FEBS openbio

FEBS PRESS science publishing by scientists

Check for updates

FEBS openbio

Volume 11 Supplement 1 July 2021

TALKS

Table of Contents

PLENARY LECTURES

Saturday 3 July

Sunday 4 July

Monday 5 July

Tuesday 6 July

Wednesday 7 July

Thursday 8 July

SYMPOSIA

Sunday 4 July

Monday 5 July

Tuesday 6 July

Wednesday 7 July

Thursday 8 July

FEBS SPECIAL SESSIONS

Sunday 4 July

Monday 5 July

Tuesday 6 July

Wednesday 7 July

SPEED TALKS

Sunday 4 July

Monday 5 July

Tuesday 6 July

Tuesday 6 July

EARLY BIRD SESSIONS

Sunday 4 July

Monday 5 July

Tuesday 6 July

FEBS Open Bio **11** (Suppl.1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

Abstracts submitted to the virtual 45th FEBS Congress from 3rd to 8th July 2021 (originally planned to be held in Ljubljana, Slovenia), and accepted by the Congress Organizing Committee are published in this Supplement of *FEBS Open Bio*. Late-breaking abstracts are not included in this issue.

About these abstracts

Abstracts submitted to the Congress are **not peer-reviewed**. In addition, abstracts are published as submitted and are **not copyedited** prior to publication.
We are unable to make **corrections of any kind** to the abstracts once they are published.

Indexing

Abstracts published in *FEBS Open Bio* Supplement for the 45th FEBS Congress will be included individually in the Conference Proceedings Citation Index published by Web of Science.

How to cite these abstracts

AuthorOne, A., AuthorTwo, B. (2021). Abstract title. FEBS Open Bio, 11: Abstract number*. doi:10.1002/2211-5463.13206

* The Abstract number begins with the letters EB, PL, S, ShT, SpT or SS or and can be found atop each abstract's title in the PDF file.

TALKS

Plenary Lectures

Saturday 3 July
18:00–19:00, Kocka Hall

Opening Plenary Lecture

PL-01-1
The Nobel Laureate campaign supporting GMOs

R. Roberts
New England Biolabs, Inc., Ipswich, United States of America

When Monsanto first tried to introduce GMO seeds into Europe there was a backlash by the Green parties and their political allies, who feared that American agri-business was about to take over their food supply. Thus began a massive campaign not against the true targets, Monsanto and the large agri-businesses, but rather against the surrogate target, GMOs. This has had disastrous consequences for one of the most promising technologies ever developed for improving food supplies. I am spearheading a campaign by the Nobel Laureates to counter the damage that is being done to the poor people in this world – notably in the developing countries – by Greenpeace and their allies who have deliberately ignored the science that underpins GMOs and have been painting horrific pictures of the dangers that might ensue. I will use Golden Rice as a clear example of the costs of these shortsighted policies. Millions of children have died or suffered developmental impairment because of a lack of Vitamin A in their diet. Golden Rice could reverse this, but has become a target of the Green parties because it is a GMO. This is foolish and dangerous. How many more children must die before this is considered a crime against humanity? I will argue that the Pope and the major religious organizations in this world could play a pivotal role in countering the pseudoscience being propagated by the so-called green parties and make a real difference to the lives of the poor in this world.

Sunday 4 July
11:30–12:30, Kocka Hall

FEBS Sir Hans Krebs Lecture

PL-02-1
RNA silencing, disease resistance and the inheritance of acquired characteristics

D.C. Baulcombe
Department of Plant Science Cambridge University, Cambridge, United Kingdom

Eukaryotes contain small regulatory RNAs that have been referred to as the dark matter of genetics. They are typically 21–24 nucleotides long and they bind to Argonaut or Piwi proteins. Some of these small RNAs guide the Argonaut/Piwi protein to a complementary RNA and they are negative regulators of gene expression acting at the level of messenger RNA turnover, translation or at the chromatin level. In plants the posttranscriptional mechanism is involved in disease resistance. It can either target the pathogen directly or it is part of a negative regulation of innate immunity. I will discuss how this process mediates the trade-off of the costs and benefits of disease resistance. The chromatin level effects of RNA silencing are also associated with defense against transposons rather than pests or pathogens. These epigenetic effects explain McClintocks "controlling elements" descriptor of transposons because they influence the expression of adjacent genes. I will describe how RNA silencing accounts for epigenetic inheritance and I will discuss its relationship to the possibility of the inheritance of acquired characteristics.

Sunday 4 July
14:30–15:30, Kocka Hall

EMBO Lecture

PL-03-1
Autophagy as an aging decelerator

G. Kroemer
Cordeliers Research Center, Paris, France

Colliding with the historically widespread idea that autophagy would be a mechanism of cellular suicide, autophagy usually has a cytoprotective function and hence avoids the premature death of stressed cells. Indeed, most if not all longevity extending manipulations, be they genetic, pharmacological or metabolic, must induce autophagy to be efficient. In this sense, autophagy may be conceived as a cytoplasmic recycling and rejuvenation mechanisms that protects cells from stress and, in addition, slows down biological clocks involved in the aging process. Lifestyle factors that favor metabolic syndrome, including excessive carbohydrate intake and obesity, likely act through the inhibition of autophagy to accelerate the manifestation of age-related diseases. The nucleocytosolic pool of acetyl coenzyme A and cytoplasmic protein acetylation repress autophagy, mechanistically linking excessive caloric intake to autophagy inhibition. 'Caloric restriction mimetics' (CRMs) are non-toxic agents that induce autophagy via protein deacetylation reactions. We developed phenotypic high-throughput screening systems to identify new CRMs and demonstrated that such agents, including several chalcones (e.g. 3,4-dimethoxychalcone and 4,4′-dimethoxychalcone) actually confer autophagy-dependent life-span extension and cardioprotection to flies and mice, respectively. We discovered that CRMs including aspirin, chalcones, hydroxycitrate and spermidine also are potent inducers of anticancer immunosurveillance, an effect that involves autophagy induction in tumor cells. Recently, we found that additional healthspan-extending agents can stimulate immunosurveillance, thus reducing the manifestation and slowing down the progression of hormone-induced mammary carcinomas. Thus, it appears that multiple CRMs can extend lifespan while improving anticancer immunosurveillance.

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

Monday 5 July
11:30–12:30, Kocka Hall

FEBS 2021 Plenary Lecture

PL-04-1
Structural and mechanistic insights into eukaryotic protein synthesis

N. Ban
Institute for Molecular Biology and Biophysics, Zurich, Switzerland

We are investigating bacterial and eukaryotic ribosomes and their functional complexes to obtain insights into the process of protein synthesis. Building on our studies that revealed the fundamental structures of eukaryotic cytosolic and mitochondrial ribosomes, we are now investigating eukaryotic cytosolic and mitochondrial ribosome assembly, translation initiation, targeting of proteins to membranes, regulation of protein synthesis, and how viruses reprogram host translation. Previously, we studied how Hepatitis C virus genomic RNA can bind mammalian ribosomes to achieve translation of viral mRNAs in the absence of several canonical cellular translation initiation factors. With our recent research activities, we contributed to the understanding of how SARS-CoV-2, the virus that is responsible for the COVID-19 pandemic, shuts off host translation to prevent cellular defense mechanisms against the virus. Furthermore, using a combination of cryo-electron microscopy and biochemical assays we also investigated the mechanism of programmed ribosomal frameshifting, one of the key events during translation of the SARS-CoV-2 RNA genome that leads to synthesis of the viral RNA-dependent RNA polymerase and downstream viral proteins.

Monday 5 July
14:30–15:50, Kocka Hall

FEBS/EMBO Women in Science Award Lectures

PL-05-1
Defining intrinsic determinants of regeneration ability and inability

E. Tanaka
Institute of Molecular Pathology, Vienna Biocenter, Vienna, Austria

Among tetrapods, regeneration shows a wide spectrum of capacities ranging from full appendage regeneration lifelong seen in salamanders, to stage-associated regeneration such as pre-metamorphic appendage regeneration in frogs, or stage dependent digit tip regeneration in mouse. We have combined genetic fate mapping and transcriptional profiling to follow successful limb regeneration in the axolotl, an excellent salamander model for regeneration. These and previous results have stressed that fibroblasts are a key participant and organizing cell type for limb regeneration, and by single cell profiling we could confirm that these fibroblasts dedifferentiate into a multipotent limb progenitor during regeneration. We are now studying whether this process "goes wrong" in non-regenerative contexts. The frog, *Xenopus laevis*, shows abortive regeneration after metamorphosis, whereby a blastema forms but an unsegmented cartilage rod regenerates rather than a full limb. We have used genetic fate mapping and single cell profiling to see that different fibroblasts subtypes behave differently during this process and cells are unable to complete the dedifferentiation process. We are ultimately interested in rescuing this abortive regeneration as a guidepost for promoting regeneration.

PL-05-2
Exploring the interface between living and non-living matter to transform health

M. Stevens
Imperial College, London, United Kingdom

The elucidation of the interfaces between living and non-living matter enable us to design and engineer biomaterials that elicit desired responses from the biological environment. We engineer simple, conceptually novel biosensing approaches using designer bio-nanomaterials for ultrasensitive diagnostic assays that are simple, cost-effective and easy deploy to the point-of-care. We are exploiting the sensing capabilities of nanoparticles to engineer paper-based lateral flow immunoassays (LFIAs) for the detection of infectious diseases such as HIV [2], Ebola, tuberculosis and Covid-19, and we can integrate our assays with smartphone technology for patient self-monitoring, geographical tagging and epidemic surveillance [3]. Harnessing our knowledge of the bio-interfaces, we also engineer complex 3D architectures with spatially arranged biochemical cues and cell interfacing nanoneedles for multiplexed intracellular biosensing at sub-cellular resolution and modulation of biological processes [4]. I will discuss how these versatile technologies can be applied to a wide range of transformative biosensing, regenerative medicine and drug delivery applications. [1] P. D. Howes, R. Chandrawati, M. M. Stevens. Science. 2014. 346: 53-63. [2] C. Loynachan, M. R. Thomas, E. Gray, D. Richards, J. Kim, B. Miller, J. Brookes, S. Agarwal, V. Chudasama, R. A. McKendry, M. M. Stevens. ACS Nano. 2018. 12(1): 279-288. [3] C. S. Wood, M. R. Thomas, J. Budd, T. P. Mashamba-Thompson, K. Herbst, D. Pillay, R. W. Peeling, A. M. Johnson, R. A. McKendry, M. M. Stevens. Nature. 2019. 566: 467-474. [4] C. Chiappini, E. De Rosa, J. O. Martinez, X. W. Liu, J. Steele, M. M. Stevens, E. Tasciotti. Nature Materials. 2015. 14: 532-539.

Tuesday 6 July
11:30–12:30, Kocka Hall

IUBMB Lecture

PL-06-1
Seeking non-coding RNA regulators of acetylcholine functioning

H. Soreq
The Hebrew University of Jerusalem, Jerusalem, Israel

Acetylcholine (ACh) signaling controls cognition, metabolism and behavior. MicroRNAs (miRs) targeting mRNAs with complementary sequence motifs ('CholinomiRs') maintain balanced ACh signaling via cooperative context-dependent activities. Specifically, the stress-inducible master regulator miR-132 limits trauma-induced cognitive impairments by targeting the ACh hydrolyzing enzyme acetylcholinesterase (AChE). Furthermore, aging ex-war prisoners without post-traumatic stress disorder

(PTSD) presented higher incidence compared to veterans with PTSD of the rare allele of the single nucleotide polymorphism (SNP) interrupting AChE blockade by the primate-specific miR-608. This allele associates with protective over-activation of prefrontal lobe reaction to stressors and with age- and sex-affected increases of anxiety, blood pressure and inflammation, and presents complex interaction patterns with another SNP in the miR-608 gene itself. Correspondingly, brains from men and women Bipolar Disorder and Schizophrenia patients and cultured human-originated neuronal cells of male and female origin under cholinergic differentiation show sex-dependent differences of cholinergic, immune and circadian pathways. Also, engineered miR-132 excess in all body tissues induces hepatic lipids accumulation, and diet-fattened mice react to antisense suppression of miR-132 by retracted hepatic hyperlipidemia. That CholinomiRs-mediated suppression of stress-inducible cognitive and metabolic impairments modulate reactions to multiple traumatic, mental and metabolic conditions highlights the links between cholinergic RNA regulators, metabolism and behavior.

Tuesday 6 July
14:30–15:30, Kocka Hall

FEBS Education Plenary Lecture

PL-07-1
Why science education is more important than most scientists think

B. Alberts
University of California at San Francisco, San Francisco, United States of America

The knowledge and the problem-solving skills of scientists are critical for every nation – no matter how rich or poor. In addition, every society needs the values of science: honesty, generosity, and an insistence on evidence while respecting all ideas and opinions regardless of their source of origin. These values – promoted as a "scientific temper" by India's first Prime Minister, Jawaharlal Nehru – are critical for the success of any democratic form of government. But to spread such values, "science education" will need to be redefined at all levels, becoming a central part of the curriculum. Scientifically based research, both on how students learn and on science education, has been critical for changing our views of how best to teach science at all levels, from kindergarten through college. Significant progress has been made, with research supporting active science learning through student inquiry in small groups, as opposed to the rote memorization of science facts. But a more rapid change from traditional teaching is needed, including a focus on the introductory college courses that are taught by scientists. How might the world's scientists band together to advance an even more ambitious strategy, in which "school science lessons" are extended to include student activities in the community, with the help of large numbers of scientist volunteers? I will introduce some prototypes that exist; how can we build on such efforts?

