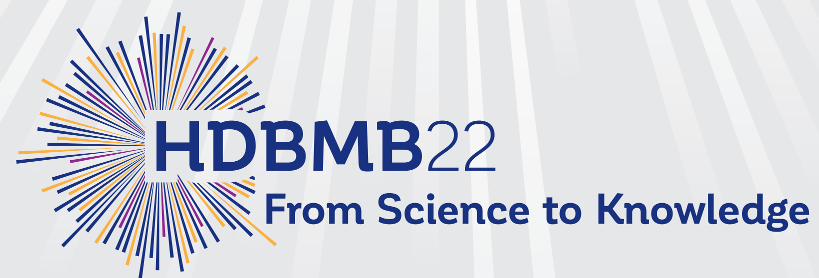
International Congress of the Croatian Society of Biochemistry and Molecular Biology

From Science to Knowledge

September 28 to October 1, 2022 | **Brela** | **Croatia**

International Congress of the Croatian
Society of Biochemistry and Molecular Biology



Book of Abstracts

Book of Abstracts of the Congress of the Croatian Society of Biochemistry and Molecular Biology

HDBMB22: From Science to Knowledge

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Morana Dulić, Nino Sinčić, Ivana Vrhovac Madunić

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Dear colleagues,

Welcome to the **Congress of the Croatian Society of Biochemistry and Molecular Biology, HDBMB22 "From Science to Knowledge"**. We are pleased that our Congress is being held in Brela on the Makarska Riviera, one of the most beautiful coastal regions of Croatia.

The congress is organised under the auspices of the University of Split, the University of Rijeka, the University of Osijek, and the University of Zagreb. We use this opportunity to express our gratitude to them all as well as to all of the exhibitors, sponsors and donors whose support is invaluable to the overall success of the Congress.

The Scientific Programme is focused on important recent developments in a wide range of life sciences including biochemistry, molecular biology, biotechnology, biomedicine, but also on education in science. The programme is headed by five outstanding plenary lecturers: Kristijan Ramadan (UK) giving IUBMB PLenary Award Lecture, Irene Diaz-Moreno (Spain) giving FEBS National Lecture, Roger Williams (UK), William F Martin (Germany), and Luciane Vieira de Mello (UK). In addition, the programme comprises 13 invited lectures, 16 short presentations and 104 posters. The best poster prize will be awarded by FEBS Open Bio.

After a bit longer time with scarce personal contacts, we hope to provide an excellent opportunity to exchange ideas and experiences with colleagues, establish new acquaintances, and renew old ones.

Enjoy a successful and stimulating HDBMB22 and have a very pleasant stay in Brela!

Damjan Franjević
Chair of the Scientific Committee

Morana Dulić
Chair of the Organising Committee

ORGANISER

Croatian Society of Biochemistry and Molecular Biology
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CONGRESS SECRETARIAT

Morana Dulić
Croatian Society of Biochemistry and Molecular Biology
Ksaverska cesta 2, 10000 Zagreb, Croatia
https://congress2022.hdbmb.hr/

VENUE

Bluesun Hotel Soline, Trg Gospe od Karmela 1, 21322 Brela, Croatia

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Ministry of Science and Education of the Republic of Croatia
FEBS - Federation of European Biochemical Societies
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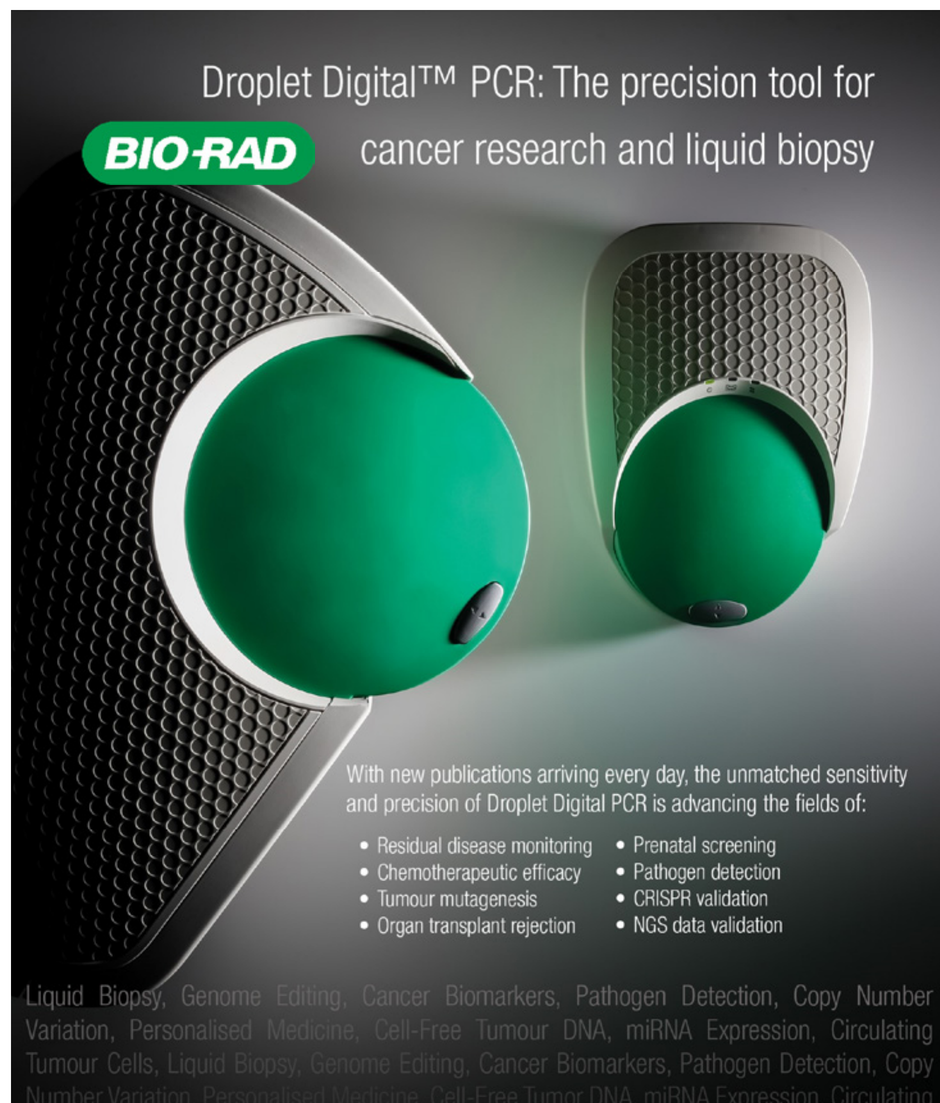

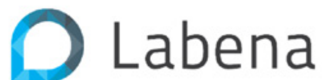

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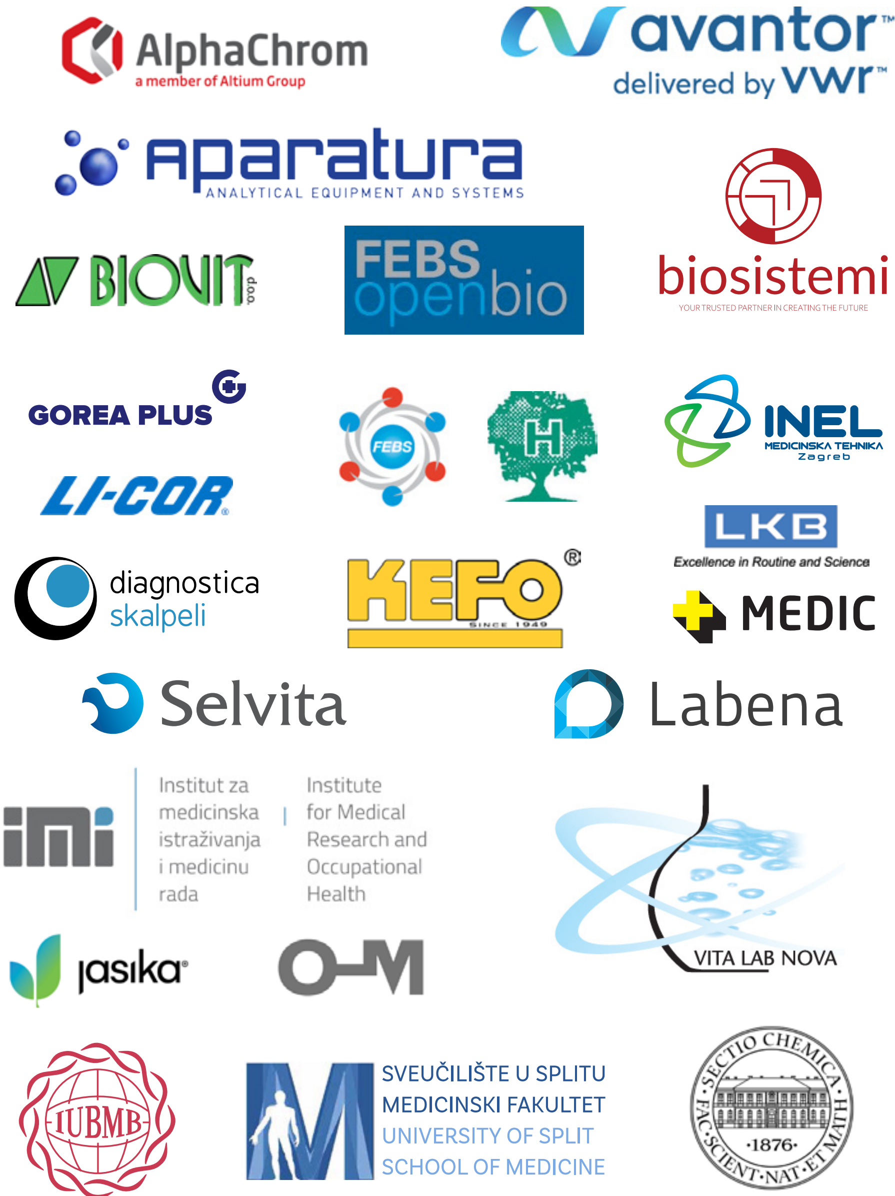

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Information

Registration

Registration will take place at the registration desk in the Congress hall of the Bluesun Hotel Soline from 14:00 on Wednesday, September 28th.

Registration fee for participants includes admission to lectures and exhibitions, Book of Abstracts, congress materials, refreshments during the congress and admission to all social events including congress dinner and excursion. Registration for accompanying persons includes all social events including congress dinner and excursion.

The certificate of attendance will be provided at the registration desk.

Language

The official language of the congress is English. There will be no simultaneous translation.

Lectures and oral presentations

Standard personal computer and LCD projector will be available. The speakers are kindly asked to deliver their presentations (USB stick) in advance to our technician, at least one hour before session start. The use of personal laptops is discouraged due to a potential compatibility and timing issues.

Congress rooms and audience interaction

Lectures and oral presentations will be possible to follow in two congress rooms. The main congress room will accommodate the lecturers, session chairs and 100 participants in F2F mode. The additional congress room will be used to follow the congress in a teleconference mode in a real time frame. Audience interaction will be enabled using Slido platform on personal cell phones.

Poster presentations

Poster presentations will take place in the additional congress room. Posters should be mounted according to the schedule and to the List of posters in the Book of Abstracts. Posters with odd numbers have to be mounted on Thursday 29th September by 9:00 and removed the same day between 19:30 and 20:30. Posters with even numbers have to be mounted on Friday 30th September by 9:00 and removed on Saturday 1st October between 13:00 and 14:00.

Poster panels and mounting accessories will be available at site.

All posters will be evaluated by the Scientific board. Poster Award will be given to the best presenters at the closing ceremony.

Social events

Wednesday, September 28th	19:00	Welcome party (Bluesun Hotel)
Thursday, September 29th	20:00	Quiz night
Friday, September 30th	14:00	*Excursion with Congress dinner

* Excursion will start with the gathering of the participants in front of the Bluesun hotel Soline main entrance. The Excursion buses will leave the meeting point at 14:00. No additional transportation will be available after 14:00 to join the Excursion and the Congress dinner. The Congress dinner will be organized in the frame of the Excursion, as a single social event. The return to the Bluesun hotel Soline will be organized for all participants and scheduled for 00:00.

Programme

Wednesday, September 28, 2022		
17:30	Opening ceremony	
18:00	The HDBMB Award for Contribution to the Development of Molecular Life Sciences	

Chair: Marta Popović

18:15	PL1	**IUBMB Plenary Award Lecture** **Kristijan Ramadan** (Oxford, UK) DNA-PROTEIN CROSSLINKS PROTEOLYSIS REPAIR: FROM HUMAN DISEASE TO CANCER THERAPY
19:00	Welcome party	

Thursday, September 29, 2022

Chairs: Ivana Novak Nakir and Vedrana Filić

9:00	L1	**Janoš Terzić** (Split, Croatia) ROLE OF GUT MICROBIOTA CARCINOGEN METABOLISM IN BLADDER CANCEROGENESIS
9:25	L2	**Andreja Ambriović Ristov** (Zagreb, Croatia) INTEGRIN ADHESION COMPLEXES IN SENSITIVITY OF TUMOUR CELLS TO ANTICANCER DRUGS
9:50	PL2	**Roger Williams** (Cambridge, UK) ACTIVATION OF PHOSPHOINOSITIDE 3-KINASE FAMILY MEMBERS IN HEALTH AND DISEASE
10:35	Poster session – Odd numbered posters Exhibition and refreshment	

Chairs: Maja Herak Bosnar and Ivica Strelec

11:20	L3	**Lada Rumora** (Zagreb, Croatia) INTERPLAY BETWEEN EXTRACELLULAR HSP70 AND NLRP3 INFLAMMASOME IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE
11:45	L4	**Vanda Juranić Lisnić** (Rijeka, Croatia) FROM CMV'S MUST ABUNDANT TRANSCRIPTS TO NOVEL CONCEPTS IN IMMUNE EVASION
12:10	SP1	**Ana Novačić** (Zagreb, Croatia) THE RNA EXOSOME COMPLEX INFLUENCES THE CELLULAR RESPONSE TO THE ANTI-CANCER DRUG 5-FLUOROURACIL IN YEAST
12:25	SP2	**Valentina Ević** (Zagreb, Croatia) BENEFICIAL EFFECT OF MISTRANSLATION ON PROLIFERATION AND VIABILITY OF BACTERIA *Escherichia coli* IN OXIDATIVE STRESS
12:40	SP3	**Uršula Prosenc Zmrzljak** (Labena d.o.o.) ddPCR IN MEDICAL-RESEARCH AND BIOTECHNOLOGICAL APPLICAITONS
12:55	Lunch Break	

Chairs: Jasmina Rokov Plavec and Maja Katalinić

14:15	L5	**Ivana Ivančić Baće** (Zagreb, Croatia) REGULATION OF THE *Escherichia coli* CRISPR-Cas SYSTEM AT TRANSCRIPTIONAL LEVEL AND CHANGE IN PROTEIN CONFORMATION
14:40	L6	**Ana Sunčana Smith** (Zagreb, Croatia) RECEPTOR AND MEMBRANE BIOMECHANICS IN IMMUNE CELL ACTIVITY
15:05	SP4	**Damir Baranašić** (London, UK) DYNAMICS AND LOCUS ORGANIZATION OF ENHANCERS REGULATING KEY FACTORS IN EARLY ZEBRAFISH DEVELOPMENT
15:20	SP5	**Andrea Gelemanović** (Split, Croatia) THE PROPENSITY OF AN ORGAN TO BECOME PRIMARY CANCER OR METASTATIC HOSTS IS ASSOCIATED WITH THEIR GENE EXPRESSION PATTERNS
15:35	SP6	**Domagoj Kifer** (Zagreb, Croatia) EVOLUTIONARY ORIGINS OF THE N-GLYCOSYLATION FROM THE PERSPECTIVE OF *HOMO SAPIENS* GENOME
15:50	SP7	**Marleen J. Meyer** (Greifswald, Germany) WHAT CAN WE LEARN ABOUT THE MECHANISMS OF OCT1 POLYSPECIFICITY USING INTERSPECIES COMPARISONS?
16:05	SP8	**Željka Pezer** (Zagreb, Croatia) GENOMIC STRUCTURAL VARIATION IN SPERM CELLS: INSIGHTS FROM OPTICAL MAPPING DATA
16:20	Poster session - – Odd numbered posters Exhibition and refreshment	

Chairs: Ljubica Glavaš Obrovac and Zrinka Kovarik

17:00	L7	**Maja Herak Bosnar** (Zagreb, Croatia) NME6-AN UNUSUAL NUCLEOSIDE DIPHOSPHATE KINASE INVOLVED IN BASIC MITOCHONDRIAL FUNCTIONS
17:25	L8	**Mirta Boban** (Zagreb, Croatia) DEGRADATION-MEDIATED PROTEIN QUALITY CONTROL BY THE UBIQUITIN-PROTEASOME SYSTEM
17:50	SP9	**Mija Marinković** (Split, Croatia) *HDBMB Young Scientist Award 2021* INTERPLAY BETWEEN C TERMINAL BNIP3L/NIX PHOSPHORYLATION AND DIMERIZATION AS A NOVEL MECHANISM OF RECEPTOR-MEDIATED MITOPHAGY REGULATION
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18:35	SP12	**Davor Šakić** (Zagreb, Croatia) MAKING RINGS FROM AMINO ACIDS. SHINING LIGHT ON POSSIBLE (CHEMO)EVOLUTION OF PROLINE
20:00	Quiz night	

Friday, September 30, 2022

Chairs: Antonija Jurak Begonja and Damjan Franjević

9:00	L9	**Ira Milošević** (Oxford, UK) TOWARDS IMPROVING ORGANELLAR ACIDIFICATION AND SYNAPTIC VESICLE (RE)FILLING
9:25	L10	**Nicholas Bradshaw** (Rijeka, Croatia) PROTEIN AGGREGATION IN SCHIZOPHRENIA AND OTHER CHRONIC MENTAL ILLNESSES
9:50	PL3	**William F. Martin** (Düsseldorf, Germany) HYDROTHERMAL VENTS HYDROGEN AND TRANSITION METALS: SYNTHESIS TOWARDS LIFE
10:35	Poster session – Even numbered posters Exhibition and refreshment	

Chairs: Tihomir Balog and Nicholas Bradshaw

11:20	L11	**Vanja Nagy** (Vienna, Austria) DISCOVERING NOVEL RECEPTORS FOR THE EXTRACELLULAR MATRIX THROUGH UNDIAGNOSED MONOGENIC NEUROLOGICAL DISORDERS
11:45	SP13	**Kristina Mlinac Jerković** (Zagreb, Croatia) THE EFFECT OF GANGLIOSIDES ON MEMBRANE ION TRANSPORT IN MOUSE BRAIN
12:00	SP14	**Teuta Opačak-Bernardi** (Osijek, Croatia) COMPOSOTION OF SPHEROIDS FORMED BY MAGNETIC LEVITATION
12:15	SP15	**Martina Šeruga Music** (Zagreb, Croatia) QUEST FOR PUTATIVE EFFECTORS OF 'CANDIDATUS PHYTOPLASMA SOLANI': TOWARDS UNDERSTANDING OF A SUCCESSFULL AND ADAPTABLE PATHOGEN
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12:45	Lunch Break	
13:50	Excursion with Congress dinner	

Saturday, October 1, 2022		
Chair: Jerka Dumić		
9:00	PL4	**FEBS National Lecture** **Irene Diaz Moreno** (Sevilla, Spain) CYTOCHROME C AS A CORNERSTONE IN THE NUCLEUS-MITOCHONDRIA CROSSTALK
9:45	Poster session – Even numbered posters Exhibition and refreshment	
Chairs: Nino Slnčić and Igor Stuparević		
10:25	PL5	**Luciane Vieira de Mello** (Liverpool, UK) EDUCATIONAL RESEARCH AND SCIENTIFIC TRAINING
11:05	L12	**Ferhan Sagin** (Ismir, Turkey) EDUCATION ANAD RESEARCH – NEW PERSPECTIVES
11:30	L13	**Boris Jokić** (Zagreb, Croatia) WHAT TO DO NEXT? – A NEW VISION FOR EDUCATION IN A TIME OF INSTABILITY AND UNCERTAINTY
11:55	panel	**Luciane Vieira de Mello**, **Ferhan Sagin**, **Boris Jokić** Panel and discussion on education and science
12:35	Closing ceremony and the Best Poster Award announcement sponsored by *FEBS Open Bio*	

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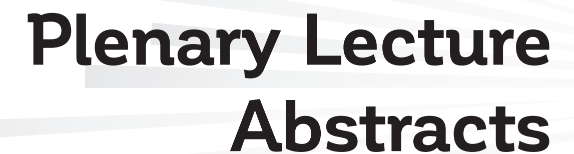

Plenary Lecture Abstracts

PL1
DNA-PROTEIN CROSSLINKS PROTEOLYSIS REPAIR: FROM HUMAN DISEASE TO CANCER THERAPY

Kristijan Ramadan
Medical Research Council Oxford Institute for Radiation Oncology, Department of Oncology, University of Oxford, UK

DNA-protein crosslinks (DPCs) and DPC-like lesions (e.g., trapped PARP1) are specific type of DNA lesions that constitute a major threat to genome stability. DPCs are constantly formed in cells either by endogenous metabolic products, e.g., formaldehyde or exogenous toxins and chemotherapeutics such as alcohol and topoisomerase inhibitors, respectively. Despite the essential importance of DPCs for genome stability and chemotherapy, the mechanisms by which DPCs are repaired are poorly understood. To study the mechanisms of DPC repair and their relevance for cancer therapy, we focus on (a) the SPRTN protease, the main enzyme for DPC repair in mammalian cells, and (b) chemotherapeutic drugs that induce DPCs or DPC-like lesions such as topoisomerase and PARP inhibitors. Here, I will discuss a recently discovered DPC proteolysis repair pathway and how this knowledge can be used to improve the current cancer therapy based on DPC-inducing drugs.

PL2
ACTIVATION OF PHOSPHOINOSITIDE 3-KINASE FAMILY MEMBERS IN HEALTH AND DISEASE

Glenn R Masson, Madhan Anandapadamanaban, Shirley Tremel, Yohei Ohashi, Olga Perišić, Maren Heimhalt, Domagoj Baretić, Alex Berndt and Roger Williams
Medical Research Council, Laboratory of Molecular Biology, Cambridge, UK

Anabolic and catabolic pathways are tuned to meet the demands of changing environments. Members of the phosphatidylinositol 3-kinase (PI3K) superfamily of enzymes are present in all eukaryotes and have closely-related catalytic domains. These enzymes regulate responses to nutrition, growth factors and stress. Activities of the enzyme complexes depend on both their residence time on membranes and the efficiency with which they form the transition state complex once they are bound to membranes. We have used hydrogen/deuterium exchange mass spectrometry (HDX-MS) and electron cryo-microscopy (cryo-EM) to gain insight into mechanisms of activation of PI3Ks and mTOR by G-proteins, membrane interactions, cancer-associated mutations and small-molecule modulators. Activators commonly increase flexibility of the enzyme complexes and release auto-inhibitory interactions.

PL3
HYDROTHERMAL VENTS HYDROGEN AND TRANSITION METALS: SYNTHESIS TOWARDS LIFE

William F. Martin
Institute of Molecular Evolution, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany

Though all theories for the origin of life require a source of energy to promote primordial chemical reactions, the nature of energy that drove the emergence of metabolism at origins is still debated. Evidence for the nature of energy at origins should be preserved in the biochemical reactions of life itself, and changes in free energy, ΔG, which determine whether a reaction can go forward or not, should help specify the source. Calculating values of ΔG across the conserved and universal core of 402 individual reactions that synthesize amino acids, nucleotides and cofactors from H_2, CO_2, NH_3, H_2S and phosphate in modern cells, reveals that 95–97% of these reactions are exergonic ($\Delta G^{\circ\prime} < 0$ kJ·mol^{-1}) at pH 7-10 and 80-100 °C under nonequilibrium conditions with H_2 replacing biochemical reductants. While 23% of the core's reactions involve ATP hydrolysis, 77% are ATP-independent, thermodynamically driven by ΔG of reactions involving carbon bonds. We identified 174 reactions that are exergonic by −20 to −300 kJ·mol^{-1} at pH 9 and 80 °C and that fall into ten reaction types: six pterin dependent alkyl or acyl transfers, ten S-adenosylmethionine dependent alkyl transfers, four acyl phosphate hydrolyses, 14 thioester hydrolyses, 30 decarboxylations, 35 ring closure reactions, 31 aromatic ring formations, and 44 carbon reductions by reduced nicotinamide, flavins, ferredoxin, or formate. The 402 reactions of the biosynthetic core trace to the last universal common ancestor (LUCA), and reveal that synthesis of LUCA's chemical constituents required no external energy inputs such as electric discharge, UV-light or phosphide minerals. The biosynthetic reactions of LUCA uncover a natural thermodynamic tendency of metabolism to unfold from energy released by reactions of H_2, CO_2, NH_3, H_2S, and phosphate.

PL4
CYTOCHROME *C* AS A CORNERSTONE IN THE NUCLEUS-MITOCHONDRIA CROSSTALK

Irene Díaz-Moreno
Institute for Chemical Research, cicCartuja, University of Seville – CSIC, Spain

Over the past decade, evidence has emerged suggesting a much broader role for cytochrome *c* in the transition of apoptotic cells from life to death.
In the lecture, I will show novel mechanistic insights into electron transfer (ET) from cytochrome c_1 to cytochrome *c*, including gated, long-range ET in aqueous solution. Remarkably, a close contact between cytochrome c_1 and cytochrome *c* is not essential for ET: when proteins are approaching each other, cation exclusion occurs between their active sites, enabling the building of a Gouy-Chapman charge conduit and the long-distance ET through the aqueous solution. Phosphorylation of cytochrome *c* not only affects its structure and dynamics, but also shortens the long-distance charge conduit between the partners, strengthens their interaction, and departs it from equilibrium. In response to DNA damage, cytochrome *c* escapes from its natural mitochondrial environment and, once in the cytoplasm, binds to Apaf-1 to form a complex—the so-called apoptosome—that triggers caspase activation and further leads to controlled cell dismantlement. Recent work from our group shows that cytochrome *c* in the cytoplasm also binds to the chaperone 14-3-3ε, which is an inhibitor of Apaf-1, to block 14-3-3ε-mediated Apaf-1 inhibition, thereby unveiling a novel function for cytochrome *c* as an indirect activator of caspase-9/3. Besides such key apoptotic roles of cytochrome *c* in the cytoplasm, its migration to the nucleus soon after DNA damage—even before caspase cascade activation and apoptosome formation in the cytoplasm—has recently been an exciting discovery. Cytochrome *c* in the nucleus actually targets a variety of well-known histone chaperones involved in chromatin remodeling and DNA damage response. Our results show that nuclear/nucleolar cytochrome *c* inhibits the nucleosome (dis)assembly activity of histone chaperones, impairs dephosphorylation events and controls p53-mediated cell cycle arrest during the repair of injured DNA. Histone chaperones do interact with cytochrome *c* lysine residues through their acidic disordered regions, which are involved in the heterotypic contacts leading to liquid-liquid phase transitions and are responsible for the assembly of nuclear condensates, including heterochromatin. Altogether, our recent data demonstrate that cytochrome *c* functions as a master, pleiotropic organellar factor, thereby playing a crucial global role in cell metabolism, both in life and death.

PL5
EDUCATIONAL RESEARCH AND SCIENTIFIC TEACHING

Luciane Vieira de Mello
School of Life Sciences, University of Liverpool, UK

In our lives as scientists, we approach research with rigour and we are constantly asking questions and self-critically evaluating what we are doing. Our active mind is essential to the development of science. Scientific teaching should involve teaching strategies / approaches that actively engage students with their learning and with scientific processes. In one way or another, we are all involved in teaching, from delivering lectures to supervising students. If the goal is to train future scientists, teaching should involve the same rigour of enquiry and evaluation that we apply to our research. Therefore, those who teach should engage with educational research (by doing it or reading about the results) with the intention of improving educational practice or policy. I invite you to discuss the claim that evidence should support teaching practice, just as evidence supports research practice.

Lecture Abstracts

L1
ROLE OF GUT MICROBIOTA CARCINOGEN METABOLISM IN BLADDER CANCEROGENESIS

Janoš Terzić
University of Split School of Medicine, Split, Croatia

The main risk factor for bladder cancer is exposure to cigarette smoke toxins, but exposure to aromatic amines (e.g. in paint and rubber industry) and arsenic in the drinking water can also promote bladder cancer development. Microbiota is of particular interest in bladder cancer since this cancer type can develop due to chronic infection with *Schistosoma haematobium*. Furthermore, the most common therapeutic intervention is an intentional infection of the urinary bladder with *Bacillus Calmette-Guerin*. Recent studies, including our own, demonstrated that urine from healthy persons is not sterile and that urinary microbiota differs between bladder cancer patients and healthy individuals. Known mechanisms of microbiota-mediated carcinogenesis include bacterial toxin production, promotion of chronic inflammation, activation of signaling pathways, and tumor cell proliferation. We investigated the role of the gut microbiota in bladder cancer development using a nitrosamine-induced bladder cancer mouse model. Microbiota depletion reduced the bladder cancer burden, which we could link to gut microbial metabolism affecting the toxicity and tissue distribution of nitrosamine. To investigate the translational potential of our findings, we tested microbial communities and isolates from gut, lung, and oral cavities from human donors and confirmed that microbial nitrosamine metabolism varies between individuals. Our data suggest that gut microbial metabolism is an important element of bladder carcinogenesis. This could give rise to new opportunities for bladder cancer risk assessment and possible preventive and/or therapeutic measures based on the patient's microbiome profile.

Lecture Abstracts

L2
INTEGRIN ADHESION COMPLEXES IN SENSITIVITY OF TUMOUR CELLS TO ANTICANCER DRUGS

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Integrins are the main cellular adhesion receptors used by cells to bind to the extracellular matrix (ECM). Integrin expression is frequently altered in tumours in which they regulate cell proliferation, migration and survival. Upon integrin activation and binding to the ECM, multimolecular integrin adhesion complexes (IACs) are formed, composed of a complex array of signalling, scaffolding and cytoskeletal proteins. To identify changes in IACs involved in sensitivity to chemotherapeutic drugs, we isolated IACs from long term culture of two tumour cell models consisting of a parental cell line and clones with altered integrin expression showing different sensitivity to chemotherapeutic drugs. In the first model the melanoma cell line MDA-MB-435S upon knockdown of integrin αV show increased sensitivity to microtubule (MT) poisons, paclitaxel and vincristine and decreased cell migration and invasion. Mass spectrometry (MS)-based proteomics showed that cells preferentially use integrin αVβ5 for adhesion and identified key components of integrin αVβ5 IACs organised as focal adhesions. Among the molecules whose quantity decreased after αV knockdown was KANK2. Its knockdown in MDA-MB-435S cells mimicked the effect of integrin αV knockdown and resulted in increased sensitivity to MT poisons and decreased migration. We concluded that KANK2 is a key molecule linking integrin αVβ5 IACs to MTs, enabling the actin-MT crosstalk important for both sensitivity to MT poisons and cell migration. In the second model the tongue squamous cell carcinoma Cal27 cells with de novo expression of integrin αVβ3 showed resistance to cisplatin, mitomycin C and doxorubicin and increased cell migration and invasion compared to Cal27 cells. MS-based proteomics analysis of these cells indicated that both cell lines preferentially use integrin α6β4 for adhesion, organised as hemidesmosomes type II. However, integrin αVβ3 expressing Cal27 cells demonstrated an increased level of α6β4 compared to Cal27. Furthermore, suppression of α6β4 expression in both lines conferred resistance to all chemotherapeutic drugs through a mechanism independent of αVβ3, identifying a role for α6β4-containing type II hemidesmosomes in regulating drug sensitivity. Taken together, our results illustrate the complexity of integrins and their adaptable and sometimes antagonistic roles in cancer cells. Since therapeutic targeting of these receptors is a challenge, the analysis of IACs profiles is a potential way forward.

