brought benefits in the learning of the contents, attributed to the following beliefs: the activation of the senses (handling and visualization) was a facilitator for the understanding of the structure of biomolecules; the use of models motivates the user to learn. These and other impressions collected in the courses offered by our group over the last 17 years motivated a previous research (Silva and Bossolan, 2019), which evaluated the contribution of these tactile models to the elaboration of mental representations about proteins with undergraduate students, under the Johnson-Laird's mental models theory. This study identified that the use of tactile models promoted the construction of more sophisticated protein models, besides the capacity to predict the functional loss of the protein from changes in its structure. We therefore intend to include in our teacher training courses a discussion about how individual mental models are formed and which didactic strategies collaborate with their formation and/or expansion. *The authors marked with an asterisk equally contributed to the work.

P-E-01-11
Protocol for evaluation of DNA damage – a transfer of knowledge from theoretical subjects into practical elective subject Student research work at the Faculty of Medicine

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The following study was performed by Jinan Fazal, a pregraduate student at the Faculty of Medicine in Hradec Kralove, as a part of the elective study subject, Student research work. The aim was to establish a protocol for the evaluation of DNA damage caused by different anticancer drugs. This protocol will enable a future student of the subject to get familiar with basic laboratory techniques and above that, it puts into practise the theoretical knowledge taught within the subjects Biology, Biochemistry and Pathobiochemistry of the cell. As a model system, the MCF-7 breast cancer cells treated with increasing concentration of doxorubicin (0.1 to 10 μM) for 2 hours, were chosen. As one of the earliest events after DNA damage is phosphorylation of histone H2AX at serine 138, the cells were stained with primary anti-phospho-histone H2A.X (Ser139) and secondary Alexa Fluor 488-AffiniPure Goat Anti-Rabbit antibody. The labelled samples were acquired by the mean of ImageXpress XLS and the percentage of cells expressing phosphorylated H2AX analysed with MetaXpress 6.3 software. Developed protocol gives the students opportunity to gain skills in cell culture cultivation and treatment, immunofluorescence staining, image acquisition and analysis. Besides, it can be routinely employed for assessing DNA damage caused by various stimuli.

P-E-01-12
Teaching of scientific research skills to Medical Doctor Program students during the first two years of study

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Teaching of scientific research skills is one of the main aims of the Medical Doctor program. European University Medical Doctor Program was modified in 2019 according to WFME standards and national medical benchmark and important changes were made in the teaching of scientific skills. Program evaluation is performed annually by all stakeholders. Students' academic performance of the fall semester of 2021-22 academic year was evaluated in the courses „scientific reasoning I" and „scientific reasoning II", which are taught in the II and IV semesters respectively. In total 73 students took „scientific reasoning I" and 82 students took „scientific reasoning II" in this period. Based on the results, 15% of students failed and 28% crossed the minimum threshold in „scientific reasoning I". In „scientific reasoning II" 5% of students failed, 67% crossed the minimum threshold, while none of the students could get the highest mark „A". Same students' academic performance was evaluated in all other subjects, but such low performance was not observed. Special survey was distributed among the Faculty members delivering the subjects „scientific reasoning I and II". In the answers, all lecturers underlined that the main reason of students' low performance is caused by the difficulty of the subject and learning material. Another possible reason indicated by the lecturers can be the early entry of scientific skills teaching in the curriculum (from II semester). Based on all above mentioned, we can suppose that first and second year MD students are more focused on basic subjects of major field, which are time and energy consuming. Accordingly, it is important to reconsider the teaching and assessment methodology of research skills in order to increase students encourage in the study process and to support them to reach learning outcomes. *The authors marked with an asterisk equally contributed to the work.

P-E-01-13
A comparison of the impact of the Covid-19 pandemic on the quality of teaching of medical students in Poland and the Czech Republic