Wednesday 7 July
11:30–12:00, Kocka Hall

FEBS Letters Award Lecture

PL-08-1
Selective brain region vulnerability in Parkinson's disease is governed by α-Synuclein conformations

T. Bartels
Dementia Research Institute/University College London, London, United Kingdom

Neuronal aggregates of alpha-synuclein (αSyn) (Lewy bodies and neurites) are a pathological hallmark of Dementia with Lewy Bodies (DLB) and Parkinson's disease (PD). We recently discovered that αSyn, a neuronal protein of unclear physiological function, normally exists in cells and brain tissue principally as a α-helically folded multimers that resists pathological aggregation. We show here that the helical form is not only resistant to time-dependent self-aggregation but also shows increased resistance (compared to unfolded monomers) to misfolding initiated by tiny amounts of fibrillar material, i.e., "seeded aggregation". Based on our new findings, it is important to identify factors that could trigger the denaturation of folded αSyn and allow its abnormal aggregation in neurons. We can demonstrate that DLB and sPD patients exhibit a region-specific reduction of αSyn multimers in brain tissue according to the classical Braak staging scheme, indicating their destabilization in the course of the disease. The results indicate the vulnerability of early affected brain regions, the importance of a balance of aSYN multimers and monomers and the functional reserve of different brain regions. A factor governing the stabilization of multimers seems to be lipid composition of the cell specific membranes since transient lipid contact acts as a catalyst for multimer formation, meaning that lipid vesicles might act as a "liposomal chaperone" capable of conferring aggregation resistance to the large cytosolic pool of αSyn.

Wednesday 7 July
12:00–12:30, Kocka Hall

The FEBS Journal Richard Perham Prize Lecture

PL-09-1
Redox-sensitive mobile protein HIC-5: a guardian of anchorage-dependent cell growth and a fortress against tumor metastasis

M. Shibanuma
Showa University, Shinagawa-ku, Tokyo, Japan

Hydrogen peroxide-inducible clone 5 (HIC-5) is a multidomain LIM protein that is homologous to paxillin. It functions as a molecular scaffold at focal adhesions and in the nucleus while shuttling between cellular compartments. However, once cells detach from substrata, HIC-5, but not paxillin, accumulates in the nucleus. This distinctive feature is endowed by its unique nuclear export signal, which is redox-sensitive and is modified by reactive oxygen species (ROS) produced upon cellular

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

detachment. Thus, HIC-5 is a specific ROS effector that changes cellular localization in response to intracellular ROS levels or the state of cell adhesion. Importantly, HIC-5 hetero-oligomerizes with LIM-only protein PINCH and CRP2, thereby regulating anchorage-dependent cell growth. Specifically, upon anchorage loss, the HIC-5-PINCH complex circumvents nuclear localization of cyclin D1, and HIC-5-CRP2 transactivates p21Cip1. Interestingly, HIC-5 also regulates ROS production. Our most recent work (FEBS J, 286: 459–478, 2019, awarded The FEBS Journal Richard Perham prize) demonstrated that HIC-5 negatively regulates NOX4-mediated mitochondria-associated ROS (mtROS) production. NOX4, a ubiquitously expressed NOX family member, is localized in various cellular organelles including mitochondria. HIC-5 suppresses NOX4 expression at the mRNA level, thereby preventing the generation of irrelevant mtROS. In cancer cells, mtROS stabilizes matrix metalloproteinase (MMP)9 mRNA. Consequently, the HIC-5-NOX4-mtROS axis suppresses the invasive properties and metastasis of cancer cells. It is possible that mRNAs other than that of MMP9 are stabilized by mtROS and implicate this axis in various pathological conditions (FEBS J, 286: 456–458, 2019). Of note, this axis is suggested to operate under the conditions where NOX4 is upregulated, such as in RAS-activated cells. In conclusion, HIC-5 is a unique protein as an effector and a regulator of ROS with potential as a therapeutic target for various diseases.

Wednesday 7 July
14:00–15:00, Kocka Hall

FEBS Datta Lecture

PL-10-1
Designing nature, from protein origami to cellular circuits

R. Jerala
National Institute of Chemistry, Ljubljana, Slovenia

Self-organizing matter evolved during evolution into highly complex molecular structures and biological systems. Synthetic biology is engineering biological and biomimetic systems in order to obtain new interesting properties but also to understand the function of natural systems in agreement with Richard Feynman's "What I cannot create I do not understand". We can design new protein folds, introduce new mechanisms of regulation and design signal processing signals in cells, which is needed for better efficiency and safety of therapeutic cells. Natural proteins represent only a tiny fraction of an almost infinite number of sequences and tertiary structures. We are starting to design protein structures based on different design principles than natural proteins and introduce new functions. Almost 70 years after the proposed structure of coiled-coils (CC) by Francis Crick and Linus Pauling we are using this motif for many new purposes – from the design of new protein folds to the regulation of the activity of natural proteins and cellular processes. We devised coiled-coil protein origami (CCPO) where the CC peptides are fused into a single polypeptide chain that self-assembles into polyhedral folds not found in nature. Moreover, use of the well understood CC modules facilitated design of the folding pathway that enabled multiple usage of the same module within the designed protein chain. CC modules are also functional inside mammalian cells, enabling multiplexing of cellular localization, tunable enhancement of transcriptional regulation, proteolysis- and CC-based logic gates that can respond within minutes and regulation of various natural proteins. References: Gradišar H. et al. Nat Chem Biol. (2013) 9:362-6.; Ljubetič A, Lapenta F. et al. Nat Biotechnol.(2017) 35:1094-1101; Lebar T, et al. Nat Chem Biol. (2018); Fink T. et al. Nat Chem Biol. (2019); Lebar et al. Nat.Chem. Biol. (2020).

Wednesday 7 July
15:15–16:15, Kocka Hall

PABMB Lecture

PL-11-1
The oxygen paradox in human biology and medicine: bioenergetics, free radicals and oxidative stress

R. Radi
Departamento de Bioquímica and Centro de Investigaciones Biomédicas (CEINBIO), Facultad de Medicina, Universidad de la República, Montevideo, Uruguay

Oxygen is an essential requirement in human biology as, among other reactions, it participates as the final electron acceptor of the mitochondrial electron transport chain that in turn is coupled to ATP synthesis. Under normal conditions more than 99.5 % of oxygen consumed by most human cells is fully reduced to water through a four-electron reduction process by cytochrome aa3. Thus, molecular oxygen fuels the redox/bioenergy axis with these processes representing central aspects of mitochondrial homeostasis and metabolism. Still, oxygen consumption is associated to the formation of partially reduced intermediates, notably superoxide radical ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2). These species are short-lived by a combination of their intrinsic reactivity with various molecular targets and also by their catabolism by enzymatic antioxidant systems that encompass superoxide dismutases, peroxiredoxins, glutathione peroxidases and catalase. Different cellular compartments emit $O_2^{\cdot -}$ and H_2O_2 as part of cell signaling processes; however, sustained increases of these reactive intermediates can lead to undesired oxidation events, and lead to the disruption of cell homeostasis and even cell death. Of particular interest to me has been how the interaction of $O_2^{\cdot -}$ with another free radical and messenger molecule nitric oxide (·NO) alters cell signaling and tilts the cellular redox status towards a pro-oxidative condition (oxidative stress) via the formation of peroxynitrite anion (ONOO). Peroxynitrite is recognized as a pathogenic mediator in inflammation and degenerative diseases and it also contributes in the aging process. Thus, the "oxygen paradox" in human biology reflects the essentiality of keeping the right balance between the beneficial effects of oxygen-dependent metabolism and signaling with the unavoidable formation of excess free radicals and reactive species that must be kept under control by dedicated catabolic and repair systems.

Thursday, 8 July
11:30–12:30, Kocka Hall

FEBS Theodor Bücher Lecture (Closing Plenary Lecture)

PL-12-1
The biology of CRISPR-Cas

E. Charpentier
Max Planck Unit for the Science of Pathogens, Berlin, Germany

Abstract unavailable.

Symposia

Sunday 4 July
9:00–11:00, Kocka Hall

Autophagy and protein recycling

S-02.2-1
Autophagy in lymphangiogenesis

P. Agostinis, O. Mece, D. Houbaert
VIB-KU Leuven/Center for Cancer Biology, Leuven, Belgium

In adult, the lymphatic system plays a central role in tissue fluid homeostasis, lipid transport, and trafficking of immune cells and regulation of immune responses. Accordingly, dysregulation of lymphatic vessels is associated to important human pathologies including lymphedema, a condition characterized by chronic tissue edema and impaired immunity. The process of lymphangiogenesis, the formation of lymphatic vessels from pre-existing ones, is triggered under inflammatory conditions, such as tissue injury, wound healing and cancer and contributes to metastatic spreading and responses to immunotherapy. Understanding the processes that regulate the vital biological function of lymphatic vessels is therefore of primary importance for the development of strategies harnessing the lymphatic vasculature in human diseases. Here we studied the role of autophagy, a major lysosomal pathway for the degradation and recycling of cytoplasmic components, in lymphatic endothelial cells both *in vitro* and *in vivo*. I will present unpublished data underscoring the crucial role of autophagy in the metabolic regulation of the differentiation program that maintains the lymphatic phenotype, in inflammation driven lymphangiogenesis and cancer growth control.

S-02.2-3
Autophagic quality control in the nervous system: physiology and pathology

N. Tavernarakis[1,2]
[1]*Foundation for Research and Technology-Hellas, Heraklion, Greece,* [2]*School of Medicine, University of Crete, Heraklion, Greece*

Numerous gene mutations and treatments have been shown to extend the lifespan of diverse organisms ranging from the unicellular *Saccharomyces cerevisiae* to primates. It is becoming increasingly apparent that most such interventions ultimately interface with cellular stress response mechanisms, suggesting that longevity is intimately related to the ability of the organism to effectively cope with both intrinsic and extrinsic stress. Key determinants of this capacity are the molecular mechanisms that link ageing to main stress response pathways. How each pathway contributes to modulate the ageing process is not fully elucidated. Mitochondrial impairment is a major hallmark of several age-related neurodegenerative pathologies, including Alzheimer's disease. Accumulation of damaged mitochondria has been observed in post-mortem brain of Alzheimer's disease patients. Although disease-associated tau and amyloid β are known to deregulate mitochondrial function, it remains elusive whether they also directly influence the efficiency of mitophagy. Mitophagy is a selective type of autophagy mediating elimination of damaged mitochondria, and the major degradation pathway, by which cells regulate mitochondrial number in response to their metabolic state. However, little is known about the role of mitophagy in the pathogenesis of Alzheimer's disease. To address this question, we developed an in vivo imaging system to monitor mitophagy in neurons. We demonstrated that neuronal mitophagy is impaired in *C. elegans* models of Alzheimer's disease. Urolithin A- and nicotinamide mononucleotide-induced mitophagy ameliorates several pathological features of Alzheimer's disease, including cognitive defects. Mitophagy stimulation restores memory impairment through PINK-1-, PDR-1 or DCT-1-dependent pathways. Our findings suggest that impaired removal of damaged mitochondria is a pivotal event in Alzheimer's disease pathogenesis highlighting mitophagy as a potential therapeutic intervention.

S-02.2-2
Organelle degradation by autophagy and autophagy-related pathways

N. Mizushima
The University of Tokyo, Tokyo, Japan

Autophagy is a major degradation system in the cell. Intracellular components are sequestered by autophagosomes and then degraded upon fusion with lysosomes. Yeast genetic studies have identified many autophagy-related genes. Most of them are conserved in higher eukaryotes, which brought about an exponential expansion of autophagy research in various organisms including mammals. We have shown that autophagy is important not only for the maintenance of the amino acid pool during starvation and preimplantation development but also for intracellular protein quality control to prevent neurodegeneration and tumorigenesis. We also identified mutations of WDR45/WIPI4, which is one of the core autophagy genes, in a human neurodegenerative disease called SENDA/BPAN. While these studies have highlighted the important role of autophagy in organelle degradation and homeostasis, we also found that massive organelle degradation occurring during lens differentiation does not depend on conventional autophagy. To identify a novel autophagy-independent mechanism of organelle degradation in the lens, we established a live imaging system of this process in zebrafish and found that matrix proteins of the ER and mitochondria were released into the cytosol during differentiation, indicating that these organelles are ruptured. By transcriptome and CRISPR/Cas9 knockout screens, we revealed that a phospholipase, which is highly expressed in the lens, is essential for this large-scale degradation in the lens. Its function is conserved also in mice. We propose a novel phospholipase-dependent and

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

macroautophagy-independent mechanism of organelle degradation in the lens.