L3
INTERPLAY BETWEEN EXTRACELLULAR HSP70 AND NLRP3 INFLAMMASOME IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Chronic obstructive respiratory disease (COPD) is a heterogeneous and complex disease characterized by airway obstruction and inflammation. Pathophysiology of COPD is associated with a long-term exposure to noxious particles and gases, particularly to cigarette smoke. Increasing evidence points to a key role of the innate immune system with its pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), in COPD. PRRs are capable of sensing different pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). Under physiological conditions, DAMPs, such as adenosine triphosphate (ATP) or heat shock protein 70 (Hsp70), are mostly present intracellularly and are therefore hidden from recognition by the host immune system. However, under conditions of cellular stress or injury, these molecules are increasingly released into the extracellular environment and trigger sterile inflammation. PAMPs and DAMPs might be also recognized by intracellular PRRs, such as nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3). The NLRP3 inflammasome consists of a sensor (NLRP3), an adaptor (apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (ASC)) and an effector (caspase-1). Upon its activation, autoactivation of caspase-1 is triggered, thus promoting the maturation and secretion of interleukin (IL)-1β and IL-18. It has been shown that concentrations of extracellular Hsp70 (eHsp70) are increased in plasma of COPD patients. However, the mechanism underlying its pro-inflammatory effects in COPD is still unclear.

In order to explore a possible interplay between eHsp70 and NLRP3 inflammasome in COPD we detected NLRP3 and IL-1β mRNA expression, and also ATP and IL-1β concentrations in supernatants of various cell lines and their primary cells counterparts after treatment with recombinant human (rh) Hsp70 protein and/or cigarette smoke extract (CSE). We also measured aforementioned parameters in blood samples of COPD patients in stable phase and age- and sex-matched healthy controls. Our findings suggested a possible involvement of eHsp70-induced activation of NLRP3 inflammasome in pathophysiology of COPD.

L4
FROM CMV'S MUST ABUNDANT TRANSCRIPTS TO NOVEL CONCEPTS IN IMMUNE EVASION

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Human cytomegalovirus (CMV) is a widespread herpesvirus, infecting majority of human population. Like all herpesviruses, it causes life-long persistent infections punctuated by occasional reactivations, usually following transient immunosuppression. As such, although CMV is usually well controlled by immunologically sufficient individuals, it may cause grave disease and even death in immunologically suppressed or immature patients such are transplant patients (major cause of death and organ loss) or newborns and fetuses (major cause of congenital developmental disabilities). Also, like all herpesviruses, it likely achieves its success and persistence due to it's large genome teeming with genes targeting nearly every aspect of our immune response. Human cytomegalovirus is strictly species specific and cannot infect experimental animals. Luckily, related murine CMV (MCMV) is not only genetically similar to human CMV; but also shares disease manifestations and pathology. We were among the first to perform transcriptional analysis of CMV's genome and identified several, until then unknown and highly abundant transcripts. Among those, the most interesting was MAT (for MOST abundant transcript) which was the first CMV's transcript demonstrated to encode two proteins and also serves as a sponge for cellular miRNA. We have further characterized MATp1 protein that fine-tunes surface levels of MHC I molecules in order to evade natural killer cells and CD8 T cells.

L5
REGULATION OF THE *Escherichia coli* CRISPR-Cas SYSTEM AT TRANSCRIPTIONAL LEVEL AND CHANGE IN PROTEIN CONFORMATION

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Many bacteria and most archaea use CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats; CRISPR-associated) systems to fight against invading DNA elements such as plasmids and phages. However, the expression of CRISPR-Cas components in *E. coli* is strongly silenced by the repressing activity of the histone-like nucleoid-structuring protein (H-NS). Due to repression by H-NS, the CRISPR-Cas system in *E. coli* does not provide protection against phage infection in *wt* cells. We will show that the H-NS paralog protein StpA is another protein involved in repression of the CRISPR-Cas system in the absence of H-NS protein. The activity of the CRISPR-Cas system is further regulated at the protein level, by regulation of the Cas3 nuclease activity. The Cas3 protein is a nuclease-helicase that cleaves single-stranded (ss) DNA and is essential in the final step of CRISPR immunity. From genetic studies we had noted that the efficacy of Cas3 in protection of bacteria against phage infection was greatly reduced when temperature was increased from 30 °C to 37 °C by unknown mechanism. We investigated the purified *E. coli* Cas3 protein and found that inhibition of the nuclease activity at higher temperature corresponds with measurable changes in protein structure and plaque numbers. We found that the change in a single highly conserved tryptophan residue (Trp406) into an alanine, changed the activity of the Cas3W406A protein. Trp406 is situated at the interface of Cas3 HD and RecA1 domains that is important for manoeuvring DNA into the nuclease active site. Molecular dynamics simulations showed temperature induced changes in Trp406 positioning that either blocked or cleared the ssDNA pathway. These results indicate that allosteric change in Cas3 structure by temperature may control the Cas3 nuclease activity.

L6
RECEPTOR AND MEMBRANE BIOMECHANICS IN IMMUNE CELL ACTIVITY

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Inhibitory receptors of T cells and natural killer (NK) cells sustain self-tolerance of the immune system and prevent collateral damage during a physiological immune response. Upon binding to their ligands expressed by target cells, these receptors initiate signaling cascades that target signaling pathways of activating and costimulatory receptors, suppressing the activation of T cells and NK cells. It has long been hypothesized that the biological function of activating and inhibitory receptors, besides on chemical recognition, depends on their biomechanical characteristics such as length, flexibility and spatial arrangement. Especially the physical proximity of activating and inhibitory receptors has been considered essential for the integration of their signals. Yet, quantitative measurements of cell activation as a function of the mentioned biomechanical parameters are challenging and largely missing. Similarly, mathematical models accounting for the biomechanics of the adherent cells are scarce and insufficient to predict or rationalize the potential dependencies. Therefore, it remains difficult to clearly relate the spatial organization of inhibitory and activating receptors, the length, the flexibility and the stability of their bonds, and the properties of the surrounding membrane with the cell performance. In this talk, I will present a synergistic experimental and modeling approach which aims at clearly identifying the role of ligand-receptor biomechanics and the effect of spatial organization and patterning of bonds in the membrane on T and NK cell activation.

L7
NME6 - AN UNUSUAL NUCLEOSIDE DIPHOSPHATE KINASE INVOLVED IN BASIC MITOCHONDRIAL FUNCTIONS

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NME6 is a member of the nucleoside diphosphate kinase (NDPK) family of enzymes with crucial roles in controlling the cellular nucleotide homeostasis, as well as cell signaling, membrane remodeling and metastasis suppression, therefore, having a major impact on basic cellular functions and human diseases. The well characterized NME1-4 proteins are catalytically active - they transfer the terminal phosphate from d(NTP)s to d(NDP)s or proteins through a high-energy phospho-histidine intermediate. On the contrary, the data on other NME proteins, including NME6, are scarce and inconsistent. In our work we aimed to clarify and extend the overall knowledge on the human NME6 which brought us to unexpected discoveries. We have found NME6 to be present in the cell in two different isoforms. The recombinant NME6 protein is largely monomeric, and consequently, it does not exhibit the NDPK activity. Immunofluorescence and cell fractioning procedures revealed that NME6 resides in the mitochondrial matrix, probably adjacent to the mitochondrial inner membrane. Overexpression of NME6 had negative influence on oxidative phosphorylation but did not alter mitochondrial potential, mass or network characteristics. A screen for NME6 protein partners revealed its interaction with RCC1L, a protein involved in mitoribosomal assembly and translation. Therefore, our findings suggest a completely novel function for one of the NME/NDPK family members, independent of their classical phophotransfer activity.

L8
DEGRADATION-MEDIATED PROTEIN QUALITY CONTROL BY THE UBIQUITIN-PROTEASOME SYSTEM

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Maintaining a functional proteome is one of the major tasks of the cell. Increased levels of protein aggregates are associated with aging and neurodegeneration, such as in Alzheimer's, Parkinson's diseases and other. A key to understanding the cause of the disease is elucidating how a functional cell deals with the problem of protein misfolding and aggregation. To prevent accumulation of aberrant proteins, cells possess an intricate network of protein quality control pathways, including the ubiquitin-proteasome system for the selective degradation of terminally misfolded proteins. Many cells, such as neurons or quiescent microorganisms, exist in a non-dividing state. In contrast to proliferating cells that can get rid of protein aggregates by asymmetric segregation in cell divisions, non-dividing cells lack this possibility. In this work we address the specificities of protein quality control in non-dividing cells using a unicellular eukaryote, yeast *Saccharomyces cerevisiae* as a model. In nature, yeast spend most of their lifetime in a non-proliferative, quiescent state that is induced by nutrient starvation. Intriguingly, in glucose-starved cells, proteasomes re-localize from the nucleus into the cytoplasmic granules, and it is unclear whether these proteasomes are active. To study how glucose-starved yeast cells deal with misfolded proteins, we express model misfolded proteins and examine their stability in a set of mutants for specific protein quality control pathways. Our results indicate that distinct pathways are involved in degradation of misfolded proteins in proliferating and quiescent cells. We believe that elucidating the mechanisms of protein quality control in quiescent yeast will contribute to understanding how non-dividing mammalian cells, such as neurons, protect the cell against accumulation of misfolded proteins, thereby preventing pathological protein aggregation.

L9
TOWARDS IMPROVING ORGANELLAR ACIDIFICATION AND SYNAPTIC VESICLE (RE)FILLING

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Membrane trafficking at the synapse is among the most complex, rapid and tightly regulated processes in cell biology. To fully comprehend how neurons communicate with each other, the detailed understanding of how synaptic vesicles (SVs) are formed, acidified, filled with neurotransmitters and 'matured' to attain fusion-competence is needed. The investigation of the SV trafficking beyond exocytosis and endocytosis has been challenging due to lack of technology required to trace SVs during their whole cycle. We have recently developed methodology to isolate SVs and their recycling intermediates from neurons of mice with early lethality and combined it with new molecular probes to measure changes in membrane potential and organellar pH. Despite its critical importance at the synapse and intracellular trafficking, the regulation of proton-pumping vacuolar ATPase (v-ATPase) that acidifies SVs remains poorly understood. We have cloned *DMXL2* gene that encodes human Rabconnectin-3a, an essential, synapse-enriched 340 kDa protein linked to neurodevelopment, mental retardation, polyendocrine-polyneuropathy syndrome (PEPNS), Ohtahara syndrome and hearing loss. We found that Rabconnectin-3a stabilizes the expression and activity of v-ATPase, and that it is present on all vesicles/organelles that acidify. Mice without Rabconnectin-3a showed early embryonic lethality. Neurons without Rabconnectin-3a developed and made synapses in culture, but showed severely impaired neuronal activity, altered SV recycling, failed to acidify SVs, and accumulated lysosome-like structures at the synapse and in the axons. These and other roles of Rabconnectin-3a in membrane trafficking will be discussed. In addition, two other new ways of mammalian v-ATPase activity regulation will be proposed.

L10
PROTEIN AGGREGATION IN SCHIZOPHRENIA AND OTHER CHRONIC MENTAL ILLNESSES

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Major mental illnesses, such as schizophrenia, are devastating and often chronic conditions, which together form the largest cause of disability worldwide. This problem is made significantly worse by the lack of biological tests available to diagnose them, and the comparatively small range of effective medications to treat them. It is therefore vital that we gain a greater understanding of what the biological events are that underlie these conditions. The genetic background to these conditions has been thoroughly investigated, but has proven to be highly complex, with few obvious targets for new drugs or therapies. We, and our collaboration partners, have therefore been attempting to complement this approach by also studying proteins involved in major mental illness. Specifically, we have been investigating the existence of misfolded protein aggregates in these disorders, comparable to similar neurotoxic aggregates in neurodegenerative diseases such as Alzheimer's or Parkinson's disease. Several proteins have now been identified that appear to specifically form aggregates in the brains of patients with schizophrenia, bipolar disorder and/or depression. These are all brain-expressed proteins that, when functioning normally, are involved in either synaptic function and/or neurodevelopment. In many instances, one isoform or variant of the protein readily forms aggregates in cell culture and/or animal models, while others do not. Many of these proteins can "co-aggregate" with each other in the same patients, and when studied in cell culture. Specific structural regions have been identified in many of these proteins that are essential for their aggregation. Work is now ongoing to determine how aggregation of these proteins occurs, and what their consequences are for the normal function of neurons and the brain. While still at a comparatively early stage, the study of protein aggregation in mental illness is showing promise. Studies in larger brain collections, as well as further biochemical characterisation of the proteins, are now required to determine their ultimate relevance for diagnosis and/or treatment of major mental illness.

L11
DISCOVERING NOVEL RECEPTORS FOR THE EXTRACELLULAR MATRIX THROUGH UNDIAGNOSED MONOGENIC NEUROLOGICAL DISORDERS

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The negatively charged, growth non-permissive extracellular matrix (ECM) makes up 25% of brain volume. The tightly regulated biophysical properties and its function during development and adulthood are poorly understood. Whole exome sequencing of two patients with idiopathic complex neurodevelopmental disorder (NDD) identified biallelic variants of unknown significance within FIBCD1, encoding an endocytic acetyl-group binding transmembrane receptor with no known function in the central nervous system. We found that FIBCD1 preferentially binds and endocytoses glycosaminoglycan (GAG) chondroitin sulphate-4S (CS-4S) and regulates GAG content of the brain ECM. In silico molecular simulation studies and GAG binding analyses of patient variants determined that such variants are loss-of-function by disrupting FIBCD1-CS-4S association. Gene knockdown in flies resulted in morphological disruption of the neuromuscular junction and motor-related behavioural deficits. In humans and mice, FIBCD1 is expressed in discrete brain regions, including hippocampus. Fibcd1 KO mice exhibited relatively normal hippocampal neuronal morphology but impaired hippocampal-dependent learning. Further, hippocampal synaptic remodelling in acute slices from Fibcd1 KO mice was deficient but restored upon enzymatically modulating the ECM. Together, we identified FIBCD1 as an endocytic receptor for GAGs in the brain ECM and a novel gene associated with an NDD, revealing a critical role in nervous system structure, function and plasticity. This study highlights the utility of rare disease patient-led studies in elucidating the evolutionary conserved roles of genes in human physiology and identification of potential therapeutic targets for a wide variety of more common diseases beneficial to a larger number of patients. Additionally, it underscores the important role of the extracellular matrix in the development and functioning of the brain.

L12
EDUCATION AND RESEARCH - NEW PERSPECTIVES

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Future teachers are those whom you see as your best students, whom you dream will get a PhD and then do what? Teach. And they are those who are going to go into business and industry and will spend a great deal of their time mentoring other people in their work places as teachers; they too, are in the midst of a teaching environment. If we don't meet this challenge of taking the pedagogy seriously, I fear that fifty years from now people will look back on our era as the period in the late 1980s and early 1990s when we had the opportunity in less than a decade to educate two-thirds of the teachers who would teach for the next thirty-five years, the period when we had this extraordinary opportunity to make a difference in education.' Lee S. Shulman (1989 National Conference of the American Association of Higher Education). PhD graduates of today compete for the rarest and most competitive jobs in academia, a job market which is shrinking in many countries, specially after COVID-19. Yet most of the training in a PhD emphasizes research, above all, and the curriculums are designed according to the professional needs for a job at a research university. The educators' role is to unlock the PhD students' creativity by broadening their vision while students' responsibility is to nurture both practically and intellectually on the career pathway by beginning with the end in mind. This can only take place if PhD training integrates other skills into the curriculum that students will need in different career paths afterwards. Thus, now is the time for educators' and students' to rethink career-diverse PhD's and to readjust mind-sets for shaping one's career also as an educator. PhD students are highly resourceful people; however, they may not be aware of the need to nurture their skills beyond research. Thus, as educators we should guide them in readjusting their mind-sets to recognize the different career pathways and equip themselves on their way to graduation. This talk is aimed to raise some awareness in young researchers and also in senior educators about the ultimate need to get/give/practice the best education content/skills to top, with the hope that participants will continue to follow this interesting area besides their research field.

L13
WHAT TO DO NEXT? – A NEW VISION FOR EDUCATION IN A TIME OF INSTABILITY AND UNCERTAINTY

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The COVID-19 pandemic, climate change and geopolitical relations are currently major global challenges, affecting education and the personal and social development of pupils and students at different educational levels around the world. The negative effects of the pandemic on educational processes were manifested in significant knowledge and skills gaps, loss of work habits and motivation for learning, and disruptions in the aspirations of young people. These negative effects of the pandemic further intensified the already present loss of relevance of formal education in the lives of children and young people. Furthermore, the pandemic and climate change have exacerbated existing inequalities between and within education systems. The education systems of low- and lower-middle-income countries experienced a greater negative impact. In all systems, learning opportunities and experiences for gifted students, those at risk or with special educational needs have changed significantly. However, the responses of educational authorities, institutions and practitioners resulted in many positive elements, opening up space for innovation and creativity. Furthermore, the situation revealed a much-needed flexibility in educational structures, a feature not often associated with such robust and inert systems. Along with the presentation of empirical results on the effects of the pandemic on students, this lecture will present a vision of how to restore the importance of education for pupils, students, teachers, professors and society as a whole. To achieve this vision, significant changes in educational structures, approaches to teaching and learning, and assessment and evaluation practices are essential. In this vision, it is important to strongly position science, including biochemistry, from the early years, as well as systematic efforts aimed at making science understandable and interesting to all children and young people. Equally important is rigorous research related to education.

Short Presentation Abstracts

SP1
THE RNA EXOSOME COMPLEX INFLUENCES THE CELLULAR RESPONSE TO THE ANTI-CANCER DRUG 5-FLUOROURACIL IN YEAST

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5-fluorouracil (5-FU) is a widely used drug for the treatment of colon and breast cancer. It is an anti-metabolite that is incorporated into DNA and RNA molecules and interferes with various cellular processes. Cancer cells can become resistant to 5-FU, which can be a major obstacle to 5-FU-based therapies, especially since the molecular mechanisms underlying such resistance remain poorly understood. The RNA-based toxicity of 5-FU is thought to be related to the RNA exosome, a highly conserved exoribonuclease complex that degrades and processes practically all classes of RNAs in eukaryotic cells. Previous studies have shown that the depletion of Rrp6/EXOSC10 catalytic subunit of the RNA exosome results in pronounced sensitivity to 5-FU in yeast *Saccharomyces cerevisiae* and human cells. In this work, we sought to gain more insight into the involvement of different RNA exosome subunits and cofactors in the cellular response to 5-FU in yeast. We demonstrated that the Dis3 catalytic subunit, as well as certain nuclear-specific RNA exosome cofactors, are required to achieve physiological level of growth upon 5-FU treatment. Surprisingly, inactivation of certain RNA exosome cofactors increased the resistance of cells to treatment with 5-FU compared with wild-type cells, suggesting possible compensatory mechanisms that might confer resistance to such mutant cells. Given the high degree of conservation of the RNA exosome complex and its cofactors from yeast to human, these results contribute to elucidation of the molecular mechanisms of 5-FU resistance which may be of general importance.

SP2
BENEFICIAL EFFECT OF MISTRANSLATION ON PROLIFERATION AND VIABILITY OF BACTERIA *Escherichia coli* IN OXIDATIVE STRESS

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Reactive oxygen species (ROS) are byproduct of bacterial aerobic metabolism, however, if the metabolism is disrupted, amount of ROS increases beyond cell's capacity which causes oxidative stress. Additionally, oxidative stress can be caused by other bacteria that produce and excrete H_2O_2 or by phagocytes that produce ROS as a defence mechanism against pathogenic bacteria. Interestingly, it was shown in some cases that one stress can preadapt cells to other stress by activating stress response. Here, we investigated the effect of specific mistranslation of isoleucine with structurally similar amino acids valine (Val) and norvaline (Nva), on bacterial response to oxidative stress. We used *Escherichia coli* strain expressing mutant variant of isoleucyl-tRNA synthetase with inactivated editing domain which is more prone to mistranslation compared to wild type. Variable mistranslation rates were induced by different concentrations of Val or Nva added in the medium and oxidative stress was induced by adding H_2O_2. We observed reduced growth rate of bacteria in the media supplemented with Val or Nva and prolonged lag phase in the media supplemented with H_2O_2. However, prior exposure to mistranslation stress shortened the lag phase in oxidative stress after which bacteria continued to grow normally. Survival assays resulted in low survival rate of nonmistranslated culture in oxidative stress, while survival rates of cultures preexposed to mistranslation stress were higher. Using light microscopy, we observed filamentation of bacteria. Under transmission electron microscope, bacterial cells grown with 1 mM Val or Nva exhibited profound morphological and ultrastructural changes, such as numerous intracellular vesicles. More pronounced filamentation of bacteria grown with higher Val or Nva concentrations implies stronger stress response activation which correlates with better proliferation and viability. The results suggest that mistranslation, specifically, mistranslation of Ile by Val and Nva, induces cellular response that increases bacterial tolerance to oxidative stress. The further research in proteomics is planned to elucidate cellular mechanisms of adaptative response induced by mistranslation.

SP3
ddPCR IN MEDICAL-RESEARCH AND BIOTECHNOLOGICAL APPLICAITONS

Uršula Prosenc Zmrzljak, Rok Košir, Saša Šterpin
Labena d.o.o.

SP4
DYNAMICS AND LOCUS ORGANIZATION OF ENHANCERS REGULATING KEY FACTORS IN EARLY ZEBRAFISH DEVELOPMENT

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Numerous long-range enhancers, which often overlap with highly Conserved Non-coding Elements (HCNEs) within genomic regulatory blocks (GRBs), control the expression of key genes regulating development. GRBs often contain other “bystander” genes that do not respond to those enhancers. The extent of GRBs coincides with those of topologically associating domains (TADs) around developmental genes. However, the exact influence of GRB spatial organization on gene regulation in the early stages of development is still poorly understood, which substantially limits our understanding of processes leading to differentiation and cell fate decision. To answer those questions, we exploited early zebrafish developmental datasets to characterize chromatin opening and interaction topology in those poorly understood loci and their regulatory role in TADs. In GRB TADs, characterized by a high density of extreme non-coding conservation, we found more promoter-proximal enhancers in early and distal enhancers in late developmental stages. We observed that enhancers in the late stages are numerous, short, and distributed throughout the entire TAD length. In contrast, fewer enhancers were active at the early stage, and they often occurred in clusters with uninterrupted H3K27ac signal connecting them, a histone mark found on active chromatin. We called those regions H3K27ac ensembles. We hypothesized that they might be associated with the lack of fully formed TADs in the early stages when enhancers are proximal to active promoters. We also investigated the relationship between the chromatin interactions and activity of H3K27ac-ensemble-associated genes during early vs late embryogenesis. We observed increased contacts within H3K27ac ensembles at an early stage which later spread throughout the entire TAD, arguing for their role in the timely opening of chromatin in their host TADs. Finally, we show that H3K27ac ensembles participate in the activation of early-acting developmental genes, including those later dependent on long-range regulation.

SP5
THE PROPENSITY OF AN ORGAN TO BECOME PRIMARY CANCER OR METASTATIC HOSTS IS ASSOCIATED WITH THEIR GENE EXPRESSION PATTERNS

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Clinical observations show a high variability in the prevalence of primary or metastatic cancers across different organs, suggesting an influence of the host tissue in the onset of cancer. Current studies are investigating this from the perspective of organs that already host cancers suggesting an important role of tumor microenvironment. However, little is known about the role of healthy organs. In this study we sought to determine the features of organs that are positively correlated with their likeliness to become a primary cancer or metastatic host. To that end, we analyzed correlation between gene expression patterns in healthy organs and organ-specific prevalence of cancers. We found that gene expression signatures are dramatically different between healthy organs that are more commonly primary cancer hosts compared to those that are more commonly metastatic hosts. In particular, we identified a negative correlation between percentage of underexpressed genes and primary cancers, while positive correlation between overexpressed genes and metastases. Further exploration revealed that immunity and apoptosis are shared mechanisms, epithelial-mesenchymal transition and hemostasis as processes associated with metastasis nesting, while various biological processes are additionally needed for the initiation of a primary cancer, such as regulation of cell cycle, DNA replication, DNA repair, transcription, metabolism. It seems that low transcriptional activity in healthy organs is protective against cancer initiation, while overexpression of in particular immune response and wound healing genes appears to be the most important metastasis-friendly feature of a healthy organ. Our analyses also identified some outliers such as spleen, small intestine and bladder which are not common metastatic sites but also show high overexpression of immune genes. We suggest that these organs should be clinically investigated even though they may not contain visible metastases, as they might represent a hidden source of premetastatic cells that can be disseminated to other sites. Our results pave new avenues for predictive diagnostics of cancer and for development of strategies that would prevent the onset or delay progression of the disease.

SP6
EVOLUTIONARY ORIGINS OF THE *N*-GLYCOSYLATION FROM THE PERSPECTIVE OF *HOMO SAPIENS* GENOME

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Glycosylation is present in all living organisms, ranging from bacteria and archaea all the way to humans. Glycan structures attached to lipids and proteins are not coded into the genome but are precisely added by enzymes involved in the glycosylation process. The observed increase in glycan structure complexity through evolution suggests that the glycosylation processes are under continuous evolutionary change. To reconstruct the evolutionary origin of the genes involved in *N*-glycosylation and their target proteins in *H. sapiens* we applied the phylostratigraphic approach. Results showed that homologs of most human genes involved in *N*-glycosylation could be traced back to the last universal common ancestor (LUCA) suggesting that the protein glycosylation is essential to all cellular life. Apart from that, we observed significant enrichment in the last eukaryotic common ancestor (LECA), probably linked to the emergence of the endomembrane system which plays important role in eukaryotic *N*-glycosylation.
Focusing on the enzymes of the *N*-glycosylation biosynthetic pathway localized on the cytosolic and luminal side of the membrane of the endoplasmic reticulum, it has been observed that these enzymes are grouped into blocks and that evolutionarily older enzymes, originated in LUCA, are oriented toward the cytosolic side, while evolutionarily newer enzymes, originated in LECA, are oriented towards the lumen of the endoplasmic reticulum. Such rearrangement supports suggested theories of eukaryogenesis which theorize the development of the endomembrane system from the cell membrane of the host cell.

SP7
WHAT CAN WE LEARN ABOUT THE MECHANISMS OF OCT1 POLYSPECIFICITY USING INTERSPECIES COMPARISONS?

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Organic cation transporter OCT1 (*SLC22A1*) is a hepatic membrane transporter that mediates the first step in hepatic metabolism or excretion of cationic or weakly basic compounds. OCT1 is polyspecific and transports a variety of structurally diverse compounds, among them numerous clinically relevant drugs. The exact amino acids involved in polyspecific substrate binding are still unknown, although a few key amino acids have been suggested. There is no crystal structure of OCT1 or a related transporter and the majority of structure-function data were obtained using mutagenesis. Species differences between OCT1 orthologs are well known. Human and rodent OCT1 differ in organ expression and in 23% of protein sequence (124 amino acids). Functional differences were only scarcely analyzed, but we recently reported strong differences in the uptake kinetics of a number of substrates between human and mouse OCT1. The differences affected both transport capacity (v_{max}) and affinity (K_M) and were highly substrate-specific. Our aim was to utilize the differences in sequence and transport between OCT1 orthologs to better understand the polyspecificity of OCT1. Using a hypothesis-free approach, we analyzed human-mouse chimeric OCT1, which enabled narrowing down regions involved in OCT1 transport. Using this strategy followed by site-directed mutagenesis of non-synonymous amino acids in those regions, we identified single amino acids – C36 and L32 – that confer the differences in fenoterol and trospium kinetics between human and mouse OCT1. Using a hypothesis-driven approach, we analyzed the effects of mutating proposed key amino acids between human and mouse OCT1. Substitution of F159/F160A (F159 in human, F160 in mouse), W217/218Y and D474/475N affected transport in a strongly species and substrate-specific manner, suggesting species and substrate-specific differences in their roles in OCT1 transport. Moreover, we extended the species comparisons by cloning and functionally characterizing OCT1 from dog. Comparison of transport kinetics between human, mouse and dog orthologs revealed distinct differences and similarities that were strongly substrate-specific. Analyses of fenoterol and trospium transport supported our findings of an important role of codons 36 and 32. This approach can further be extended to other species. In conclusion, interspecies comparisons can be utilized successfully to better understand OCT1 transport and the mechanisms conferring OCT1 polyspecificity. The strategies outlined are powerful tools and can be extended to other species or proteins as needed.

SP8
GENOMIC STRUCTURAL VARIATION IN SPERM CELLS: INSIGHTS FROM OPTICAL MAPPING DATA

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Genomic structural variation represents a major source of genetic diversity in mammals. Structural variants (SVs) appear either as somatic variations (common in tumors) or can arise in the germline. Genome optical mapping (OM) is a technique that can be used for detecting large-scale SVs by comparing the patterns of high-resolution restriction maps from single molecules of DNA. Due to its ability to resolve different alleles, OM is often deployed for detecting genome-wide structural aberrations in genetically heterogeneous tumor tissues. Here we explore the utility of OM for SV detection in another kind of population of cells with heterogeneous genomes - sperm cells. To the best of our knowledge, this is the first set of OM data successfully produced on germline cells. Studying SVs in the germline allows direct detection of *de-novo* events which present heritable mutations that may be associated with various disorders or confer an adaptive advantage. Moreover, OM of the germline enables us to explore shifts in frequency of recurrent SVs that are brought about by environmental factors. These are otherwise deduced indirectly from population studies on much larger timescales (*i.e.* over many generations compared to within single generation in germline). In essence, OM of the germline provides the unprecedented means to study genome-environment interaction at the level of SVs that may affect progeny's health predisposition as well as evolutionary trajectory. We deal with these facets in a high-fat diet experiment on mice of the C57BL/6 strain. The comparison of genome-wide SVs in the experimental and control group will be presented.

SP9 *HDBMB Young Scientist Award 2021*
INTERPLAY BETWEEN C TERMINAL BNIP3L/NIX PHOSPHORYLATION AND DIMERIZATION AS A NOVEL MECHANISM OF RECEPTOR-MEDIATED MITOPHAGY REGULATION

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Autophagy is an essential and a conventional regulation mechanism in all eukaryotic cells, conserved throughout the evolution and indispensable for optimal function of each cell. To maintain homeostasis, autophagy balances the biosynthesis and catabolism of different macromolecules to protect against diverse pathologies, including cancer or neurodegeneration. As the most important energetic organelles, mitochondria play a pivotal role in intracellular homeostasis, thus the maintenance of mitochondrial function and integrity is crucial for normal physiology. A form of autophagy specialized for selective removal of mitochondria, mitophagy, is needed for elimination of dysfunctional mitochondria whose accumulation can lead to the development of various diseases. Conversely, programmed mitophagy of healthy mitochondria is prerequisite for differentiation of particular cell types and autophagy receptor BNIP3L/NIX is shown to be a key for mitochondrial removal during erythropoiesis. We have uncovered completely novel mechanism of selectivity underlying BNIP3L/NIX-mediated mitophagy. This mechanism involves the interplay between C terminal BNIP3L/NIX dephosphorylation and consequently receptor dimerization. Stable BNIP3L/NIX homodimers provide the formation of strong interactions between the receptor and autophagosomal proteins, more robust recruitment of autophagosomes and more efficient mitochondrial removal. Analysis of C terminal intermembrane part of BNIP3L/NIX has revealed that receptor dimerization is achieved by specific Ser212 dephosphorylation and has the same effect on mitophagy process as LIR phosphorylation described earlier. We have observed the interplay between BNIP3L/NIX phosphorylation and dimerization, together with LIR phosphorylation, is needed for proper BNIP3L/NIX-dependent mitophagy initiation and progression. Currently, the focus of our research is in detailed analysis of interactions between BNIP3L/NIX and identified kinases/phosphatases to unveil upstream signaling pathways that trigger and regulate mitophagy especially in erythroid cell lines. This knowledge is crucial for better understanding the mechanisms of particular cell`s differentiation and the development of pathological conditions that underlie the disturbed mitophagy process.

SP10 (HDBMB Annual Young Scientist Award 2021)
GASTROINTESTINAL ALTERATIONS IN RAT MODELS OF ALZHEIMER'S AND PARKINSON'S DISEASE

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The gastrointestinal system plays an important role in the etiopathogenesis and progression of neurodegenerative disorders. Accumulating epidemiological, clinical, and mechanistic *in vivo* and *in vitro* evidence supports the current working model of a bi-directional involvement of the gut in neurodegeneration via the gut-brain axis. Unfortunately, most current evidence suggesting the importance of the gut-brain axis in neurodegeneration is based on observational studies and/or indirect associations that make causal relationships and critical pathophysiological events challenging to identify let alone exploit as pharmacological targets. The role of the gut-brain axis has been explored, particularly in the context of maintenance of redox homeostasis of the gastrointestinal tract using two toxin-induced brain-first models of neurodegeneration to dissect the temporal patterns of pathological changes in the gut and elucidate its potential importance in the context of the progression of neurodegeneration. Intracerebroventricular streptozotocin administration (STZ-icv) has been used as a brain-first model of sporadic Alzheimer's disease, and bilateral intrastriatal administration of 6-hydroxydopamine (6-OHDA) has been used as a model of progressive Parkinson's disease. The results suggest that in the STZ-icv model there is a pronounced indirect effect on the gastrointestinal tract with structural and functional alterations, pronounced redox dyshomeostasis, and insensitivity to homeostatic regulation mediated by the brain-gut pathways already at the time-point resembling early stages of the disease. Conversely, in the 6-OHDA-induced rat model of Parkinson's disease, gastrointestinal dyshomeostasis seems to be dependent on the degeneration of the nigro-vagal pathways that occurs relatively late in the intrastriatal administration-based models characterized by slow retrograde damage propagation.