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The Covid-19 pandemic has changed many areas of life. It influenced the students' well-being and the ways of learning. Universities have taken up online learning, introducing innovations in many fields. How were students in different countries perceived? How did these changes affect the teaching process? The study compares traditional learning methods in Poland and the Czech Republic. But also the changes and innovations introduced in the learning process during a pandemic. The similarities and differences in teaching models are described. Two Universities where the subject of Biochemistry was taught to medical students (Charles University, Prague, and Jagiellonian University Medical College, Krakow) were compared. The study was attended by students of the second year of medicine. As a research tool, a questionnaire created for the study was used to measure the mood of the respondents and the impact on behavior while studying during a pandemic. 276 people participated in the study (111 from Poland, 165 from the Czech Republic, including 192 women, 82 men, 2 others). The results are presented in the form of graphs as % of the number of answers given. The survey was divided into 2 parts, one referring to mental health and the other to the quality of education. The study revealed several significant differences between the two countries. Analysis shows that lockdown in Poland did not have such a negative impact on students' extra-curricular activities as in the Czech Republic, and Poles also felt less isolated. Czechs seem to show lower levels of stress in the pandemic situation, and they also indicated significantly more often that the University was able to provide them with psychological support. Charles University students rate the quality of education better than UJ CM students after switching to online learning, which is expressed in such indicators as the standard of materials prepared by instructors, lecturer involvement, and participation during classes. *The authors marked with an asterisk equally contributed to the work.

P-E-01-14
Arousing students' interest in the subject of classes with the use of activating techniques during online classes

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Currently, academic education is characterized by a practical approach to acquiring knowledge. Fewer and fewer people prefer the theoretical approach. This form of education could not be fully implemented without innovative methods and techniques to activate students, as well as without the proper preparation of educators. Due to the COVID-19 pandemic, distance learning has been introduced in universities. This required the teaching staff to quickly adapt to the new working conditions. Conducting classes in a remote form is quite a challenge. For people conducting practical classes, this can be a great difficulty, especially when it comes to laboratory exercises. In this work, we present a few examples of activating techniques prepared with students from the Student Science Club, based on their learning experience in times of a pandemic. These include various laboratory experiments performed by students at home, mind maps and presentations. They are used not only to convey information, but also to develop skills, deepen reflection, stimulate the participants' own creativity and help in making contacts. We will also present the results of the student survey regarding online classes conducted in this way. They showed that the activating techniques used during the classes increased the interest in the subject, broadened the knowledge and made the classes more attractive. *The authors marked with an asterisk equally contributed to the work.

P-E-01-15
BioMolViz: a community to improve biomolecular visual literacy

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Biochemistry relies heavily on the use of images and diagrams to make the abstract tangible. Yet, as biochemistry instructors, how do we know whether our students see the same things we instructors see? BioMolViz (biomolviz.org) is a community of practice dedicated to the instruction and assessment of biomolecular visual literacy. To this end, BioMolViz created the Biomolecular Visualization Framework, an assessment tool that identifies more than 200 learning objectives clustered under twelve overarching themes, such as Structure-Function and Alternate Renderings. The team is currently assembling a searchable repository to host assessment instruments for each of these learning objectives. To aid in its construction, BioMolViz is recruiting participants to help write, revise, and pilot these instruments in the classroom. Here, we introduce the Framework, share various BioMolViz projects, and invite like-minded individuals to work with us to build students' biomolecular visual literacy.

P-E-01-16
Implementation of a gymkhana to learn Sequence Analysis and Molecular Phylogeny

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The great development of high-throughput molecular biology techniques and the consequent generation of biological data has forced bioinformatics to become an essential subject for undergraduate natural science students. The importance of this scientific discipline is evidenced by the enormous number of specialized publications, tools and databases available. In recent years, it is common to see in the teaching programs of academic institutions subjects focused on the acquisition of bioinformatics skills. We have implemented a two-day practical class of the

Bioinformatics course in the Biology degree, whose main objective is to introduce students to molecular databases and systems for searching information on genes, proteins and phylogenies. In the first day, students carry out an outdoor "sequence sampling" hidden in a QR code in the format of a Gymkhana, which they then analyze in the Computer Science Classroom. In the second day, they analyze the phylogenetic relationships of the identified sequences. We present the content and procedure of the practical class, the evaluation method and the results of a student survey to assess their opinions.