ShT-02.2-2
Mitophagy is downregulated upon thermogenic stimulus in human beige adipocytes

A. Shaw[1,*], M. Szatmári-Tóth[1,*], I. Csomós[2], G. Mocsár[2], Z. Balajthy[1], F. Győry[3], E. Kristóf[1], L. Fésüs[1]
[1]*Department of Biochemistry and Molecular Biology, University of Debrecen, Debrecen, Hungary,* [2]*Department of Biophysics and Cell Biology, Debrecen, Hungary,* [3]*Department of Surgery, Faculty of Medicine, University of Debrecen, Debrecen, Hungary*

Adipocytes are classified into white, brown and beige. Brown and beige adipocytes are important targets to combat obesity, as they are capable to dissipate energy in the form of heat, while the white adipocytes are primarily for energy storage. UCP1, an inner mitochondrial membrane protein mediates thermogenesis by uncoupling the mitochondrial respiratory chain from ATP synthesis. Hence, mitochondria are important for the thermogenic and metabolic functions of adipocytes. $UCP1^{+}$ mitochondria in human adipocytes are mostly fragmented (Pisani et al, 2017). Mitophagy plays a vital role in beige to white adipocyte transition in mouse (Altshuler-Keylin et al, 2016). We intend to characterize the role of mitophagy in the thermogenic activation of primary human abdominal subcutaneous adipocytes and SGBS cells. Isolated preadipocytes were differentiated into white and browning adipocytes, which were treated with dibutyryl-cAMP (6, 10 and 14 hours). Genes related to parkin-dependent and independent mitophagy were downregulated upon thermogenic stimulus; the parkin dependent ones being the most downregulated. The lipidated form of LC3, LC3-II is recruited on the outer membrane of the autophagosome which indicates ongoing autophagy. TOM20 is an outer mitochondrial membrane protein and marks the mitochondria. LC3 and TOM20 immunostaining were performed, followed by quantification of LC3 punctae, which was high in untreated control adipocytes but decreased significantly upon thermogenic stimulus, suggesting repressed autophagy/mitophagy. Colocalization of TOM20 and LC3 can indicate mitophagy. Decreased colocalization was observed upon thermogenic stimulus, which further proved repressed mitophagy. TOM20 quantification showed increased number of fragmented mitochondria upon thermogenic stimulus suggesting prompt inhibition of mitophagy, thereby protecting many fragmented mitochondria from degradation and boosting thermogenesis. This work was supported by GINOP-2.3.2-15-2016-00006. *The authors marked with an asterisk equally contributed to the work.

ShT-02.2-1
Lipid droplets and autophagy cooperate in the protection of cancer cells against metabolic stress

E. Jarc Jovičić[1,2], M. Jusović[2], **Š. Koren**[1], P. Starič[1], E. Guštin[1], A. Kump[1], D. Lainšček[3], R. Jerala[3,4], T. Petan[1]
[1]*Jozef Stefan Institute, Department of Molecular and Biomedical Sciences, Ljubljana, Slovenia,* [2]*Jozef Stefan International Postgraduate School, Ljubljana, Slovenia,* [3]*Department of Synthetic Biology and Immunology, National Institute of Chemistry, Ljubljana, Slovenia,* [4]*EN-FIST Centre of Excellence, Ljubljana, Slovenia*

Lipid droplets (LDs) are lipid storage organelles present in most eukaryotic cells. They are composed of a core of neutral lipids surrounded by a phospholipid monolayer and proteins. LD biogenesis is induced in cells exposed to excess nutrients and lipids and is characteristic of many diseases, such as obesity, diabetes and cancer. Intriguingly, their formation occurs also in cells fully deprived of nutrients and oxygen, suggesting that LDs are an integral part of the cellular stress response. LDs engage in a complex and as yet poorly defined relationship with autophagy, the major cellular recycling machinery and stress response pathway. First, autophagy may drive LD biogenesis by providing lipids recycled from other membranous organelles. Second, autophagy may participate in LD breakdown through a selective form of autophagy named lipophagy. Third, LDs may promote autophagy by providing lipids or signals that support the formation of autophagosomal membranes. We aim to discover the principal ways in which LDs and autophagy cooperate to promote the resistance of cancer cells to stress. We have found that lipid droplets are dynamically synthesized and broken down in cancer cells depending on the length and severity of nutrient deprivation. Autophagy is required for their biogenesis under acute starvation conditions, whereas lipolysis seems to be involved in their breakdown under milder conditions of starvation. By manipulating the activities of the major enzymes involved in LD metabolism in the context of activated or inhibited autophagy, we are currently examining the links between these two processes and their roles in cancer stress resistance. Our work may open new perspectives in cancer research by providing important clues on the function of the recently recognized stress-associated organelle – the lipid droplet.

Sunday 4 July
9:00–11:00, Marmorna Hall A

Genome structure and regulation

S-01.1-3
Epigenetic regulation of chromatin structure and function: the role of linker histones

F. Azorin
IRB Barcelona an IBMB-CSIC, Barcelona, Spain

Linker histones H1 constitute an evolutionarily conserved family of chromosomal proteins that play an important structural role in regulating chromatin compaction and higher order chromatin organization. In metazoan species, histones H1 usually exist as

multiple variants, some of which are specifically expressed in the germline (reviewed in (1)). For instance, four of the eleven mice/human H1 isoforms are germline specific, of which three are expressed in males (H1T, HILS1 and H1T2) and one in females (H1oo). Female-specific variants usually accumulate in the oocyte and are retained during early embryogenesis (1). In comparison to most metazoa, H1 complexity in Drosophila is much reduced since it contains a single somatic dH1 variant (reviewed in 2), which is ubiquitously expressed throughout development, and a single germline specific variant dBigH1, which is expressed in both the female and male germlines, and it is retained in the early embryo (3). Embryonic H1s persist as long as the zygotic genome remains transcriptionally silent, being replaced by somatic variants when transcription begins during zygotic genome activation (ZGA) (1). In Drosophila, dBigH1 is present during early embryogenesis until ZGA onset at cellularization (3). At this stage, dBigH1 is replaced by somatic dH1 in somatic cells, whereas it is retained in the primordial germ cells (PGC) (3), which remain transcriptionally silent. In this presentation I will review recent advances in the functional analysis of linker histones dH1 and dBigH1 in Drosophila, their contribution to development and their role in maintaining genome stability (3-8). 1.Pérez-Montero et al. (2016) Chromosoma 125:1. 2. Bayona-Feliu et al. (2016) Biochim Biophys Acta 1859:526. 3. Pérez-Montero et al. (2013) Dev Cell 26:578. 4. Vujatovic et al. (2012) Nucleic Acids Res 40:5402. 5. Carbonell et al. (2017) Cell Rep 21:3178. 6. Bayona-Feliu et al. (2017) Nat Commun 8:283. 7.Climent-Cantó et al. (2020) Nucleic Acids Res. https://doi.org/10.1093/nar/gkaa122

S-01.1-2
Modulation of gene expression by satellite DNAs: physiological and evolutionary implications

Đ. Ugarković[1], I. Feliciello[2]
[1]*Ruder Boskovic Institute, Zagreb, Croatia,* [2]*Universita' degli Studi di Napoli Federico II, Naples, Italy*

Non-coding repetitive DNAs constitute a considerable portion of most eukaryotic genomes and their function is intensively investigated. Among most investigated non-coding repetitive DNAs are mobile transposable elements which represent an important source of regulatory sequences while the influence of another abundant class of non-coding repetitive elements such as satellite DNA on genome regulation has been also proposed. Satellite DNAs are tandemly assembled within constitutive heterochromatin at the (peri) centromeric and subtelomeric regions. However, some satellite DNAs are not only clustered within heterochromatin but are dispersed as short arrays within euchromatin, in the vicinity of genes. Examples of satellites partially dispersed within euchromatin include the major satellite DNA of the red flour beetle *Tribolium castaneum* and the human alpha satellite DNA. Here we reveal that dispersed repeats of the human alpha satellite as well as those of the major *T. castaneum* satellite can influence neighbouring gene expression by modulating the local chromatin environment in cis, under specific environmental conditions such as heat stress. Namely, upon heat stress the level of silent histone modification H3K9me3 is increased on dispersed repeats of both satellites and spread to the neighbouring regions, resulting in the suppression of nearby genes. Our results reveal dispersed satellite DNA repeats as nucleation cores for repressed chromatin and indicate their contribution to variation in the gene expression response to heat stress as well as their role in the evolution of gene regulatory networks.

S-01.1-1
DNA methylation, satellite repeats and genome stability: challenges and perspectives from rare genetic disorders

P. Mihic[1], E. Boyarchuk[2], G. Velasco[1], **C. Francastel**[1]
[1]*Epigenetics and Cell Fate - UMR7216 - CNRS - Université de Paris, Paris, France,* [2]*Epigenetics and Cell Fate, Paris, France*

The vast majority of mammalian genomes does not code for proteins. Repeated sequences actually represent about half of the human genome; they are scattered throughout the genome, or repeated in tandem and referred to as satellite sequences. Large arrays of satellite DNA define specialized chromosomal loci like centromeres, which are essential for faithful chromosome segregation during cell division, and their adjacent heterochromatin domains, implicated in the functional organization of genome functions. Yet, DNA repeats represent a substantial threat to genome stability as they are prone to recombination. Satellite repeats are also transcriptionally competent, although at low levels in normal somatic cells, and the resulting transcripts participate in the assembly of specialized chromatin complexes at their loci of origin. In turn, unscheduled transcription or accumulation of satellite transcripts is a signature of physiopathological cases characterized by genomic instability like in stress, aging or cancer. We explore functional links between transcription of repetitive sequences and the (de)regulation of molecular and cellular programs, in various cellular contexts and pathophysiological situations. We question mechanisms and actors that promote their (de)regulated transcription, with a special interest in DNA methylation (DNAme), the major mechanism responsible for silencing repetitive elements and for maintaining genome stability. We also focus on a rare human genetic disease with chromosomal instability, the ICF (Immunodeficiency, Centromeric instability, Facial anomalies) syndrome, a remarkable case where compromised centromere integrity is directly linked to constitutive alterations of DNAme of the underlying repeated sequences. We identified unsuspected factors to be necessary for DNAme at repeated sequences, with important consequences for our understanding of the basic mechanisms that control genome function and preserve its integrity.

ShT-01.1-2
New insights into direct functions of BET proteins in transcription regulation

A. Mayer
Max Planck Institute for Molecular Genetics, Berlin, Germany

Transcription elongation has emerged as a regulatory hub in gene expression of metazoans and its misregulation has been implicated in human disease. A major control point occurs during early elongation before RNA polymerase II (Pol II) is released into productive elongation. Prior research has linked the bromodomain and extra-terminal domain (BET) protein BRD4 with transcription elongation. Here, we use rapid BRD4-selective degradation along with a set of quantitative genome- and proteome-wide approaches to investigate direct functions of BRD4 in transcription regulation. Notably, as an immediate

consequence of acute BRD4 loss, promoter-proximal pause release was impaired and transcribing Pol II past this checkpoint underwent readthrough transcription. An integrated proteome-wide analysis revealed 5′-elongation and 3′-RNA processing factors as core BRD4 interactors. Further mechanistic studies show that loss of BRD4 disrupted the recruitment of 3′-RNA processing factors prompting RNA cleavage and transcription termination defects. These studies, performed in human cells, reveal a general BRD4-mediated transcription checkpoint and establish a molecular link between 5′-elongation control and 3′-RNA processing.

ShT-01.1-1
The bacteriophage GIL01 take-over mechanisms of the host's SOS response

A. Pavlin[1], N. Fornelos[2], N.C.J. Strynadka[3], D.F. Browning[4], N.A. Caveney[3], G. Caballero[3], L. de Castro[3], Z. Podlesek[1], V. Hodnik[1], M. Bahun[1], M. Salas[5], M. Butala[1]
[1]University of Ljubljana, Biotechnical Faculty, Department of Biology, Chair of Biochemistry, Ljubljana, Slovenia, [2]The Broad Institute of MIT and Harvard, Massachusetts, United States of America, [3]Department of Biochemistry and Molecular Biology and the Centre for Blood Research, University of British Columbia, Vancouver, British Columbia, Canada, [4]Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, Birmingham, United Kingdom, [5]Centro de Biologia Molecular Severo Ochoa (CSIC-UAM), Madrid, Spain

The GIL01 bacteriophage, is a tectiviral temperate phage, which infects the insect pathogen Bacillus thuringiensis. Unlike most temperate phages, GIL01 lysogeny is not established by a dedicated phage repressor, but rather by the host's regulator of the SOS response, LexA. However, LexA is unable to maintain lysogeny, unless the small phage-encoded protein gp7 is also present. Gp7 directly interacts with LexA to enhance its DNA binding to phage promoter. We obtained the crystal structure of the 50-amino acid gp7 protein and according to SAXS data, we generated a structural model of gp7 in complex with LexA, which illustrates that gp7 positions LexA in a DNA bound conformation. By applying surface plasmon resonance approach we also recently discovered, that gp7 homologs identified in other tectiviruses, that infect a diverse range of bacteria, exhibit similar mode of action as gp7. Our recent results furthermore show that a small protein non-homologous to gp7 in an important human pathogen, analogously to gp7, interacts with its cognate DNA damage response repressor to modulate the SOS response. Thus, these results lead us to think that this kind of mechanism is widely spread to enable the SOS control beyond the LexA/RecA regulation. To determine how GIL01 establishes the lytic cycle, we examined the regulatory mechanisms at the lytic promoter. We show that lytic promoter is also repressed by LexA/gp7 complex and that the second phage-borne small protein, gp6, is the key activator of the lytic cycle. Surprisingly, gp6 is homologous to LexA itself and, thus, is a rare example of a LexA homologue directly activating transcription. We propose that the interplay between these two LexA family members, with opposing functions, ensures the timely expression of GIL01 phage late genes.