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SP11
THE OCULOCEREBRORENAL SYNDROME OF LOWE PROTEIN CONTROLS ACTIN AND MICROTUBULE REARRANGEMENTS DURING HUMAN PLATELET SPREADING

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Lowe syndrome (LS) is a rare X-linked disorder that affects the brain, the eyes, and the kidneys leading to central hypotonia, mental retardation, glaucoma, congenital cataracts, and renal Fanconi syndrome. LS is caused by mutations in the oculocerebrorenal syndrome of Lowe protein (OCRL) which is a 5-phosphatase that dephosphorylates phosphatidylinositol-4,5-bisphosphate [PI(4,5)P_2] to phosphatidylinositol-4-monophosphate (PI4P). Numerous roles of PI(4,5)P_2 include the regulation of actin nucleation and reorganization which is a crucial step during the activation of the smallest blood cells, platelets (PLTs). Upon vessel wall injury, PLTs adhere to the exposed molecules of the extracellular matrix, activate, aggregate, and with the help of fibrinogen form a clot. Furthermore, it has been shown that PLTs have impaired wound closure times in some LS patients. Therefore, we hypothesized that OCRL has an important role during PLT spreading. We show by immunocytochemistry that inhibition of OCRL with the YU142670 inhibitor impairs PLT spreading on fibrinogen, resulting in the extensive formation of actin nodules as opposed to the actin stress fibres of control PLTs. These actin nodules colocalize with proteins implicated in actin dynamics (ARP2/3, vinculin, SNX9) and with phospho-tyrosines showing they are sites of active signalling. Although OCRL-inhibited PLTs have impaired actin reorganization, the flow cytometry analysis revealed no change in the net F-actin levels. However, Western Blot analysis showed that OCRL inhibition decreases the levels of myosin light chain (MLC) phosphorylation upon stimulation with thrombin and TRAP-6. OCRL inhibition also impairs the disassembly of the microtubular coil (not in PLTs spread on collagen or glass) and increases the levels of acetylated tubulin. Impaired cytoskeletal rearrangements were also confirmed by electron microscopy. Interestingly, the OCRL inhibition did not alter the release of PLT granules and activation of integrins upon stimulation of thrombin or collagen receptors. We conclude that OCRL has an important role in the reorganization of actin and tubulin cytoskeleton during PLT spreading but does not affect PLT degranulation or integrin activation which could explain the mild bleeding problems of LS patients.

SP12
MAKING RINGS FROM AMINO ACIDS. SHINING LIGHT ON POSSIBLE (CHEMO)EVOLUTION OF PROLINE

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Proline is an unusual proteinogenic alfa amino acid, where the side chain is connected to the amino group, forming a pyrrolidine ring and secondary amine. Due to its unique internal bonding it has different structural properties compared to other amino acids in peptides and proteins, including different ratio of cis/trans isomerism in peptide bonds, conformational rigidity and losing hydrogen-donating characteristics. Biosynthesis of proline starts from L-glutamate, proceeds through gamma-semialdehyde, that cyclizes to pyrrolidine-ring that is then reduced to proline. Generation of proline in *primordial soup* is not well understood. While it is possible that generation through Schiff base mechanism is possible, here we provide an alternative. Pyrrolidine rings can be formed through Hofmann-Löffler-Freytag (HLF) reaction. This reaction starts from halogenated amine, which under illumination or heating cleaves homolytically, generating *N*-centred radical. Rearrangement of *N*-centred radical to C-centered radical, and subsequent ring formation can be one of the ways proline is formed from nor-leucine. This reaction can also be used for synthesis of pharmacologically active compounds, such as nicotine derivatives. Here presented are simulations of thermodynamic and kinetic profile, synthesis, kinetic studies, and characterisation of proline derivatives utilising HLF reaction. Results can be used to describe structural changes and degradation in proteins in oxidative stress and high UV radiation conditions.

SP13
THE EFFECT OF GANGLIOSIDES ON MEMBRANE ION TRANSPORT IN MOUSE BRAIN

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Gangliosides, the most complex of all glycosphingolipids, carry approximately 75% of the brain's sialic acid. They are prominent constituents of neuronal membranes that serve not only as glycolipid scaffold of the membrane, but ligand-recognition partners as well. Gangliosides recognize and modulate the function of a multitude of specific proteins, including myelin-associated glycoprotein, TrkA receptor for the nerve growth factor and insulin receptor. In the last decade, several human diseases caused by the impaired biosynthesis of gangliosides have been reported. These congenital disorders of ganglioside biosynthesis include one form of hereditary spastic paraplegia and infantile-onset symptomatic epilepsy syndrome with symptoms ranging from motor deficits to cognitive deficits, epileptic seizures, hearing loss and intellectual disability. The molecular chain of events starting from aberrant ganglioside synthesis and leading towards this phenotype has not yet been explained. The long-standing goal of our research group is to clarify specific roles of gangliosides by studying their interactions with specific proteins. By using *in vitro* model systems, cell culture models as well as mouse models with deficient ganglioside synthesis, we identified several membrane ion transporters and their protein partners as being heavily influenced by ganglioside microenvironment of the membrane. Those include Na^+/K^+-ATPase (NKA), plasma membrane Ca^{2+}-ATPase (PMCA), and the PMCA partner neuroplastin. We used gene expression analysis, protein expression analysis by Western blotting and immunohistochemistry, enzyme activity determination, submembrane localization analysis in mouse models with deficient ganglioside biosynthesis, in addition to analysis of the effects of exogenously added gangliosides and anti-ganglioside antibodies in cell culture and tissue homogenates. By using this comprehensive approach which encompasses all the crucial stages in the regulation and function of a protein - gene expression level, protein expression level, correct positioning of the protein and the ultimate enzyme activity - we unequivocally show that specific gangliosides contribute to brain ion homeostasis by regulating the expression and micro-positioning of specific ion transporters.

SP14
COMPOSITION OF SPHEROIDS FORMED BY MAGNETIC LEVITATION

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Ball shaped cellular structures called spheroids are the basis of 3D cell culture. Shift from 2D to 3D cell culture was necessary in various fields since 3D structures mimic live tissue better. There are many different ways to form spheroids from single cells. One of the methods is magnetic levitation where cells are grown under the influence of a magnetic field after taking up ferrous nanoparticles. Spheroids formed this way are well defined and compact. Our work set out to establish the baseline composition of these spheroids to better characterize them for further use in toxicity assays. Spheroids in question were formed from glioblastoma cells (namely D54 cell line) by magnetic levitation. They were formed and grown for up to 15 days prior to analysis with the growth media changed as needed. Completely grown spheroids were harvested and dissolved in 0.5% trypsin before staining with Annexin and PI. Samples were analyzed by flow cytometry to determine the ratio of live, apoptotic and necrotic cells in the samples. Additional samples were frozen, cryostat cut and stained to observe changes in morphology and collagen production. Results showed that the ration changes over time with spheroids of different size having different proportions of apoptotic and live cells, but relatively constant number of necrotic cells. The ratio of apoptotic and live cells rises and falls respectively during the growth period. The intent is to determine how spheroids behave in control conditions as to better interpret toxicity results. Longer growth time resulted in bigger, less compact spheroids that show beginnings of ECM synthesis.

SP15
QUEST FOR PUTATIVE EFFECTORS OF '*CANDIDATUS* PHYTOPLASMA SOLANI': TOWARDS UNDERSTANDING OF A SUCCESSFULL AND ADAPTABLE PATHOGEN

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During the co-evolution of microbial pathogens and their hosts, pathogens developed a wide array of effectors – powerful molecular weapons increasing their virulence by disturbing hosts' developmental processes and immune system. The term effector usually denotes a small secreted protein interacting with host cell; however, effectors can include toxins, elicitors, analogues of (phyto)hormones and different enzymes. Phytoplasmas (genus '*Candidatus* Phytoplasma') are endocellular wall-less bacteria with small genomes affecting numerous plant species and causing significant damage in agriculture worldwide. They have a dual parasitic cycle colonizing plant phloem and insect cells. Due to the inability of obtaining a pure culture *in vitro*, studies related to their pathogenicity mechanisms are still hindered. However, the ascent of metagenomic era and new technologies delivered new tools and possibilities for finding potential effectors. '*Ca.* P. solani' is a cosmopolitan pathogen with a broad host range transmitted by many insect species and one of the most important phytoplasmas in Croatia. The aim this study was to sequence two '*Ca.* P. solani' strains in order to get insight into their pathogenic potential and the repertoire of putative effectors. Whole plant samples of periwinkle infected with '*Ca.* P. solani' were used for generation of libraries sequenced on Illumina MiSeq and MinION nanopore sequencing platforms. Raw reads were mapped to the reference genome and reads originating from periwinkle were filtered out. *De novo* assembly was performed as well as whole genome alignment and comparative analyses. Two draft genomes were generated with total size of 647,316 bp (ST19) and 668,749 bp (STOL) and longest contigs of 140,634 bp and 158,078 bp, respectively. Whole genome alignment with reference SA-1 revealed synteny and conserved gene order. Prediction and search for putative effectors showed the presence of homologues of SAP11 and SAP54 effectors as well as some putative effectors that were strain specific. Nonetheless, sequencing and comparative genome analyses of '*Ca.* P. solani' genomes confirmed their highly repetitive nature and the presence of diverse putative effectors as a mechanism for successful adaptation and pathogenicity of this versatile pathogen.

SP16
MEET QIAGEN QIAcuity DIGITAL PCR SYSTEM

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Company QIAGEN with long expertise in sample preparation, PCR and sequencing would like to introduce a new instrument- QIAcuity Digital PCR System. The nanoplate-based digital PCR system provides a more familiar workflow, just like in qPCR experiments with capability of transforming PCR experience with new addition: absolute quantification without standard curve, increase precision and reproducibility, simultaneous quantification of multiple targets. The QIAcuity Digital PCR System is designed to deliver precise and multiplexed quantification results for mutation detection, copy number variation (CNV), gene expression studies, gene-editing analysis, and many more. The system integrates partitioning, thermocycling, and imaging into a single fully automated instrument that takes users from sample to result in under two hours. Main Features: Fully integrated system, scalable format (1-, 4- and 8-plate instruments), advanced multiplexing capabilities with up to 5 channels (5 plex), flexible sample throughput and comprehensive results in under 2 hours.

Poster Abstracts

P1
MIR-182-5P AND MIR-375-3P AS BIOMARKERS FOR DIFFERENTIATING PROSTATE CANCER FROM BENIGN PROSTATE HYPERPLASIA

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Prostate cancer (PCa) represents a malignancy with the highest prevalence and very high incidence among men worldwide. Clinical challenge is to differentiate localized PCa from benign prostate hyperplasia (BPH) due to the lack of specificity of routinely used biomarker PSA. Epigenetic biomarkers in liquid biopsies, especially miRNA, could address this challenge. The absolute expression of miR-375-3p, miR-182-5p, miR-21-5p, and miR-148a-3p were quantified in blood plasma and seminal plasma of 65 PCa and 58 BPH patients by digital droplet PCR. The sensitivity and specificity of these microRNAs were determined using ROC curve analysis. The higher expression of miR-182-5p and miR-375-3p in the blood plasma of PCa patients was statistically significant as compared to BPH ($p = 0.0363$ and 0.0226, respectively). Their combination achieved a specificity of 90.2 % for predicting positive or negative biopsy results, while PSA cut-off of 4 μg/L performed with only 1.7 % specificity. In seminal plasma, miR-375-3p, miR-182-5p, and miR-21-5p showed a statistically significantly higher expression in PCa patients with PSA >10 μg/L compared to ones with PSA >10 μg/L. MiR-182-5p and miR-375-3p in blood plasma show higher performance than PSA in differentiating PCa from BPH. Seminal plasma requires further investigation as it represents an obvious source for PCa biomarker identification.

P2
THE ROLE OF TYROSIL PHOSPHODIESTERASE 2 IN DPC REPAIR *in vivo*

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DNA-protein crosslinks (DPCs) are formed when a protein irreversibly covalently binds to DNA, physically disrupting all DNA transactions. DPCs cause genomic instability that can lead to cancer, accelerated aging, and neurodegeneration. These lesions are caused by different proteins, so it is no coincidence that cells have evolved multiple repair pathways. One of the most common cellular DPCs is Topoisomerase 2 (TOPO2), an enzyme important for generating double-strand breaks to reduce the torsional stress on DNA during replication, transcription and chromatin remodeling. Several drugs such as etoposide have been developed to stimulate the formation of Topoisomerase 2 DPCs (TOPO2-DPCs), leading to cancer cell death. TOPO2-DPCs can be repaired (1) by SPRTN protease and Tyrosyl phosphodiesterase 2 (TDP2), (2) by MRE11 nuclease during DNA end resection, and (3) by the synchronized action of zinc finger protein ZATT and TDP2. To date, the mechanisms of TOPO2-DPCs repair are partially unknown, whereas their repair at the organism level has not been investigated. Therefore, we set out to determine the role of TDP2 in TOP2-DPCs repair in to the context of SPRTN protease and MRE11 nuclease, which have previously been implicated in repair *in vitro*. Mutations of TDP2 in humans have been associated with intellectual disability, seizures, and spinocerebellar ataxia. To investigate the role of TDP2 protein in DPC repair at the organismal level, we decided to generate zebrafish strains lacking TDP2 protein and strains with enzymatically inactive protein. Since zebrafish has two orthologs of the TDP2 protein, Tdp2a and Tdp2b, we used CRISPR/Cas genome editing to mutate both genes. We successfully introduced a premature STOP codon into the Tdp2a protein and were able to delete the enzymatically important Glu at position 232. For the Tdp2b protein, we created a fish line into which we introduced the STOP codon and a strain carrying a point mutation at position A4860C that produces an enzymatically impaired Tdp2b. These animal models will reveal (1) which TOPO2 repair pathway is predominant in the organism and (2) whether TDP2 can help eliminate other DPCs. In addition, our study will help to better understand diseases associated with TDP2 deficiency.

P3
ASSOCIATION OF OPTICAL PARAMETERS WITH TSH, THYROID HORMONES AND THYROGLOBULIN LEVELS

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The scope of this study was to determine if 24 optical parameters were associated with thyroid-stimulating hormone (TSH), thyroid hormones and thyroglobulin (Tg) levels. It is well known that in some pathological thyroid conditions, eyes can be affected, too. The example of such condition is Thyroid Eye disease (or Graves' eye disease). This condition that is caused by the hyperactive thyroid is characterised by the attack of the eye tissues by the immune system that consequently leads to the expansion of the eye muscles or fat. In this study we wanted to test the association of various optical parameters with TSH, thyroid hormones and Tg levels in big cohort of healthy individuals. This cross-sectional study included 4848 healthy adults recruited within the "10,001 Dalmatians project" which is a part of the Croatian Biobank program. Plasma TSH, free triiodothyronine (fT3), free thyroxine (fT4), and Tg levels were measured by an immunoassay. Additionally, we determined 24 optical parameters (left and right eye posterior chamber length, eyeball length, IOL1, IOL2, IOL3, corneal thickness, lens thickness, cylinder power, spherical power, angle, anterior chamber depth, corneal radius) for each participant. Two statistical methods (simple correlation and unsupervised machine learning algorithm; principal component analysis [PCA]) confirmed the association of various optical parameters with TSH, thyroid hormones and Tg levels in healthy individuals This study should be replicated in individuals with hyperthyroidism and hypothyroidism that could elucidate the physiological background of our results and give us explanations of obtained associations.
Funded by: Croatian Science Foundation under the project "Regulation of Thyroid and Parathyroid Function and Blood Calcium Homeostasis" (No. 2593).

P4
OVEREXPRESSION AND PURIFICATION OF THE C-TERMINAL DOMAIN OF SH2D3C IN *ESCHERICHIA COLI*

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Protein-protein interactions are crucial for many cellular processes and their research allows us to understand these processes from a biochemical point of view. Since changes in the regulation of signaling pathways in the cell often lead to the development of various diseases, studying protein-protein interactions can help us better understand their origin, development and impact on human health. SH2D3C acts as an adapter protein in signaling pathways involved in cell adhesion and migration, tissue organization, and regulation of the immune response. The protein contains a Ras GEF-like C-terminal domain that has no significant GEF activity, but may interact with other proteins. Analysis of the cellular proteome by SILAC-MS revealed a potential interaction of SH2D3C with dipeptidyl peptidase III (DPP III) involved in the regulation of oxidative stress by competitively binding to the KEAP1 protein which is a key participant in the Nrf2/KEAP1 signaling pathway. This would mean that their interaction represents a possible link between the Nrf2/KEAP1 signaling pathway and cell migration regulation. To confirm the interaction by other methods such as micro-scale thermophoresis (MST), the C-terminal domain of SH2D3C was overexpressed and purified in *E. coli* with two different tags, GST and MBP.

P5
NATURAL REMEDY OF MODERN AGE - ANTIOXIDATIVE AND ANTIPROLIFERATIVE POTENTIAL OF MEDITERRANEAN PLANTS

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The aim of this study was to assess the antioxidative and antiproliferative potential of mediterranean plants, *Pinus halepensis*, *Pinus pinea*, *Pinus brutia*, *Cichorium intybus*, *Artemisia annua* and *Anacyclus clavatus*, traditionally used for medicinal purposes. Plant hydrolates were isolated by microwave extraction method from dried plants. Antioxidative properties of the hydrolates were assessed using DPPH method with trolox as a reference compound. The antiproliferative activity of plant hydrolates was examined on HepG2 cell line using the MTS assay. Afterwards, the expression of apoptotic gene *BCl2* in HepG2 cells was determined using quantitative PCR after cells were exposed to two different concentrations (20% and 40%) of hydrolates for 24 hours. Each hidrolate, with exception of *Artemisia annua* showed some antioxidative potential in comparison with trolox. *Anacyclus clavatus*, *Pinus halepensis* and *Pinus pinea* had mild proliferative effect on HepG2 in contrast to *Pinus brutia* whose hydrolate was cytotoxic. Expression of *BCL2* was significantly decreased after HepG2 cells were exposed to 40% hydrolates of *Artemisia annua*, *Anacyclus clavatus* and *Pinus brutia*. *Pinus halepensis* and *Pinus pinea* lowered *BCL2* expression in both concentrations. Lower expression of *BCl2* gene indicates that plant hydrolats have proapoptotic activity. In conclusion, some of these traditional plants could be used as a potential antioxidative and antitumor remedy but their impact on human health should be investigated in more detail in the future.

P6
THE ROLE OF SPRTN IN HEPATOCELLULAR CARCINOMA PATHOGENESIS

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SPRTN protein mediates the proteolytic cleavage of covalent DNA-protein cross-links (DPCs) during DNA synthesis, thereby maintaining genomic integrity. The involvement of SPRTN in hepatocellular carcinoma (HCC) was discovered in three patients with different SPRTN mutations but the exact mechanism remains to be elucidated. The relationship between the endoplasmic reticulum (ER) stress response, as well as the DNA damage and the pathogenesis of HCC was proven but the correlation of SPRTN in ER stress response was never tested. We aim to evaluate the involvement of SPRTN, DNA damage and ER stress in the pathogenesis of HCC by measuring the genomic expression of SPRTN and other candidate genes and to observe the difference in amounts and localization of SPRTN and other candidate proteins in HCC samples. Additionally, we aim to investigate the possible functional link between SPRTN, DNA damage and ER-stress-response proteins. Research was performed on human samples of HCC and adjacent (control) liver tissue, collected in the Merkur clinical hospital during liver transplantation surgery procedure. These samples were dissected, embedded in paraffin or used for RNA, DNA and protein isolation. The expression of SPRTN and other candidate genes was evaluated by qPCR and RT2 profiler PCR array for DNA damaging signaling pathway. Protein levels in tissues were evaluated by western blot and immunohistochemistry. Samples were evaluated for the SPRTN gene and candidate gene expressions and protein level alterations. Out of 24 ER stress response candidate genes evaluated by qPCR, we found a significant alteration of 22 genes. Immunohistochemistry staining of tissue sections showed an alteration in tested protein level amount. SPRTN protein is involved in the ER stress response pathogenesis of hepatocellular carcinoma development and further study is needed to elucidate the mechanism of this response.

P7
DIFFERENT DYNAMICS OF DIABETIC WOUND HEALING IN CONDITIONS OF DIPEPTIDYL-PEPTIDASE IV (DPP IV/CD26) DEFICIENCY IN MICE

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Diabetes mellitus and its comorbidities are one of the major health issues worldwide. Diabetes-related complications such as chronic ulcerations are the leading causes of hospitalization of diabetic patients. Dipeptidyl-peptidase IV or CD26 molecule (DPP IV/CD26) is widely known, besides the involvement in a broad range of physiological and pathological processes, for its role in the regulation of glycaemia. DPP IV/CD26 inhibitors are known therapeutics in diabetes. It has been previously shown that DPP IV/CD26 inhibition improves the healing of chronic diabetic ulcers. However, the mechanisms of its actions in the processes and mechanisms of wound healing are unknown. Our hypothesis was that DPP IV/CD26 plays a significant role in the process of cutaneous wound healing in hyperglycaemia through a mechanism of induction enhanced angiogenesis. We aimed to research the process of wound healing in CD26 deficient diabetic mice in order to broaden the knowledge on the role of DPP IV/CD26 in processes of cutaneous reparation and regeneration. A streptozotocin-induced model of diabetes was established in CD26-deficient as well as C57BL/6 (wild-type) mice. Experimental wounds were made on the dorsal region of the mice. Animals were sacrificed in scheduled time periods according to the protocol of wound healing monitoring. Tissue samples were subjected to pathohistological, histomorphometrical, immunohistochemical and immunochemical analyses. Serum samples were analyzed for DPP IV/CD26 activity and concentration of target vascularization factors. The results of this study confirm the hypothesis that DPP IV/CD26 plays an important role in the regulation of glycaemia. Moreover, parameters of wound healing in hyperglycaemia were found to be improved in CD26 deficient mice, indicating that inhibition of DPP IV/CD26 has beneficial effects on the wound healing process in diabetes. Therefore, the significance of DPP IV/CD26 inhibition as a therapeutic strategy for the treatment of diabetes and diabetic wounds is emphasized. DPP IV/CD26 inhibitors are proposed as potential therapeutics in chronic diabetic ulcers.

P8
BIG, GBF AND BRAG ARF GEFS ARE REQUIRED FOR THE PROGRESSION OF IMMEDIATE-EARLY AND EARLY PHASE OF MCMV INFECTION

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Arf proteins are small GTPases that cycle between active (GTP) and inactive (GDP) forms. Their activation is mediated by guanine nucleotide exchange factors (GEFs), and their inactivation is facilitated by GTPase-activating proteins (GAPs). Since we have previously shown that Arf1 and Arf6 proteins are required for the establishment of murine cytomegalovirus (MCMV) infection, the aim of this study was to determine importance of Arf GEFs in regulation of Arf´s activity during the immediate-early and early phase of MCMV infection. Balb 3T3 murine fibroblasts treated with small inhibitors of Arf GEFs were infected with MCMV and the expression of viral proteins was determined by Western blot and immunofluorescence microscopy after 6 and 16 hours of infection. In addition, cells were infected with C3X-GFP MCMV, in which a GFP expression cassette was inserted upstream of the ie2 gene, and the intensity of green fluorescence as well as percentage of GFP positive cells was monitored by flow cytometry as an indicators for progression of the immediate-early phase of infection. The expression of IE1, E1 and m06 proteins was reduced in cells treated with Brefeldin A (BFA), Golgicid A (GCA), NAV2729 or EXO2, in contrast to cells treated with SecinH3 or EXO1 in which the expression of the viral proteins was similar to that in control cells. In cells infected with C3X-GFP MCMV, percentage of GFP positive cells and the green fluorescence intensity were significantly decreased in cells treated with BFA, GCA and EXO2 compared with control cells, suggesting that inhibition of Gbf1, Big1, Big2 and BRAG2 Arf GEFs prevents entry into the immediate–early/early phase of MCMV gene expression. Our results suggest that Arf GEFs from Big, Gbf and Brag2 family have important role in regulation of Arf protein activity during the earliest stages of MCMV infection and are required for the progression of immediate-early and early phase of MCMV infection.

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P9
DNA METHYLOME CHANGES PRECEDE BLADDER CANCER INVASIVENESS

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Understanding the molecular details of bladder cancer (BC) progression from a non-invasive to an invasive form is of paramount importance since the invasive BC, despite aggressive therapy, has a very high mortality rate. The leading risk factor for BC is tobacco smoke, thus approximately half of all BC cases have been attributed to tobacco exposure. Epigenetic changes accompanied by deregulated gene expression are an important element of cancer pathogenesis. It has been demonstrated that aberrant DNA methylation, followed by chromatin remodeling, could be a driver event in BC pathogenesis. To further investigate BC methylome, we used a mouse model for BC, based on N-butyl-N-(4-hydroxybutyl) nitrosamine, a carcinogenic chemical similar to compounds from tobacco smoke. With genome-wide approaches, we profiled methylome and transcriptome of invasive and non-invasive tumors. Analyses highlighted enrichment in genes involved in muscle contraction and neuronal system, as determined with Reactome pathways. Methylation deregulation of these pathways was detected in invasive and non-invasive groups, while these alterations have been reflected on the transcriptome level only across the invasive BC. Moreover, with additional validations, we identified biomarkers that could be used for risk stratification of BC invasiveness. Our findings suggest that BC progression could be revealed through methylation profiling at its pre-invasive stage, which could improve the monitoring of BC patients.

P10
CD26 DEFICIENCY MODULATES MACROPHAGE POLARIZATION VIA TARGETING OF STAT PROTEINS IN A MOUSE MODEL OF ULCERATIVE COLITIS

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Inflammatory bowel disease represents a chronic inflammation of the gastrointestinal tract and includes ulcerative colitis (UC), a disease characterized by the destruction of intestinal mucosa followed by an excessive and dysregulated activation of the immune system. In recent years, macrophages have been identified as essential elements that not only maintain intestinal homeostasis but also control the inflammatory response and mucosal healing. Macrophage polarization into either classically activated proinflammatory (M1) or alternatively activated anti-inflammatory (M2) subclass occurs as a response to the downstream signal of different cytokines so the regulation of M1/M2 balance has recently been targeted as a potential therapeutic strategy for UC. The inhibition of CD26, a multifunctional glycoprotein that regulates the immune response via its dipeptidyl peptidase (DP) 4 enzyme activity, was proven to have beneficial effects in various autoimmune inflammatory diseases but the role of DP8 and DP9, proteins with DP4-like activity, are not characterized in detail. We aimed to investigate the impact of CD26 deficiency on the process of macrophage polarization as well as DP8/9 expression profiles in the dextran sulfate sodium (DSS)-induced model of UC. Our results revealed that mRNA expression of M2 markers arginase 1 and Fizz were increased, while the expression of M1 marker inducible NO synthase was downregulated in CD26$^{-/-}$ mice with acute UC. Decreased STAT1 mRNA, as well as upregulated pSTAT6 and pSTAT3, additionally support the demonstrated activation of M2 macrophages under CD26 deficiency. Furthermore, we concluded that CD26 deficiency is not a key factor for the noted upregulation of DP8 and DP9 expression in UC development and resolution. In conclusion, we demonstrated that CD26 deficiency regulates macrophage polarization toward the anti-inflammatory M2 phenotype, which is driven by STAT6/STAT3 signaling pathways. This process is additionally enhanced by the reduction of M1 differentiation via the suppression of proinflammatory STAT1. Further studies should be done to investigate the clinical potential of CD26 inhibitors in the treatment of UC.

P11
SESQUITERPENE LACTONES PHARMACOLOGICAL POTENTIAL

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Sesquiterpene lactones (SLs) are the active constituents of a variety of medicinal plants used in traditional medicine. In recent years, the interest in SLs has risen due to their vast array of biological activities beneficial for human health. Anti-cancer property of various SLs has attracted a great deal of interest and extensive research work has been carried out to characterize the anti-cancer activity, the molecular mechanisms, and the potential chemo preventive and chemotherapeutic application of SLs. The anti-inflammatory potential of these compounds results from their ability to target and inhibit various key pro-inflammatory molecules enrolled in diverse inflammatory pathways and prevent or reduce the inflammatory damage on tissues. Sesquiterpene lactones (SLs) are composed of a large and diverse group of highly bioactive plant secondary metabolites, characterized by a 15-carbon backbone structure and a common functional structure, an α-methylene-γ-lactone group. This important chemical characteristic means that the thiol-reactivity of SLs is an underlying mechanism responsible for their bioactivities. In particular, the SL-thiols reaction, the effect of SLs on cell signaling pathways such as nuclear transcription factor-kappa B (NF-κB) and mitogen-activated protein kinases (MAPK). Besides their anti-inflammatory and anticancer potential, their cytotoxicity, structure–activity relationships, and pharmacokinetics is in focus of various research. In our work we will focus on comparison of biological activity of selected sesquiterpene lactones. The selected sesquiterpene lactones were tested on cell viability, demonstrated their induction of autophagy, and discussed their mode of activity in correlation with biological activity of selected plant extracts.

P12
ROLES OF ADAR PROTEINS IN HSV-1 PRODUCTIVE INFECTION

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HSV-1 is an important human pathogen that causes a lifelong latent infection and occasionally induces cold sores. During latency HSV-1 expresses only a small subset of miRNA and we have recently identified that a miRNA-H2 is edited by adenosine deaminases acting on RNAs (ADAR protein) within its seed sequence. Several transcriptomic and proteomic analysis identified ADAR proteins to be upregulated during HSV-1 infection. Here we confirmed that ADAR1 is upregulated early in infection and decreases at later time points in HSV-1 infected cells. Moreover, we have observed a substantial misslocalization of ADAR1 in HSV-1 infected cell that aggregate in the perinuclear region. ADAR1 is an important player in modulating the innate immune proinflammatory responses to viral infection, therefore using CRISPR Cas9 system we are planning to knockout ADAR1 to dissect the ADAR1 signaling pathway during HSV-1 infection and understand its effect on viral replication. Functions of ADAR proteins are very well documented in RNA viruses but only limited information is available concerning DNA viruses. Thus, the importance of our work is to get new insights into HSV-1 biology but also to reveal novel molecular mechanism with a broader significance.

P13
PHARMACOLOGICAL TARGETING OF PHOSPHATIDYLINOSITOL-4-KINASE A REVEALS ITS IMPORTANT ROLE IN PLATELET SPREADING

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Phosphatidylinositol-4-kinase A (PI4KA) stimulates the conversion of phosphatidylinositol (PI) to phosphatidylinositol 4-monophosphate (PI4P), considered to be the major source of phosphatidylinositol (4,5) bisphosphate (PIP_2) at the plasma membrane (PM). Since PIP_2 is known to be a crucial component of platelet adhesion and shape change following cytoskeletal reorganization, we hypothesized that PI4KA could have a role in cytoskeletal reorganization during platelet spreading. PI4KA expression and signaling pathways were analyzed in human blood platelets (hPLTs) using Western blot. PI4KA was pharmacologically inhibited and platelets were spread on either collagen or fibrinogen. Fluorescent microscopy was used to observe PI4KA expression, polymerized actin (spreading area, lamellipodia formation) and β1-tubulin, as well as the levels of PM PI4P and PIP_2 during spreading. Flow cytometry was used to investigate the activation markers P-selectin and integrin αIIbβ3 activation upon stimulation with collagen-related peptide (CRP) that targets GPVI receptor. PI4KA was shown to be highly expressed in hPLTs and its inhibition resulted in a dose-dependent decrease in both PI4P and PIP_2, indicating that PI4P is a major source of PIP_2 in hPLTs. Consequently, PI4KA inhibition reduced the spreading of hPLTs, with aberrant F-actin and decreased lamellipodia on both matrices. Interestingly, PI4KA inhibition prevented the rearrangement of the PLT microtubules. The flow cytometry analysis revealed no effect on P-selectin granular release, however, it significantly and dose-dependently decreased integrin αIIbβ3 activation in CRP-activated hPLTs. Finally, total tyrosine-phosphorylation in PI4KA-inhibited hPLTs folowing CRP activation revealed several upregulated candidates. Further analysis confirmed increase in phospho- PLCγ2 and Syk, proximal signaling targets of GPVI receptor. In contrast, downstream signaling pathways, such as Akt (phospho-serin), were downregulated. Our results suggest that PI4KA has a major role in maintaining not only PI4P, but also PIP_2 PM levels in hPLTs. Therefore, PI4KA inhibition during GPVI mediated platelet activation causes aberrant cytoskeletal rearragments, diminishes integrin αIIbβ3 activation, and leads to the impaired platelet spreading.