Postgraduate teaching/learning

P-E-02-01
Collaborative project design as a strategy for meaningful learning in postgraduates. Student and teacher thoughts

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Meaningful learning is a common goal, pursued by students and instructors. In postgraduates, the development of skills and competencies related to professional performance remains essential. In the experience developed, postgraduates participated in the workshop on the subject Methods of Design and Selection of Biologically Active Molecules (Master in Therapeutic Targets in Cell Signaling: Research and Development). Students self-organised into multidisciplinary teams to design a project according to the following phases: i) choice of an alteration or disease; ii) literature search integrating the contents reviewed so far; iii) selection of a therapeutic target; iv) design of an experimental development that supports an original therapeutic approach; v) presentation of the projects and discussion of different aspects about them. This action contextualizes in the classroom the professional tasks that these postgraduates will carry out in the foreseeable future. Thus, they had to work collaboratively with their teammates, making decisions and providing innovative solutions in order to achieve the success of the group. The joint thinking of students and instructors have led us to consider continuity in the implementation of this action. Furthermore, certain improvements will be introduced, such as reconsidering the sequence of the contents in the master's degree. Teaching Innovation Group InDoBio 5.0, Universidad de Alcalá (UAH)

P-E-02-02
Educational regimen and innovative solutions applied in biological sciences education

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Since the beginning of Molecular Biology in the mid of the last century, it experienced continuous development. Besides the pioneer university that teaches this specialization at undergraduate and graduate levels, the number of universities offering such courses increased annually with taking into account the relative difference in curricula offered by each Institution, Teaching experiences from other institutions applied in part but they were examined carefully and further developed due to the impact that might enhance the quality of knowledge acquisition by the molecular undergraduate or graduate students. Among these experiences were; the use of the 3D models to describe 2D biological and biochemical structures and pathways, learning of developmental biology, molecular diagnostics, genomics, and personalized medicine curriculum development based on the molecular knowledge available.[1] The aspect of distance learning especially during the COVID-19 pandemic proved to be a type of educational experience that should be supported in the future due to its role in teaching and exchanging ideas and thought between tutors and students and the students from the different institutions. The use of virtual laboratories for molecular biology teaching with the simulation of experimental methods is an important choice for this aspect. Experience can help us to further enhance the capacities related to the teaching curricula to be more responsive to the demands of the different working sectors whether it is in academy, industry, or decision making. Further, related extracurricular knowledge and soft skills help the prospective graduates to have better chances for employment and an important specialized function in their societies References: 1. Barthet, MM, 2021, Biochem Mol Biol Educ, 49(4):598-604. 2. Jiang X, Ning Q, 2021, Biochem Mol Biol Educ, 2021 May;49(3):346-352 3. Ashkanani, A., al, 2022, Biochem Mol Biol Educ.2022;50:104–113 4. Dal NE et al 2020, Gene. 724:143923.

P-E-02-03
Using Role-Plays in review scientific articles with postgraduate students

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The current university system is constantly updating the teaching strategies used in teaching. University students must develop competences that enable them to carry out their professional practice by being able to solve problems in different contexts. In this sense, the role-playing methodology allows the creation of a simulation where students adopt different perspectives, bringing them closer to the professional reality. This active learning technique brings a multitude of benefits to the training process as students become involved in a specific task that is key to their professional future. Our experience has been developed with postgraduate students of the Master's Degree in Therapeutic Targets in Cell Signalling, during three academic years. The students have to become critics of scientific articles, simulating one of the most important stages that all scientific research goes through, peer review. Throughout the whole experience, the teacher becomes a guide for the achievement of the final objective, indicating to the students how to carry out the review and what aspects they must take into account in order to do so. The following phases were carried out: i) selection of an indexed published scientific article; ii) in-depth analysis and understanding of the selected scientific article; iii) proposal for constructive improvement of the article; iv) presentation of the findings and possible criticisms to the rest of the classmates, and debate with interventions by the participants; vi) self-evaluation, co-evaluation and evaluation by the teacher with delivery of comments for improvement. The results of the action implemented in the three academic years showed an improvement in the acquisition of professional competences by the students, with respect to the courses prior to its implementation. Likewise, the comments made by the students in the evaluation of the activity, referring to the development of competences and skills

as true professionals, were very positive. For all these reasons, we consider that role-playing in this context is a fundamental part of meaningful learning. Teaching Innovation Group InDoBio 5.0 of Universidad de Alcala (UAH).