Sunday 4 July
9:00–11:00, Marmorna Hall B

Molecular evolution and phylogenetics

S-01.3-2
A hydrogen dependent geochemical analog of primordial carbon and energy metabolism

W. Martin
Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany

Hydrogen gas, H_2, is generated in alkaline hydrothermal vents from reactions of iron containing minerals with water during a geological process called serpentinization. It has been a source of electrons and energy since there was liquid water on the early Earth, and it fuelled early anaerobic ecosystems in the Earth's crust. H_2 is the electron donor for the most ancient route of biological CO_2 fixation, the acetyl-CoA (or Wood-Ljungdahl) pathway, which unlike any other autotrophic pathway simultaneously supplies three key requirements for life: reduced carbon in the form of acetyl groups, electrons in the form of reduced ferredoxin, and ion gradients for energy conservation in the form of ATP. The pathway is linear, not cyclic, it releases energy rather than requiring energy input, its enzymes are replete with primordial metal cofactors, it traces to the last universal common ancestor and abiotic, geochemical organic syntheses resembling segments of the pathway occur in hydrothermal vents today. Laboratory simulations of the acetyl-CoA pathway's reactions include the nonenzymatic synthesis of thioesters from CO and methylsulfide, the synthesis of acetate and pyruvate from CO_2 using native iron as the electron source. However, a full abiotic analogue of the acetyl-CoA pathway from H_2 and CO_2 as it occurs in life has not been reported to date. Three hydrothermal minerals — awaruite (Ni_3Fe), magnetite (Fe_3O_4) and greigite (Fe_3S_4) — catalyse the fixation of CO_2 with H_2 at 100°C under alkaline aqueous conditions [1]. The product spectrum includes formate (100 mM), acetate (100 μM), pyruvate (10 μM), methanol (100 μM), and methane [1]. With these simple catalysts, the overall exergonic reaction of the acetyl-CoA pathway is facile, shedding light on both the geochemical origin of microbial metabolism and on the nature of abiotic formate and methane synthesis in modern hydrothermal vents. [1] M. Preiner, K. Igarashi, K. Muchowska, M. Yu, et al. bioRxiv 682955 (2019)

S-01.3-1
Evolutionary analysis of the *Bacillus subtilis* genome reveals new genes involved in sporulation

I. Mijakovic
Chalmers University of Technology, Gothenburg, Sweden

Bacilli can form dormant, highly resistant, and metabolically inactive spores to cope with extreme environmental challenges. In this study, we examined the evolutionary age of *Bacillus subtilis* genes using the approach known as genomic phylostratigraphy. We found that *B. subtilis* sporulation genes cluster in several groups that emerged at distant evolutionary time-points, suggesting that the sporulation process underwent several stages of expansion. Next, we asked whether this evolutionary stratification of the genome could be used to predict involvement in sporulation of presently uncharacterized genes (y-genes). We individually inactivated a

representative sample of uncharacterized genes that arose at the same time as the known sporulation genes and tested the resulting strains for sporulation phenotypes. Sporulation was significantly affected in 16 out of 37 (43%) tested strains. In addition to expanding the knowledge base on *B. subtilis* sporulation, our findings suggest that evolutionary age could be used to help with genome mining.

S-01.3-3
Phylogeny–ontogeny correlations: lessons from microbial biofilms

M. Futo[1,*], L. Opašić[2,*], S. Koska[1,*], N. Čorak[1,*], T. Široki[3], V. Ravikumar[4], A. Thorsell[5], D. Kifer[6], M. Domazet-Lošo[3], K. Vlahoviček[7], I. Mijaković[8], **T. Domazet-Lošo**[1,9]
[1]Ruder Boskovic Institute, Zagreb, Croatia, [2]Max Planck Institute for Evolutionary Biology, PLÖN, Germany, [3]Faculty of Electrical Engineering and Computing, University of Zagreb, Zagreb, Croatia, [4]The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kongens Lyngby, Denmark, [5]Proteomics Core Facility, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, [6]Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia, [7]Bioinformatics Group, Division of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia, [8]Systems and Synthetic Biology Division, Department of Biology and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden, [9]Catholic University of Croatia, Zagreb, Croatia

Many bacteria species form biofilms. These multicellular communities display organised behaviours that resemble to some extent developmental processes in truly multicellular organisms like animals and plants. Ontogenies in multicellular eukaryotes were recently studied by phylo-transcriptomic approaches that uncover phylogeny–ontogeny correlations during development. Underlying causes of these correlation patterns are still debated; however, they readily demonstrate that true developmental processes harbour macroevolutionary imprints that could be found at the molecular level. To test if similar phylogeny–ontogeny correlations exist in bacterial forms of multicellularity we studied expression patterns in growing *Bacillus subtilis* biofilms. Our results revealed clear macroevolutionary imprints suggesting that *B. subtilis* biofilm growth is more complex than previously thought. We discuss these findings in relation to the origin of life, development and major macroevolutionary transitions. *The authors marked with an asterisk equally contributed to the work.

ShT-01.3-1
Investigation of functional annotations to enzyme classes reveals an extensive annotation error

E. Rembeza, M. Engqvist
Chalmers University of Technology, Gothenburg, Sweden

Only a small fraction of genes deposited to databases has been experimentally characterised. The majority of proteins have their function assigned automatically, which can result in erroneous annotations. The reliability of current annotations in public databases is largely unknown, and we lack experimental attempts to validate accuracy of existing annotations. In our study we performed an overview of functional annotations to the BRENDA enzyme database. We first applied a high-throughput experimental platform to verify functional annotations to an enzyme class of S-2-hydroxyacid oxidases (EC 1.1.3.15). We chose 122 representative sequences of the class and screened them for their predicted function. Based on the experimental results, predicted domain architecture and similarity to previously characterised S-2-hydroxyacid oxidases, we inferred that at least 78% of sequences in the enzyme class are misannotated. We experimentally confirmed four alternative activities among the misannotated sequences and showed that misannotation in the enzyme class increased over time. Finally, we performed a computational analysis of annotations to all enzyme classes in BRENDA database, and showed that nearly 18% of all sequences are annotated to an enzyme class while sharing no similarity to experimentally characterised representatives. We showed that even well-studied enzyme classes of industrial relevance are affected by the problem of functional misannotation.

ShT-01.3-2
Pre-LUCA protein evolution reconstructed from an imprint conserved within the genetic code

B. Davis
Research Foundation of Southern California, La Jolla, United States of America

Identification of structural features conserved within the genetic code has revealed insights into protein evolution prior to the Last Universal Common Ancestor (LUCA). Amino acid residue hydrophobicity, specified by the free energy of partitioning between an aqueous solution and non-polar medium with a dielectric constant of 2, was, notably, found to exhibit a linear correlation with the synthesis path-distance of fourteen amino acids formed in 1 to 8 reaction steps from a precursor in central metabolism. Since increases in amino acid path-distance have been linked to the advancement of code evolution, this finding revealed that hydrophobic attractors had contributed to the direction of protein evolution during expansion from a small, initial code. With standard protein phylogenetics, reliant on interspecies mutation distances, these pre-LUCA findings were evidently beyond its scope. Code formation was found to have proceeded by successive recruitment of sets of base triplets with a common mid-base, resulting in a stepwise pattern, NAN → NCN → NGN → NUN (N, unrestricted base site), of codon recruitment. Encoded amino acids with progressively longer mean synthesis paths, extending 1.5 → 4 → 5 → 7 steps, exhibited hydropathy free energy trending toward elevated residue hydrophobicity, +6.8 → −0.8 → −1.2 → −3.2 kcal/mol; as previously reported by Davis BK (2020) Biorxiv: doi.org/10.1101/2020.07.29.227728. Two forms of hydrophobic attractor arose: (i) in a 23 residue ferredoxin antecedent, a hydrophobic 17 residue segment, linked to a 4Fe-4S cofactor, served to prolong attachment (Fajan-Paneth principle) to a cationic mineral surface, in a primal pre-cell system, and (ii) non-polar residues of the H^+ ATPase proteolipid helix-1 subunit led to it partitioning within the membrane phospholipid bilayer.

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

Sunday 4 July
9:00–11:00, Gallery Hall

Protein biosynthesis and expansion of genetic code

S-02.1-1
Keeping translation canonical: lessons from aminoacyl-tRNA synthetases

I. Gruic Sovulj
University of Zagreb Faculty of Science, Zagreb, Croatia

Compromised fidelity of protein synthesis may disrupt protein function and homeostasis and lead to impaired cell viability and growth. We focus on the accuracy of aminoacylation – the reaction by which amino acids are covalently coupled to their cognate tRNAs for delivery to the ribosome. This reaction, that translates the genetic code, is catalyzed by aminoacyl-tRNA synthetases (AARSs). What is the weakest link in keeping aminoacylation accurate? And how do AARSs control this link? Traditionally, the main threats for the accuracy of aminoacylation were sought among twenty canonical proteinogenic amino acids. We showed, however, that the editing domains of isoleucyl-, valyl-, and leucyl-tRNA synthetases (IleRS, ValRS, LeuRS) are highly efficient in preventing non-canonical amino acids, like norvaline and alpha-aminobutyrate, from participating in translation (Previously published in: Bilus et al. (2019) J Mol Biol. 431, 1284-1297 and Zivkovic et al (2020) FEBS J. 287, 800-813). We further demonstrated that incorporation of norvaline to *Escherichia coli's* proteome at isoleucine positions promotes higher toxicity than the same level of incorporation of valine. Taking into account that norvaline and alpha-aminobutyrate were likely abundant in abiotic conditions, and the common evolutionary origin of IleRS, ValRS and LeuRS, an intriguing hypothesis, that the editing domain was acquired by the ILVRS ancestor to ensure participation of only twenty canonical amino acids in modern translation, will be discussed.

S-02.1-2
Recent advances in genetic code engineering in *Escherichia coli*

N. Budisa
University of Manitoba, Winnipeg, Canada

Systematic reprogramming of the genetic code to incorporate non-canonical amino acids (ncAAs) into proteins is a universally important tool for systems bioengineering at the interface of chemistry, biophysics, and biotechnology. In other words, by integrating synthetic biology, chemistry, and genome manipulation, we can expand the scope of protein biosynthesis, deepen our understanding of the translation process, and create new classes of functional protein-based scaffolds. Expanding the genetic code by adding new ncAAs to the existing amino acid repertoire is already giving proteins and cells new and unusual functions for specific applications. For example, it enables the functionalization (e.g., cross-linking) of protein structures in combination with genetic and chemical methods (e.g., click chemistry). Aminoacyl-tRNA synthetases (aaRSs) are an important class of enzymes crucial for maintaining accuracy during translation of the genetic code. To introduce novel chemistries into proteins site-specifically in both live cells and cell-free extracts, many orthogonal pairs (o-pairs) consisting of an engineered aaRS and its cognate tRNA have been created in recent years. As part of a so-called orthogonal translation system (OTS), various aaRS/tRNA pairs allow installation of more than 200 non-canonical amino acids (ncAAs) into proteins, commonly in response to amber (i.e., UAG) stop codons. To change the substrate specificity towards a desired ncAA, the active site of a given natural aaRS is redesigned, usually via directed evolution methods coupled with a positive/negative selection system. I will exemplify these opportunities here by presenting the design and performance of orthogonal pairs for (i) fine-tuning of protein functions by caged ncAAs as chemo-optogenetic tools, (ii) vibrational energy transfer in proteins, and (iii) non-invasive determination of local electric fields in protein structures.

S-02.1-3
Dynamic changes in tRNA modifications and abundance during T-cell activation

Y. Pilpel
Weizmann Inst. of Science, Rehovot, Israel

The tRNA pool determines the efficiency, throughput, and accuracy of translation. Previous studies have identified dynamic changes in the tRNA supply and mRNA demand during cancerous proliferation. Yet, dynamic changes may occur also during physiologically normal proliferation, and these are less characterized. We examined the tRNA and mRNA pools of T-cells during their vigorous proliferation and differentiation upon triggering their antigen receptor. We observe a global signature of switch in demand for codons at the early proliferation phase of the response, accompanied by corresponding changes in tRNA expression levels. In the later phase, upon differentiation, the response of the tRNA pool is relaxed back to basal level, potentially restraining excessive proliferation. Sequencing of tRNAs allowed us to also evaluate their diverse base-modifications. We found that two types of tRNA modifications, wybutosine and ms2t6A, are reduced dramatically during T-cell activation. These modifications occur in the anti-codon loops of two tRNAs that decode "slippery codons", that are prone to ribosomal frameshifting. Attenuation of these frameshift-protective modifications is expected to increase the potential for proteome-wide frameshifting during T-cell proliferation. Indeed, human cell lines deleted of a wybutosine writer showed increased ribosomal frameshifting, as detected with a reporter that consists of a critical frameshifting site taken from the HIV gag-pol slippery codon motif. These results may explain HIV's specific tropism towards proliferating T-Cells since it requires ribosomal frameshift exactly on the corresponding codon for infection. The changes in tRNA expression and modifications uncover a new layer of translation regulation during T-cell proliferation and exposes a potential trade-off between cellular growth and translation fidelity.