P14
PROOXIDATIVE EFFECTS OF METAL IONS ELUTED FROM ORTHODONTIC APPLIANCES TO HUMAN CELL LINES

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Orthodontic appliances have been used for decades as a primary way of treating orthodontic problems and achieving an aesthetically pleasing look. Each appliance consists of metal parts: arches, brackets, ligatures and rings, which are connected to the teeth with a special glue. During the period of wearing, such device will cause some damage to the oral cavity and will show local aggression towards the mucosa. In addition to physical abrasion, chemical corrosion and the release of metal ions (nickel, chromium, titanium, cobalt, iron) is also stimulated. The aim of this research was to test prooxidative effects of metal ions released by appliance parts into saliva on four human cell lines: Hep G2, CAL 27, AGS and Caco2. Concentrated artificial saliva solutions (Toni-Zucchi artificial saliva, pH 4.8), in which parts of orthodontic appliances (brackets, arches, ligatures, rings and all parts together) were immersed for 3, 7 and 14 days, respectively, were used as test samples. The effect of all samples at four different concentrations (0.1; 0.5; 1.0 and 2.0) was examined on all tested cell lines. Prooxidative effects were tested using the DCFH-DA method and ϕX-174 RF I plasmid linearization assay. Gel Analyser was used for measurement of the band intensity. TIBCO Statistica program was use for data analysis. The elution time affects the content of metal ions of the saliva and biological effects can be measured. Metals in saliva samples caused a slight prooxidative effect on DNA - lower ratio of supercoiled and linear plasmid form compared to the negative control, but the ratio of the supercoiled/coiled DNA was higher in comparison to the positive control. The highest toxic effect of all tested samples was achieved on CAL 27 cell line. The results show that a certain amount of those effects on cells is visible, but that it is not worrying to the point of saying that orthodontic appliances are harmful for human health.

P15
OPTIMIZATION OF THE METHOD FOR DETERMINATION OF SELECTED EXOSOMAL MICRO RNAS IN LIQUID BIOPSY SAMPLES OF COLORECTAL CANCER PATIENTS

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Liquid biopsy offers a promising non-invasive approach for determination of the molecular profile of various tumours, including colorectal cancer (CRC). Exosomal microRNA represents one of most promising biomarkers for diagnostic screening, as well as for determining a response to therapy and evaluating disease progression and outcome. Since handling of liquid biopsy specimens is demanding and nonstandardized, in this study we aim to evaluate and optimise a method for determining selected microRNAs expression in exosome-derived microRNA samples from CRC patients. Exosomal microRNA was isolated from peripheral blood liquid biopsy samples obtained from 18 CRC patients using miRCURY Exosome Serum/Plasma kit and miRNeasy Serum/Plasma Advanced kit (Qiagen). cDNA was obtained using miRCury RT LNA kit (Qiagen) while expression of eight selected microRNAs was assessed with miRCURY LNA SYBR GREEN PCR system (Qiagen) on 7500 Real-Time PCR System (Applied Biosystems) with UniSp6 as internal control. Initial examination of expression of three microRNAs with four different starting sample volumes showed satisfactory results with the starting microRNA volume of 2 μl. miR-103a-3p was shown to be the best reference microRNA candidate, with Ct values ranging from 27.40 to 36.05 and ΔCt (corrected to UniSp6) from 13.36 to 22.31, since miR-1228-3p and miR-520d-3p were not detected. miR-125-3p was detected in three samples with Ct ranging from 33.58 to 35.86, and ΔCt from 20.01 to 22.40; miR-193a-3p was detected in seven samples with Ct ranging from 36.13 to 42.17, and ΔCt from 21.15 to 27.70; miR-210-3p was detected in 14 samples with Ct ranging from 31.15 to 42.91 and ΔCt from 17.71 to 29.18, whereas miR-19a-3p and miR-92a-3p were detected in all samples with Ct ranging from 32.20 to 32.67 and 23.87 to 31.96, and ΔCt ranging from 9.90 to 16.74 and 10.35 to 16.97, respectively. The described optimisation of the method for the detection of selected exosomal microRNAs in samples obtained by the peripheral blood biopsy will contribute to the further profiling of CRC utilizing liquid biopsy through comparison with expression in tumour tissue samples.

P16
ORTHODONTIC BRACKETS AND BANDS - A SOURCE OF HARMFUL METAL IONS?

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Brackets and bands make up a major part of fixed orthodontic appliances. Nowadays, an increasing number of (young) people are starting to wear orthodontic appliances, unaware of the potential harmful effects that its components could have on their organism. The release of metal ions from metal parts of orthodontic appliances is becoming a growing concern of the orthodontic world because, in addition to contact dermatitis, acute or chronic toxicity is also possible to occur. The aim of this study was to examine the effects of ions released from brackets and bands on the vitality and viability of yeast cells. Two strains of *Saccharomyces cerevisiae* yeast were used: W303 and wild type. Two types of experimental solutions with released ions were prepared – in artificial saliva and YPD media, with three different leaching periods (3,7 and 14 days). The change in the cell size and number, and type of growth, of both yeast strains, provoked by the 24 hours growth in differently prepared solutions, were measured by LUNA-FL™ (Logos, Biosystems) cell counter. Vitality was observed by the change in cell's metabolic activity using the LIVE/DEAD Yeast Viability Kit (Thermo Fisher Scientific, USA) and XTT test. Also, an intracellular oxidation determined by the amount of ROS generated in yeast, using the H_2DCFDA fluorescence method was measured. Changes/damages on the surface of used brackets and bands, were measured by SEM-EDS method. Metal ions released from orthodontic brackets and bands (Cr^{3+}, Ni^{2+}, Mo^{4+}, Fe^{3+} and even Co^{2+}) affect the vitality and viability of both strains of the yeast *Saccharomyces cerevisiae*. Ions released from bands show a higher potential to disrupt the viability of cells, while the ions from brackets show a higher potential for metabolic disturbances of both yeast strains. Strain W303 is more sensitive than wild strain (prototroph). There is a good correlation between brackets and bands surface damage, released metal ions and provoked changes in vitality and viability, and intracellular oxidation in yeast cells.

P17
CYTO/GENOTOXIC AND OXIDATIVE STRESS RESPONSE IN BISPHENOL A TREATED ONION (*Allium cepa* L.) ROOT MERISTEM CELLS

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The aim of this study was to test cytotoxicity, genotoxicity, and oxidative stress as a mechanism of toxicity of bisphenol A (BPA) using *Allium* test. *Allium* test is considered as a good model for detecting environmental mutagens and good correlation in cytogenetic studies of *Allium* test with other test organisms is confirmed. Onion bulbs (*Allium cepa* L.) were germinated for 24 h, and afterwards, rooted bulbs (n = 10) were exposed to BPA in concentration range 1-50 mg/L or to distilled water (negative control) for 72 h. Following 72 h exposure, morphometry (the root length and the fresh weight) was assessed. In root meristem cells mitotic index (MI) and frequency of micronuclei (MNi) were determined using a light microscope analysing minimum 500 cells per slide. In root homogenate as oxidative stress parameters the level of glutathione (GSH) and protein carbonyls (PC) was assessed spectrophotometrically. The results were analysed by use of one-way ANOVA followed by Dunnett's test. Statistical significance level was set at $p < 0.05$. After 72 h exposure to BPA (1-50 mg/L) a concentration-dependent decrease in the root length, root fresh weight as well as decrease in the percentage of MI of meristem cells was observed. Even the lowest concentration of BPA (1 mg/L) inhibited the root growth while the root fresh weight was reduced after exposure to BPA at 5 mg/L compared to negative control. The lowest BPA concentration (1 mg/L) decreased the percentage of MI as well as the number of cells in each of the cell cycle phase. With the increase of BPA concentration, an increase in MNi formation was observed. A decrease in GSH level was observed already after exposure to the lowest BPA concentration (1 mg/L) while an increase of PC was observed after exposure to BPA in concentration 10 mg/L. Based on the obtained results, it can be concluded that BPA induces cyto/genotoxic effects in root meristem cells and that oxidative stress is involved in its toxicity.
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P18
THE ANTITUMOR EFFECT OF NICLOSAMIDE ON BLADDER CANCER CELLS

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Niclosamide is an antihelmintic drug which acts as a mitochondrial uncoupler that inhibits oxidative phosphorylation and reduces ATP production. Recent studies suggest that niclosamide exhibits antitumor activity in breast and lung cancer cells. The antitumor effect of niclosamide is achieved through induction of apoptosis and inhibition of several signalling pathways, such as WNT/CTNNB1, mTORC1, STAT3, NFκB, Notch and Hedgehog. Bladder cancer is the most common neoplasm of the urinary system and it is the ninth most common cancer worldwide. Concerning detrusor muscle invasion, bladder cancer presents distinctive clinical outcomes and muscle-invasive bladder cancer (MIBC) has a very low survival rate even with immune checkpoint-directed therapy. The aim of our study was to examine the mechanism of the antitumor effect of niclosamide on bladder cancer cells. Our results demonstrate that niclosamide exerts an antitumor effect by inhibiting cell proliferation in addition to inducing apoptosis and autophagy in bladder cancer cells.

P19
MOLECULAR PATHWAYS MEDIATING SELECTIVE DEGRADATION OF MISFOLDED PROTEINS IN GLUCOSE STARVED YEAST *SACCHAROMYCES CEREVISIAE*

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Accumulation of misfolded proteins is associated with aging and diseases, such as Alzheimer's and Parkinson's. To prevent the negative impact of misfolded protein accumulation, cells have developed protein quality control pathways, including selective protein degradation by the ubiquitin-proteasome system (UPS). Previous research on the UPS has been predominantly done using proliferating cells, however, many cells in metazoan organisms, as well as unicellular microorganisms, spend a considerable part of their lifetime in a non-dividing or quiescent state, that is characterized by a distinct cellular organization and protein degradation pathways. For instance, in quiescent yeast *Saccharomyces cerevisiae* a large pool of the proteasomes is sequestered within cytoplasmic granules in an inactive form. Therefore, it has been unclear how misfolded proteins, which are normally proteolytic substrates of the UPS in proliferating cells, are managed by the quiescent cells. To address this question, we investigate the specificities of degradation-mediated protein quality control in quiescent cells, by studying glucose starved yeast *S. cerevisiae*. We expressed model misfolded proteins tGnd1 and stGnd1, and a respective wild-type protein Gnd1 in yeast and examined their stability. While wild-type protein was stable, misfolded variants were degraded fast in both proliferating and glucose starved cells. Next, we set to determine which molecular pathway mediates selective degradation of tGnd1 and stGnd1. Our data show that degradation of stGnd1 in glucose starved cells required E3 ubiquitin ligase Ubr1, similarly as in proliferating cells. In contrast, degradation of tGnd1 was solely dependent on Ubr1 in glucose starved cells, while in proliferating cells it was dependent on two E3 ubiquitin ligases, San1 and Ubr1. Together our results indicate that glucose starved yeast cells are able to recognize misfolded proteins and selectively target them for degradation. Furthermore, our finding that selective degradation of misfolded tGnd1 depends on a different set of E3 ubiquitin ligases in proliferating and glucose starved cells suggests that distinct pathways operate in protein quality control of certain types of misfolded proteins during proliferation and quiescence.

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P20
IMMUNOMODULATORY ROLE OF MAST CELLS IN BLADDER CANCER MICROENVIRONMENT

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Bladder cancer (BC) is the fourth most common cancer in men across Europe. Tumor microenvironment in bladder cancer plays an important role in its development and progression, therefore immunotherapy has a great potential in bladder cancer treatment and this was previously demonstrated with successful use of immune checkpoint inhibitors in muscle-invasive BC. As a part of an immune milieu, mast cells (MC) also play a role in the shaping of the tumor microenvironment, presenting a dichotomous role in cancers which is cancer-and stage-specific. The mechanisms by which MC influence the formation and progression of bladder cancer remain unclear. To address the role of MC in BC, we used the most common preclinical mouse model of bladder cancer induced with the carcinogen N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN). BBN-induced BC resembles the human basal-like BC subtype with great similarities in pathohistology in addition to high mutational burden, specifically Trp53 mutation. In this study, we investigated the role of MC in modulating the bladder cancer microenvironment by analysing MC-deficient mice ($Cpa3^{Cre/+}$) in the early stages of carcinogen administration (2 weeks) as well as at the stage of fully formed tumors (20 weeks). Pathohistological analysis of BBN-treated bladder specimen was performed to include a differential analysis of degenerative changes, inflammation and oedema at 2 weeks, as well as tumor invasiveness at 20 weeks. To further investigate the consequences of MC activation in BC onset and progression, the use of MC activators and stabilizers was implemented in BBN-induced BC mouse model.

P21
PROTECTIVE ROLE OF EOSINOPHILS IN BLADDER CANCER MICROENVIRONMENT

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Bladder cancer (BC) is the ninth most common malignancy worldwide. It is considered an immunogenic type of tumor responsive to immune checkpoint inhibitors. Tumor-infiltrating immune cells have prognostic and predictive roles which can help determine successful cancer therapy. Eosinophils contribute to the formation and shaping of the tumor microenvironment. Even though their exact role in tumors remains uncertain, eosinophils are important for tissue repair and the normalization of tumor vasculature. Administration of carcinogen N-butyl-N-(4- hydroxybutyl)-nitrosamine (BBN) in mice induces tumor formation which recapitulates the human basal-like subtype of BC. This is the most common mouse model of BC due to histological similarities and high mutational burden typical of human BC. In this study, we examined the role of eosinophils in the shaping of the BC microenvironment with the use of eosinophils-deficient male mice (ΔdblGATA1). The bladders of BBN-treated mice were analysed prior to tumor formation, after 2 weeks of BBN administration, as well as after the tumor formation (20 weeks). Pathohistological analysis was performed to characterize the degenerative and carcinogenic changes in bladder tissue in addition to the presence of inflammation, oedema and immune cell composition in tumors. Specific eosinophil stabilizers and activators were used during the BBN treatment to investigate the mechanism by which eosinophils impact tumor formation.

P22
THE STAR ASSAY: A NEW SENSITIVE, SELECTIVE, AND VERSATILE METHOD FOR ISOLATION OF DNA-PROTEIN CROSSLINKS

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DNA-protein crosslinks (DPCs) are toxic DNA lesions created by the covalent conjugation of proteins onto DNA molecule. They can be created endogenously or exogenously by exposing DNA or surrounding proteins to physical or chemical insults or created by faulty enzymatic reactions. Because of their bulky nature and chemical changes to DNA molecule, they interfere with DNA-linked processes such as replication and transcription. With the recent discovery of proteases and nucleases that participate in the repair of such lesions, the development of an accurate biochemical assay for DPC isolation became a priority for the further mechanistic understanding of their repair. We have developed a novel assay for direct quantification of DPCs, with high sensitivity to physiologically relevant treatment conditions. The STAR assay is a sensitive, selective, and versatile method for direct quantification of DPCs and is efficient in detecting physically, chemically, and enzymatically created DNA-protein adducts. The STAR assay is the first assay to demonstrate that nuclear presence is necessary for proteins to become cross-linked to DNA molecule. Employing the STAR assay, we have demonstrated that DPC repair is a two-step process, starting with the removal of the protein part of the lesion by dedicated DNA-dependent protease SPRTN, followed by the repair of the DNA molecule itself. The STAR assay is fast and simple and is applicable for studying the formation, repair, and biological significance of DPCs.

P23
THE COMPARISON OF EXPRESSION OF TARGET GENES *TNF-α*, *IFN-γ*, *cREL*, *CXCL10*, *CXCR3*, *PD-L1* AND PD-1 BETWEEN PRIMARY MEDIASTINAL B-CELL LYMPHOMA AND NON-TUMOUR B- AND T-CELLS

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Interactions between tumour cells and tumour microenvironment cells affect tumour development and progression. The aim of this research is to explore one of the possible mechanisms of communication between primary mediastinal B-cell lymphoma tumour cells and tumour microenvironment cells, specifically T lymphocytes. The first part of the research included tissue samples from patients diagnosed with primary mediastinal B-cell lymphoma and control non-tumour tonsils fixed with formalin and embedded in paraffin. Expression of target genes *TNF-α*, *IFN-γ*, *cREL*, *CXCL10*, *CXCR3*, *PD-L1* and *PD-1* in tumour samples were compared to the expression of these genes in non-tumour T lymphocytes and B lymphocytes isolated from non-tumour tonsils. No significant differences in the expression of target genes were observed, but this might be due to the fact the genes' expression could not be traced in time. The next part of the research will include the analysis of the expression of target genes in model cell lines following the treatment with various concentrations of TNF-α and IFN-γ proteins over various periods of time.

P24
MOLECULAR APPROACHES FOR MULTIMODAL IMAGING OF THE BRAIN

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Treatments and diagnoses are severely limited by our inability to visualize the biochemical processes underlying disease. Nowhere is this issue more limited than in the brain, due to the high level of complexity and presence of the blood-brain barrier preventing the vast majority of molecules from entering the brain. Our ability to understand the biochemical processes underlying disease processes is often limited to histological methods, which deliver a frozen snap-shot of conditions. Fluorescence is an invaluable tool with the ability for real-time studies, but largely limited to *in vitro* models that struggle to replicate the complex environment found in the body. New approaches are needed that combine biochemically specific *in vivo* real-time imaging with *in vitro* and *ex vivo* approaches. Here, we present our ongoing efforts to develop molecular imaging approaches for understanding the biochemical processes underlying disease, with a particular focus on the design and application on novel contrast agents for magnetic resonance imaging of the brain. Key applications include detection of key neuroinflammatory biomarkers, tracking drug delivery, and targeted agents for studying neurodegeneration. Our approach involves the synthesis of molecular and nanoparticle-based contrast agents, conjugation to targeting vectors (antibodies/proteins/polysaccharides), and application in a variety of *in vitro*, *ex vivo*, and *in vivo* models. We are particularly focused on the use of multimodal approaches, including spectroscopic magnetic resonance methods and correlation with microscopy and mass spectrometry. Through this combinatorial imaging approach, we aim to deepen understanding of biological processes across length scales - from subcellular to whole organ levels.

P25
PRENATAL PITFALLS OF 5-HYDROXYTRYPTOPHAN – A SEROTONIN PRECURSOR WIDELY OFFERED AS A SAFE ALTERNATIVE TO ANTIDEPRESSANTS

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Serotonin is a biogenic amine that serves as a signaling molecule in the mammalian organism. During brain development it regulates axon branching and synaptogenesis, hence a disbalance of the optimal concentrations in the particular brain regions could lead to structural and biochemical alterations resulting in the development of behavioral disorders. Being a mood-regulating neurotransmitter, serotonin serves as a target for the action of antidepressants – medications which, besides the beneficial actions, have many side-effects. The versatility of potential therapeutic effects, easy availability, and the convenience of unsupervised use have increased the popularity of the serotonin precursor 5-hydroxytryptophan (5-HTP) as a natural and safe alternative to antidepressants, especially for pregnant women. With an aim to investigate possible consequences of an exposure to 5-HTP during brain development, we treated an experimental group of Wistar rats with 25 mg/kg of 5-HTP, and a control group of rats with the same volume of saline, from gestational day 13 to post-natal day 21. In comparison to the control rats, 5-HTP treated pups displayed several statistically significant differences: reduced survival rate and neonatal body mass, increased blood serotonin concentrations, and increased separation anxiety. Several serotonin-related changes were also observed in adulthood: decreased number of serotonin-producing cells in the small intestine, decreased serotonin concentrations in the frontal cortex, overexpression of the serotonin-degrading enzyme in the brain stem, and increased exploratory behavior. The results implicate that perinatal treatment with 5-HTP induced long-lasting changes in serotonin homeostasis at the genetic, neurochemical, structural and behavioral levels. Considering that the period of brain development of rats during the treatment corresponds to the 2nd and 3rd trimester of human pregnancy, and that the applied 5-HTP concentration is comparable to therapeutic doses in humans, our results call for thorough examination of the potential neurological and behavioral effects in children prenatally exposed to 5-HTP treatment.

P26
PERFORMANCE OF TWO DIFFERENT METHODS FOR MICRORNA EXTRACTION FROM EXOSOMES OF COLORECTAL CARCINOMA PATIENTS

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Exosomes, extracellular vesicles, and their cargo, such as microRNAs, are among the most promising emerging biomarkers of various diseases, including colorectal cancer (CRC). Since their handling and isolation are not standardised, in this study we aimed to evaluate the performance of two different methods for isolation of microRNA from CRC patients' exosomes. Exosomes and exosomal microRNA were isolated from peripheral blood of 11 CRC patients using miRCURY Exosome Serum/Plasma kit and miRNeasy Serum/Plasma Advanced kit (Qiagen) or Total Exosome isolation reagent and Total Exosome RNA and Protein isolation kit (Invitrogen). Concentration of microRNA was determined using DS-11 spectrophotometer (Denovix), and quality of the samples was determined on Bioanalyzer 2100 (Agilent). cDNA for microRNA expression analysis was obtained using miRCURY RT LNA kit (Qiagen), while expressions of mir-103a-3p and mir-19a-3p were assessed with miRCURY LNA SYBR GREEN PCR system (Qiagen) on 7500 Real-Time PCR System (Applied Biosystems) with UniSp6 as internal control. Wilcoxon matched pairs test was used for statistical analysis in GraphPad Prism 6.01 software. There was no significant difference in RNA concentrations between the two methods ($P=0.102$), and both methods isolated RNAs less than 200 nt in size. ΔCt (corrected to UniSp6) for mir-103a-3p ($P=0.123$) and mir-19a-3p ($P=0.413$) was similar between the two methods. mir-19a-3p was also corrected to mir-103a-3p (usually used as reference gene) and we found no difference between the used methods ($P=0.083$). We concluded that both assessed methods showed similar performance and could be used for determination of microRNA expression from CRC patients' exosomes.

P27
DNA METHYLATION PROFILE IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE FROM TWO DIFFERENT REGIONS IN CROATIA.

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Chronic obstructive pulmonary disease (COPD) is a third leading cause of death worldwide, presenting as an abnormal inflammatory response of the lungs to inhaled noxious particles or gases. The influence of tobacco smoke can lead to epigenetic changes such as DNA methylation that represents a reversible regulatory gene modification. Previous studies has shown that global DNA methylation could be useful as a biomarker in COPD. The goal of this work was to investigate if there is a difference in the global DNA methylation between the control group and the group of COPD patients from Osijek and Zadar respected to different climate and lifestyle. Based on the calculation of the mean effect in different numerical variants between the two independent groups, 136 patients with COPD, 69 patients treated in Clinical Hospital Center Osijek, and 67 patients treated in General Hospital Zadar, were included in this research. The control group consists of 64 subjects, 32 from each center. DNA for global DNA methylation measurement was isolated from leukocytes using the commercial set *NucleoSpin Blood L (Macherey Nagel, Germany)*, and after that absolute quantification of global DNA methylation was done with colorimetric method by commercial set *Methylated DNA Quantification Kit (Colorimetic) (ab117128) Abcam (Cambridge, United Kingdom)* according to the manufacturer's instructions. The obtained results were analyzed using various statistical methods. The COPD patients were found to have lower values of global DNA methylation than the control group, but a satistically significant difference was found only between male patients with COPD and male control subjects in Zadar. Further research on global DNA methylation on a larger sample of healthy and COPD subjects from both observed regions should be done.

P28
VPS34 LOCALIZES TO THE NUCLEOLUS AND MEDIATES EARLY MEGAKARYOPOIESIS *VIA* RIBOSOMAL BIOGENESIS

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Platelets, the smallest blood cells, are produced in the bone marrow by their precursors, megakaryocytes (MKs). One of the most characteristic features of MK maturation is a substantial increase in size, together with the polyploidization of the nucleus. At the end of the maturation process, MKs generate prolonged cytoplasmic protrusions, proplatelets, which extend through the vascular sinusoids of the bone marrow and release platelets into the bloodstream. Recently, we have shown that phosphatidylinositol 3-monophosphate (PI3P), a small membrane phospholipid mainly produced by the Vps34 kinase, significantly contributes to the final steps of MK maturation by late endosomal/lysosomal fusion to the plasma membrane mediating proplatelet formation. Vps34 is ubiquitously expressed sole member of the class III PI3-kinase subgroup. In the present study, we demonstrate for the first time that Vps34 kinase localizes to the nucleolus of primary mouse bone marrow-derived MKs. Vps34 colocalized with upstream binding factor (UBF), a transcription factor required for ribosomal RNA (rRNA) expression that controls a rate limiting step of ribosome biogenesis. Vps34 nucleolar localization was further observed in human mononuclear cells and in the Balb3T3 cell line. In addition, pull-down of GFP-UBF co-immunoprecipitated Vps34 from Balb3T3 cells. In MKs, inhibition of RNA polymerase I (Pol I) with actinomycin D abolished Vps34 nucleolar localization and Vps34 binding to UBF. Moreover, pharmacological inhibition of Vps34 reduced the production of nascent RNA and inhibited the transcription of 45S rRNA, indicating aberrant ribosomal biogenesis. Additionally, inhibition of RNA Pol I function with Vps34 inhibitor correlated dose-dependently with the decreased cellular size and the expression of MK maturation marker GPIb, indicating reduced MK development. Taken together, these data indicate that Vps34 might play an important, still undescribed, role in the nucleolar function that could control the early stages of MK development *via* ribosome biogenesis.

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P29
OPTIMIZED PROTOCOL FOR WHOLE-MOUNT IMMUNOHISTOCHEMISTRY IN ZEBRAFISH EMBRYOS (*Danio rerio*)

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In the last couple of decades, the use of zebrafish model in biomedical and toxicological research is exponentially increasing. Many studies investigate the physiological/ecotoxicological function of different proteins localized across different zebrafish organs/tissues. Whole-mount preparations coupled with fluorescence microscopy are widely used for this task. To date there is no single approach for conducting whole-mount immunohistochemistry in zebrafish embryos. In this study we used zebrafish embryos (96 hpf) to determine key parameters in preparation of zebrafish embryos for the use in whole-mount immunohistochemistry using two antibodies detecting Na+/K+ATPase and ZN-8 membrane proteins. Two fixatives (4% paraformaldehyde/4% PFA and Dents fixative) and three different incubation temperatures (37 °C/30 min, 70 °C/15 min, 95 °C/5 min) were tested to optimize the whole-mount immunohistochemistry approach. Results showed that the use of Dents fixative compared to 4% PFA fixative enables better binding of the tested primary antibodies and reveals the known localization of Na+/K+ATPase and ZN-8 proteins in zebrafish embryos. However, the use of different incubation temperatures was not crucial for the binding of the tested antibodies. Overall, this work presents a unified approach for whole-mount immunohistochemistry that can be used to test optimal conditions for localization of proteins in zebrafish embryos.

P30
IN A PURSUIT OF NEW GLYCAN FLUORESCENT LABEL FOR NEGATIVE MS MODE

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Glycans are molecules that contain multiple monosaccharides linked by glycosidic bonds and often bind to proteins or lipids to form various glycoconjugates. Rising awareness of the vital role that glycosylation plays in protein functioning governs the need for further advances in glycan analysis. Because free glycans lack chromophore or fluorophore properties, and do not ionize well, they are often derivatized, to facilitate their separation or detection, and to enhance the sensitivity of the analysis. Released glycans are usually derivatized using a fluorescent tag, which enables their optical detection in LC profiling. Some fluorescent labels can also improve ionization efficiency, which then facilitates MS detection. For this reason, there is a need to design fluorophores that will contribute more to the fluorescence and ionization of glycans and the need to quantify these contributions to improve glycan analysis methods in negative MS mode as these methods are more informative than methods involving positive MS mode, allowing for a less ambiguous elucidation of detailed glycan structures. Therefore, eleven fluorescent labels of different chemo-physical properties were chosen in order to study the influence of label hydrophobicity and presence of a negative charge on glycan ionization in negative MS mode. N-glycans released from IgG sample were labeled with one of the eleven labels, purified with HILIC-SPE and analyzed with HILIC-UHPLC-FLR-MS. The results show that hydrophobicity significantly contributes more to signal-to-noise intensity ratios in the mass spectrum than the negative ion charge in the case of negative MS mode. With this in mind, further research and development of fluorophores that will be suitable for glycan analysis in the negative MS mode are proposed.

P31
PREDICTION OF QUALITY OF BISCUITS WITH GRAPE POMACE ADDITION BY A MODIFIED METHOD OF FLOUR SOLVENT RETENTION CAPACITY TESTING

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Grape pomace is a by-product of wine production and is rich in natural antioxidants, fiber and phenolic components. Therefore, it is used, among other things, in the development of various food products that acquire new functional properties through the addition of grape pomace. In this paper, the influence of using different sample amounts (5 g, 1 g and 0.2 g) on the prediction of the quality of biscuits made from wheat flour with the addition of *Cabernet Sauvignon* grape pomace was studied. The methods used were Solvent Retention Capacity (SRC) and Alkaline Water Retention Capacity (AWRC). These are methods used to test the quality of wheat flour, measuring the ability of the flour to retain various solutions after centrifugation of the suspension. The test results showed that there is a significant correlation between the retention values of the solutions used, regardless of the sample weight used. The best results for the retention capacity of flour were obtained for samples of 5 g. The obtained results show that we can use with high statistical significance ($p < 0.01$) the AWRC, SRC-H_2O, SRC-Sucrose and SRC-Na_2CO_3 methods to predict the biscuit volume, the SRC-Sucrose and SRC-Na_2CO_3 methods to predict the width and the SRC-Sucrose method to predict the spreading factor. The prediction of the height of the biscuit with the addition of *Cabernet Sauvignon* grape pomace can be performed with high statistical significance ($p < 0.01$) for 1 g samples using the AWRC method. According to the obtained results, SRC and AWC are methods for determining the quality of wheat flour that can be used to predict the quality of biscuit with grape pomace addition when we need quick results or when we have a small amount of samples.

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P32
INTERACTIONS AND KINETICS OF ACETYLCHOLINESTERASE WITH SELECTED PESTICIDES

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Organophosphate compounds (OPs) comprise an important class of pesticides, chemical agents used in agriculture for management of diseases and pests. Due to their widespread and frequent use as insecticides and herbicides during the last 80 years, they are considered as an increasing environmental and public health concern. The uptake of contaminated food and water or exposure through agricultural activities is harmful to humans and other species because of their low target selectivity and severe toxicity. OPs act mainly by inhibiting enzyme acetylcholinesterase (AChE) which hydrolizes essential neurotransmitter acetylcholine (ACh), leading to a paralysis of cholinergic synaptic transmission in the central and peripheral nervous system which may induce convulsions, respiratory failure, loss of consciousness and death. In this study we investigated *in vitro* and *in silico* kinetics of inhibition and interactions of human AChE with four OP herbicides (anilofos, bensulide, butamifos and piperophos) and compared them to a potent AChE inhibitor, insecticide ethoprophos. Their overall rate constants of inhibition (k_i) were up to 300-fold lower than k_i of ethoprophos and they achieved a maximum inhibition of 85% of enzyme activity. Since medical therapy for restoration of AChE activity in case of poisoning includes oxime reactivators which dephosphylate AChE's active center, we also evaluated potential of standard pyridinium oximes (2-PAM and HI-6) and a novel zwitterionic oxime RS194B to reactivate selected herbicide- and ethoprophos-inhibited AChE. Our results provide insight into neurotoxic effects of pesticides and facilitate further development of antidotes directed against OP pesticide poisoning.