P-E-02-04
Participating in scientific meetings as part of PhD training: 6 years of educational and scientific data

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One of the ignored elements in the hierarchy of scientific achievements for obtaining PhD degree is participation in academic events. Interestingly, these have the potential to impact not only the scientific but also professional development of the PhD candidate. The aim of this abstract is to show an example of integrating attendance of a scientific event to the PhD training. We aim to provide 6 years of quantitative and qualitative data of young scholars' participation and gains from a continuing series of symposium. Launched in 2016 by the Turkish Society of Biochemistry, Izmir Branch, the *In Vitro* Diagnostics Symposia bring together biochemists each year for a 3-day event with a different theme. we proposed the scientific program to the Graduate School of Health Sciences, Dokuz Eylul University as an elective 2-credit course to which PhD students can attend. About 15–20% of the students to whom we award nearly 50 scholarships each year preferred to take the symposium as a course and they were required to participate actively >80% of the sessions. The assessment was performed via an online exam and feedback questionnaire. The exams (open-ended evaluation-application questions) targeted the scientific content covered in the symposium. The questionnaires not only revealed qualitative data of opinions on different scientific program elements but also aimed to trigger reflection (relevance of the symposium theme with their research field, most exciting new information they learned, their short- and long term-plan for activities & research on the symposium theme, etc.). The results are promising and prove that the use of scientific events as a tool in PhD education is a strategy that encourages the motivation and engagement of students. In conclusion, continuous well-defined scientific event programs can enrich the young scholar scientifically and contribute to the culture of research, exchange, and integration that graduate education seeks to achieve.

P-E-02-05
A training strategy from a research problem to a collaborative research environment and to building an innovation culture

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Today, PhD education has reached a whole new dimension by leaving its classical structure. It is not only academic and career-oriented, but now trains individuals capable of conducting independent research, fostering a culture of innovation, and having a voice in any academic or industrial field. The aim of the study is to describe the roadmap of a collaborative research, innovation, and entrepreneurship process that served as an ideal training strategy. Specific info about patent development and its relevance to education is also discussed. The project titled "Development of a Biosensor for Characterization of Human Primary Fibroblast Cells," which served as a tool for this training, arose in response to a PhD thesis problem. The research question, hypothesis, and study design were formulated and discussed through creative thinking and problem-solving sessions among the research team formed from 3 different institutes. The research was successfully conducted in collaboration and resulted in entrepreneurial exploitation of a new product. At this stage, research evolved from a scientific discovery to an innovation process. The entrepreneurial, creative spirit of the PhD students and postdocs was fostered through informative discussion sessions on innovation process with senior researchers and administrators. The innovative spirit, coupled with considerable persistence in following application procedures (preparing patent application documents for the developed product in accordance with college legislation), led to success after 9 months. After the decisions of the relevant institutions, including independent "Patent" institute which examined the specification and originality of the patent application, the product of the original research led to a patent and publication. In summary, this journey from a scientific question in academic research culture to a patent in innovation culture formed an ideal hands-on training strategy for the PhD students and post-docs.

Faculty development

P-E-03-01
Implementation of OSPE in basic sciences of the Medical Doctor Program, students' general satisfaction and future challenges

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Undergraduate Medical Doctor programs in Georgia are required to be accredited according the WFME standards and national benchmark. One of the recommended assessment methods in basic sciences is objectively structured practical examination (OSPE), which was implemented in the 2021–22 academic year at the European University. OSPE was conducted for the Medical Doctor program students as the part of the final exam of the integrated subjects „Body Systems I" and „Gene, Cell, Tissues 1". Several meetings were conducted for the lecturers and students about the key points of OSPE during the semester. Aim of our research was to evaluate the general satisfaction among the students after they gave their first OSPE for its' better planning and implementation in future. In total 50 first semester students attended OSPE. All of them received the OSPE satisfaction survey after the exam session and 33 (66%) filled questioner. Based on the results 60.6% of students had full information about the OSPE before the exam, 33.3% had almost enough information and for 2.6% given information was not enough. 78.8% of students mentioned that instructions on each OSPE stations were clear, 18.2% answered that instructions were somehow clear and for 3% was not clear. For 51.5% of students time given at each station to perform the task was enough, for 30.3% somehow enough and for the 18.2 % was not enough. According to 72.7% of students the feedback which they received after the exam helped them to identify their mistakes, for 42.4% feedback

was constructive. 72.7% of students answered that OSPE increased their motivation to improve their practical skills and for 81.8% of students OSPE increased their interest toward the laboratory classes. Finally, we can conclude that implementation of the OSPE increased the interest and motivation of students to laboratory classes, however more detailed OSPE instructions should prepared and exam time must be reconsidered. *The authors marked with an asterisk equally contributed to the work.

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