ShT-02.1-1
Deciphering the mechanism of action of the bacterial translational inhibitor synthesized by *Actinoplanes* sp. VKM Ac-2862

I. Lisevich[1,*], D. Lukianov[1,*], E. Razumova[2], I. Osterman[1], P. Sergiev[1,2], O. Dontsova[1,2]
[1]*Skolkovo Institute of Science and Technology (Skoltech), Moscow, Russia,* [2]*Lomonosov MSU, Moscow, Russia*

The bacterially induced infections have become one of the most important global biological challenges due to rapidly developing resistance to the commonly used antibiotics. In the era of the constantly emerging superbugs speeding up the search for novel antibacterial agents is of great practical importance. Applying the mode of action-based platform devised in our laboratory, we detected antibacterial activity and ribosome stalling induction ability of the molecule (so-called 49252) produced by *Actinoplanes* sp. VKM Ac-2862. Having observed the concentration-dependent effect on translation inhibition in vitro and in vivo we directly proved that protein biosynthesis machinery is the target of 49252. A primary analysis of the resistant mutants provided us with some inklings of the 49252 mechanism of action. The first group of clones carries the mutations in the region of 16S rRNA called loop 560, with C564G substitution providing the highest level of resistance. There are no available literature data on the functional role of most of the identified mutations and these nucleotides do not overlap with binding sites of the existed small subunit inhibitors. This lets us assume that the molecule is likely to have a novel site of action on the ribosome. Another target, revealed in the second group of clones, was the S4 protein, which plays a pivotal role in the decoding process. Interestingly, all of the described mutations belong to the so-called ram or ribosomal ambiguity category, which means that such ribosomes are error-prone. Using *in vivo* miscoding assay, we succeeded in confirming that the second group of mutants indeed have a higher rate of misreading during translation. Surprisingly, the C564G mutant variant of the 16S rRNA group also appeared to have error-prone ribosomes. All these facts led us to formulate the hypothesis that 49252 is likely to have an impact on translation fidelity. This work was supported by RSF- 20-74-10031 grant (I. Osterman). *The authors marked with an asterisk equally contributed to the work.

ShT-02.1-2
Emerging noncanonical functions of plant aminoacyl-tRNA synthetase

J. Rokov-Plavec[1], M. Kekez[1], V. Evic[1], V. Zanki[1], R. Soic[2], D. Matković-Čalogović[2], I. Kekez[2]
[1]*Division of Biochemistry, Department of Chemistry, Faculty of Science, University of Zagreb, HR-10000 Zagreb, Croatia,* [2]*Division of General and Inorganic Chemistry, Department of Chemistry, Faculty of Science, University of Zagreb, HR-10000 Zagreb, Croatia*

Aminoacyl-tRNA synthetases (aaRSs) participate in translation, catalyzing formation of aminoacyl-tRNAs. Many aaRSs are involved in cellular processes beyond translation but reports on noncanonical functions of plant aaRSs are scarce. We have identified protein BEN1 as protein interactor of seryl-tRNA synthetase (SerRS) from plant *Arabidopsis thaliana*. BEN1 is involved in metabolism of brassinosteroid hormones that regulate variety of physiological processes, including stress response. Interaction interface involves SerRS globular catalytic domain and N-terminal extension of BEN1. The partnership between SerRS and BEN1 indicates a link between protein translation and steroid metabolic pathways of the plant cell. Structural studies revealed that Arabidopsis SerRS contains intrasubunit disulfide bridge. Cysteines (at positions 213 and 244) involved in disulfide link are conserved in all SerRSs from green plants, indicating their plant-specific functional importance. In order to determine whether disulfide link plays structural or alosteric role we have substituted cysteines with serine. C213S mutant showed lower stability compared to the wild type. Unexpectedly, C244S mutant showed higher stability than wild type, while double mutant had the same stability as the wild type. Crystal structure of C244S mutant and modeled structures of two other mutants showed that number of hydrogen bonds involving residues at positions 213 and 244 correlated with protein stability. The results imply that cysteines involved in the disulfide link are important but not essential for SerRS stability. Future studies are aimed at determination of enzyme activity of the wild type and mutant proteins. Considering that disulfide bonds in cytosolic proteins are usually linked to response mechanisms to oxidative stress, the disulfide link in plant SerRSs may be involved in regulation of translation under oxidative stress conditions.

Sunday 4 July
9:00–11:00, Povodni mož Hall

Protein folding and misfolding

S-02.4-1
Molecular chaperone functions in protein folding and proteome surveillance

U. Hartl
Max Planck Institute of Biochemistry, Martinsried, Germany

The past two decades have witnessed a paradigm shift in our understanding of cellular protein folding. While the three-dimensional structures of functional proteins are determined by their amino acid sequences, we now know that in the crowded environment of cells newly-synthesized polypeptides depend on molecular chaperone proteins to reach their folded states efficiently and at a biologically relevant time scale. Assistance of protein folding is provided by different types of chaperone which act to prevent misfolding and aggregation, often in an ATP-dependent mechanism. Once folded, many proteins continue to require chaperone surveillance to retain their functional states, especially under conditions of cell stress. Failure of the chaperone machinery to maintain proteostasis, i.e. the conformational integrity and balance of the cellular proteome, facilitates the manifestation of diseases in which proteins misfold and form toxic aggregates. These disorders include Parkinson's, Huntington's and Alzheimer's disease. I will provide a brief overview of chaperone functions in protein folding and then discuss recent findings from model systems suggesting that toxic protein aggregation in neurodegenerative disease is both a consequence and a cause of proteostasis decline.

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

S-02.4-3
Rational design of oligomer-specific antibodies as diagnostic tools for Alzheimer's and Parkinson's diseases

M. Vendruscolo
University of Cambridge, Cambridge, United Kingdom

The accurate quantification of the amounts of small oligomeric assemblies formed by Aβ and α-synuclein represents a major challenge in Alzheimer's and Parkinson's diagnostics. There is therefore great interest in the development of methods to specifically detect these oligomers by distinguishing them from larger aggregates. The availability of these methods will enable the development of effective diagnostic and therapeutic interventions for these and other diseases related to protein misfolding and aggregation. I will describe an antibody discovery method to generate single domain antibodies able to selectively probe Aβ and α-synuclein oligomers in isolation and in complex protein mixtures.

S-02.4-2
Protein folding stability in the cell and its possible implication for phase separation

S. Ebbinghaus
Technische Universität, Braunschweig, Germany

Proteins fold and function in the densely crowded and highly heterogeneous cell, which is filled up to a volume of 40% with macromolecules. That under such conditions cells can keep their proteome folded and organized without uncontrollable aggregation is a remarkable aspect of cell biology. First, molecular aspects of how cosolutes in the cellular milieu such as ions, crowders and osmolytes govern the protein folding equilibrium are discussed. Thereby, a novel classification scheme of cosolute effects based on their thermodynamic fingerprints is presented. In the cell, this scheme is less well defined including cofactors, metabolites or chaperones that play an important role. The cellular environment is also subject to spatiotemporal changes and cells respond to stress or stimuli. Protective cellular mechanisms are highly adapted to minimize the impact on the proteome, but certain proteins tend to accumulate under such conditions forming aggregates or condensates. Are these the least stable ones?

ShT-02.4-1
Identification of pharmacological chaperones for human alanine:glyoxylate aminotransferase as therapeutic strategy for primary hyperoxaluria type I

G. Pampalone[1], S. Grottelli[1], G. Annunziato[2], M. Pieroni[2], M. Dindo[3], G. Costantino[2], B. Cellini[1]
[1]University of Perugia, Perugia, Italy, [2]University of Parma, Parma, Italy, [3]Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan

Primary Hyperoxaluria Type I (PH1) is a rare disease due to the deficit of liver peroxisomal alanine:glyoxylate aminotransferase (AGT), a pyridoxal-5′-phosphate (PLP)-dependent enzyme responsible for glyoxylate detoxification. Inherited mutations of the AGXT gene often cause AGT misfolding, thus reducing the amount of functional enzyme at hepatic level and resulting in oxalate accumulation and precipitation as calcium oxalate stones in the urinary tract. The use of pharmacological chaperones (PCs) represents a promising approach for misfolding diseases. PCs are small molecule ligands that acts as competitive inhibitors and promote the correct folding of a mutant enzyme, thus restoring activity. In order to identify ligands acting as PCs for AGT, we performed an *in silico* screening of commercially-available compounds. Each molecule was analysed using a two-fold approach aimed at defining (1) the inhibition potency on purified AGT, and (2) the chaperone activity in mammalian model cells expressing wild-type AGT and a misfolded variant associated with PH1. On the basis of the results, a chemical optimization campaign was performed and the resulting synthetic molecules were tested using the same experimental approach described above. Overall, the results allowed to identify one hit compound active as PC for AGT able to increase by 1.5-2.5-fold the specific activity and expression of the enzyme, an important step toward a future preclinical development.

ShT-02.4-2
A tryptophan 'gate' in the CRISPR-Cas3 nuclease controls ssDNA entry into the nuclease site in *Escherichia coli*

L. He[1], Z. Jelić Matošević[2], D. Mitić[3], D. Markulin[3], T. Killelea[1], M. Matković[4], B. Bertoša[2], E.L. Bolt[1], **I. Ivančić Baće**[5]
[1]School of Life Sciences, University of Nottingham, Nottingham, United Kingdom, [2]University of Zagreb, Faculty of Science, Department of Chemistry, Zagreb, Croatia, [3]University of Zagreb, Faculty of Science, Department of Biology, Division of Molecular Biology, Zagreb, Croatia, [4]Ruder Boskovic Institute, Zagreb, Croatia, [5]Faculty of Science, Department of Biology, HR-10000 Zagreb, Croatia

Cas3 is a ssDNA-targeting nuclease-helicase essential for class 1 prokaryotic CRISPR immunity systems. Cas3-DNA crystal structures show that ssDNA follows a pathway from helicase domains through to a HD-nuclease active site, requiring protein conformational flexibility during DNA translocation. In genetic studies we had noted that the efficacy of Cas3 in CRISPR immunity was drastically reduced when temperature was increased from 30°C to 37°C, by unknown mechanism. Here using *E. coli* Cas3 proteins we show that inhibition of nuclease activity at higher temperature corresponds with measurable changes in protein structure. This effect of temperature on Cas3 was alleviated by changing a single highly conserved tryptophan residue (Trp-406) into an alanine. The Cas3W406A protein is a hyperactive nuclease that functions independently from temperature and from the interference effector module Cascade. Trp-406 is situated at the interface of Cas3 HD and RecA1 domains that is important for maneuvering DNA into the nuclease active site. Molecular dynamics simulations based on the experimental data showed temperature-induced changes in Trp-406 positioning that either blocked or cleared the ssDNA pathway. We propose that Trp-406 forms a 'gate' for controlling Cas3 nuclease activity via access of ssDNA to the nuclease active site. The effect of temperature in these experiments may indicate allosteric control of Cas3 nuclease activity caused by changes in protein conformations.

Sunday 4 July
16:00–18:00, Kocka Hall

Proteolytic processing

S-02.3-1
Matrix remodeling as early pathophysiological biomarker

I. Sagi, E. Shimshoni, I. Adir, I. Solomonov
The Weizmann Institute of Science, Rehovot, Israel

The majority of research efforts on complex diseases are invested in studying cellular molecular processes and cellular signaling molecules, e.g., cytokines, chemokines and growth factors, which often represent only a subset of factors responsible for tissue pathology. All of these processes and molecular signals are embedded within the extracellular matrix (ECM), which comprises a large portion of the tissue. Tissue damage, manifesting in the extracellular matrix, is a hallmark of many invasive diseases including inflammatory bowel disease. We used colitis models for investigating extracellular-matrix remodeling dynamics during disease onset. We reveal that even prior to the first inflammatory symptoms, the colon displays a unique extracellular-matrix signature in terms of composition, morphology and stiffness. Specifically, the matrix becomes perforated at pre-symptomatic states, which is mediated by sub-clinical infiltration of neutrophils and monocytes bearing remodeling enzymes. The unique proteomic composition of this newly discovered state is also marked by specific biomarkers. Remarkably, whether the inflammation is chronic or acute, its matrix signature converges at pre-symptomatic states. Our work showcases the importance of an integrative approach to extracellular matrix (ECM) analysis. We suggest that the existence of a pre-symptomatic extracellular-matrix is general and relevant to a wide range of tissue damage associated diseases. E Shimshoni, R Afik, I Solomonov, I Adir, A Shenoy, M Adler, L Puricelli, bioRxiv, 665653

S-02.3-2
Extracellular cysteine cathepsins: from signalling to matrix degradation

B. Turk
Department of Biochemistry, Molecular and Structural Biology, Jožef Stefan Institute, Ljubljana, Slovenia

Endolysosomal system contains over 50 hydrolases, including a number of proteases, which have a major role in numerous processes, in addition to intracellular protein turnover. Among these proteases, the most abundant are cysteine cathepsins. In human genome there are 11 cysteine cathepsins, and although structurally similar, they do not have the same roles. Several of them are also associated with MHC II-mediated antigen presentation, prohormone processing and bone resorption. In a number of inflammation-associated diseases, including cancer, arthritis and atherosclerosis, they have been found to be secreted in the extracellular milieu. Early in vitro work suggested that their primary extracellular role is the degradation of the extracellular matrix. Several of cathepsins were suggested to be involved, but the major roles seem to have cathepsins B, K, S and L. In addition, there is increasing evidence that the cathepsins are, through the cleavage of different extracellular or membrane proteins, including various CAMs, CD44, EGFR and plexins involved in the regulation of many other processes, such as regulation of Ras GTPase activity. Moreover, there is evidence that cathepsins are also involved in the regulation of complement activation, which may be of major importance in cancer. This is probably linked also with cathepsin-mediated chemokine processing that may be of major importance for generation of a feedback loop to attract immune cells, which in turn secrete more cathepsins. Identification of their physiological substrates is therefore of major importance for understanding their signaling pathways linked with disease progression and will be further discussed.

S-02.3-3
Neutrophil proteases: anti-microbial agents or key instigators of inflammation?