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P33
ACUTE PLATELET NEEDS MEDIATED THROUGH INTERLEUKIN-1-ALPHA AFFECT VPS34 KINASE IN MOUSE MEGAKARYOCYTES

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Megakaryocytes (MKs) are the largest cells in the bone marrow (BM) and precursor cells of blood platelets. While proplatelet formation is the dominant model of physiological platelet release, in conditions of acute platelet needs MKs undergo a rupture process mediated mostly by interleukin-1-alpha (IL-1α) *in vivo*, releasing high number of platelets to rapidly compensate their low counts. Vacuolar sorting protein 34 (Vps34) is a kinase that catalyzes the production of phosphatidylinositol-3-monophosphate (PI3P) and has a role in vesicular trafficking. Although mostly localized in the cytoplasm and plasma membrane, our recent work revealed that Vps34 also localizes within nucleoli with upstream binding factor (UBF), a nucleolar transcription factor (Bertović *et al*, unpublished). Nucleoli are small membranelles compartments and sites of ribosome biogenesis involved in cellular stress response. Here, we investigated Vps34 localization in *in situ* mouse BM MKs and in conditions of high platelet demand *in vivo* and *in vitro*. Firstly, we depleted circulating platelets in mice using an anti-GPIbα antibody which was confirmed by flow cytometry. Confocal microscopy analysis of MKs in BM sections showed Vps34 staining in cytoplasm and nuclei, within DAPI-void areas resembling nucleoli, indicating Vps34 localization in nucleoli *in vivo*. In platelet-depleted sections, a significant increase in Vps34 staining was observed, however, in the cytoplasm. *In vitro* culturing of BM MKs in the presence of thrombopoietin (TPO), the major regulator of thrombopoiesis, together with IL-1α resulted in larger MKs as compared to only TPO-cultured MKs. Combined TPO/IL-1α treatment increased the number of MKs with visible UBF/Vps34 colocalization and the number of colocalizing foci within nucleoli per MK, indicating higher nucleolar activity as compared to only TPO-cultured MKs. The presence of a potent and selective Vps34 inhibitor (Vps34-IN1) dose-dependently reduced the size and expression of MK maturation marker GPIbβ in both TPO and TPO/IL-1α conditions. Interestingly, this was coupled with reduced UBF/Vps34 colocalization mostly in TPO/IL-1α-derived MKs. Taken together, these results point to a new role of nucleolar Vps34 in MK response to cellular stress induced by high platelet needs.

P34
CYTOTOXICITY AND GENOTOXICITY OF TYROSINE KINASE INHIBITORS IN EXPERIMENTAL MODEL WITH ZEBRAFISH LIVER CELLS

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Over the past two decades, tyrosine kinase inhibitors (TKIs) have been increasingly used to treat various types of malignancies. TKIs are being excreted through patients' body fluids and thus enter the aquatic environment through wastewater treatment plant effluents, posing a potential risk to non-target aquatic organisms. In our study we investigated cytotoxic and genotoxic effects of three frequently used TKIs, namely sorafenib (0.25-4 μM), regorafenib (0.5-4 μM), and dasatinib (0.08-0.12 μM) on zebrafish (*Danio rerio*) liver cell line (ZFL). Cytotoxicity was determined using the MTS assay and cell cycle analysis by flow cytometry. Genotoxicity was assessed using two modifications of the micronucleus assay: (i) flow cytometric enumeration of micronuclei, and (ii) cytokinesis block micronucleus (CBMN) assay. After 72-h exposure, ZFL cell viability was reduced at the higher concentrations of sorafenib (4 μM), regorafenib (4 μM), and dasatinib (0.06 μM) tested, along with cycle arrest in the G0/G1 phase. However, the TKIs did not affect the genomic instability of the cells, as no micronuclei formation was detected under the applied experimental conditions. Moreover, none of the TKIs induced the formation of nucleoplasmic bridges and nuclear buds. In conclusion, the results demonstrated that TKIs affect cell proliferation by arresting ZFL cells in G0/G1 phase but have no genotoxic effect.

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P35
SYNTHESIS AND BIOLOGICAL EVALUATION OF 4-AMINO-7-CHLOROQUINOLINES AS NOVEL REVERSIBLE INHIBITORS OF HUMAN CHOLINESTERASES

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4-Aminoquinolines are a class of compounds known mostly as antimalarials, among which chloroquine, hydroxychloroquine and amodiaquine are the most used drugs. These compounds also have anti-infective and anti-inflammatory activity and have been successfully used for the treatment of certain autoimmune diseases. Due to their ability to cross the blood-brain barrier, they have attracted attention as potentially CNS active agents. In our pilot study, we determined the ability of eight 4-aminoquinolines differing in the substituents attached to the C(4)-amino group and C(7) to inhibit the activity of human acetyl- and butyrylcholinesterases, the main targets in developing drugs for treating Alzheimer's disease (AD). In continuation of our studies, we pointed out two major goals: the first to explore the impact of length, conformational flexibility, steric demands, basicity and electronic density of the linker between aminoquinoline moiety and terminal amino group, and the second to explore the impact of different *N*-terminus substituents, as are adamantane or substituted benzyl, on the inhibition of human acetyl- and butyrylcholinesterase. Starting from the corresponding 4-chloroquinoline and using different alkyl- (normal or branched) or aryl-diamines, in various solvents (net diamine, phenol, NMP or EtOH) and heating in the inert atmosphere, corresponding 4-aminoquinolines were obtained. Further modifications of the terminal amino group by reductive amination yielded two series of derivatives that have *N*-benzyl or *N*-adamantyl moieties. Alternatively, previously synthesized side chains were directly coupled with the corresponding 4-chloroquinoline. Structure-activity analysis revealed that *n*-octenyl as a linker is favourable for the inhibition of both acetyl- and butyrylcholinesterase, without pronounced selectivity toward either. Docking studies rationalized our kinetic results and provided us insight into potential ligand-enzyme interactions. An *in silico* evaluation of ligand's ability to cross the blood-brain barrier and their drug-likeness was also evaluated.

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P36
THE ANTITUMOR EFFECT OF SHIKONIN IN MOUSE URINARY BLADDER CANCER MODEL

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Bladder cancer is one of the most common malignancies in man. Although great progress has been achieved in terms of bladder cancer management, survival rates for muscle-invasive bladder cancer are still poor, which warrants the search for new therapeutic approaches. Shikonin, a natural compound isolated from the roots of the plant *Lithospermum erythrorhizon*, has long been used in traditional Chinese medicine to treat dermatitis, wounds, measles and bums, and today is known for its anti-inflammatory, antioxidant and antitumor effects. In this study, the antitumor effect of shikonin was studied on murine bladder cancer induced by carcinogen N-butyl-N-(4- hydroxybutyl)-nitrosamine (BBN). Our results demonstrate that shikonin slows down tumor growth by induction of necroptosis and apoptosis and by changing immune cell composition in the tumor microenvironment. Altogether, shikonin is a potential new therapeutic approach in bladder cancer management.

P37
THE cfDNA FRAGMENTATION PROFILE OF TESTICULAR TUMORS

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While being the gold standard in oncology tissue biopsies have multiple issues in need of resolving. They are invasive, have difficulty detecting tumor heterogeneity, and certain tumors are simply unapproachable or too risky to perform biopsies on. Testicular tumors are such a tumor, where tissue biopsies can not be performed due to the risk involved with the procedure, leading to potentially unnecessary surgical procedures. 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.

P38
THE ROLE OF GCNA (ACRC) PROTEIN IN THE DNA-PROTEIN CROSSLINK REPAIR IN ZEBRAFISH

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DNA is a molecule in a chemically reactive environment and under these conditions various DNA lesions are constantly formed in cells. Proteins that interact with DNA or are in its proximity can become irreversibly covalently bound to DNA and form lesions called DNA-protein crosslinks (DPCs). DPCs can be induced by exogenous (UV and ionizing radiation, transition metals, chemotherapeutic agents) and endogenous factors (reactive oxygen and nitrogen species, aldehydes, DNA helix alterations). They affect cellular processes that include DNA (DNA replication, transcription, repair, recombination, and chromatin remodeling) and are obstacles to genome stability maintenance. Several groups have characterized the proteins Wss1 in yeast and SPRTN in metazoans as the first identified proteases involved in DPC repair. At the cellular level, impaired DPC repair can lead to DNA double-strand breaks, genomic instability, and consequently cell death, while at the organismal level it can cause cancer, premature aging, and neurodegenerative diseases. Recent studies have shown that there is a second potential DPC protease in metazoans: GCNA (germ cell nuclear acidic) or ACRC (acidic repeat-containing). Phylogenetic analysis of the SprT and WLM domains and structural modeling of the protease cores of SPRTN and GCNA have revealed similarities between these two proteins. The aim of our study was to determine whether GCNA is involved in DPC repair *in vivo* and whether its putative protease core is important for GCNA function. We used zebrafish (*Danio rerio*) as a model organism, CRISPR/Cas9 gene manipulation to generate zebrafish mutant strains, and the RADAR (rapid approach to DNA adduct recovery) method to isolate DPCs from mutant embryos. We introduced a mutation in the putative protease active site of GCNA that includes a deletion of E451 (putative catalytic glutamate). Our results showed that the mutant embryos had 100% mortality within the first 24 hours of development due to maternal effect. Primarily, they had an increased total amount of cellular DPCs. Our study demonstrates the importance of GCNA for embryonic development and DPC repair at the organismal level, as well as the importance of the putative protease core for GCNA function.

P39
INFLUENCE OF ENGINEERED DISULFIDE BRIDGE ON PROPERTIES OF METAL SENSING TRANSCRIPTION FACTOR MntR FROM *Bacillus subtilis*

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Transition metals are essential for a variety of biological processes in the living cell: they serve as centres of enzyme catalysis, structural constituents of proteins and stabilizers of protein structure. However, they can be toxic if present in high concentrations. Manganese is an essential metal that is used in bacteria for basic cellular processes such as DNA replication and resistance to oxidative stress. Its cytoplasmic concentration and effect on virulence in pathogens varies greatly between species, but it has been identified as necessary for survival and infection in multiple bacteria. The most common mechanism for regulating metal ion homeostasis in bacteria involves metal sensing transcription factors. These proteins reversibly bind metal ions, which modulate their DNA binding affinity. The main goal of our research is to understand details of the molecular mechanism through which bacterial metallosensors accomplish their role in regulating the concentration of manganese ions. We started our investigations with the manganese-responsive metallosensor MntR from *B. subtilis*. This protein is a homodimer both in its apo- and metal-bound form, and binds two metal ions per monomer which form a binuclear cluster at the dimer interface. Molecular dynamics simulations have shown that even without metal ion present, DNA binding and dimerization domains of the protein come in close contact (that is usually stabilized by Mn^{2+}). To see if we can stabilize this contact differently, we created a double mutant D8C/E99C of *B. subtilis* MntR with the goal of establishing disulfide bridge that would replace Mn^{2+} ion in connecting these domains. Both native and mutant protein were overexpressed in *E. coli* cells and purified *via* affinity chromatography and size-exclusion chromatography. Their secondary structure properties and DNA binding properties were determined *via* CD and DSC measurements. Obtained results are discussed, with aim of better understanding of MntR mechanism of action.

P40
PROTEOLYTIC PROCESSING AND COVALENT BINDING OF YEAST CELL WALL PROTEIN Scw10

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The proteins Scw4, Scw10, and Scw11 form a group of putative glucanases found in the yeast cell wall. *SCW4* and *SCW10* are paralogous genes with very high sequence identity. It has been previously shown that Scw4 undergoes complex proteolytic processing that affects its biological function. Scw4 is first processed by the Kex2 protease in the Golgi and then by yapsin proteases located in the cell membrane and/or cell wall. This two-step processing has been reported to affect its activity and cell wall binding. Scw10 is known to have a Kex2 recognition site, but its proteolytic processing has not been thoroughly investigated. It has also been shown that both Scw4 and Scw10 can form non-covalent and covalent bonds with the wall. The covalent bond is of particular interest because it differs from other previously known bonds in the cell wall. Although it has similar properties to the bonds formed by Pir family proteins, both Scw4 and Scw10 lack the typical Pir binding sequence. Therefore, the binding sequence and mechanism of Scw4 and Scw10 remain unknown. Here we present results obtained by studying the processing of Scw10. They show that the processing is similar in complexity to that of Scw4 but differs in terms of processing sites. The role of several putative Scw4/Scw10 binding sequences in forming a covalent bond to cell wall structures has been discussed.

P41
NEUROPATHY TARGET ESTERASE-RELATED ENZYME AND ITS KINETIC CHARACTERIZATION

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The object of our study was the kinetic characterization of the neuropathy target esterase-related enzyme (NRE, PNPLA7) in different cell lines. NRE enzyme is a member of the PNPLA (*patatin-like phospholipase domain containing proteins*) family and a membrane protein associated with endoplasmic reticulum or lipids droplets and is highly expressed in insulin-targeted tissues. Its crystal structure has not been resolved, yet, but according to the sequence analysis and homology modelling it consists of an N-terminal domain with a transmembrane segment and three cyclic nucleotide binding sites, and a C-terminal domain where the active site with catalytic dyad Ser-Asp is located. NRE has been identified as a lysophospholipase that hydrolyses *sn*-1 esters in lysophosphatidylcholine and lysophosphatidic acid, but its physiological roles are not fully understood. NRE enzyme shares high homology with neuropathy target esterase (NTE, PNPLA6), which suggests that NRE might also be a target of highly toxic organophosphorus compounds (OP) implying involvement of NRE in OP caused pathological conditions such as poorly defined intermediate myopathy syndrome. In our research, we used two commercially available donors of human skeletal muscle cells and the liver hepatocellular carcinoma cell line HepG2 to kinetically characterize NRE activity using known substrate *p*-nitrophenyl valerate (*p*-NPV). Firstly, we confirmed presence of NRE in myotubes, differentiated human skeletal muscle cells, and HepG2 by Western blot. Afterwards, esterase activity measurement was optimised for cell lysates. In order to confirm esterase activity, we incubated cell lysates with different OP compounds and measured esterase activity with *p*-NPV. Our results showed a difference in the NRE activity in different donors of human skeletal muscle cells, probably due to difference in the protein expression. Also, we have concluded that optimal *p*-NPV concentration for activity measurement is 1 mM with the K_m value of 0.4827 mM. Since little is known about this enzyme's physiological role and biological relevance, any findings would most certainly contribute to the understanding of the importance of NRE, which still calls for a detailed clarification.

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P42
THALLIUM TOXICITY AND POTASSIUM PROTECTIVE EFFECT *IN VITRO*

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Thallium is a naturally occurring, highly toxic trace element. Human exposure to thallium is mainly connected to occupational exposure, environmental contamination and food accumulation. The exact mechanism of thallium toxicity is still poorly understood, however its toxic effect is often connected to disruption of potassium-dependent processes and development of oxidative stress. The aim of this study was to access the cytotoxic effect of thallium on several cell culture lines and relate the obtained results with its specific toxicity to a particular organ. In addition, possible protective effect of potassium on toxicity of thallium was also examined. Human keratinocytes (HaCaT), human hepatocellular carcinoma (HepG2), porcine kidney epithelial cells (PK15), human neuroblastoma (SH-SY5Y) and Chinese hamster lung fibroblast cells (V79) were treated with thallium (I) acetate in a wide concentration range (3.9-500 μg/ml) for 24h, 48h and 72h. Based on cytotoxicity results, four concentrations (15.6, 31.25, 62.5, 125 μg/ml) were chosen to be tested in combination with potassium acetate (500 μg/ml) to assess their competitive interaction during 24h treatment. Cell morphological changes were monitored on inverted microscope, and cytotoxic effect was assessed by 3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium bromide (MTT-test). Thallium toxicity for each cell line tested was both, time- and dose-dependent. For each measurement point SH-SY5Y cells had the lowest IC_{50} values and appeared to be most sensitive, while HepG2 were most resistant to thallium exposure. Simultaneous treatment of cells with thallium and potassium (I) acetate resulted with higher viability compared to treatment with thallium (I) acetate alone. Results of our study indicate that tissue origin contributes to cell's susceptibility to thallium toxicity. Different toxic effect of thallium could be attributed to cell's antioxidant capacity as well as transport systems through which thallium and potassium compete for entering the cell. The obtained results point to importance of monitoring thallium concentrations in the environment and call for further research into the mechanism of its toxicity.

P43
CD15s EXPRESSION IN DIFFERENT CD44CD24 BREAST CANCER CELL SUBPOPULATIONS AFTER THIENO[2,3-*b*]PYRIDINE TREATMENT

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Depending to the extent of CD44/CD24 ratio in breast cancer cells, they are more related to cell proliferation and tumorigenesis then to metastasis. $CD44^+CD24^-$ cells are well known as cancer stem cell subpopulation. Recently, three different breast CD44CD24 non-cancer stem cell subpopulations have been described as relevant for cancer proliferation and drug resistance: $CD44^-CD24^+$ and $CD44^-CD24^-$ as epithelial, and $CD44^+CD24^+$ as hybrid cells with both mesenchymal and epithelial features. The aim of this study was to investigate novel anticancer compound 1, 3-amino-5-oxo-*N*-naphthyl-5,6,7,8-tetrahydrothieno[2,3-*b*]quinoline-2-carboxamide, effect upon expression of CD15s, the ligand that enables cancer cell to metastasize, in three different triple negative breast cancer cell subpopulations. MDA-MB-231 triple negative breast cancer cells were incubated 48 h with compound 1 alone or in combination with paclitaxel. Flow cytometric analysis was performed to detect the number of cells in each subpopulation as well as their CD15s expression and percentage of each $CD15s^+$ subpopulation. Compound 1 decreased the number of $CD44^-CD24^+$ cells but without statistical significance (p=0.123). $CD44^+CD24^+$ subpopulation was halved (p=0,021). Compound 1 lowered CD15s expression only in $CD44^-CD24^+$ epithelial cells. Treatment with paclitaxel was without effect. In addition to our previous findings of compound 1 effects in $CD44^+CD24^-$ cancer stem cells, this study proved its anticancer effects in $CD44^+CD24^+$ and $CD44^-CD24^+$ breast cancer cell subpopulations, superior in relation to paclitaxel.

P44
DIPEPTIDYL PEPTIDASE III INHIBITION TESTS BY METAL DICATIONS

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It has long been known that the peptidase activity of human dipeptidyl peptidase III (hDPP III) is inhibited by excess zinc ions. The aim of my paper is to determine the effects of different concentrations of metal dication: Zn^{2+}, Mn^{2+}, Co^{2+} and Cu^{2+} on the activity of hDPP III and to identify the inhibitory binding site of the metal. The existence of an inhibitory binding site was hypothesized based on a decrease in hDPP III activity at higher zinc concentrations and the similarity of hDPP III active site with those of carboxypeptidase A and thermolysin in whose crystallographic structures binding of another metal ion was observed in the immediate vicinity of the catalytically active ion. Inhibition of hDPP III by metal dications (Zn^{2+}, Mn^{2+}, Co^{2+} and Cu^{2+}) is being investigated using various experimental and computational methods. For the purposes of experimental research, we prepare holoenzymes by incubating hDPP III with different concentrations of Zn^{2+}, Mn^{2+}, Co^{2+} and Cu^{2+} ions. In the samples thus prepared, metal-protein stoichometry is determined by high-resolution mass spectrometry with inductively coupled plasma (HR-ICP-MS) and microcalorimetric methods (isothermal titration calorimetry, ITC). Using the stopped-flow method, we monitor the decrease in hDPP III enzymatic activity caused by excess metal dications. Finally, using molecular dynamics combined with free binding energy calculus and quantum mechanical - molecular mechanical calculations we identified the zinc binding site and its effect on hDPP III structure and dynamics. Also, following the path of zinc ion exchange, we concluded that, as in the case of anthrax lethal factor, it is the so-called associative mode of modification described by relations:

. enzyme – metal A + metal B ↔ enzyme – metal A – metal B

i. enzyme – metal A – metal B ↔ enzyme – metal B +metal A .

The results of this study allowed us to propose a mechanism by which the inhibitory zinc ion reduces enzyme activity.

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P45
DESIGN, STRUCTURE PREDICTION AND MOLECULAR DYNAMICS SIMULATIONS OF THE NEW VACCINE CANDIDATES AGAINST INFLUENZA INFECTION

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The rational vaccine design approach involves antigen, delivery system and adjuvant integration with the aim of immune response induction. The new vaccine candidate(s) against influenza infection consists of human parainfluenza virus type 2 nucleoprotein (HPIV2 NP) fused with influenza epitopes. HPIV2 NP binds viral RNA, forming filamentous nano-sized particles, therefore displaying delivery system and adjuvant properties. HPIV2 NP is used as an adaptive noninfectious carrier of antigenic determinants. Selected antigenic determinants from influenza virus include those from the stalk domain of the hemagglutinin (HA) protein and the whole extracellular domain of the M2 protein. Natural HA epitopes with minimal (stalk20) and maximal (stalk55) length are considered. The artificial epitope of stalk20X2 was designed by linking two short stalk20 epitopes together. Retaining the active conformation of the pathogen epitope within the structure of the recombinant protein is a vital aspect to consider for developing promising epitope-based vaccine candidates. Here, we investigate the conformational behavior of the incorporated influenza epitopes within the fused nucleoprotein using computational methods. Firstly, comparative modelling was carried out to obtain HPIV2 NP 3D structure model using HPIV5 NP structure as a template, and HPIV2 NP 3D structure models with incorporated influenza epitopes. Analyzing the HPIV2 NP structure model, the spot for inserting pathogen epitopes was chosen to maximize exposure for interaction with the protective immune system. Computational analysis was employed to evaluate the final fusion construct by picking the one with the highest overall score out of all the predicted structures. Molecular dynamics (MD) simulations analysis provided a closer insight into the conformational dynamics behavior of the inserted epitope and overall protein structure stability. Computational evaluation of the potential influenza vaccine candidates is performed in order to determine candidates suitable for in vivo expression and further structural analysis.

P46
STUDY OF THE INTERACTION OF DIPEPTIDYL PEPTIDASE 3 AND SH2 DOMAIN-CONTAINING PROTEIN 3C

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Dipeptidyl peptidase 3 (DPP3) is a zinc metallopeptidase that cleaves dipeptides from unsubstituted amino-termini of 3 to 10 residues long peptides *in vitro*. Its physiological role is still unconfirmed, however, it is presumed that it has a role in the final stages of protein turnover in cells and in the regulation of blood pressure and pain. These proposed functions are related to its peptidase activity, while it also has a role in the NRF2-KEAP1 oxidative stress response signaling pathway through its interaction with KEAP1 protein. SH2 domain-containing protein 3C (SH2D3C) was identified as a putative interactor of DPP3 in SILAC-MS screening of HEK293T cells stably overexpressing HA-tagged DPP3. SH2D3C is one of three members of the NSP family of proteins which contain both, SH2 domain and a domain similar to guanine nucleotide exchange factor domains of Ras family GTPases (Ras GEF-like domain). SH2D3C interacts with the phosphorylated cytoplasmic domain of small Ras family GTPases, EphB2, R-Ras, and Rap1A, but does not exhibit GTP exchange activity *in vitro*. It is involved in the regulation of cell adhesion and migration, tissue organization, and the regulation of the immune response. Therefore, the interaction of DPP3 and SH2D3C may be a link between these processes and the response to oxidative stress. The interaction between DPP3 and SH2D3C-isoforms 2 and 3 and C-terminal Ras GEF-like domain was confirmed by GST-DPP3 pulldown, indicating that it binds DPP3 through its C-terminal domain. Molecular docking revealed two representative modes of binding, one in the interdomain cleft of DPP3 and another resembling KEAP1-DPP3 binding. The interaction was confirmed by bimolecular fluorescence complementation (BiFC) in NIH 3T3 cells and detected in the cytoplasm and membrane ruffles. The interaction was also confirmed by co-immunoprecipitation of endogenous proteins in HeLa and SH-SY5Y cells, and overexpression of SH2D3C isoform 3 was also found to downregulate mRNA expression of NQO1 and NRF2. Further experiments to determine the role of DPP3-SH2D3C interaction are in progress.

P47
THE RASGAP PROTEIN IQGC REGULATES CELL-SUBSTRATUM ADHESION IN AMOEBOID CELLS

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Proper cell-substratum adhesion is crucial for motility, phagocytosis and cytokinesis of single cells, and is also essential for complex biological processes such as embryonic development, wound healing, and the maintenance of tissue integrity. Amoeba *Dictyostelium discoideum* is a unicellular model organism that possesses homologs of many proteins involved in cell-substratum adhesion in higher eukaryotes, for instance, talin, paxillin, and small GTPase RapA. IqgC is a RasGAP protein that inactivates small GTPase RasG and negatively regulates phagocytosis and macropinocytosis. We observed that cells lacking IqgC have impaired attachment to the substratum. Indeed, when the detachment of cells from the bottom of petri dishes was assessed using a shaking assay, *iqgc*-null cells were more easily detached than wild-type cells, and the expression of recombinant IqgC in mutant cells rescued this defect. We also observed the localization of fluorescently tagged IqgC to the punctiform adhesion structures on the ventral part of moving cells using TIRF microscopy. To determine which protein domain is important for the localization and function of IqgC in adhesion, truncated variants of the protein were expressed in mutant cells. IqgC consists of two main functional domains – a GRD (GAP-related domain) and an RGCt (RasGAP C-terminal domain). The GRD domain binds small GTPases in homologous proteins, for example in human IQGAP1, while the RGCt domain is important for binding PtdIns(4,5)P2, E-cadherin and β-catenin. In order to compare the localization of these two domains with the full-length protein, the *iqgc*-null cells were transfected with the fluorescently labeled YFP-IqgC, YFP-GRD and YFP-RGCt constructs. YFP-IqgC and YFP-RGCt localized to the ventral punctiform adhesion structures, while YFP-GRD did not. Furthermore, we tested the ability of these constructs to rescue the adhesion defect of *iqgc*-null cells. Only YFP-IqgC, and none of the truncated constructs, rescued the impaired adhesion. Therefore, we conclude that the RGCt domain is sufficient for the localization of IqgC to the adhesion structures, but it needs additional parts of the full-length protein (possibly the GRD domain) to fully exert its role in the cell-substratum adhesion.

P48
A SCREEN OF NATURALLY OCCURRING COMPOUNDS FOR ACTIVITY AGAINST RHINOVIRUSES

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Human rhinoviruses (HRVs) are some of the most widely prevalent viral pathogens in humans and are estimated to be responsible for up to 50% of cases of the relatively mild, acute respiratory disease known as the common cold. In addition to causing the common cold, it has been established that rhinoviruses are the main causative agents of more serious lower respiratory tract infections that can lead to severe illness in vulnerable individuals, such as small children and the elderly, which may require emergency treatment or admission to intensive care units. Notwithstanding the more severe rhinovirus infections, common colds place a significant socio-economic burden on individuals, families, healthcare systems, and society. It has been estimated that adults have an average of four to six, whereas children have an average of six to eight colds every year, and such a high number of common cold cases resulted in yearly productivity losses exceeding 24 billion dollars in the USA alone. Regrettably, despite numerous efforts, there are no effective antiviral therapies or vaccines against rhinoviruses. Here, we performed a systematic, *in vitro* screen of more than 100 carefully selected, naturally occurring compounds for their cytotoxic and antiviral properties against HRV-16, a representative member of HRV-A species. Our analysis revealed several candidate compounds that exhibited significant antiviral activity while also having a favorable cytotoxicity profile. These compounds will be used to design an intranasal pharmaceutical formulation that will reduce the symptoms and progression of colds caused by rhinoviruses.

P49

GRAPE POMACE AS AN ACTIVE BIOLOGICAL RESISDUE OF GRAPES AGAINST CACO-2 AND SW-620 CELLS GROWTH AND RESPONSE TO UV INDUCED OXIDATIVE STRESS

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Grape pomace (GP) is the residue of winemaking, rich in polyphenolic compounds. Polyphenols have antioxidant, anti-inflammatory, anti-allergic and regenerative potential, which makes them a subject of medical and pharmaceutical research. The aim of this study is to investigate the effect of GP extracts on Caco-2 and SW-620 cells, focusing on their antiproliferative and antioxidant properties. The cytotoxic effect of 5 samples of the GP extracts (Merlot 2018 (GP1); Cabernet Sauvignon 2018 (GP2); Cabernet Sauvignon 2016 (GP3); Frankovka 2017(GP4); Mix GP 2016: Graševina, Sauvignon Blanc, Muscat Blanc, Incrocio Manzoni (GP5)) at three concentrations (0.02; 0.1; 0.5 mg/mL) was tested by MTT assay comparing 2D and 3D cell growth. The antioxidant capacity of the GP extracts was determined spectrophotometrically after exposure to UV light for 20 minutes. The absorption rate was evaluated on the Caco-2 cell model after 20 days of cultivation. Polyphenols were quantified by the HPLC method. Data were analysed using Statistica 13.1 ($P < 0.05$). GP extracts at the highest concentration (0.5 mg/mL) were most effective in suppressing cell growth. Caco-2 was suppressed from 53.7 – 79.2 % (0.5 mg/mL) in the 2D model ($P < 0.05$), whereas SW-620 was inhibited from 41.3 – 74.5 % at (0.5 mg/mL; $P < 0.05$). The efficacy of GP extracts on the 3D model of cell growth was statistically insignificant. Measurement of total cell antioxidant capacity showed sensitivity of Caco-2 to UV treatment and a reduction in cell antioxidant status of 17.0% (GP2) -20.0% (GP4) at 0.5 mg/mL ($P < 0.05$). The Caco-2 absorption model shows an absorption rate for polyphenols of 30 to 45 minutes, resulting in an overall absorption efficiency of 85 to 95%. HPLC analysis of the GP extracts revealed catechin as the most abundant polyphenol, followed by epicatechin, procyanidin B1 and B2, gallic acid, resveratrol, caffeic acid and *p*-coumaric acid. GP is a good source of polyphenols that show significant antiproliferative effect on Caco-2 cells depending on the model used (2D vs 3D). The most effective concentration of the studied GP extracts was 0.5 mg/mL.

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P50

N-GLYCOSYLATION OF TOTAL SERUM PROTEINS IN ADULTS WITH TYPE I DIABETES MELLITUS

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Aberrant plasma protein N-glycosylation has been reported in children newly diagnosed with type 1 diabetes (T1D) and N-glycosylation has also been associated with poor glycaemic control and nephropathy in T1D. N-glycosylation features distinct for adult patients with T1D, however, remain to be assessed. In this study we compared the serum protein N-glycosylation between adult patients and corresponding healthy controls as well as examined the association of N-glycans with common diabetic complications, duration of the disease, glycaemic control, and smoking. Serum N-glycans were analysed by UPLC in 201 patients with T1D (18-70) and 298 healthy controls (18-79). N-glycome was divided into 39 glycan groups and 16 derived traits calculated based on structural similarities. Serum N-glycome in T1D subjects exhibited significant decrease in monogalactosylated and an increase in digalactosylated, monosialylated and antennary fucosylated structures, as well as changes in 19 directly measured N-glycan traits. None of the N-glycosylation features, however, correlated with the duration of the disease nor with the development or progression of microvascular complications, similarly to glycaemic parameters, excluding HbA1c. HbA1c was positively associated with sialylated and highly branched glycans, while the effect of smoking was observed through increased complexity of plasma N-glycome.This study shows that T1D associates with a multitude of changes in the N-glycome, reflecting the complexity of the disease. Some of the changes were previously described in newly diagnosed children, but a number of new are found to be present only in the adult patients: e.g., an increase in antennary-fucosylated structures commonly originating from alpha-1-acid glycoprotein, or a decrease in oligomannose structures associated with immunoglobulin M. Interestingly, glycans did not associate with the duration of the disease or with microvascular complications, while conversely poor glycaemic control and smoking were strongly reflected in the N-glycome. In general, this study provides a more comprehensive portrayal of N-glycan changes in adult patients with T1D and highlights changes in certain plasma proteins, but nevertheless warrants further research on N-glycosylation in T1D.