S. Martin
Department of Genetics, Trinity College, Dublin, Ireland

Neutrophil granule proteases are thought to function as anti-microbial agents, hydrolyzing microorganisms within phagosomes, or upon liberation into the extracellular space. However, evidence also suggests that the latter proteases play an important role in the coordination and escalation of inflammatory reactions, but how this is achieved has been obscure. IL-1 family cytokines are important initiators of inflammation but require proteolytic processing for activation. Our recent work has shown that proteases liberated from activated neutrophils can positively or negatively regulate the activity of multiple members of the extended IL-1 cytokine family (IL-1alpha, IL-1beta, IL-33, IL-36alpha, IL-36beta and IL-36gamma) with exquisite sensitivity. In contrast, neutrophil proteases displayed very poor bactericidal activity, exhibiting 100-fold greater potency towards cytokine processing than killing of multiple bacterial species. We propose that neutrophils function as key regulators of inflammatory responses through deployment of their granule proteases to process IL-1 family cytokines liberated from dead cells.

ShT-02.3-1
From ubiquitin-independent degradation to ubiquitin degradation – a signature activity of emergency 20S proteasome in hypoxia

I. Sahu[1], S.M. Mali[2], P. Sulkshane[1], R. Morag[1], A. Rosenberg[1], C. Xu[3], M.P. Sahoo[1], S.K. Singh[2], Z. Ding[3], Y. Wang[3], S. Day[4], Y. Cong[3], O. Kleifeld[1], A. Brik[2], M.H. Glickman[1]
[1]Faculty of Biology, Technion—Israel Institute of Technology, Haifa, Israel, [2]Schulich Faculty of Chemistry, Technion—Israel Institute of Technology, Haifa, Israel, [3]National Center for Protein Science Shanghai, University of Chinese Academy of Sciences, Shanghai, China, Shanghai, China, [4]Medical School, University of Michigan, MI-48109, USA, Michigan, United States of America

Eukaryotic cells harbor fully assembled free 20S proteasome in addition to active 30S/26S proteasomes. 20S proteasome efficiently degrades unstructured proteins *in vitro* however; its cellular proteolytic activity has been a long-standing debate. Although partial evidences support the *in vivo* function of 20S proteasome, indistinct contribution from both 26S and 20S proteasomes blur this hypothesis due to common core enzymes. In this study, we unravel the unique contribution of 20S proteasome towards intracellular degradation and proteostasis. By following series of systematic approaches including chemical synthesis of a

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

set of synoptic ubiquitin-conjugates, single particle cryo-EM analysis and intracellular peptidomics, we defined "Four signature activities" of 20S proteasome that are distinct from 26S proteasome. Under condition of hypoxia or human Failing heart, we discovered these signature activities of 20S proteasome due to its elevated levels. Taking Cyclin B1 as a model substrate and a genetically modified Hi20S mammalian cell model, we revealed that 20S proteasome cleavage activity and product outcomes are different from 26S proteasomes. One of the signatures is the participation of 20S proteasome in degradation of polyubiquitin conjugates under hypoxic stress. A further single particle Cryo-EM study elucidated the ubiquitin conjugate driven asymmetric conformational changes for 20S proteasome function. In conclusion, we could determine 20S proteasome signature function in mammalian cells that can be exploited as a prognostic marker for hypoxia associated diseases. Moreover, Hi20S state under hypoxia might be an adaptive response for emergency proteasome activity to alleviate proteotoxic-load and provide better survival.

ShT-02.3-2
Identifying PRRSV CD^{8+} T cell epitopes using a tailored vector system

M. Mötz[1], C. Riedel[2], A. Saalmüller[2], T. Rümenapf[1]
[1]*Institute of Virology, University of Veterinary Medicine Vienna, Vienna, Austria,* [2]*Institute of Immunology, University of Veterinary Medicine Vienna, Vienna, Austria*

Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped, single stranded RNA virus of positive polarity within the family *Arteriviridae*. It is one of the most relevant porcine pathogens and has a huge economic impact on the swine industry worldwide. Clinical signs of infected animals are respiratory disease, reproductive failure in sows, and the birth of weak, congenitally infected piglets. PRRSV is highly immunosuppressive and virus induced adaptive immune responses are yet poorly understood. $CD8^+$ T cells are considered an important correlate of protection in infected pigs. Therefore our aim is to identify viral peptides bound to class I swine leukocyte antigen (SLA-I) molecules, which present these antigens to stimulate $CD8^+$ T cells and trigger an effector response. Since PRRSV downregulates the expression of SLA-I, we tailored a classical swine fever virus based vector system to deliver defined PRRSV genome fragments to its target cells and mark them for proteasomal degradation. Furthermore, we immunoprecipitated SLA-I molecules and isolated their bound viral epitopes. Finally, we identified these 9-mer to 11-mer peptides with mass spectrometry. With this novel vector system for the delivery of viral proteins for immunopeptidome analysis, we are able to gain insight into the processing and presentation of viral antigens to $CD8^+$ T cells.

Sunday 4 July
16:00–18:00, Marmorna Hall A

RNA function

S-01.2-3
The molecular mechanisms of cytoplasmic RNA quality control in human cells

D. Zigáčková, N. Varadarajan, Z. Feketova, T. Skalický, **Š. Vaňáčová**
CEITEC, Masaryk University, Brno, Czech Republic

The 3′-terminal RNA uridylation catalyzed by the terminal uridyltransferases (TUTases) mediates degradation of various RNAs and processing of some ncRNAs. DIS3L2 is mammalian oligo(U) specific exonuclease, that is involved in the decay of uridylated precursors of let-7 miRNAs, tRNAs, and cleaved mRNAs (Previously published in: 1. Ustianenko D., et al. (2016), EMBO J 35, 2179-2191, Pirouz M., et al. (2016), Cell Rep 16, 1861-73, and Reimão-Pinto MM.,et al. (2016), EMBO J 35, 2417-2434). Mutations in TUTases and DIS3L2 have been linked to cancer and developmental defects (7,8). We and others have recently demonstrated, that TUT-DIS3L2 (TDS) is a conserved cytoplasmic pathway for surveillance of aberrant transcripts (9,10,11,12). TDS targets mostly aberrant noncoding RNAs, such as snRNAs, rRNA, tRNAs, YRNAs, and also transcripts originating from pseudogenes. Interestingly, we also uncovered a fraction of transcripts corresponding to 5' termini of protein-coding genes (5′ mRNA fragments, 5′ mRFs). In our follow-up study, we investigate the process leading to 5'mRFs formation and uridylation. We show, that 5′ fragments of mRNAs are formed in the nucleus, exported to the cytoplasm, where they are targeted by the TDS and additional factors that we identified by RNA-based protein precipitation. In summary, our data elucidate in greater detail TDS mechanisms and its impact on cellular physiology. This work was supported by the CEITEC 2020 (LQ1601) project with financial contribution made by the Ministry of Education, Youths and Sports of the Czech Republic (MEYS CR) within special support paid from the National Programme for Sustainability II funds.

S-01.2-2
TDP-43 condensation properties specify its RNA binding and regulation

M. Hallegger[1], A. Chakrabarti[1], F. Lee[1], A. Amalietti[1], K. Kuret[1], I. Huppertz[1], N. Luscombe[1], **J. Ule**[1,2]
[1]*The Francis Crick Institute & UCL, London, United Kingdom,* [2]*National Institute of Chemistry, Ljubljana, Slovenia*

Mutations that cause amyotrophic lateral sclerosis (ALS) have been shown to affect condensation properties of many RNA binding proteins (RBPs). Less is known, however, about the role of RBP condensation in the selection of endogenous RNA binding sites, and thereby in the regulation of specific RNAs. To address this question, we created cell lines for inducible expression of TDP-43, an RBP that is central to the pathogenesis of ALS. We mutated the C-terminal intrinsically disorder region (IDR) of TDP-43 that act on a gradient from negative to positive condensation effects, as evident by changes in the formation of nuclear foci and TDP-43 mobility. Using UV crosslinking and immunoprecipitation (iCLIP) we found that condensation properties modulate the binding of TDP-43 to a

subset of RNA sites. Condensation is important particularly for TDP-43 to be able to bind to long binding sites, which contain highly multivalent RNA motifs. Moreover, our findings demonstrate that distinct forms of IDR-mediated condensation are important for binding of TDP-43 to distinct subsets of RNA sites. We show that such condensation-mediated RNA binding is essential for TDP-43 to regulate processing of a subset of its RNA partners, including the auto-regulation of the TDP-43 mRNA itself. In conclusion, we show that IDR-mediated condensation is important for specific functions of TDP-43 in RNA regulatory networks, with implications for evolution and disease.

S-01.2-1
Understanding tissue-specific protein composition of RNA-based enzymes

Y. Liu, J. Orozco, Y. Livelo, R. Franklin, S. Cheloufi, **J. Murn**
University of California-Riverside, Riverside, CA, United States of America

Ribonuclease P (RNase P) is a deeply conserved and essential ribozyme that cleaves the 5′ leader sequence from precursor tRNAs in organisms from all domains of life. During the evolution, the nuclear RNase P gradually transformed from an RNA-dominated ribozyme into a protein-rich ribonucleoprotein complex, while maintaining a catalytic RNA core. The reasons for the increased protein content and complexity of RNase P in higher organisms remain incompletely understood. We find that the protein composition of mammalian RNase P varies between different tissues, with important consequences for cellular function. Specifically, we find two alternative variants of nuclear RNase P, one that is highly enriched in pluripotent stem cells (stem cell variant), and another, which is present ubiquitously (constitutive variant). Deletion of the constitutive variant inhibits differentiation of mouse embryonic stem cells (ESCs), whereas lack of the stem cell variant stimulates differentiation of ESCs towards endodermal lineages. I will present our insights into the biochemistry of the protein subunits that distinguish both RNase P variants, including their association with the catalytic RNA and their impact on the activity of the ribozyme. I will also discuss potential non-canonical roles of these protein subunits and their general requirement for mammalian development.

ShT-01.2-1
NAD-capping: a universal RNA modification in Mycobacteria, Archaea and *Escherichia coli*

O. Ruiz-Larrabeiti[1], R. Benoni[2], V. Zemlianski[3], N. Hanišáková[4], A. Suder[5], M. Schwarz[6], B. Brezovská[1], B. Svojanovská[2], M. Vítězová[4], H. Cahová[2], M. Převorovský[3], L. Krásný[1]
[1]*Institute of Microbiology, The Czech Academy of Sciences, Prague, Czech Republic,* [2]*Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic,* [3]*Department of Cell Biology, Charles University, Prague, Czech Republic,* [4]*Faculty of Science, Masaryk University, Brno, Czech Republic,* [5]*Malopolska Centre of Biotechnology, Jagiellonian University, Krakow, Poland,* [6]*Laboratory of Bioinformatics, Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic*

The 5′ end status is key to the life of a transcript. NAD^+ (nicotinamide adenine dinucleotide) universal redox cofactor has been detected covalently attached to the 5'-end of RNA in bacterial and eukaryotic cells, where RNA polymerase uses NAD^+ instead of ATP to initiate transcription. This new RNA modification has been termed NAD-capping. Until now, NAD-capping had not been investigated in the mycobacterial genus, which contains important pathogens. At the same time, the nature of NAD^+ cofactor and the discovery of NAD-capping in a widespread variety of organisms raised up the possibility that NAD-capping may be a universal RNA modification, a hypothesis that remained unproven until now. Likewise, a cohesive view of the biological role of NAD-cap and its effect on the transcript and the cell is still being formed. Here we show that NAD-capping exists in mycobacteria, and that mycobacterial NAD-RNAs are mRNAs involved in membrane and redox processes and some small RNAs (sRNAs), like the characterized Ms1 and rnpB. We have also analysed archaeal RNA by mass spectrometry and discovered NAD^+ covalently attached to it, proofing that NAD-capping is universal to all domains of life. Finally, we have studied the biological role of NAD-cap in RNAI, the most abundantly modified sRNA in *Escherichia coli*. We found that preventing NAD-capping on RNAI caused it to have a longer half-life, and changed the balance between RNAI and its regulatory target, which affected the ability of the cell to exit LAG phase at high ampicillin concentrations, as compared to the wild-type. In this talk I will explain how our discoveries on NAD-capping in Mycobacteria, Archaea and *Escherichia coli* correlate to previous findings, and how they add to our knowledge on transcriptomic modifications. O.R.L. was supported by the grant POS_2019_1_0033 from the Basque Government.

ShT-01.2-2
New targets for drug design: importance of nsp14/nsp10 complex formation for the 3′-5′ exoribonucleolytic activity on SARS-CoV-2

M. Saramago, C. Bárria, V.G. Costa, C.S. Souza, S.C. Viegas, S. Domingues, D. Lousa, C.M. Soares, C.M. Arraiano, R.G. Matos
Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

SARS-CoV-2 virus has triggered a global pandemic with devastating consequences. The understanding of fundamental aspects of this virus is of extreme importance. In this work, we studied the viral ribonuclease nsp14, one of the most interferon antagonists from SARS-CoV-2. Nsp14 is a multifunctional protein with two distinct activities, an N-terminal 3′-to-5′exoribonuclease (ExoN) and a C-terminal N7-methyltransferase (N7-MTase), both critical for coronaviruses life cycle, indicating nsp14 as a prominent target for the development of antiviral drugs. In Coronaviruses, nsp14 ExoN activity is stimulated through the interaction with the nsp10 protein. We have performed a biochemical characterization of nsp14-nsp10 complex from SARS-CoV-2. We confirm the 3′-5′ exoribonuclease and MTase activities of nsp14, and the critical role of nsp10 in upregulating the nsp14 ExoN activity. Furthermore, we demonstrate that SARS-CoV-2 nsp14 N7-MTase activity is functionally independent of the ExoN activity and nsp10. A model from SARS-CoV-2 nsp14-nsp10 complex allowed mapping key nsp10 residues involved in this interaction. Our results show that a stable interaction between nsp10 and nsp14 is required for the nsp14-mediated ExoN activity of SARS-CoV-2. We studied the role of conserved DEDD catalytic residues of SARS-CoV-2 nsp14 ExoN. Our results show that motif I of ExoN domain is essential

for the nsp14 function, contrasting to the functionality of these residues in other coronaviruses, which can have important implications regarding the specific pathogenesis of SARS-CoV-2. This work unravelled a basis for discovering inhibitors targeting specific amino acids in order to disrupt the assembly of this complex and interfere with coronaviruses replication.

Sunday 4 July
16:00-18:00, Marmorna Hall B

Epigenetics

S-01.4-3
DNA demethylation and aging

C. Niehrs
Institute of Molecular Biology (IMB), Mainz, Germany

Changes in DNA methylation are among the best-documented epigenetic alterations, which accompany aging. However, if and how altered DNA methylation is causally involved in aging has remained elusive. GADD45α and ING1 are adapter proteins for site-specific demethylation by TET methylcytosine dioxygenases. We show that Gadd45a/Ing1 double knockout mice (DKO) display premature aging and phenocopy impaired energy homeostasis and lipodystrophy, characteristic of Cebp (CCAAT/enhancer binding protein) mutants. Correspondingly, GADD45α occupies C/EBPβ/δ- dependent super-enhancers, and cooperatively with ING1 promotes local DNA demethylation to permit C/EBPβ recruitment. Our study reveals a causal nexus between DNA demethylation, metabolism and organismal aging.

S-01.4-1
Mass spectrometry-based epigenetic mapping of clinical samples for biomarker discovery and (breast) cancer patient stratification

T. Bonaldi, R. Noberini, E.O. Savoia, F. Bedin
European Institute of Oncology, Milano, Italy

Although cancer has been traditionally considered as the result of the accumulation of genetic defects, striking evidence has now shown that epigenetic changes also contribute to cancer initiation and progression. Aberrations in histone post-translational modifications (PTMs) have been reported as hallmarks of cancer and as prognostic markers, and many inhibitors of histone modifying enzymes are currently under investigation for cancer treatment. Therefore, profiling histone PTM in cancer may have important implications for the discovery of both biomarkers for patient stratification and novel epigenetic targets. I will present the battery of mass spectrometry-based approaches that we have developed to carry out a comprehensive and quantitative analysis of up to 58 histone modified peptides from >200 cancer patient tissues of different origin. By comparing tumor and normal tissues for various cancer models – including different breast cancer subtypes, and lung, prostate, head and neck and ovarian cancers – we identified histone modification changes that represent general hallmarks of cancer, in addition to those previously reported, as well as tumor- and subtype-specific changes. Moreover, we carried out the MS-profiling of histone PTMs in different breast cancer subtypes, with a special focus on triple negative breast cancers (TNBCs), which comprise a highly diverse group of tumors that lack well-defined molecular targets and targeted therapies. We identified a panel of epigenetic marks that distinguish TNBCs from the other subtypes and that differentiate TNBC patients with and without relapse after chemotherapy. These histone PTM signatures offer insights into potential epigenetic mechanisms underlying cancer in general, and TNBC in particular, not only providing potential biomarkers useful for TNBC patient stratification and prediction of response to therapy, but also suggesting possible novel epigenetic pathways targetable for therapy.

S-01.4-2
Epigenome deregulation in cancer

Z. Herceg
International Agency for Research on Cancer, Lyon, France

Recent years have witnessed a remarkable pace of discoveries in epigenetics which have revolutionized our understanding of complex human diseases. The spectacular advances in epigenomics that allow the analysis of the epigenome with unprecedented resolution in high throughput and genome-wide settings have further accelerated investigations in this area. The challenge posed by major international sequencing efforts is to identify changes in the (epi)genome that precede and promote tumour development, and to differentiate functionally important ("drivers") from non-functional "passenger" events. In addition, there is little understanding about whether epigenetic changes can be used as biomarkers for exposure assessment, risk stratification, and early detection. The epigenome has been proposed to function as an interface between environmental factors and the genome, therefore, the identification and functional characterization of epigenetic events deregulated by specific environmental and lifestyle stressors should enhance our understanding of mechanisms of carcinogenesis linked to risk-factor exposures. The intrinsic reversibility of epigenetic changes represents a tremendous opportunity for the development of novel strategies for cancer treatment and prevention. I will discuss recent conceptual and technological advances in epigenetics and ongoing efforts aiming to identify epigenetic targets that could be exploited in cancer prevention and therapy as well as molecular epidemiology.

ShT-01.4-1
Investigating the link between DNA replication, chromatin change and transcriptional regulation during *in vivo* erythroid differentiation

R.A. Beagrie[1,2], A.M. Oudelaar[1], M. Gosden[1], D. Hidalgo[2], J.R. Hughes[1], M. Socolovsky[2], D.R. Higgs[1]
[1]*Weatherall Institute of Molecular Medicine; University of Oxford, Oxford, United Kingdom,* [2]*University of Massachusetts Medical School, Worcester, MA, United States of America*

In mouse red blood cell differentiation (erythropoiesis), early progenitors transition to terminal differentiation by passing through a highly specialised cell cycle[1]. During this cell cycle, DNA is replicated faster than in preceding or following cycles due to accelerated replication forks[2]. We have performed an integrated analysis of matched chromatin accessibility and single-cell expression data, revealing that this cell cycle also coincides with widespread changes in gene expression and chromatin

accessibility. We analysed chromatin folding of erythroid gene loci at various stages in red blood cell differentiation and show that specific enhancer-promoter loops are formed concomitantly with both progressive upregulation of gene expression and changes in histone post-translational modifications. We used SeqGL[3], a quantitative model using a k-mer feature representation and group lasso regularization to identify transcription factor motifs enriched in enhancer sequences at each stage of red blood cell differentiation. SeqGL highlighted the erythroid transcription factor GATA1 as highly enriched at enhancers that first become accessible during the transition to terminal differentiation. Using CUT&RUN, we are able to show that this specialised cell cycle is indeed the point at which GATA1 first binds to chromatin. Finally, we inhibit DNA replication and measure the effects of this perturbation on Gata1 recruitment and on chromatin composition. Our findings demonstrate that chromatin architecture and gene activation are tightly linked during development and provide insights into the distinct mechanisms contributing to the establishment of tissue-specific chromatin structures. References: 1. Pop *et al*. PLoS Biol. 8, e1000484 (2010). 2. Hwang *et al*. Sci. Adv. 3, e1700298 (2017). 3. González *et al*. Nat. Genet. 47, 1249–1259 (2015).

ShT-01.4-2
Novel approach for genome-wide high-resolution profiling of 5-hydroxymethylcytosine and its application for neuroblastoma analysis

M. Narmontė[1,*], P. Gibas[1,*], Z. Staševskij[1], K. Daniūnaitė[2], J. Gordevičius[1], S. Klimašauskas[1], E. Kriukienė[1]
[1]*Department of Biological DNA Modification, Institute of Biotechnology, Life Sciences Center, Vilnius University, Vilnius, Lithuania,* [2]*Human Genome Research Group, Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania*

The epigenetic DNA modification 5-hydroxymethylcytosine (5hmC) has a crucial role in development and gene regulation and is associated with complex human diseases including cancer. Neuroblastoma (NB) is the most common solid extracranial pediatric tumor. Its low mutational burden points out the need to find new epigenetic markers for better diagnosis and treatment monitoring. Analysis of 5hmC changes in tumor samples requires cost-effective high-resolution techniques. We developed a bisulfite-free approach for 5hmC profiling at single-nucleotide resolution, named hmTOP-seq (5hmC-specific tethered oligonucleotide-primed sequencing) (Gibas et al. (2020) PLoS Biol 18(4): e3000684), which is based on the direct sequence readout primed at covalently labeled 5hmC sites from an in situ tethered DNA oligonucleotide. hmTOP-seq was validated on a model bacteriophage genome and mouse embryonic stem cells, indicating quantitative, single-base resolution detection of 5hmC. We showed that hmTOP-seq is capable to detect subtle differences in the strand-specific CG hydroxymethylation and also allows 5hmC identification in a non-CG context. After extensive validation we employed this new approach for genome-wide 5hmC profiling of different NB cells grown under atmospheric or hypoxic conditions. A combined analysis of 5hmC and transcriptome demonstrated hypoxic signatures of NB cells and defined a link between gene expression and hypoxic 5hmC changes, suggesting epigenetic 5hmC functions in response to oxygen deprivation. Furthermore, we demonstrated that 5hmC well characterizes cell identities of various NB cells, proposing their different malignant transformations and diverse involvement in NB progression. Altogether, hmTOP-seq is a valuable cost-effective technique for detection of 5hmC profiles in various tissues and cell types that could help explore tumor heterogeneity. *The authors marked with an asterisk equally contributed to the work.

Sunday 4 July
16:00–18:00, Gallery Hall

Protein localization and dynamics

S–02.5-1
ER–Golgi contact sites

M.A. De Matteis
TIGEM, POZZUOLI (NA), Italy

The last decade has witnessed an enormous interest in membrane contact sites (MCS), i.e. sites where two organelles come closer than 30 nm. Now we know that this type of contacts can be established between the ER and almost every other organelle: i.e. mitochondria, endolysosomes, PM, peroxisomes and Golgi complex. The location of membrane contact sites between the endoplasmic reticulum (ER) and the trans-Golgi-network (TGN) in the crowded perinuclear area has hampered their analysis as well as their mechanistic study. Therefore, there is a lack of conclusive information about their composition and function. To overcome these limits, we developed a FRET-based approach to study ER-TGN contact sites (ERTGoCS), and analyzed candidate proteins possessing dual targeting motifs for the TGN and the ER. We found that OSBP1 and ORP9 play a redundant tethering role while ORP10 is required for ERTGoCS integrity due to its ability to transfer phosphatidylserine to the TGN. We exploited these results to assess whether and how ERTGoCS control the TGN levels of PI4P, a phosphoinositide with key roles in the Golgi complex. We found that they do so by providing a spatial setting suitable for the 4-phosphatase Sac1 to dephosphorylate PI4P in trans at the TGN. However, the ERTGoCS, though necessary, are not sufficient for the in trans activity of Sac1 since this needs the phosphatidyl-Four-phosphate-AdaPtor-Protein-1 (FAPP1). FAPP1 interacts with Sac1 and promotes its in trans phosphatase activity in vitro. We envision that FAPP1, acting as a PI4P detector and adaptor, positions Sac1 at TGN domains with elevated concentrations of PI4P allowing its in trans phosphatase activity. In fact, FAPP1 depletion induces an increase in TGN PI4P, which in turn leads to increased secretion of selected neosynthesized cargoes (such as ApoB100 in hepatocytes) indicating that FAPP1, by controlling Sac1 activity at the ERTGoCS and thus TGN PI4P levels, acts as a gatekeeper of Golgi exit.

S-02.5-2
Oscillatory dynamics of Rac1 activity in amoeboid cell motility

M. Šoštar[1], M. Marinović[1], V. Filić[1], N. Pavin[2], **I. Weber**[1]
[1]*Division of Molecular Biology, Ruder Boškovic Institute, Bijenicka 54, HR-10000 Zagreb, Croatia,* [2]*Department of Physics, Faculty of Science, University of Zagreb, Bijenicka 32, HR-10000 Zagreb, Croatia*

Migration of eukaryotic cells is governed by segregation of the cortical actin cytoskeleton into structurally and functionally distinct domains, protrusive at the cell front and retractile at the back. Dictyostelium amoebae can change their polarity within 20 seconds, which is the fastest among eukaryotic cells. This repolarization is heralded by an enhancement of Rac1 activity at the incipient leading edge of the cell, where it stimulates Arp2/3-mediated actin polymerization. At the cell back, Rac1-GTP promotes stability of the cell cortex by initiating formation of a complex containing IQGAP-related protein DGAP1 and actin-bundling cortexillins. We present and compare results of experimental and modelling approaches to investigate the dynamics of Rac1 in Dictyostelium cells. Rac1 activity was monitored using a specific fluorescent probe based on the GTPase-binding domain from DPAKa kinase. Besides Rac1-GTP, fluorescently labelled DGAP1 was also monitored. We observed that active Rac1 and DGAP1 in the cell cortex exhibit anti-correlated oscillations in the form of standing and travelling waves. To gain insight into the molecular mechanisms underlying the observed dynamics, we formulated a reaction-diffusion model that incorporates interactions between Rac1, DGAP1 and a Rac1-inactivating protein GAP. The model was able to reproduce detailed features of obtained experimental results and to predict new types of dynamics that were subsequently observed in experiments, such as colocalization of active Rac1 and DGAP1 during directed cell migration. Dynamics of Rac1-GTP often changed over from rotation to oscillation and vice versa, which was successfully simulated by a stochastic version of the model. Dissection of the model showed that the occurrence of oscillatory patterns depended on the signalling network topology used to describe the Rac1-GAP interaction, whereas the polarized states were governed by the interaction of activated Rac1 with its effector DGAP1.