P51
TOXICITY ASSESSMENT OF THREE TYROSINE KINASE INHIBITORS IN HEPG2 CELLS

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Tyrosine kinase inhibitors (TKIs) are effective in the targeted treatment of various malignancies; more than 30 drugs are used in the clinic. These drugs are orally active small molecules that target specific overexpressed and/or mutated tyrosine kinases to interfere with signalling pathways that are deregulated in certain cancers. Most of these drugs are designed to compete with ATP for the ATP-binding pocket of the mutated kinase, thereby inhibiting differentiation, proliferation, growth and migration of cancer cells. Successful clinical therapy with rare, usually mild or moderate and generally reversible side-effects has made them one of the most commonly used anticancer drugs.. Therefore, indirect exposure of healthy healthcare workers and family members of patients to the residues of these drugs is expected to increase. Although TKIs act as signal transduction inhibitors, and do not directly affect DNA structure, the limited data available show that some TKIs induce genotoxic effects in animal models and non-target cells *in vitro*. However, information to assess the risk and characterise the hazards of indirect exposure of the healthy population to most TKI residues is limited. Therefore, the cytotoxicity and genotoxicity of regorafenib, dasatinib, and sorafenib, one of the most commonly used TKIs, were studied in HepG2 cells. Genotoxic potential was evaluated using two complementary methods, single cell gel electrophoresis (comet assay) and the γH2AX assay (marker of DNA double strand breaks, evaluated by flow cytometry). In addition, the effects of selected TKIs on cell cycle (Hoechst staining), cell proliferation (Ki67), and phosphorylation of histone H3 (Ser28, marker for mitotic cells) were examined. The results of the study showed variable cytotoxic potential, with regorafenib being the most toxic followed by sorafenib and dasatinib. The results of our study suggest that certain TKIs may interfere with DNA of non-target cells, and thus pose a higher risk to indirectly exposed healthy populations. It is therefore urgent to assess their risk to healthy populations.
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P52
ELUCIDATING POST-TRANSCRIPTIONAL MODIFICATIONS OF LATENT HSV-1 TRANSCRIPTS

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Herpes Simplex Virus 1, also known as Human Alphaherpesvirus 1 (HSV1) is well known as the causative agent of cold sores. After productive infection in epithelial cells, virus is carried to neurons where it establishes a lifelong latent infection. During this period, latency associated transcripts (LATs) are the only abundantly expressed transcript in infected neurons and the main hallmark of latent infection. The LAT expression helps in preserving the viral reservoir and in some cases, allows subsequent reactivation leading to secondary infections. But the exact mechanism of controlling LAT expression is not fully explored yet. We hypothesize that the post transcriptional modifications, mainly editing of LAT molecule and subsequent products arising from the LAT transcript, such as miRNA, might play important role in these phenomena. To validate the concept and upstage the understanding, we designed a bioinformatic workflow to identify editing events using STAR as a mapping tool and REDItools to identify variations. The interface is outlined to filter out reads highly specific to human genome increasing precision while mapping to viral reference. In comprehensive investigation of publicly available RNA-seq data of human trigeminal ganglia (TG, the main site of latency), we observed consistent variation in reported RNA sequence than mapped DNA reference albeit its high conservation. Though it is hard to absolutely conclude that the variation is post transcriptional modification and not change occurred in DNA of infected virus, due to absence of corresponding DNA sequence. To address this issue and further substantiate our view, we collected human lately infected trigeminal ganglia (TG, the main tissue of latency) and sequenced both DNA and RNA using specially designed primers predominantly amplifying HSV1 LAT region. We are currently in the process of confirming the results.

P53
SIGNALING PATHWAYS CHANGES CAUSED BY S-ADENOSYLHOMOCYSTEINE-HYDROLASE DEFICIENCY IN MCF7 CELLS

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S-adenosylhomocysteine hydrolase (AHCY) deficiency is a disorder caused by lowered enzymatic activity of AHCY protein due to the mutations in distinct coding regions of the *AHCY* gene. AHCY has a key role in proper functioning of the methionine cycle in the cell, therefore the lack of AHCY function causes severe metabolic and multisystem disorder. S-adenosylhomocysteine hydrolysis is an important step in a series of transmethylation and transsulfuration reactions, and AHCY indirectly participates in the regulation and maintenance of the celular methylation potential. Clinical presentation of this potentially lethal disorder includes a combination of muscular, neurological and hepatic disorders most likely due to inhibition of S-adenosyl methionine-dependent trans methylation reactions. The aim of this research was to analyse and understand molecular roles of AHCY in distinct cellular model systems such as the breast cancer cell line MCF-7. To do so, differential expression analysis was performed on AHCY-deficient MCF7 cells using NGS based approaches. RNA-Seq data was analyzed by IPA (Ingenuity Pathway Analysis) Software and Core analysis was performed. Fifteen differentially expressed networks were identified. Subsequently, we found a predicted indirect interaction of AHCY protein with Histone H3, which bodes well for its role during epigenomic reprogramming. Top canonical pathways with predicted inhibitory activity (negative z score) were a) EIF2 signaling (EIF2, Eukaryotic Initiation Factor 2), b) G-protein coupled receptor signaling, c) ID1 signaling (ID1, Inhibitor of differentiation 1), and d) cAMP mediated signaling (cAMP, cyclic adenosine monophosphate). The most interesting differentially expressed signaling pathway includes EIF2. Phosphorylation of eIF2L by upstream kinases leads to sequestering GAP eIF2B, preventing recycling and inhibiting translation initiation from normal capped mRNAs. Stalled ribosomes enter stress granules, which results in apoptosis, endoplasmatic reticulum stress response and unfolded protein response, all coordinated with a negative z score of canonical pathways. We can conclude that AHCY directly and/or indirectly affects a large network of signaling molecules, unsurprisingly indicating AHCY as a crucial actor in a multisystem disorder.

Poster Abstracts

P54
CHARACTERIZATION OF OPTINEURIN INSUFFICIENCY MICE DURING AGEING

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Amyotrophic lateral sclerosis (ALS) is genetically and clinically exceptionally heterogenous neurodegenerative disease targeting cortical, bulbar and spinal motor neurons. Common hallmarks of ALS include chronic neuroinflammation and proteinopathy, both of which are exacerbated during ageing. A small subset of ALS patients harbours mutations in the optineurin (*OPTN*) gene. OPTN is an adaptor protein in many processes, including inflammatory signalling and autophagy. To understand the role of OPTN in neurodegeneration, we analysed a mouse model carrying $OPTN^{470T}$ truncation, which lacks ubiquitin-binding domain, thus mimicking some ALS patient mutations. Since ALS occurs late in life, we analysed tissues obtained from two-year-old mice by immunofluorescent staining, flow cytometry and protein arrays. In the motor cortex of the brains of $OPTN^{470T}$ mice we found no signs of neurodegeneration, while we detected neuronal loss in the ventral horns of the spinal cords. The latter were not accompanied by microgliosis or astrocytosis. We have also analysed spleen and lymph nodes for the presence of regulatory T cells (Tregs), which were shown to negatively correlate to disease progression. Interestingly, in the spleens and lymph nodes from 2-year-old $OPTN^{470T}$ mice, we found a lower percentage of Tregs, which also had a lower level of their master transcription factor FoxP3. Protein array analysis did not show a difference in cytokine and chemokine profiles of $OPTN^{470T}$ brains and spinal cords. Based on neurological tests, $OPTN^{470T}$ female mice did not show overt motor or memory impairments during ageing, which suggests that OPTN insufficiency-mediated neuronal loss is not sufficient for phenotype. However, 2-year-old $OPTN^{470T}$ male mice showed a significant drop in the motor coordination compared to 1-year-old mice, which was not observed in WT mice. Overall, OPTN insufficiency did not lead to classical ALS symptoms or neuroinflammation in aged mice, but was coupled with neuronal loss in the spinal cords and decreased peripheral Treg numbers. Given that unmanipulated $OPTN^{470T}$ aged mice did not show any major phenotype (apart from the mild motor deficits in male mice), we are currently developing two-hit ALS models to further elucidate the effect of optineurin insufficiency.

P55
THE INTERPLAY BETWEEN TUBULIN β ISOTYPES AND DRUG RESPONSE IN OVARIAN CANCER CELL LINES RESISTANT TO CARBOPLATIN

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Ovarian cancer (OC) is the seventh most common cancer type in women worldwide. Unfortunately, lack of early detection methods leads to diagnosis of already advanced disease tied with highest fatality-to-case ratio among gynecological malignancies. Conventional therapy for OC consists of cytoreductive surgery followed by chemotherapy with carboplatin (CBP) and paclitaxel (TAX). However, 80% of late-diagnosed patients experience relapse due to the acquired resistance and frequently develop metastases. It is important to emphasize that resistance mechanisms to CBP and TAX involve mostly different pathways and up till now these two processes have been studied separately. Here, we explored CBP-induced TAX cross-resistance with aim to identify possible linkers of CBP and TAX drug response and potential use of those molecules as predictive biomarkers. For this purpose, we used two different mesenchymal-like OC cell models developed by stepwise CBP treatment of parental MES-OV and SK-OV-3 cell lines, CBP-resistant and TAX cross-resistant MES-OV CBP cell line and CBP-resistant but TAX sensitive SK-OV-3 CBP cell line. Interestingly, both cell lines showed elevated levels of class III β-tubulin (TUBB3) however, transient silencing of TUBB3 only impacted MES-OV CBP response to CBP while SK-OV-3 CBP did not show any sensitization. Additionally, reduced levels of TUBB3 in MES-OV CBP cell line disturbed the trafficking of RAD50, MRN complex protein involved in DNA damage repair, and increased whole-cell platination levels. Both of these processes implied involvement of TUBB3 silencing in CBP sensitization of MES-OV CBP cells. The difference between these two cell lines was the compensation of TUBB3 silencing with other β-tubulin isotypes noticed in SK-OV-3 CBP, but absent in MES-OV CBP cell line. Based on this we proposed that TUBB3, despite upregulation in both cases, can be used as a predictive marker for treatment efficacy only when β-tubulin isotype compensation is lacking.

P56
HEDGEHOG-GLI SIGNALING PATHWAY IN PROSTATE CANCER AND STROMAL CELL CO-CULTURE

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Prostate cancer is the second most frequent cancer diagnosed in men worldwide. Although, radical prostatectomy and radiation have been proven quite effective at the early stages of the disease while tumor is still localized, management of advanced stages is limited due to the development of biological mechanisms of resistance to medication. One of the relevant pathways in prostate cancer progression is the Hedgehog-GLI (HH-GLI) signaling pathway which is crucial for normal embryonic development, stem cell maintenance and tissue homeostasis in adult organisms but its aberrant activation in adult cells has been linked with the development of various tumors, including prostate cancer. The aim of this research was to better understand the differences in HH-GLI signaling pathway in co-culture of prostate cancer and stromal cells in two-dimensional (adherent) and three-dimensional (spheroid) models. We used adenocarcinoma prostate cell line LNCaP and cancer-associated prostate fibroblasts WPMY-1. For the two-dimensional models, cells were grown together in different ratios (5:1, 2:1, 1:1, 1:2 and 1:5). For the generation of spheroid models, we employed the hanging drop system. The response to HH-GLI signaling pathway inhibition was investigated using four different treatments and observing these cell lines with MTT assay. We have also investigated differences in expression of key genes involved in HH-GLI signaling pathway between cancer and stromal cell lines, both grown in 2- and 3-dimensional system by Real-time PCR. From the results that we obtained by MTT assay, we observed that the best seeding ratio for both cell lines is 1:1, so this ratio was chosen for 3D co-cultures. Cancer and stromal cells are forming spheroids of different shapes and sizes. HH-GLI signaling pathway inhibition showed that the cell death is delayed in the cancer-fibroblast co-culture compared to monocultures. From the analysis of the expression of key genes involved in HH-GLI signaling pathway it is visible that stromal cells are changing their characteristics to the higher extent compared to the cancer cell lines. Results obtained so far are pointing out the importance of interaction between the cancer cells and cancer associated fibroblast in the prostate cancer progression. It is known that tumor-stroma interaction is crucial for Hedgehog signaling in the prostate, but here we demonstrate the resistance of co-culture compared to monocultures on an in vitro model.

P57
PROTECTION AGAINST HARMFUL EFFECTS OF HIGH FAT DIET IN FEMALE MICE IS ATTRIBUTED TO THE COMBINED EFFECT OF FEMALE SEX HORMONES AND SIRT3

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The prevalence of each risk factor contributing to the metabolic syndrome differs by sex. By understanding how and why metabolic processes differ, we can develop targeted and personalized therapies based on sex. In females, ovarian hormones play a critical role in metabolism, food intake and body weight regulation. In addition, pre-menopausal women have fewer obesity-related metabolic disorders, but their prevalence increases dramatically after menopause, indicating the influence of sex hormones on body adiposity and the occurrence of metabolic syndrome. A high- fat diet (HFD) is an important factor in the development of many metabolic diseases, and the liver, as a metabolic center, is particularly exposed to this influence. In addition, sirtuin 3 (Sirt3), an important mitochondrial NAD^+ dependent deacetylase, plays a role in preventing the metabolic syndrome, but its role in relation to sex differences in the development of the metabolic syndrome is still unknown. Here, we investigated the effect of Sirt3 in the liver of ovariectomized and sham female mice after 10 weeks of feeding with standard fat diet (SFD) or HFD. Liver was examined by Folch, gas chromatography and lipid hydroperoxide analysis, histology and oil red staining, RT-PCR, Western blot, antioxidant enzyme analyzes, and oxygen consumption. Ovarectomy in WT females results in increased expression of Sirt3, followed by maintained mitochondrial function and decreased levels of lipid hydroperoxides under SFD conditions. The combination of ovarectomy and Sirt3 depletion leads to dysregulation of lipid metabolism and increases lipid oxidative damage under SFD conditions. HFD in combination with ovarectomy and Sirt3 depletion results in increased body weight gain, higher expression of NAFLD- and oxidative stress-inducing genes, and impaired antioxidant system response, suggesting that protection against the harmful effects of HFD in female mice is due to the combined action of female sex hormones and Sirt3. Overall, this study provides evidence that protection against harmful effects of HFD in female mice is attributed to the combined effect of female sex hormones and Sirt3, thus contributing to preclinical research on possible sex-related therapeutic agents for metabolic syndrome and associated obesity-related diseases.

P58
PRIMARY CILIA - A POTENTIAL LINK BETWEEN HEDGEHOG-GLI AND MAPK SIGNALING IN GANT61-RESISTANT MELANOMA CELL LINES

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Hedgehog-GLI and MAPK signaling are known signaling partners. BRAF and NRAS can activate GLI proteins directly regardless of the upstream membrane events, but the exact signaling order in which this occurs, other components involved in this interaction and final outcomes are still not fully understood. This interaction may be crucial in establishment and maintenance of drug resistance, a known issue for metastatic melanoma. We established three melanoma cell lines with different *BRAF/NRAS* mutation background resistant to GANT61, a GLI specific inhibitor, to explore in more depth the mechanism that underlays HH-GLI and MAPK signaling interaction. Cells were treated with increasing GANT61 concentrations in duration 8-12 months and validated with MTT viability test. HH-GLI and MAPK protein expression was examined by Western blot. Potential transcription targets connected to MAPK signaling were identified with ChIP-seq for endogenous GLI1, GLI2 and GLI3 proteins in parental cell lines and validated by qPCR. By gaining GANT61 resistance, cell lines changed response to other HH-GLI inhibitors beside GANT61, migration and colony forming capacity. In comparison to the *BRAF/NRAS* wild type cell line, the *NRAS* mutated resistant cell line showed upregulated MAPK signaling, downregulated HH-GLI signaling and a newly identified GLI transcription target RAB34. RAB34 is essential for primary cilia formation and plays a central role in signal transduction of several signaling pathways, including HH-GLI and MAPK. Therefore, we focused on primary cilia visualisation using immunofluorescence. We successfully induced primary cilia with serum deprivation in the *NRAS* mutated parental cell line, but only few primary cilia were detected in the resistant cell line, suggesting primary cilia loss in the resistant cell line. Based on our current results, we believe that primary cilia present a potential link which can explain the switching from HH-GLI on MAPK signaling. Established GANT61-resistant cell lines present valuable *in vitro* models which can bring new insights into HH-GLI-MAPK interplay. Our future work will be based on proteins involved in ciliogenesis to see if primary cilia restoration can resenzitize cells to GANT61.

P59
IgG N-GLYCOSYLATION IS NOT INFLUENCED BY SEVERITY OF COMPLICATIONS IN TYPE 1 DIABETES

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N-glycosylation of immunoglobulin G (IgG) is known to influence the antibody function. Changes in IgG N-glycome associate with many inflammatory conditions and were reported in early stages of type 1 and type 2 diabetes mellitus (DM). IgG N-glycosylation was also studied in relation to disease progression in type 2 DM and was found to associate with diabetes complications. Although type 1 and type 2 DM share complications, these findings may not apply to type 1 DM due to different pathophysiology involved and a longer disease duration prior to complication manifestation. In this study we investigated IgG N-glycosylation of 190 patients (age 18-70, median 46, 81 M, 109 F) with type 1 complications. Complications included: hypertension, albuminuria and retinopathy. Patients were differentiated by the levels of complications but were all in later stages of disease progression. N-linked glycans from IgG were released, fluorescently labelled and analysed using HILIC-UPLC. Twenty-four glycan structures were identified and relatively quantified. In addition, nine derived traits, corresponding to different structural characteristics of glycans were calculated. Data was analysed using multiple linear regression with age and sex as covariates. Glycan traits were log transformed prior to analysis. We observed no statistically significant changes of IgG N-glycosylation with respect to type of complication or severity level. Previously reported changes with respect to age, sex and lifestyle aspects, e.g. smoking were replicated in this study, confirming their influence on the glycome. IgG N-glycosylation changes previously reported as connected to the severity of complications in type 2 DM were not replicated for type 1 DM patients in our study. This can be explained by the fact that pathophysiological changes leading to type 1 DM and influencing the N-glycome occur much earlier in life and are not further influenced by disease progression and development of complications in the adult age. Absence of differences with respect to type of complications may also be due to fact that they share some common pathophysiological processes which prevent their distinction based on IgG N-glycosylation profile.

P60
INTERPLAY OF CISPLATIN-INDUCED MITOPHAGY, AUTOPHAGY AND APOPTOSIS IN RENAL TUBULAR EPITHELIAL CELLS AND MICE KIDNEYS: INVOLVEMENT OF ERK SIGNALING PATHWAY

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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 and 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. Seahorse XF Cell Mito Stres analysis was performed to clarify the impact of ERK signaling pathway on CP-induced disruption of key parameters of mitochondrial function. Smart targeting of molecules involved in CP nephrotoxicity should enable a new approach to nephrotoxicity prevention and potential application in the pharmaceutical industry.

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P61
MISTRANSLATION UPREGULATES CHAPERONE PRODUCTION AND IMPAIRS METABOLISM AND GROWTH

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Aminoacyl-tRNA-synthetases (aaRSs) pair tRNAs with their cognate amino acids (aa). To ensure faithful protein biosynthesis, aaRS needs to bind its substrates with high specificity. However, it can be challenging to differentiate cognate aa from other similar competing substrates. For example, cognate isoleucine (Ile) is recognised 160 times better than non-cognate valine (Val) in the active site of isoleucyl-tRNA synthetase (IleRS), which is not enough to ensure minimal translational fidelity. Therefore, IleRS has developed additional editing domain for hydrolysis of mischarged tRNA. To investigate consequences of inactivated IleRS editing, we triggered proteome-wide Ile-to-Val mistranslation and carefully monitored its effect on cellular proteostasis. We constructed the strain MG1655 *ileS* T243R/D342A (ED^-) expressing editing-deficient IleRS and monitored its growth in the presence of 1 mM Val to artificially induce Ile-to-Val mistranslation in *Escherichia coli.* We investigated *in vivo* effects of Ile-to-Val mistranslation and by methods of quantitative proteomics compared expression profiles of proteomes isolated from ED^- strain in mistranslating and non-mistranslating conditions. We isolated the clients of DnaK, the central chaperone in *E. coli*, to determine which proteins are structurally most compromised by mistranslation. Ile-to-Val mistranslation impairs bacterial growth rate and within 24 h causes filamentation of cells. Mistranslation upregulates main chaperone systems in *E. coli* (DnaK/DnaJ/GrpE, GroES/EL and HtpG). On the other hand, it downregulates amino acid biosynthesis. Number of isolated DnaK clients at 37 °C was surprisingly low. We imposed higher temperature (42 °C) to further destabilise already unstable mistranslated proteins and provoke higher number of clients. In this way, isolated DnaK clients in mistranslating conditions were enriched in biological processes most impaired by mistranslation - translation and metabolism. Furthermore, combined effects of heat-shock (42 °C) and mistranslation downregulated other metabolic processes such as carbohydrate metabolism. To conclude with, in mistranslating conditions *E. coli* upregulates production of chaperones to regulate cellular proteostasis, while lowering down metabolism and growth rate.

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DICTYOSTELIUM DISCOIDEUM IQGD REGULATES ACTIN CYTOSKELETON IN LARGE-SCALE ENDOCYTOSIS

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IqgD protein from amoeba *Dictyostelium discoideum* belongs to an evolutionarily conserved family of IQGAP proteins. IQGAPs are multidomain proteins that act as scaffolds to integrate diverse signalling pathways. They regulate cellular processes that require extensive rearrangement of the actin cytoskeleton, such as migration, adhesion and vesicle trafficking. IQGAPs bind actin filaments directly via their calponin homology domain (CHD) and can further cross-link them into bundles due to their oligomerization. The oligomerization of IQGAPs is facilitated by Rho family GTPases Rac1 and Cdc42, that bind via GAP-related domain (GRD). IQGAPs also regulate actin dynamics via interactions with actin-assembly (Arp2/3, Dia1) and nucleation-promoting (N-WASP) factors responsible for the generation of protrusive structures at the cell leading edge. IqgD is the only *Dictyostelium* IQGAP that harbours an actin-binding domain. It also contains a coiled-coil region, a GRD and a RasGAP_C-terminal (RGCt) extension, the latter two forming a typical domain architecture of an IQGAP. Using confocal microscopy of live *D. discoideum* cells we showed that fluorescently labelled IqgD localizes to the entire cell cortex. However, it was significantly enriched at the endocytic cups during macropinocytosis (bulk fluid uptake) and phagocytosis (particle uptake). This localization suggests its involvement in both types of large-scale endocytosis. Hence, we investigated whether IqgD also interacts with small GTPases from the Rho family, in particular those known to be involved in large-scale endocytosis. Using yeast two-hybrid assay we demonstrated that IqgD interacts with Rac1A and Rac1C. Next, we demonstrated that IqgD co-immunoprecipitates with endogenous actin. Finally, we observed that fluorescently labelled IqgD co-localizes with a probe for active Rac1 GTPases and a probe for filamentous (F-) actin in live cells during macropinocytosis, throughout the evolution of a macropinosome.
Overall, these results strongly suggest that IqgD acts as a Rho-regulated IQGAP protein that, similar to its mammalian counterparts, regulates actin cytoskeleton remodelling, in particular during the formation of large protrusions in macropinocytosis and phagocytosis.

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THE HUMAN MITOCHONDRIAL NME6 INTERACTS WITH RCC1L AND IMPACTS RESPIRATION

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NME6 is a member of the nucleoside diphosphate kinase (NDPK/NME/Nm23) family, a group of proteins known to catalyze the transfer of gamma phosphate from NTPs to NDPs (NDPK activity). The ping-pong reaction involves the phosphorylation of a histidine residue within the catalytic site, strictly conserved among NME members. The family is divided in two groups based on the structure and phylogenetic analysis. The well-studied Group I members (NME1-4) are enzymatically active while the less-described Group II members (NME5-9) fail to display the NDPK activity. To date, only three NMEs are targeted to mitochondria: NME3 localizing at the mitochondrial outer membrane, NME4 localizing at the mitochondrial inner membrane, either facing inter-membrane space or matrix, and NME6. Mitochondria are the center of aerobic respiration, where Krebs cycle's products, oxygen and ADP are used by the electron transport chain (ETC) to produce ATP. Mitochondria carry their own DNA and encode for 13 proteins, all constituent of the ETC complexes and indispensable for ETC function. We focused our efforts on studying the barely explored human NME6 protein. Using confocal microscopy and cell fractionation, we confirmed NME6 to be a mitochondrial, matrix facing protein. Co-immunoprecipitation experiments revealed an interaction between NME6 and RCC1L, another matrix facing protein involved in mitochondrial ribosome biogenesis and associated with *de novo* biosynthesis of mitochondria-encoded protein. Our oxygraphy analysis revealed a strong negative impact of NME6 overexpression on mitochondrial respiration. Accordingly, western blot analysis uncovered a downregulation of respiratory complex III protein UQCRC2 and the mitochondria-encoded complex IV protein COXII. Altogether, our research shed new light on the human NME6 protein, indicating its fundamental role in the regulation of mitochondrial respiration, independent of its enzymatic activity, and probably in concert with RCC1L. Our results provide solid basis for further investigation of the NME6 function within mitochondria.

P64
IQGC PROTEIN INTERACTS WITH RASG AND RAB5A GTPASES DURING MACROPINOCYTOSIS IN AMOEBA *DICTYOSTELIUM DISCOIDEUM*

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Dictyostelium discoideum protein IqgC is a GAP (GTPase activating protein) specific for the small GTPase RasG. GTPases from the Ras superfamily regulate macropinocytosis, the endocytic process for non-selective uptake of extracellular fluid. By deactivating RasG, IqgC negatively regulates macropinocytosis in amoeba *D. discoideum*. IqgC strongly localizes to forming macropinosome, where it colocalizes with active Ras. However, Ras dissociates from the internalized macropinosome prior to IqgC[1], which suggests RasG-independent roles of IqgC during macropinosme maturation. First, we examined the role of RasG in IqgC recruitment to macropinocytic cups. By monitoring the localization of IqgC in *rasG* null cells, or localization of IqgC mutants unable to bind Ras in *iqgC* null cells, we observed the loss of IqgC localization to macropinosomes. These results demonstrate that RasG is indispensable for the recruitment of IqgC to forming macropinosomes. Next, we searched for novel IqgC interactor(s) that could mediate IqgC functions in early macropinosome maturation, after detachment of Ras. Potential protein candidates, previously identified by mass spectroscopy[1], were selected according to their known function in the early endosome maturation. From those, early endosome marker, GTPase Rab5A was shown to colocalize with IqgC on the primary macropinocytic vesicle in live cells that co-express both fluorescently labelled proteins. Furthermore, by performing GST-Rab5A pull-down assay with purified IqgC, we identified Rab5A as a direct interactor of IqgC. Using lipid dot blot assay with lysates of cells expressing full length or truncated IqgC variants, we demonstrated that IqgC binds to various phosphoinositides (PIs). Although its binding was generally of low specificity, IqgC bound with the highest affinity to PI(4,5)P_2 via its N-terminus and RasGAP domain. Nevertheless, the binding was abrogated for RasG-binding mutants, confirming the necessity of RasG for IqgC loading to macropinosome membrane. Altogether, these results show that RasG is necessary for IqgC recruitment to the forming macropinosome, but PIs and Rab5A likely mediate its retention on the RasG-vacated vesicle. The biological significance of Rab5-IqgC interaction is under investigation.

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P65
COMPOSITION OF FOCAL AND RETICULAR ADHESIONS IN MELANOMA CELL LINES MDA-MB-435S AND RPMI-7951

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Cell adhesion mediated by integrins, cell surface heterodimers composed of α and β subunits, which integrate signalling between the extracellular matrix (ECM) and cells, and control many aspects of normal and tumour cell behaviour. Molecular mechanisms related to cell adhesion have been extensively studied, especially in cancer in which mutations and/or changes in expression of these proteins have been observed contributing to proliferation, migration and invasion. Through binding to the ECM and clustering, integrins form multimolecular adhesion complexes (IACs) that are connected to, and can regulate, the cell cytoskeleton. These complexes are termed nascent adhesions, focal adhesions (FAs), fibrillar adhesions and hemidesmosomes. A new class of IACs, named reticular adhesions (RAs), were initially described as clathrin lattices composed of hexagonal clathrin structures, enriched in dynamin and AP2 (adaptor protein complex 2), both of which are involved in clathrin-mediated endocytosis. RAs are formed by integrin αVβ5, lack association with actin and are devoid of vinculin (which is marker of FAs). There is only limited evidence for other integrins than αVβ5 being localized to these sites as well as presence of other components especially talins whose binding is necessary for integrin activation. While FAs are depleted during mitosis, RAs are maintained to enable effective mitosis and also transmit spatial memory from pre-mitotic to post-mitotic daughter cells. We have previously analysed IACs of two melanoma cell lines MDA-MB-435S and RPMI-7951, grown in long term culture, using biochemical isolation and mass spectrometry (MS)-based proteomics, and demonstrated that both cell lines preferentially use integrin αVβ5 for adhesion. Immunofluorescence analysis has shown that integrin αVβ5, in both cell lines, was localized in vinculin-positive FAs and vinculin-negative, ring-like or reticular structures thus resembling RAs. To determine the composition of these IACs, we exposed MDA-MB-435S and RPMI-7951 cells grown in long term culture to an inhibitor of actin polymerisation, cytochalasin D, to disrupt FAs, thus enabling the isolation of RAs. The composition of RAs was then analysed by (MS)-based proteomics. Our results demonstrate the absence of FA components such as talin-1, filamins and alpha-actinins and enrichment of proteins such as AP-2, disabled homolog 2 (DAB2) and Numb. Interestingly, in RAs isolates we observed the presence of talin-2. To validate these results, Western blot analysis of RA isolates and immunofluorescence analysis were performed, and this demonstrated colocalisation of TLN2, DAB2 and Numb proteins. The comprehensive analysis of FA and RA compositions in two cell lines, sharing the same integrin αVβ5, will enable us to analyse their regulatory interplay/relationships.

P66
GC-MS BASED COMPARATIVE ANALYSIS OF MDA-MB-231 AND MCF-7 CANCER CELLS TREATED WITH NOVEL THIENO[2,3-b]PYRIDINE DERIVATIVE

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Metabolic profiling of cancer cells has ever growing role in elucidation of molecular base of cancer development, progression and prognosis. In this study, we investigated the effect of a novel thieno[2,3-b]pyridine derivative on the metabolic profile of two breast cancer lines with different hormonal status; MDA-MB-231 (triple negative) and MCF-7 (ER, PR, HER2-). The thieno[2,3-b]pyridines were initially discovered as potential inhibitors of phospholipase C (PLC) isoforms by virtual high throughput screen (vHTS). GC-MS coupled system was used for separation and identification of metabolites. The objective was to identify the compound effect on each metabolite and to find metabolites significantly different between treated and control cells in each line. Data processing, data normalization, statistical analysis and high-level functional interpretation was performed using MetaboAnlayst, a web-based platform for comprehensive analysis of quantitative metabolomics data. Test of statistical significance shows that results for MDA-MB-231 cells are more significant than for MCF-7 cells. PCA results show that drug treatment causes metabolic alternations in both cell lines. Quantitative enrichment analysis was employed to identify patterns of metabolite concentrations and to help elucidate possible biological mechanisms. The results of analysis reveal that treatment has a major impact in glucose/energy metabolism specifically in glycolysis/gluconeogenesis, pyruvate metabolism, Warburg effect, inositol metabolism for both lines. Metabolic profiling of cancer cells plays an increasingly important role for research on the mechanism of action of anticancer drug candidates.

P67
DETECTED CNV HOTSPOTS AS POTENTIAL SEMINOMA BIOMARKERS FROM SEMINAL PLASMA

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Testicular seminoma (SE) represents the most common type of testicular germ cell tumours, which are affecting young men. Thus, it is important to find novel biomarkers for early detection which could improve the life and reproductive health of the patients after the diagnosis and treatment. Copy number variation (CNV) has already been associated with various cancers as well as with SE. In this study, we selected two genes, *NANOG* and *KITLG* for CNV analysis which are located on chromosomes susceptible to gains, and whose aberrant expression was already detected in SE. CNV analysis was performed on genomic DNA (gDNA) and cell-free DNA (cfDNA) from seminal plasma as liquid biopsy. The aim of the study was to investigate the CNV of selected genes and determine their potential as a possible SE biomarker. Twenty-four SE patients and thirty-five healthy volunteers (HV) were recruited for this study. CNV analysis was performed by droplet digital polymerase chain reaction (ddPCR) on gDNA from SE and nonmalignant testicular tissue. Seminal plasma cfDNA from SE patients before and after surgery was analysed, as well as from healthy volunteers. Statistically significant gains were detected in gDNA for both analysed genes. Comparison between CNVs detected in cfDNA from seminal plasma of HV, preoperative, and postoperative samples disclosed that CNV of *NANOG* and *KITLG* was increased in preoperative samples, indicating the reflection of increased *NANOG* and *KITLG* CNV from gDNA SE tissue in cfDNA from seminal plasma. Although statistically not significant, a decrease in CNV trend in analysed genes in postoperative compared to preoperative seminal plasma samples indicates that operational treatment induces at least slight normalization in CNV to an HV level. Although clinical value is yet to be determined, presented data emphasize a potential use of CNV as a potential SE biomarker from a liquid biopsy.

P68
AUTOMATION OF IgG N-GLYCAN SAMPLE PREPARATION METHOD FOR HIGH THROUGHPUT ANALYSIS

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Role of protein N-glycosylation and especially changes in IgG N-glycosylation pattern has repeatedly been confirmed as crucial in different physiological and pathological processes. Expanding knowledge requires more affordable, reliable, and higher throughput methods. New approaches to glycosylation analysis would allow its optimization and application in the biopharmaceutical industry, epidemiology as well as advance clinical diagnostics that would rely on glycan biomarkers. Though there were successful attempts to develop new automated strategies for glycan research, many laboratories still leverage manual sample preparation protocols that limit their throughput. To help address this issue, automated methods for glycosylation analysis should be developed further. This research proposes one possible approach to automation of IgG glycan sample preparation using the Tecan Freedom Evo liquid handling automated platform. To improve throughput and robustness, the manual method was substituted by the automated system that was equipped with a liquid handling arm (LiHa) and a robotic manipulator arm (RoMa), while the vacuum manifold operations were replaced with A200, a positive pressure unit which allows for higher pressure and flow-through. The adapted automated protocol features IgG isolation on a protein G plate, IgG deglycosylation, and glycan 2-AB labeling. This automated protocol allows the samples to be analyzed using ultra-high-performance liquid chromatography (UPLC). UPLC analysis showed that peaks' number, retention time, and peak area corresponded with the manual method. Peaks with the largest area showed small variation, while the smaller peaks exhibited larger variation. Method has yielded promising results and could encourage additional automation efforts in other protocols with development.

P69
CARCINOGEN METABOLISM BY THE GUT MICROBIOTA TRIGGERS BLADDER CARCINOGENESIS

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Exposure to toxins from cigarette smoke is a well-recognized risk factor for cancer development. Furthermore, microbiome composition was recently shown to influence gut, liver, and lung carcinogenesis. Several mechanisms for microbiota-facilitated carcinogenesis are proposed including bacterial toxin production, promotion of chronic inflammation as well as carcinogen toxicokinetics. In the present study, we investigated the role of the gut microbiota in urinary bladder tumor development using a nitrosamine-induced bladder cancer model in mice. We found that antibiotic depletion of the gut microbiota significantly reduces the cancer burden in the bladder, which we then causally link to gut microbial metabolism affecting the toxicity and tissue distribution of the nitrosamine. Further, we tested microbial gut, lung, and oral cavity communities and isolates from different human donors to demonstrate that microbial nitrosamine metabolism strongly varies between individuals. Our findings demonstrate that gut microbial metabolism is an important element of bladder carcinogenesis. This could give rise to new opportunities for urinary bladder cancer risk assessment and preventive interventions based on the patient's gut microbiome profile.

P70
CONTRASTING EFFECT OF EVOLUTIONARY CONSERVED CYSTEINES ON THE STABILITY OF PLANT SERYL-tRNA SYNTHETASE

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In general, cytosolic proteins do not contain disulfide bonds due to high reducing power of the cytosol. Previously, we have identified a unique disulfide bond between Cys213 and Cys244 in the crystal structure of cytosolic seryl-tRNA synthetase (SerRS) from plant *Arabidopsis thaliana*. SerRS belongs to family of aminoacyl-tRNA synthetases, essential houskeeping enzymes that covalently link amino acid to cognate tRNA, preparing aminoacylated tRNA substrates for translation on the ribosome. Cysteines involved in disulfide link are evolutionary conserved in all green plants, including unicellular green algae. In order to decipher the role of disulfide link in plant SerRS we have prepared mutant variants comprising substitution of cysteines with serine residues. Aminoacylation activity of all variants were comparable to the activity of the wild type (wt) protein. C213S mutant showed lower thermal stability compared to the wt which can be atributed to the fact that it can not form disulfide link. Unexpectedly, C244S mutant showed higher stability than wt, although it can not form disulfide link either. Interestingly, double mutant had the same stability as the wt, indicating that mutation C213S conferring lower stability and mutation C244S conferring higher stability compensate each other in the double mutant. We have solved the crystal structure of C244S variant and modeled 3D-structures of the C213S and C213S/C244S variants. The major differences in crystal structure of C244S variant with respect to the wt SerRS is the orientation of the region Phe136-Leu143 in the catalytic core and a slightly shifted coiled-coil region in the tRNA binding domain. Ser244 forms a hydrogen bond network with the surrounding residues Cys213 and Ala214 of the loop region. Consequently, loop Ala214-Asp220 that is flexible in wt SerRS is now structured in C244S variant which is in accordance with observed higher stability of the variant. We conclude that conserved cysteines involved in the disulfide bond have contrasting effect on the stability of plant SerRS. Considering that disulfide bonds in cytosolic proteins are usually linked to cellular response mechanisms to oxidative stress, disulfide link in plant SerRS may be involved in regulation of translation during oxidative stress.

P71
INITIAL CHARACTERIZATION OF MECHANISMS CONFERRING SUBSTRATE SPECIFIC VARIATION IN OCT1*2 ACTIVITY

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Organic cation transporter 1 (OCT1) is localized in the sinusoidal membrane of human hepatocytes and mediates the uptake of clinically relevant drugs and endogenous compounds. OCT1 is genetically highly variable. Five alleles - OCT1*2, *3, *4, *5 and *6 - result in a loss of OCT1 function and are associated with altered pharmacokinetics and efficacy of clinically relevant drugs like sumatriptan, fenoterol, tramadol and morphine. With a MAF of 14.1%, OCT1*2 is the most common genetic variant of OCT1. In contrast to other known alleles, OCT1*2 shows strong substrate-specific effects. OCT1*2 is characterized by a deletion of Met_{420}. As Met_{420} is not located in the proposed substrate binding cleft, the mechanisms conferring these substrate-specific changes in activity cannot be explained by direct interaction with the substrate. To analyze these mechanisms, we first identified substrate-specific effects on different OCT1 substrates, using HEK293 cells stably overexpressing OCT1*2. While OCT1*2 results in a loss of transport for substrates like tramadol, morphine or metformin, other substrates like sumatriptan or fenoterol are transported comparable to wild-type. By analyzing the OCT1*2 dependent uptake of different triptans, we observed strong substrate-specific effects even between structurally highly similar compounds. OCT1*2 reduced the CL_{int} of zolmitriptan by 4-fold
Second, we analyzed the effects of OCT1*2 on the protein itself. We investigated the role of the deletion at codon 420 and its possible effects on the nearby located extracellular loop. Therefore, we relocated the deletion after codon 420. This did not decrease OCT1 activity compared to Met_{420}del. Further, we analyzed possible interactions of Met_{420} within the protein by analyzing the effects of substituting Met_{420} with four different amino acids on transport. Each of these substitutions resulted in transport characteristics comparable to the deletion of Met_{420}, indicating a crucial role of the Met_{420} side chain for the OCT1*2 phenotype. As next steps, we will analyze the influence of OCT1*2 on its correct membrane localization by immunohistochemistry and targeted-proteomics. Further, effects on oligomerization and lipid interactions will be analyzed by FRET assays and using proteoliposomes.

P72
GENETIC ASSOCIATION STUDY OF PLASMA PROTEOME AND IgG *N*-GLYCOSYLATION SUGGESTS IMPORTANCE OF *N*-GLYCOSYLATION AND COMPLEMENT SYSTEM AT THE ONSET OF TYPE 1 DIABETES

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N-glycosylation is a ubiquitous protein modification which impacts both protein structure and function. We previously showed that plasma proteome and IgG *N*-glycosylation changes in children with new-onset type 1 diabetes (T1D) and differs from both their healthy siblings and adult T1D patients with unregulated blood glucose. Herein, we conducted genetic association study in same recent-onset T1D cases in order to search for genetic variants driving those *N*-glycosylation changes. Plasma samples of 1105 children (0-18 years) collected within three months of T1D diagnosis through the Danish Registry of Childhood and Adolescent Diabetes were obtained and DNA samples were genotyped for 183,546 single nucleotide polymorphisms on the Immunochip. In the follow-up cohort, 455 samples from the same registry were used to validate significant genetic associations from the discovery phase. Genetic association study identified four genome-wide significant loci associated with *N*-glycans. All identified loci, except for the complement C3 gene locus (*C3*), had been previously associated with *N*-glycosylation (candidate genes include *MGAT3*, *MGAT5*, and *ST6GAL1*). *N*-glycosyltransferase *MGAT3* showed novel association with different sialylated IgG *N*-glycans of which many also contain bisecting GlcNAc and the strongest *N*-glycan association out of all identified loci, which was not the case in previous GWAS on general European population comprising more than 8000 individuals. We previously showed that proportions of those *N*-glycans associated herein with *MGAT3* significantly differ between children with new-onset T1D and their healthy siblings. Second novel association is for chromosome 19 *C3* gene, encoding the pivotal protein of the complement activation pathway, the C3 protein. Missense and synonymous variants within C3 exons were associated with plasma Man9 glycan levels. Since such Man9 is attached to the C3 protein domain involved in pathogen binding, and implicated *C3* variant is often coinherited with another variant associated with increased T1D risk, this alteration might interfere with complement activation in T1D. Novel associations were confirmed in the follow-up cohort. This all suggest importance of further research of *N*-glycosylation role at the onset of T1D.

P73
N-GLYCOSYLATION OF IgA IN TYPE I DIABETES MELLITUS IN CHILDREN

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Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease with a large surge in the last few decades, especially among the young population. The etiology of T1DM is still unclear and includes both genetic and environmental factors, as well as their interaction. Numerous studies have shown that the glycosylation of plasma proteins is altered in various diseases, especially those with inflammatory elements. Our recent studies have shown that changes in glycosylation of total plasma proteins, as well as IgG, are present at the early stage of T1DM, suggesting that glycans are involved in the development of this disease and thus carry diagnostic and prognostic potential. Imunnoglobulin A (IgA) is one of proteins that might be implicated in these identified N-glycosylation changes. To confirm this hypothesis, using high performance liquid chromatography based on hydrophilic interactions (HILIC-UPLC), we analyzed N-glycosylation of IgA in children with early onset T1DM and compared it to their healthy siblings. Statistically significant increase in the level of high-mannose glycans composed of five mannose units, as well as the decrease in the highly branched trigalactosylated and trisialylated structures was found in T1DM. Change in oligomannose structure corresponds to that previously observed on immunoglobulin G. These results suggest the possible role of IgA N-glycosylation in the early course of T1DM and the need for further exploring its predictive value. The existence of a marker for early screening of T1DM risk would have an enormous value in the T1DM management.

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DNA METHYLATION AND PROTEIN EXPRESSION OF *APC* GENE IN PROSTATE CANCER PATIENTS

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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.

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TOLL-LIKE RECEPTOR 2 DEFICIENCY IS ASSOCIATED WITH SPECIFIC ALTERATIONS OF SYNAPTIC PROTEOME

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Synaptic glycoprotein neuroplastin (Np) is a crucial synaptic junction (SJ) proteome member. Evidenced functions of Np include regulating Ca^{2+} clearance and its involvement in long-term potentiation. We have previously reported differential immunohistochemical pattern of Np across brain regions in Toll-like receptor 2 (TLR2) knock-out (KO) mice and assumed that detected alterations might accompany large-scale changes in the synaptic environment in TLR2KO mice. These findings guided us on a journey to address whether the absence of TLR2 affects the SJ proteome. The study was conducted on female and male mice with 4 wild types (WT) and 4 TLR2 KO per group. Tissue samples of the cortex, cerebellum, and hippocampus were isolated by neuroanatomical dissection and subjected to membrane fractionation protocol to obtain synaptic junction enriched fractions. Tryptic in-gel digestion was applied to the collected fraction, and generated peptides were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Data analysis revealed significant changes in protein abundance across all isolated brain regions and supported our previous results demonstrating alterations of Np and P-Type ATPases expression in TLR2KO mice. The most remarkable and sex-dependent differences in protein content were observed in the cortex of TLR2KO animals compared to WT, while a lesser extent of proteome disruption was shown in the hippocampus and cerebellum of TLR2KO animals. The protein systems that underwent significant modifications are particularly those implicated in neurotransmission, overall pre-and postsynaptic arrangements, synaptic energy metabolism, axon/dendrite cytoskeleton, and myelin sheath modifications. In conclusion, investigating a synaptic proteome in the TLR2KO mouse model enables a fresh look at the potential new roles of TLR2 in intercellular communication and neuronal connectivity in a sex-specific manner.

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ROLE OF α-ACTININs IN αVβ5 FA MATURATION, ACTIN ORGANISATION AND MIGRATION IN MELANOMA CELL LINE RPMI-7951

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Changes in expression of integrins, heterodimeric transmembrane cell adhesion molecules used by cells to bind to the extracellular matrix and control cytoskeletal network organization, are frequently associated with cancer. Integrins foster tumour cell survival, proliferation and migration during invasion and metastasis through bidirectional signalling via multimolecular integrin adhesion complexes (IACs). Our previously published data in melanoma cell line RPMI-7951 showed that knockdown of integrin αV decreased migration and invasion. Mass spectrometry (MS) analysis of biochemically isolated IACs revealed that this cell line preferentially uses integrin αVβ5 for adhesion forming FAs, and we confirmed its exclusive role in regulating cell migration. MS also revealed that αVβ5 IACs contain actin cross-linking proteins α-actinin 1 (ACTN1) and α-actinin 4 (ACTN4). These are ubiquitously expressed cytoskeleton proteins that cross-link actin filaments, and through interaction with a number of IAC proteins and integrins anchor them to focal adhesions at the leading edge of migrating cells. As such, α-actinins can influence actin cytoskeleton organization and dynamics, focal adhesion maturation and cell migration. To investigate their localisation, elucidate their individual role in formation of IACs, actin dynamics and migration, we performed immunofluorescence and western blot analyses of ACTN1 and ACTN4 localisation/ expression, actin and FAs visualisation as well as migration assay upon knockdown of each ACTNs. Both knockdown of ACTN1 and ACTN4 resulted in diminished cell size and decreased expression of integrin αVβ5 FAs. However, the integrin αVβ5 FAs size only changed upon ACTN1 knockdown, indicating its possible role in maturation of αVβ5 FAs. Additional data pointing to the role of ACTN1 in FAs maintenance was the increased expression of ACTN1 upon ACTN4 knockdown. Also, we observed a transition from dorsal actin fibers with the transverse arc to ventral stress fibers upon ACTN4 knockdown. We are currently performing migration assays and live cell imaging upon each ACTN knockdown using fluorescently labelled ACTN4. These data will elucidate the differential role of ACTN isoforms in αVβ5 FAs which contribute to actin organisation and migration in melanoma.

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RHODANINE DERIVATIVES AS POTENT MUSHROOM TYROSINASE INHIBITORS

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Tyrosinases are enzymes belonging to the type-3 copper protein family which contains two copper ions in the active site. These oxidoreductases perform two sequential enzymatic reactions: hydroxylation of monophenols followed by oxidation of diphenols forming quinones which polymerize spontaneously to melanin. Tyrosinases are found in different prokaryotes as well as in plants, fungi, arthropods, and mammals. Besides its positive role in various organisms, like wound healing, sclerotization and pigmentation of insect cuticles, defence against herbivores and pathogens in plants, tyrosinases also can have negative impacts on humans (*senile lentigo*, melisma, freckles and pigmented ace scars) and plants (browning in fruit and vegetables following cell damage). Therefore, development of new potent tyrosinase inhibitors in medicine and cosmetic industry, as well in the food industry, is of interest. In this research 30 differently substituted rhodanine derivatives as possible mushroom tyrosinase inhibitors were tested with both, L-tyrosine and L-DOPA as substrates. Rhodanine derivatives were synthesized with green method in deep eutectic solvents without usage of organic solvents or harmful catalysts. Synthesised rhodanines inhibited tyrosinase in the range 4.2 - 100.0 % and 1.1 - 100.0 % with L-DOPA and L-tyrosine as substrates, respectively. Compound 3-allyl-5-(3-hydroxy-4-methoxybenzylidene)-2-thioxothiazolidin-4-one showed 100.0 % of tyrosinase inhibition when using both substrates. Tyrosinase inhibition of 100.0 % was also achieved with compounds 3-allyl-5-(4-(benzyloxy)-2-hydroxybenzylidene)-2-thioxothiazolidin-4-one and 3-amino-5-(3-hydroxy-4-methoxybenzylidene)-2-thioxothiazolidin-4-one but with L-tyrosine as substrate. The lowest IC_{50} values of 0.30 μM in case of L-DOPA and 0.23 μM in case of L-tyrosine were determined for compounds 3-allyl-5-((E)-3-phenylallylidene)-2-thioxothiazolidin-4-one and 3-amino-5-(3-hydroxy-4-methoxybenzylidene)-2-thioxothiazolidin-4-one, respectively.

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PALINDROME-INITAITED GENOME INSTABILITY IN EUKARYOTIC CELLS

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Palindromes in DNA are symmetrical motifs consisting of two inverted repeats, i.e. having the same nucleotide sequence in the two complementary strands when read in the same direction of polarity. The inverted repeats can be completely identical and adjacent to each other which is referred to as a perfect palindrome, or have mismatches and/or a spacer region in between. If a palindrome is long enough, this symmetry allows for intrastrand base pairing to occur and secondary structures can form in dsDNA. Although palindromes are often found as important functional elements of *cis*-acting regions and protein binding sites in the genomes, they are also known as potentially dangerous elements which can lead to double strand breaks and thus instigate genetic recombination. In turn, this can lead to various rearrangements - deletions, duplications, translocations or amplifications which are all common features of cancer cells. In particular, palindromic amplifications which alter expression levels of certain genes are linked to poor disease outcome since they can make cancer cells more proliferative and more resistant to drugs. The aim of our research effort is to elucidate molecular mechanisms of palindrome recombinogenicity in the eukaryotic genome using yeast *Saccharomyces cerevisiae* as a model organism. Within this aim, we are pursuing several areas of research: first one is focused on the features of the palindrome sequence (e.g. the relationship between palindrome recombinogenicity and its length as well as the spacer length); second, on the physical conditions of the environment (e.g. the influence of growth temperature on palindrome recombinogenicity); and third, on the proteins and pathways involved in occurrence and repair of palindrome-initiated double strand break and its potential consequences for the genome stability. Taken together, the knowledge collected in *S. cerevisiae* can help better understand the underlying causes of palindrome-initated genetic instability in cancer cells.

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PREPARATION OF AMINO ACID-FUNCTIONALIZED CARBON QUANTUM DOTS USING CLEMENTINE PEEL – POTENTIAL APPLICATION IN BIOMEDICAL ANALYSIS AND AS FLUORESCENT PROBE FOR Fe^{3+} DETECTION

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Luminiscent carbon quantum dots (CQDs) are widely known as zero-dimensional nanomaterials which have attracted extensive attention, especially in green chemistry and biomedicine. Due to their excellent biocompatibility and low toxicity, water solubility, stability in high ionic media and great optical properties, CQDs have been widely used as functional optical materials in fluorescence sensing. In this study, preparation and modification of CQDs using clementine peel as carbon precursor and amino acids with different chemical complexity (glycine and arginine - nitrogen dopants) has been presented. It has been demonstrated that increasing nitrogen content in CQDs samples has increased the quantum yield percentage of prepared CQDs. Some differences in sample properties were observed regarding structural and chemical diversity, biological and antioxidant activity. The antiproliferative effect of CQD@Gly against pancreatic cancer cell lines (CFPAC-1) was demonstrated. Based on the DPPH assay results, the CQD@Arg demonstrated the highest antiradical activity 81.39 ± 0.39%, and EC_{50} was determined to be EC_{50} = 53.78 ± 0.97 μg/mL (R^2 = 0.9357). Furthermore, due to the highest determined quantum yield, CQD@Arg sample was further used for the ion sensing and cellular imaging of cancer cells. The CQD@Arg was applied as a fluorescent nanoprobe for Fe^{3+} detection, with a good linear correlation in the concentration range from 7.0 μmol dm^{-3} to 50.0 μmol dm^{-3} with R^2 = 0.9931 and limit of detection (LOD) of 4.57 ± 0.27 μmol dm^{-3}. In order to investigate the applicability of prepared CQDs in cell imaging, MCF-7 cells were incubated with CQD@Arg and imaged by confocal microscopy. This study implies the potential application of the prepared CQDs in bioimaging and ion sensing, and also as a fluorescent probe with diverse biological and pharmacological activities in general.

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Poster Abstracts

P80
BIVALVES DISCLOSE NOVEL PATTERN OF SATELLITE DNA ORGANIZATION, HIGHLY DISPERSED AND CLOSELY CONNECTED TO HELITRON MOBILE ELEMENTS

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Several features have already qualified commercially and ecologically important bivalve species from the family Ostreidae as valuable non-standard model organisms, confirmed by an exponentially increasing number of genome sequencing projects and genome research on this group of organisms. The ubiquitous and in the same time still the least understood DNA components of every eukaryotic genome are repetitive DNA sequences. They cause significant technical problems in DNA sequencing and assembly and frequently are omitted or underrepresented in genome assemblies, consequently being called the "dark matter of the genome". Repetitive DNA sequences are divided into two major groups, satellite DNAs (satDNAs) composed of sequences repeated in tandem, and transposable elements (TEs) interspersed throughout the genome. In this work we have revealed a completely new principle of satDNA organization in respect to the generally accepted paradigm that satDNAs typically form long arrays of monomers, composing heterochromatic chromosomal compartments. We have identified complete inventory of satDNAs, the satellitome, of the invasive Pacific oyster *Crassostrea gigas*, consisting of 52 satDNAs. While heterochromatin in this species is extremely scarce, satellitome analysis disclosed novel and unusual, highly scattered arrangement of relatively short satDNA arrays across the whole genome. The inspection of the organizational forms of the most abundant satDNAs displayed their association with constitutive parts of Helitron TEs. In addition, Helitron-related satDNAs exhibit an advantage in the number of chromosomes occupied, indicating that these TEs are a significant factor in their genome-wide propagation, as well as in forming of genome architecture of *C. gigas*.

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LOSS OF SIRTUIN 3 HAS AN ADVERSE MALE-SPECIFIC EFFECT ON MITOCHONDRIAL FITNESS AND SURVIVAL OF MOUSE EMBRYONIC FIBROBLASTS

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Sexual dimorphism in metabolism has been observed in many animal species during early development. However, little is known about the molecular mechanisms underlying these differences. Sirtuin 3 (Sirt3) is the major mitochondrial deacetylase that plays an important role in regulating metabolic processes, but there is little information on its role in the context of sex differences in metabolic regulation. To test our hypothesis that the role of Sirt3 in metabolic regulation is sex-dependent *in vitro*, we measured proliferative, metabolic, antioxidant, and mitochondrial parameters in Sirt3 wild-type (WT) and knockout (KO) mouse embryonic fibroblasts (MEFs). We observed that depletion of Sirt3 results in reduced proliferation in both sexes, which is exacerbated in male MEFs. This suggests that Sirt3 is essential for cell viability, especially in male MEFs. Furthermore, we have shown that Sirt3 is upregulated in female MEFs, consistent with their higher energy status. In general, female MEFs had higher ATP production and more mitochondria than male MEFs in both genotypes. Loss of Sirt3 decreased mitochondrial membrane potential as well as CI-driven respiration coupled to ATP production in both sexes. Furthermore, we observed increased phosphorylation, i.e. activation of AMPK in Sirt3-depleted MEFs, and decreased levels of FASN and Scd-1, proteins involved in the anabolic process of *de novo* lipogenesis (DNL), whereas the levels of enzymes involved in fatty acid oxidation (HADHB) were increased. Overall, these results suggest that loss of Sirt3 results in lower energy levels in both sexes, with greater effects on male MEFs, contributing to their lower survival. In this study, we report for the first time unique sex-specific consequences of Sirt3 depletion and sex-specific patterns of mitochondrial function, energy status, and metabolic parameters in MEFs.

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INTERACTION OF WGA PLANT LECTIN AND GLYCOLIPOSOMES FUNCTIONALIZED WITH THE COMPONENTS OF THE BACTERIAL CELL WALL PEPTIDOGLYCAN

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Molecular nanotechnology includes different and powerful new tools for understanding biological processes and the treatment of human diseases. Liposomes are non-toxic and biocompatible; thus they are used as delivery systems for biologically active substances and models for testing ligand-receptor interactions. Peptidoglycan (PGN) is the major component of bacterial cell walls recognized by the innate immune system through a series of pattern recognition receptors (PRR), which play a key role in the first-line defense of the body. Lectins, naturally occurring carbohydrate-binding proteins, are involved in numerous biological processes. Some of them act as PRRs and bind significantly to PGN. Therefore, extensive studies are carried out on the development of functional nanosystems for PRR targeting in the fields of infectious diseases and cancer research. In our study, we were primarily interested in the study of interactions of PGM with lectins. Therefore, we designed and prepared glycosylated liposomes whose surfaces are decorated with peptidoglycan monomer, the disaccharide pentapeptide β-D-Glc*N*Ac-(1→4)-D-Mur*N*Ac-L-Ala-D-isoGlu-*meso*A_2pm-(εNH_2)-D-Ala-D-Ala, (PGM). PGM is a repeating unit of *B. divaricatum* cell wall peptidoglycan. To incorporate the PGM into the lipid bilayer, a lipophilic derivative, PGM-oleyl was synthesized. PGM-modified liposomes with various amounts of entrapped PGM-oleyl were prepared and characterized. It was shown that incorporation of PGM-oleyl into liposomes affects the size and surface charge of liposomes, the size and zeta potential was decreased in regards to PC liposomes. The presence of PGM, i.e. Glc*N*Ac on the surface of the liposomes was confirmed by measuring an increase of the mean liposome size in suspensions after the addition of model lectin, wheat germ agglutinin (WGA), specific for terminal *N*-acetylneuraminic acid and Glc*N*Ac units. It was demonstrated that PGM was effectively recognized by WGA. Since the peptidoglycan recognition by PRRs involves moderate to high-affinity interactions with the carbohydrate moiety as well as the peptide moiety of peptidoglycan, the established platform could be successfully employed in analyses of lectin-carbohydrate interactions but also as a targeted drug delivery system.

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COMPLEMENT COMPONENT C3 N-GLYCOSYLATION IS CHANGED IN EARLY ONSET TYPE 1 DIABETES MELLITUS

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The most abundant and central component, glycoprotein C3, contributes to the development of type 1 diabetes by enhancing the organ-specific autoimmune inflammatory processes. It is known that changes in glycosylation can modulate inflammatory responses and we recently showed that children at the onset of type 1 diabetes have a higher proportion of oligomannose glycans in plasma N-glycome compared to their healthy siblings. Due to the fact that C3 contains two N-glycosylation sites occupied by this type of glycans, our aim was to establish a novel workflow for C3 N-glycoprofiling in order to reveal the possible role of C3 glycosylation in type 1 diabetes development. Therefore, we developed a novel high-throughput and cost-effective glycoproteomic workflow for a site specific N-glycosylation LC-MS analysis of human C3. Our newly developed method includes C3 enrichment from human plasma in a 96-well format using Concanavalin A lectin affinity matrix, Glu-C digestion of enriched glycoproteins and nano-LC-MS analysis of purified Glu-C glycopeptides. We used our method on plasma samples from 61 children and adolescents (1-16 years) newly diagnosed with T1D and their 84 (4-22 years) unaffected siblings. Our study showed that C3 N-glycan profiles are significantly changed in type 1 diabetes patients compared to their healthy siblings. Type 1 diabetes was associated with an increase in the proportion of unprocessed glycan structures with more mannose units on both N-glycosylation sites, with glycan $Glc_1Man_9GlcNAc_2$ showing largest difference (OR = 1.28, $p = 1.78\times10^{-8}$). Finally, model including C3 N-glycans showed notable discriminative power between children with type 1 diabetes and their healthy siblings with AUC of 0.879. This study confirmed our previous findings of total plasma N-glycosylation changes of high-mannose glycan levels in a cohort of recent onset type 1 diabetes cases, and empowered the hypothesis that C3 is the real cause of these changes. Here we defined C3 N-glycan changes accompanying onset of type 1 diabetes and developed glycan-based discriminative model that could have valuable potential in risk assessment, confirming the relevance of C3 N-glycosylation in type 1 diabetes.

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GENOTOXIC ACTIVITY OF BENZO(A)PYRENE EVALUATED IN HEPATIC 3D CELL MODEL

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Xenobiotic safety assessment is an area that impacts multiple different industry sectors, such as medical drugs, industrial chemicals, cosmetics and environmental contaminants, therefore it is important to improve a safety assessment process. For this purpose, there is a high demand for the development of physiologically more relevant *in vitro* cell-based systems that can provide more predictive results for human exposure. In the present study, we developed a hepatic *in vitro* HepG2 3D cell model with the forced floating method. HepG2 cells in 3D (spheroids) were cultured under static conditions for 72 hours and validated for genotoxicity assessment by testing genotoxic activity of indirect acting compound, benzo(a)pyrene (BaP). Spheroids were exposed to BaP (0.1, 1, 10, 20 μM) for 24h. The influence of BaP on spheroid growth was monitored by planimetry, while live/dead cells were determied by FDA/PI staining and evaluated by confocal microscopy. The results revealed that BaP decreased spheroid surface area at ≥ 10 μM and affected cell viability at 20 μM. The effect of BaP on cell proliferation (Ki67 marker) and cell cycle alterations was assessed by flow cytometry, and its genotoxic activity was determined with the comet and γH2AX assays. At applied conditions, BaP (10 μM) reduced the number of Ki67 positive cells and arrested HepG2 cells in the S phase of the cell cycle. Moreover, BaP induced the formation of DNA single (comet) and double (γH2AX) strand breaks. On the mRNA level, BaP deregulated the expression of phase I (*CYP1A1, CYP1A2)* and II (*UGT1A1, SULT1B1* and *NAT2)* enzymes and DNA damage responsive genes (*P53, GADD45α, CDKN1A*). In summary, the newly developed hepatic HepG2 3D cell model provides a suitable experimental model for genotoxicity assessment due to its improved metabolic capacity.
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XANTHOHUMOL ATTENUATES THE HAZARDOUS EFFECTS OF THE CARCINOGENIC MYCOTOXIN AFLATOXIN B1

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Xanthohumol (XN), a prenylated flavonoid found in hops, the female inflorescences of the plant *Humulus lupulus* L., is known for its antioxidant properties and various other health benefits. However, the mechanisms underlying its protective effects against chemically induced carcinogenesis are not well understood. The bioavailability of XN is limited and in addition, it is nonenzymatically isomerized to isoxanthohumol (IXN) and metabolized by cytochrome P450 enzymes to 8-prenylnaringenin (8-PN), desmethylxanthohumol, which is further transformed to 6-prenylnaringenin (6-PN). We investigated the chemoprotective effects of XN against the carcinogenic mycotoxin Aflatoxin B1 (AFB1), a common food and feed contaminant, *in silico* and *in vitro*. The chemical reactions between XN and its derivatives IXN, 8-PN and 6-PN, with the carcinogenic metabolite of AFB1, aflatoxin B1 exo-8,9-epoxide (AFBO), were studied by calculating activation free energies ($\Delta G^{\ddagger}$) at the Hartree-Fock level of theory in conjunction with the 6-311++G(d,p) basis set and two implicit solvation models. The chemoprotective effects of XN were investigated in the metabolically competent HepG2 cell line, by analyzing its influence on AFB1-induced cytotoxicity and genotoxicity using the MTS assay, the Comet assay, the γH2AX assay, and by evaluating cell cycle modulation using flow cytometry. Our results provide evidence for a possible additional mechanism involved in the chemoprotective activity of XN, respectively that XN and its derivatives (IXN, 8-PN and 6-PN) could act as scavengers for the ultimate carcinogen AFBO, as the $\Delta G^{\ddagger}$ required for the reactions of XN and its derivatives with AFBO are comparable or lower than the $\Delta G^{\ddagger}$ required for the reaction of AFBO with the most reactive DNA base – guanine. The proposed scavenging activity of XN would result in a reduction in AFBO-DNA interactions and consequently reduction of DNA damage after AFB1 exposure. The latter was confirmed *in vitro* in HepG2 cells, where our results showed a dose-dependent reduction in AFB1-induced cytotoxicity and DNA single- and double-strand breaks in cells, exposed to combinations of AFB1 and graded concentrations of XN, highlighting the chemoprotective effects of this phytochemical.

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YEAST CELL WALL PROTEIN Pir3 IN RNA EXOSOME MUTANTS

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The cell wall of yeast is an extracellular organelle important for shape, communication with the environment and protection, especially under stress conditions. It consists of mannoproteins covalently or noncovalently bound to a polysaccharide, composed mainly of mannoproteins, β-glucan and chitin. Pir proteins are cell wall proteins (Pir – protein containing internal repeats) required for cell wall stability and are directly linked to the β-1,3-glucan network through an alkali-sensitive linkage via ester bonds. The cell wall is continuously remodeled as yeast cells undergo various life processes that are thought to involve changes in the expression of genes encoding cell wall proteins. Another important complex for maintaining cell wall stability is the nuclear RNA exosome, a 3′→5′ exonuclease multiprotein complex consisting of the EXO-9 core and two catalytic subunits, Dis3 and Rrp6. More specifically, the RNA exosome mediates the levels of specific mRNAs in response to environmental changes affecting the cell by processing and degrading RNA substrates. In this work, we would like to obtain information about Pir3 protein levels in different RNA exosome subunit mutants (*rrp6Δ, rrp47Δ, mpp6Δ, air1Δ, air2Δ, air1air2, trf4, trf5*) in logarithmic and stationary growth phase. In addition, this information may allow us to gain more insight into the role of the Pir3 protein, a structural component required for cell wall stability, and other members of the PIR familly.

P87
STEMNESS-INDUCING 3D CULTURING HAS A DIFFERENT IMPACT ON CARBOPLATIN RESISTANT HIGH GRADE SEROUS OVARIAN CANCER INVASIVENESS

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Ovarian cancer (OC) remains one of the deadliest forms of gynecological cancers to this day, due to its heterogeneity and the absence of specific symptoms in early stages. Thus, it is almost always detected as an advanced and metastatic disease. High grade serous ovarian cancer (HGSOC) is the most lethal type of OC which quickly acquires the resistance to the conventional first line chemotherapy protocols with carboplatin (CBP) and paclitaxel (TAX). It is essential to find novel therapeutical options as well as diagnostic and prognostic markers to successfully combat HGSOC and improve patient outcomes. Three-dimensional (3D) cell cultures where cells aggregate and exhibit cell-cell and cell-extracellular matrix interactions have emerged as a much better *in vitro* model than conventional two-dimensional (2D) monolayer cell cultures wich have been an *in vitro* standard for testing drugs and for studies of cancer cell biology for almost 80 years. We have grown several patient-derived HGSOC 3D organoid cultures in solubilized basement membrane matrix and in specific stemness-inducing OC medium, observed them for several passages and tested their resistance to CBP in such conditions. Two OC cell lines, MES-OV CBP and SK-OV-3 CBP, established previously by the treatment of the parental, MES-OV and SK-OV-3 cell lines with long-term, stepwise selection in CBP were cultured as 3D spheroid cultures in the same stemness-inducing OC medium, alongside their parental cell lines. The CBP resistance of MES-OV CBP and SK-OV-3 CBP cells was confirmed in 3D conditions. Further it was noticed that both CBP resistant cell lines expressed different 3D formations and migration patterns in the OC stemness-inducing medium in comparison with not only their parental cell lines, but also between one another, indicating differences in the underlying molecular mechanisms. Further steps will be taken toward exploration of signaling pathways and molecular mechanisms involved in regulations of invasive capacity of CBP resistant cells in 3D spheroid stemness-inducing OC culture conditions.

P88
DIVERSED EXPRESSION OF SHORT p53 FAMILY ISOFORMS MAY AFFECT MELANOMA AGGRESSIVENESS

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Metastatic melanoma is the most aggressive form of skin cancer. Despite currently available therapy targeting BRAF and MEK kinases, as well as immunotherapy, the treatment of melanoma remains a challenge due to resistance to therapy. Thus, it is of outmost importance to investigate the molecular pathways crucial for melanoma development and therapy resistance. The TP53 gene, the guardian of the genome, is altered in more than 50% of human cancers but is rarely mutated in melanoma. Shorter p53 family isoforms, whose significance has just recently become evident, can act as modifiers of the p53-dependent responses including its tumor suppressive function. We have analyzed the gene and protein expression of p53 and p73 isoforms in a panel of human melanoma cell lines with different TP53 and BRAF status, in normal conditions or after the treatment with common DNA-damaging agents or targeted therapy. We generated stable clones of H1299 p53 *null* cells over-expressing the less characterized short isoforms Δ160p53α, Δ160p53β, and Δ160p53γ. Furthermore, we developed two human melanoma cell lines resistant to the BRAF inhibitor vemurafenib and examined whether there was a change in the expression of the p53 family isoforms with the acquisition of resistance. Our results show that human melanoma cell lines express wide array of p53 and p73 isoforms. We demonstrated for the first time that Δ160p53α, and to a lesser extent Δ160p53β, can be recruited on chromatin, and that Δ160p53γ can localize in perinuclear foci. Importantly, H1299 cells stably expressing Δ160p53 isoforms demonstrated higher proliferation and *in vitro* migration. Finally, melanoma cells resistant to vemurafenib exhibited an altered expression of p53 and p73 isoforms, specifically increased expression of potentially pro-oncogenic Δ40p53β and a decreased level of tumor-suppressive TAp73β. Therefore, we propose that p53 family isoforms play a role in the aggressiveness of melanoma cells and could be a potential marker and target for melanoma therapy.

P89
ROLE OF TALINS AND KANKS IN INTEGRIN αVβ5 FOCAL ADHESIONS, ACTIN-MICROTUBULE CROSSTALK AND RESPONSE TO PACLITAXEL TREATMENT IN MDA-MB-435S CELLS

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Multiple signalling pathways that can control cytoskeletal network organisation, as well as cell proliferation, differentiation, survival and motility are triggered by cell binding to the extracellular matrix (ECM) via integrins. Upon binding to the ECM, these heterodimeric cell surface receptors cluster and form multimolecular integrin adhesion complexes (IACs). IAC composition analysis in MDA-MB-435s, previously described that integrin αVβ5 is the predominant integrin used in long term culture and showed increased sensitivity to microtubule (MT) poisons, paclitaxel (PTX) and vincristine, upon integrin αV knockdown. The analysis also revealed that αVβ5 IACs contain talins (TLN) 1 and 2, and KANKs 1 and 2. Out of these, KANK2 has already been shown to have a key role in connecting the αVβ5 focal adhesions (FAs) to MTs, and influencing cell sensitivity to PTX. Since talins and KANKs bind, our goal was to investigate their localisation, distinguish the mutual binding of their isoforms as well as elucidate their role in formation of IACs and MT dynamics, and subsequently their effect on cell sensitivity to PTX. We performed immunofluorescence and western blot analyses of TLN1, TLN2, KANK1, KANK2 localisation/ expression, actin and MTs visualisation as well as survival and proliferation assay upon knockdown of each TLNs and KANKs. TLN1 knockdown resulted in decreased expression and changed localisation of KANK2 in cell, eliminated integrin αVβ5 FAs, changed cell morphology and proliferation as well actin and MT appearance; however, it did not increase cell sensitivity to PTX. On the other hand, knockdown of TLN2 did not affect cell morphology and proliferation, nor KANK2 expression and localisation, but it slightly changed FA size, increased sensitivity to PTX and altered the MT appearance. Interestingly, knockdown of TLN1 or TLN2 did not change the localisation/ expression of KANK1, and KANK1 knockdown does not affect sensitivity to PTX. We are currently performing microtubule dynamics measurements upon each TLN and KANK protein using fluorescently labelled microtubule end-binding protein 3. These data will elucidate the differential role of TLN and KANK isoforms in αVβ5 FAs which contribute to actin-MT crosstalk and consequential response to MT poisons in the melanoma.

P90
NEUROPEPTIDES - SUPSTRATES OF DIPEPTIDYL PEPTIDASE III

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Dipeptidyl peptidase III (DPP III) is a monozinc peptidase that catalyzes the hydrolytic cleavage of dipeptides sequentially from the N-terminus of peptides which consist of three or more amino acids. It is widely distributed in mammalian tissues and is thought to be involved in the final steps of normal intracellular protein degradation. However, its marked affinity for some bioactive peptides (angiotensin II and III, opioid peptides) suggests more specific functions, such as its role in blood pressure regulation and its involvement in the mammalian pain regulatory system. Cruz-Diaz et al showed that DPP III degrades angiotensin (1-7) in human renal epithelial cells and suggested a role of DPP III in blood pressure regulation. Colocalization of DPP III in the superficial laminae with enkephalins and endomorphins and the finding that DPP III can degrade these opioid peptides in vitro support the role of DPP III in the endogenous pain regulatory system. We have investigated a number of different (endogenous) neuropeptides as potential substrates and inhibitors of human DPP III. The determined binding affinities and kinetic data in combination with fluorimetric measurements allowed us to distinguish the substrates of human DPP III: Leu-valorphin-Arg and hemorphin-4 from the slow substrates valorphin and β-casomorphin, while four of the total 14 peptides tested did not appear to interact with DPP III as determined by three complementary experimental methods. For the other peptides, the obtained data were mostly consistent with those previously determined.

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P91
CAVEOLIN-1 IN PROSTATE CANCER

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High prevalence and mortality of prostate cancer (PCa) is well known global health issue. Ongoing search upon new diagnostic, prognostic and predictive marker is always current. There is need of markers which could identify patients with PCa against benign conditions like prostate hyperplasia (BPH) or inflammation. Another issue dealing with indolent PCa course against aggressive forms of the disease is also topic of the intense research. CAV1 is protein whose expression differs among various cancer types and exhibits the dual role of this protein, acting as tumor promoter and tumor suppressor in different tumor settings. We estimated CAV1 could improve PCa diagnostics and aim of this study was to explore CAV1 methylation of cfDNA in liquid biopsy samples, blood and semen, as potential minimally invasive marker. In our study eighty patients with PCa and BPH were analyzed for CAV1 gene expression and methylation in tissue. *CAV1* cfDNA methylation from blood and seminal plasma was accessed as potential PCa biomarker. Protein expression of CAV1 in tissue showed changed pattern in the PCa tissue. Methylation in seminal plasma showed potential as biomarker. Discrimination of BPH and Gleason grade group 1 PCa patients from patients with higher Gleason grade groups revealed very good performance as well. Based on our results CAV1 methylation may be useful biomarker with potential for further seminal plasma cfDNA research but its diagnostic accuracy should be improved.

P92
SATELLITOME ANALYSIS OF THE BLACK FLOUR BEETLE *TRIBOLIUM MADENS*

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Satellite DNA (satDNA) sequences have for long been underrepresented in the genomic studies, primarily due to their non-coding nature and repetitive organization in a genome. Nevertheless, with the advances in third-generation sequencing technologies and tailored bioinformatic tools, it has become possible to characterise a large number of satDNA families in the genomes. Therefore, the term satellitome has been introduced to signify a collection of all satDNA families in one species. Species of the genus *Tribolium* are known to have a high content of satDNAs in their genomes. The black flour beetle *Tribolium madens* has two major satDNAs characterised, together comprising one third of the genome. The aim of this research was to characterise the *T. madens* satellitome comprehensively by discovering hitherto unknown low-copy satDNAs in order to unmask the setup of tandemly repeated sequences in the genome of this species. To achieve this goal, we sequenced the *T. madens* genome using two different high-throughput sequencing technologies. Using Illumina platform, 151 bp short reads were obtained and analysed by the computer pipeline TAREAN which defined low-copy satDNAs and their consensus sequences. Sequencing by PacBio HiFi technology generated 15-kb-long, highly accurate reads which were used to study the genomic organisation of the newly defined satDNAs. By this approach, over 100 new satDNAs in the genome of *T. madens* were defined, while 10 of the most prominent satDNA families were further characterised experimentally. Fluorescence *in situ* hybridisation revealed that the newly detected satDNAs are scattered throughout the genome, not present on all the chromosomes of the complement (2n = 20 + supernumerary chromosomes). Orthologous sequences of the *T. madens* low-copy satDNAs were disclosed in the genomes of related species *Tribolium castaneum* and *Tribolium freemani*, revealing that some of the satDNAs in the genus *Tribolium* evolve according to the concept of concerted evolution. Although still underestimated in relation to coding DNA studies, comparative satellitome analyses advance our understanding of the genome divergence between closely related species and may provide valuable insights into their evolution and speciation.

P93
IMMUNOSTAINING OF MAIT AND γδT CELLS IN PSORIATIC LESIONS

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Psoriasis vulgaris (PV) is a common, chronic inflammatory skin disease driven by aberrant activation of T cells. In addition to autoreactive conventional T cell lines, innate-like lymphocytes, such as MAIT (mucosal-associated invariant T cells) and γδT cells, have been recently implicated in the initiation and progression of PV. Both MAIT and γδT lymphocytes seed multiple epithelial tissues, and demonstrate various functional capacities in host tissue repair, pathogen clearance, tumor surveillance, and inflammation. In case of injury or antigen encounter in the skin, these innate-like players exhibit rapid effector responses and directly contribute to local IL-17 production, a key pro-inflammatory mediator of epithelial, stromal, and vascular remodeling in psoriasis. Emerging evidence suggest an important role for MAIT and γδT cells in PV immunopathology, but their diversity, distribution and trafficking in psoriatic skin plaques remain less well understood. To determine the number and distribution of IL-17-producing MAIT and γδT cells in psoriatic and healthy skin, a multiplex immunofluorescence analysis was performed with MR1 5-OP-RU PE-conjugated tetramer in combination with primary monoclonal antibodies against CD3, IL-17A and γδTCR. Psoriatic (n=6) and healthy (n=3) skin biopsies were initially fixed in 4% paraformaldehyde, cryoprotected in sucrose gradient and snap frozen, before being cut on the cryostat. Primary antibodies were detected with fluorescently labeled secondary antibodies, and nuclei stained with DAPI. All slides were covered with a VectaShield covering media and imaged under a fluorescent microscope. Compared to healthy skin, psoriatic skin sections were significantly enriched in CD3+ and IL-17+ T cells, of which only a minor fraction were either γδTCR+ or MR1-restricted lymphocytes. Although rarely evident in examined skin sections, both MR1-tet+IL-17+ and γδTCR+IL17+ T cells were more frequently observed in psoriatic sections, predominantly in dermal area, between elongated rete ridges of the epidermis. In conclusion, our results provide novel insights on MAIT and γδT cell distribution in healthy and psoriatic skin, and warrant further research on their phenotype and function in PV.

P94
REPURPOSING OF A COMPOUND: MECHANISMS OF SIRAMESINE'S ACTION AGAINST CANDIDA ALBICANS

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Repurposing of already known drugs is a strategy to overcome the slow process and high costs of a development of new compounds with therapeutic potential and its further evaluations in clinical trials. As fungal infections are raised in number and its control due to limited number of medicinal products is limited, using includes machine learning and molecular docking, we found that siramesine can be good antifungal compound (Vlainic et. al 2021). Using reference methods, we identified membrane of C. albicans as a molecular target of siramesine with MIC at concentration 12.5 μg/mL and MBEC range of 50-62.5 μg/mL for biofilm and mature biofilm formation, respectively. Our study showed interference of siramesine with ergosterol biosynthesis *in vitro* causing modulation of membrane permeability and consecutive leakage of intracellular content to extracellular compartments. In addition, siramesine caused depolarisation of plasma membrane, increased level of oxidative processes with consecutive lipid peroxidation. Protoplasts of *C. albicans* were destroyed and cytochrome C released following siramesine treatment. All these mechanisms triggered cell death of *C. albicans*, one fungal species that is a leading cause of infections in animals and humans. Siramesine was developed as sigma-2 receptor ligand for anxiety and depression but failed at clinical trials due to low efficacy. On the other hand, siramesine is nontoxic and well-tolerated. Taken this together with the results of our study, the possibility of siramesine repurposing as antifungal has strong background and further investigation, to elucidate its in vivo effectiveness against *Candida albicans* species is proposed.

P95
CYTOTOXIC EFFECT OF IMIDAZOLIUM OXIMES ON PROSTATE CANCER CELLS (PC-3)

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Prostate cancer is one of the most common types of cancer and is still difficult to cure with currently available antitumor agents. New compounds showing more efficient antitumor properties are being intensively developed. One of these compounds are imidazolium oximes, since these heterocyclic aromatic structures have numerous pharmacological properties, achieved through various modes of action and interactions with many cellular targets.
In view of the therapeutic importance of oximes in suppressing tumor growth, the present study focused on newly synthesized imidazolium oximes; hydroxyimino-methyl imidazolium bromides (compounds IV, VI, VII and X) in PC-3 prostate cancer cells. Cells were exposed to oximes in a concentration range of 6.25–800 µM for 1 h, 4 h, and 24 h. The effect on cell viability was investigated by monitoring mitochondrial succinate dehydrogenase activity in metabolically active cells by MTS assay. It was then examined whether the tested oximes impaired cell membrane integrity by measuring the activity of the enzyme lactate dehydrogenase (LDH). Finally, flow cytometry was used to elucidate if the tested oximes could induce programmed cell death, apoptosis. The results indicate that tested hydroxyimino-methyl imidazolium bromides caused a significant cytotoxic effect on PC-3 cells, in a time- and dose-dependent manner. Compounds VII and X, having in their structure aromatic side branch, exhibited the highest inhibitory effect on cell viability and induced a significant release of LDH into the medium, which points to the induction of necrosis. Conversely, none of the compounds induced apoptosis, which implies the need for further modification of these oximes in order for them to be suitable in future studies as potential antitumor drugs.
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P96
FUNCTIONAL CHARACTERIZATION OF THE NEWLY DISCOVERED TRUNCATION MUTATION OF S-ADENOSYLHOMOCYSTEINE HYDROLASE

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S-adenosylhomocysteine hydrolase (AHCY) catalyses the hydrolysis of S-adenosylhomocysteine (AdoHcy) to adenosine and homocysteine. Since AdoHcy is the product of all adenosylmethionine-dependent transmethylation reactions and their competitive inhibitor, its removal is crucial for maintaining normal cell methylation potential. So far, several mutations have been found that cause a decrease in AHCY activity (R49C, R49H, G71S, D86G, A89V, Y143C, Y328D and W112Ter). In addition to these known mutations, a new N403Ter mutation was recently discovered, leading to premature translation termination due to the introduction of a stop codon. Significantly elevated plasma AdoHcy (3 to 30-times) and S-adenosylmethionine (AdoMet, 2 to 20-times) have been measured in the effected individual. Similar aversive metabolic changes have been observed in previously identified patients. In order to assess its enzymatic capabilities, mutant AHCY N403Ter was overexpressed in bacterial strain *E.coli* BL21 and purified by Ni-NTA affinity chromatography. The enzymatic activity of the mutant was measured as shown before. Kinetic parameters were calculated using the Michaelis-Menten equation, using Lineweaver-Burk linearization. Truncated AHCY N403Ter exhibited ± 20% catalytic activity when compared to wild type enzyme, and showed significant changes to its quaternary structure as shown by native PAGE. Interestingly, enzymatic activity of the AHCY N403Ter mutant with the addition of thiol modifying components such as DTNB (5,5'-dithio-bis-(2-nitrobenzoic acid), and DTT (dithiothreitol) could be boosted to achieve almost full recovery of the AHCY hydrolytic activity when compared to wild type enzyme. This might enable the development of disease mitigation strategies for effected patients.

P97
INTERACTION OF ENVIRONMENTAL CONTAMINANTS WITH ZEBRAFISH (*Danio rerio*) MULTIDRUG AND TOXIN EXTRUSION PROTEIN 3 (Mate3/Slc47a3)

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Multidrug and toxin extrusion proteins belong to the superfamily of solute carriers. They function as bidirectional transporters (efflux of substrates is linked to proton-coupled electroneutral exchange) and they primarily mediate the elimination of cationic compounds. Phylogenetic analysis revealed six Mate transporters in teleost fishes annotated as Mate3-8. Because knowledge of non-mammalian Mates is still scarce, for molecular characterization of Mate transporters we used zebrafish (*Danio rerio*) as an established model organism. The zebrafish *mate3* gene, encoding the transmembrane protein Mate3, is constitutively expressed during embryonic development, followed by marked tissue-specific expression in adulthood. Mate3 is highly expressed in the kidney, intestine, testis, and brain of males, while expression is very low to moderate in females. It has been shown to interact with xenobiotic compounds, suggesting a role in efflux of toxic compounds. The aim of this study was to analyze the interaction of environmental pollutants with zebrafish Mate3 using a high-throughput screening assay. For this purpose, we have developed a stable zebrafish Mate3 cell line (Flp-In-293 / drM3 cell line) and standardized a cellular uptake assay using DAPI and ASP + as fluorescent model substrates. The developed assay was used to identify interactors of the zebrafish Mate3 transporter and to discriminate the nature of the interaction with a broad spectrum of 67 different environmental contaminants, including industrial chemicals, pesticides, and pharmaceuticals. As a result, highly potent Mate3 interactors were identified in all of the above groups, with the pharmaceuticals pyrimethamine (IC50 = 1 μM) and mitoxantrone dihydrochloride (IC50 = 1 μM) being the most potent interactors. Some of the identified interactors could be of environmental concern and their interaction with Mate3 could lead to impairment of its normal efflux function, making the fish more sensitive to environmental pollutants. Furthermore, knowledge on pharmaceuticals that act as Mate3 substrates and inhibitors may be relevant for biomedical applications.

P98
NINFE - A NEW CORRECTION METHOD FOR INNER FILTER EFFECT IN MICROPLATES

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Inner filter effect (IFE) arises from the absorption of excitation and/or emission light during fluorescence measurements. Therefore, fluorescence intensities are proportional to fluorophore concentration only in a limited, dilute range. Numerous mathematical models and experimental techniques have been used to extend the range of linear response of the fluorescence signal versus concentration, but most are inappropriate for measurements in microplates. A common method for IFE correction requires separate absorbance measurements and can only be used in flat-bottomed transparent microplates. Here we present a Numerical INner Filter Effect (NINFE) method that takes advantage of the variable position of the optical element perpendicular to the sample well (z-position) available in modern microplate readers. By using at least two fluorescence measurements at different z-positions, the linear range can be extended with NINFE. We have successfully applied the presented method to various plate well geometries, including the flat-bottom and round-bottom 96-well microplates (transparent, white, and black) and PCR microplates. NINFE corrections have been performed for protein solutions (BSA and human transferrin) and fluorescent amino acids (tyrosine and tryptophan) to demonstrate that this correction method is applicable to biological systems. Current investigations are aimed at integration with real-time PCR measurements.

P99
TRIGGERING APOPTOSIS IN HUMAN CELLS BY 3-HYDROXY-2-PYRIDINE OXIMES

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Pyridinium core-based oximes are primarily investigated as reactivators of synaptic acetylcholinesterase inhibited by organophosphorus nerve agents and pesticides. In the search for efficient antidotes, numerous structures have been synthesised, but for many of them side-effects and cytotoxicity are observed in their early-stage testing. Therefore, we aimed to investigate what causes this cytotoxicity for one of the oxime series, and whether it could be related to small differences in structural motives. We tested the effect of five 3-hydroxy-2-pyridine oximes on the viability of neuroblastoma SH-SY5Y cells, representing nerves as the main target of oxime antidotes action. The cytotoxic effect was monitored in a time- and dose-dependent manner. The results indicated apoptosis induction via the mitochondrial-dependent pathway by caspase 9 and/or 3 activation, accompanied by DNA damage, increased phosphorylation of MAPK kinase or acetyl-CoA carboxylase (ACC) and decreased phosphorylation of the transcription factor STAT3. We assume that 3-hydroxy-2-pyridine oximes target mitochondria and cellular metabolism of the fatty acids, due to increased phosphorylation of ACC. Furthermore, a hydroisoquinoline moiety in the structure seems to be responsible for triggering apoptosis, where dimethylamino-phenyl group had the most significant effect on cytotoxicity and activation of additional apoptosis initiator - caspase 8. In conclusion, even though these oximes cannot be considered as candidates in organophosphorus antidote research due to such influence on cells, they could be introduced in studies on other specific targets and as potential new drugs for different conditions.

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P100
A YEAST MODEL FOR STUDYING HUMAN TAU PROTEIN

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Age-dependent protein aggregation is a conserved phenomenon that is associated with many neurodegenerative diseases, including Alzheimer's disease (AD). AD is characterized by aggregation of Tau, a microtubule-binding protein that is normally soluble and mainly localized to neuronal axons, but which can form oligomers and higher order amyloid-like aggregates that accumulate in soma and dendrites and eventually lead to neuronal death. Although the main risk factor for the onset of AD is aging, the exact causes of Tau protein aggregation are still largely unclear. To investigate factors that influence Tau protein aggregation, we expressed human Tau protein fused with fluorescent proteins in yeast *Saccharomyces cerevisiae*. We examined its intracellular localization in young, logarithmically growing cells, in chronologically aged cells, and under different stress conditions, such as glucose starvation, hyperosmotic stress, elevated temperature and proteotoxic stress caused by a toxic amino acid analogue. Furthermore, to study the factors affecting Tau oligomerization, which is considered to be an early step in Tau pathology, we used luminescent reporter NanoBiT in which protein-protein interaction results in the complementation of the luciferase NanoLuc. Our results show basal levels of Tau-NanoBiT reporter signal in logarithmically growing wild-type cells, suggesting that Tau oligomerization does not occur under normal growth conditions.

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P101
DYNAMIC ADP-RIBOSYLATION REGULATES HSV-1 REPLICATION

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ADP-ribosylation (ADPr) is a posttranslational modification involving the covalent binding of an ADP-ribose unit from nicotinamide adenine dinucleotide (NAD^+) to target proteins. It is catalyzed by poly(ADP-ribose) polymerases (PARPs), PARP1 being the most abundant, together with histone PARylation factor 1 (HPF1) and reversed by ADPr-acceptor hydrolases (ARHs) and the macrodomain-containing enzymes such as poly(ADP-Ribose) glycohydrolase (PARG). It has been shown that the herpes simplex virus (HSV-1) triggers massive ADPr followed by the depletion of NAD^+ levels. The major HSV-1 regulatory protein, ICP4, has been found to be ADP-ribosylated. Moreover, there are several lines of evidence indicating ADP-ribosylation of other viral proteins. In addition, several studies investigating the roles of ribosylation in other herpesviruses have shown that PARP1 has a role in transcriptional repression and negative regulation of productive infection, and thus it might promote latency. Nonetheless, the biological relevance of these modifications for the HSV-1 replication is unknown. In this study, we aim to perform a broad functional screen to test the roles of PARPs and ARHs in HSV-1 infection and to investigate the underlying mechanisms for the observed phenotypes. Our preliminary results show that HSV-1 replication was remarkably reduced in cells deficient for the expression of PARP1, PARP2, and HPF1. These results indicate that ADP-ribosylation has a proviral role. However, using a PARG inhibitor that blocks removal of poly(ADP-ribose), and cells deficient for ARH3 (removal of mono(ADP-ribose)), we observed a reduction (i.e. proviral role), and enhancement of the HSV-1 replication (i.e. antiviral role), respectively. The molecular mechanisms behind these phenotypes are yet to be revealed, however, our results strongly indicate the importance of the dynamics of ADP-ribosylation (additions and removals) in virus infection.

P102
VALUE OF GALECTIN-3 IN COVID-19 DIAGNOSIS AND OUTCOME PREDICTION

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The major cause of fatality in SARS-CoV-2 patients was shown to be a “Cytokine Storm Syndrome” (CSS), a direct result of excessive immune activation and release of inflammatory cytokines, such as IL-1, TNF-α, and IL-6 by macrophages and dendritic cells, which are also the main source of galectin-3 (Gal-3). Therefore, we investigated if there is an additional value of Gal-3 serum concentration in predicting the course and outcome of COVID-19 patients. For that purpose, leftover serum samples were collected from two groups of patients: (*i*) with mild symptoms who were dismissed immediately after examination in the emergency department (N=122), and (*ii*) who were hospitalized (N=130). For longitudinal analysis of hospitalized patients, serum samples were collected on the 3rd and 6th day of hospitalization. Gal-3 and CRP concentrations, as well as leukocyte count, and differential blood count parameters were measured by standard routine laboratory methods. Statistical analysis was performed using Minitab software. Significantly higher Gal-3 ($P<0.001$) and CRP ($P<0.001$) concentrations were observed in hospitalized patients’ samples as compared to non-hospitalized patients’ samples, as well as leukocyte count ($P<0.001$) and the absolute number of neutrophilic granulocytes ($P<0.001$) and neutrophil to lymphocyte ratio (NLR) ($P<0.001$). Contrary, the absolute number of lymphocytes ($P=0.005$) and monocytes ($P=0.043$) were significantly higher in non-hospitalized patients’ samples. We found that the Gal-3 cut-off value for predicting hospitalization is 7.59 ng/mL with diagnostic sensitivity of 73.1% and specificity of 69.9%. Multivariable logistic regression analysis showed that the area under the ROC curve (AUC) for routine laboratory parameters (CRP, Lkc, Neu, Ly, NLR) was 0.728 (95% CI: 0.643-0.803) for predicting death and 0.890 (95% CI: 0.845-0.926) for predicting hospitalization. When Gal-3 concentration was added to routine laboratory parameters AUC for predicting death was 0.746 (95% CI: 0.661-0.819) and 0.896 (95% CI: 0.852-0.931) for predicting hospitalization.
In conclusion, measurement of serum Gal-3 concentration in COVID-19 patients gives additional value to conventional laboratory parameters in predicting the course and outcome of COVID-19 patients.

P103
NEGATIVE CATALYSIS BY ISOLEUCYL-tRNA SYNTHETASE: HOW BROAD SUBSTRATE SELECTIVITY DOES NOT PROMOTE FUTILE EDITING CYCLES

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Aminoacyl-tRNA synthetases (AARS) activate amino acids and transfer them to cognate tRNAs. Some AARS cannot establish the required specificity in the initial amino acid recognition at the synthetic site and thus may erroneously activate non-cognate amino acids and transfer them onto tRNA. To ensure faithful protein biosynthesis, those aminoacyl-tRNA synthetases evolved a separate editing domain aimed to hydrolyse erroneously aminoacylated tRNAs (post-transfer editing). To understand what shaped the selectivity of the editing site we used *Escherichia coli* isoleucyl-tRNA synthetase as a model enzyme. We investigated the kinetics of synthetic and editing reactions of IleRS using a wide range of amino acids, from proteinogenic to synthetic: Val, Nva, Leu, Thr, Met, Ser, Ala, Abu, Nle, F_2Abu and F_3Abu. Among these, only Val and Nva were poorly discriminated (well activated and transferred to the tRNA) and thus can be considered as a threat to the fidelity of translation. To our surprise, tRNAs misaminoacylated with all tested amino acids were rapidly hydrolysed at the editing domain, showing that amino acid's physicochemical features or how well they are discriminated at the synthetic site and consequently whether or not they can jeopardize the translational fidelity, does not make a significant difference. Only the hydrolysis of cognate Ile-tRNA^{Ile} (misediting) was slow, suggesting that the need to keep the cognate product out of editing strongly shaped the specificity of the editing domain. Detailed kinetic analysis revealed that isoleucyl-tRNA synthetase employs Thr246 and His333 for specific destabilization of Ile-tRNA^{Ile} hydrolysis (*i.e.* negative catalysis). Such design enabled isoleucyl-tRNA synthetase to have broad substrate acceptance at the editing site whilst maintaining a high specificity towards preventing the futile post-transfer editing cycles. This was the first time such broad substrate specificity, paired with negative catalysis, was observed for an AARS, and as such, it marks a new moment in the understanding of these vital and ancient enzymes.

P104
THE ROLE AND DIVERSITY OF PIR PROTEINS IN CELL WALLS OF BUDDING YEASTS

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Pir proteins (proteins with internal repeats) are the most prominent proteins that yeast *Saccharomyces cerevisiae* covalently binds to its cell wall through β-1,3-glucan residues. As such, they are regularly used for yeast surface display when the displayed protein needs to be bound close to its N-termini. However, the role of Pir proteins in cells remains unclear, and their diversity in closely-related species unexplored. These unknowns hamper rational engineering of Pir-based surface display, a technique with the potential to convert the entire yeast cell wall into enzymatically active self-regenerating living material. To address this issue, we used a deep-learning Alphafold2-based algorithm to *in silico* predict the 3D protein structures of *S. cerevisiae* Pir proteins and compare them with structures deposited in the Protein Data Bank (PBD). Through this approach, we were able to infer proteins' function solely through 3D homology. Moreover, we searched through the genomes of 77 species closely related to *S. cerevisiae* and identified over 300 Pir homologues, with which we mapped Pir evolution throughout the last 110 million years. Finally, by comparing homologous genomic loci, we show that *S. cerevisiae* is the only species within the *Saccharomyces* clade to lose one of its Pir proteins. As such, our findings point towards the role and diversity of Pir proteins in budding yeasts, thus opening novel avenues in yeast surface engineering.

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