S-02.5-3
Control of microtubule organization and dynamics: seeing proteins and drugs in action

A. Akhmanova
Utrecht University, Utrecht, Netherlands

Microtubules are dynamic cytoskeletal filaments that control different aspects of cell architecture. Microtubules are intrinsically asymmetric polymers, with fast-growing plus ends, which in cells serve as major sites of microtubule assembly and disassembly, and slow-growing minus ends, which are often stabilized and attached to different cellular structures. Tight regulation of microtubule dynamics is essential for many cellular processes, including cell division, migration and morphogenesis. Using *in vitro* reconstitution experiments, single molecule assays and live cell imaging we explored the detailed mechanisms of such regulation by microtubule plus end binding proteins. We found that Kinesin-4 family molecules can induce microtubule pausing, a function that appears to be important to prevent microtubule overgrowth in different cellular contexts. Furthermore, we used assays with fluorescent analogues of microtubule-stabilizing and destabilising agents to directly visualize their effects on microtubule growth. We found that a single molecule of the microtubule-depolymerizing drug eribulin bound to the microtubule tip was sufficient to trigger a catastrophe. Microtubule rescue and stabilization by taxanes was more complex, required multiple drug molecules and involved profound local changes in microtubule structure.

ShT-02.5-1
Self-organization in a Rab GTPase regulatory network

U. Bezeljak[1,2], L. Kowalski[1], H. Loya[3], B. Kaczmarek[1], B. Wu[1], J. Aguilera Servin[1], I. Prieto Gonzalez[1], J. Merrin[1], T.E. Saunders[4], M. Loose[1]
[1]*Institute of Science and Technology Austria (IST Austria), Klosterneuburg, Austria,* [2]*Center of Excellence for Biosensors, Instrumentation and Process Control - COBIK, Ajdovscina, Slovenia,* [3]*Indian Institute of Technology Bombay, Mumbai, India,* [4]*Mechanobiology Institute, Singapore, Singapore*

The eukaryotic vesicular trafficking is orchestrated by Rab small GTPases, which are activated at defined times and locations in a switch-like manner. While this switch is well understood for an individual protein, how their regulatory networks produce emergent and self-organized activity patterns is currently not known. Here, we combine in vitro reconstitution with computational modeling to study a minimal Rab5 activation circuit. We find that the molecular interactions between the network components give rise to a positive feedback and bistable collective switching of Rab5. Furthermore, the Rab5 activity switch is highly tunable by the amount of freely diffusing GDP-bound GTPase. The state transition near the critical point is intrinsically stochastic, triggering a traveling wave of Rab5 activation on the membrane surface, which spreads from self-assembled protein clusters through positive feedback. Together, our findings reveal ways the non-equilibrium properties of biochemical networks define the spatiotemporal organization of the cell. These general principles can be applied also to other dynamic small GTPase circuits from Ras, Rho, Rac and Arf families.

ShT-02.5-2
Ceramide chain length–dependent protein sorting into selective endoplasmic reticulum exit sites

S. Rodriguez-Gallardo[1,*], K. Kurokawa[2,*], S. Sabido-Bozo[1], A. Cortes-Gomez[1], A. Ikeda[3], V. Zoni[4], A. Aguilera-Romero[1], A.M. Perez-Linero[1], S. Lopez[1], M. Waga[2], M. Araki[3], M. Nakano[3], H. Riezman[5], K. Funato[3], S. Vanni[4], A. Nakano[2], M. Muñiz[1]
[1]*Departamento de Biología Celular, Facultad de Biología, Universidad de Sevilla, Sevilla, Spain,* [2]*Live Cell Super-Resolution Imaging Research Team, RIKEN Center for Advanced Photonics, Saitama, Japan,* [3]*Graduate School of Integrated Sciences for Life, Hiroshima University, Hiroshima, Japan,* [4]*Department of Biology, University of Fribourg, Chemin du Musée 10, 1700, Fribourg, Switzerland,* [5]*NCCR Chemical Biology, Department of Biochemistry, University of Geneva, Geneva, Switzerland*

Protein sorting in the secretory pathway is crucial to maintain cellular compartmentalization and homeostasis. In addition to

coat-mediated sorting, the role of lipids in driving protein sorting during secretory transport is a longstanding fundamental question that still remains unanswered. Here, we conduct 3D simultaneous multicolor high-resolution live imaging to demonstrate in vivo that newly synthesized glycosylphosphatidylinositol-anchored proteins having a very long chain ceramide lipid moiety are clustered and sorted into specialized endoplasmic reticulum exit sites that are distinct from those used by transmembrane proteins. Furthermore, we show that the chain length of ceramide in the endoplasmic reticulum membrane is critical for this sorting selectivity. Our study provides the first direct in vivo evidence for lipid chain length–based protein cargo sorting into selective export sites of the secretory pathway. Previously published in: Rodriguez-Gallardo et al. (2020) Sci Adv 6, eaba8237. *The authors marked with an asterisk equally contributed to the work.

Monday 5 July
9:00–11:00, Kocka Hall

New approaches in structure determination

S-04.1-1
Structural biology *in situ*: the promise and challenges of cryo-electron tomography

W. Baumeister
Max Planck Institute of Biochemistry, Martinsried, Germany

Traditionally, structural biologists have approached cellular complexity in a reductionist manner by characterizing isolated and purified molecular components. This 'divide and conquer' approach has been highly successful. However, awareness has grown in recent years that only rarely can biological functions be attributed to individual macromolecules. Most cellular functions arise from their acting in concert. Hence there is a need for methods developments enabling studies performed in situ, i.e. in unperturbed cellular environments. *Sensu stricto* the term 'structural biology in situ' should apply only to a scenario in which the cellular environment is preserved in its entirety. Cryo electron tomography has unique potential to study the supramolecular architecture or 'molecular sociology' of cells. It combines the power of three-dimensional imaging with the best structural preservation that is physically possible.

S-04.1-2
Serial crystallography with X-ray free-electron lasers

H. Chapman[1,2,3]
[1]*CFEL DESY, Hamburg, Germany,* [2]*Centre for Ultrafast Imaging, University of Hamburg, Hamburg, Germany,* [3]*Department of Physics, University of Hamburg, Hamburg, Germany*

Using X-ray free-electron laser pulses it is possible to outrun the effects of radiation damage, allowing macromolecular structures to be obtained from room-temperature crystals that are too small for conventional analyses. Since an X-ray FEL pulse ultimately destroys the sample, measurements are carried out in a serial fashion, one crystal at a time. This has led to the paradigm of serial crystallography, requiring rapid sample delivery, high frame-rate detectors and software to aggregate data into what is essentially a three-dimensional powder diffraction pattern collected one microcrystal at a time. It is now possible to record thousands of crystal diffraction patterns per second and obtain enough data for a structure in less than a minute. High-resolution diffraction can also be recorded from 2D macromolecular crystals or single fibrils. The method is especially useful for time-resolved crystallography, radiation-sensitive samples, small crystals, and studies of the dependence of structure on physical conditions and environments. The opportunities for this method have not been fully explored, and all aspects of the method are still under active development. I will outline some of these opportunities and developments.

S-04.1-3
Eliminating specimen movement in electron cryomicroscopy of biological molecules

C.J. Russo
MRC Laboratory of Molecular Biology, Cambridge, United Kingdom

Most high-resolution information loss in electron cryomicroscopy (cryoEM) stems from particle movement during imaging, which remains poorly understood. I will describe our recent efforts to develop a complete physical theory of specimen movement in cryoEM. Further, I will show that a new specimen support design can be used to reduce specimen movement to less than a 1 Ångström. I will show how this allows us to improve methods for atomic resolution structure determination, including extrapolation of the structure factors to zero dose.

ShT-04.1-2
Determination of intermediate state structures in the opening pathway of SARS-CoV-2 spike using cryo-electron microscopy

Z.F. Brotzakis, T. Lohr, M. Vendruscolo
Department of Chemistry, Cambridge University, Cambridge, United Kingdom

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of COVID19, a highly infectious disease that is severely affecting our society and welfare systems. In order to develop therapeutic interventions against this condition, one promising strategy is to target spike, the trimeric transmembrane glycoprotein that the virus uses to recognise and bind its host cells. Here we use a meta-inference cryo-electron microscopy approach to determine the opening pathway that brings spike from its inactive (closed) conformation to its active (open) one. The knowledge of the structures of the intermediate states of spike along these opening pathways enables us to identify a cryptic pocket that is not exposed in the open and closed states. We then identify compounds that bind the cryptic pocket by screening a library of repurposed drugs. These results underline the opportunities offered by the determination of the structures of the intermediate states populated during the dynamics of proteins to allow the therapeutic targeting of otherwise invisible cryptic binding sites

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

ShT-04.1-1
Abolishing "structural blindness" in metalloproteins: PioC, a NOE-less protein structure

I.B. Trindade[1], M. Invernici[2], F. Cantini[2], M. Piccioli[2], R.O. Louro[1]
[1]*Instituto de Tecnologia Química e Biológica António Xavier (ITQB-NOVA), Universidade Nova de Lisboa, Lisboa, Portugal,* [2]*Magnetic Resonance Center and Department of Chemistry, University of Florence, Florence, Italy*

Almost half of all known enzymes are metalloproteins where the metal center(s) are essential for catalysis, electron transfer, metal storage/transport, or provide stability and structural properties. NMR is a privileged method for characterizing metalloproteins providing the structure at atomic resolution, information about amplitude and time-scale of internal dynamics, hints on electronic structure and oxidation states in conditions that mimic the physiological context. However, in a significant part of the metalloproteome the metal ion is paramagnetic and, in its vicinity, a "blind sphere" exists where nuclear relaxation is enhanced and signal detection becomes a challenge. This challenge may be circumvented by substituting the paramagnetic metal with a diamagnetic analogue. Yet, this strategy often fails since it leads to unfolded proteins or the diamagnetic analogue may not mimic adequately the native paramagnetic metal. Using recent developments in pulse sequences, here we present a strategy for achieving structure determination in paramagnetic proteins [1]. PioC from *Rhodopseudomonas palustris* TIE-1 is the smallest High Potential Iron-Sulfur Protein (HiPIP) ever isolated. The paramagnetism from the [4Fe-4S] cluster affects 60% of the protein, making it the perfect example of the dual nature of paramagnetic NMR. On one side relaxation precludes signal detection, and on the other it provides unique sets of information. The structure of PioC was determined by NMR using two different sets of restraints, one containing Nuclear Overhauser Enhancements (NOEs) and another containing Paramagnetic Relaxation Enhancements (PREs). These were used independently and then combined revealing that under favorable conditions, PREs can efficiently complement and eventually replace NOEs for structural characterization [1]. 1. Trindade IB, Invernici M, Cantini F, et al (2020) PRE-driven Protein NMR Structures: an Alternative Approach in Highly Paramagnetic Systems. FEBS J https://doi.org/10.1111/febs.15615

Monday 5 July
9:00–11:00, Marmorna Hall A

Neurobiochemistry

S-03.1-1
The nicotinic receptor modulation of higher brain functions: from chemistry to cognition

J.P. Changeux
Institut Pasteur, Paris, France

A critical event in the history of both biological chemistry and neuroscience was the chemical identification of the first receptor for a neurotransmitter. It happened to be the nicotinic acetylcholine receptor. The nicotinic receptor has since then become the founding father of a broad family of brain receptors, paving the way for their identification, including that of the GABAA receptor, of glutamate receptors and subsequently G-protein linked receptors. Moreover, the nicotinic receptor appears as a typical "allosteric machine" which mediates indirect allosteric interactions between a set of topographically distinct sites – the ACh binding site, the ion channel and several allosteric modulatory sites – through a discrete and reversible conformational change of the protein. The model emerging from these studies has led to the conception and development of new pharmacological agents. The knowledge acquired with the nicotinic receptor has been further exploited to reach higher levels of brain organization, including conscious processing. The contribution of nicotinic receptors to the action of nicotine on addiction and on cognitive enhancement is explored, in particular, using a novel experimental strategy that combines nicotinic receptor genes knock-out and stereotaxic gene re-expression in the mouse. The presently available data illustrate that different brain circuits are involved in the dual use of a drug and such is the case of nicotine. Last the specific contribution of nAChRs to conscious access is evaluated in the framework of the Global Neuronal Workspace model developed by Dehaene, Kerszberg, Changeux (1998). These data and relevant theoretical models create a striking landmark in the thinking of brain sciences by causally and reciprocally linking the molecular to the cognitive levels both, within the individual brain and, between brains, in the social and cultural environment, thus suggesting new bridges between brain sciences and the humanities.

S-03.1-3
Modulation of synaptic signalling by adenosine and cannabinoids

A. Sebastiao
Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal

Cannabinoid receptors 1 (CB1R) are widely distributed in neurons and astrocytes. Exogenous activation of CB1R inhibit excitatory and inhibitory synaptic transmission and plasticity to disrupt memory. Adenosine is another ubiquitous neuromodulator of synaptic signalling. We focused on (1) understanding the role of endocannabinoids (eCBs) to modulate synaptic plasticity phenomena; (2) understanding how the two neuromodulators, cannabinoids and adenosine, control each other. We found that eCBs have a dual role upon hippocampal long-term potentiation (LTP), inhibiting weak LTP while facilitating strong LTP (Silva-Cruz et al., 2017 - Front Pharmacol. 8:921.eCollection), likely acting as a high pass filter to reduce signal to noise ratio of synaptic strengthening during memory consolidation. Exogenous activation of CB1Rs consistently inhibit LTP at the hippocampus and, probably by disrupting the fine-tune homeostatic control exerted by eCBs upon synaptic plasticity, consistently disrupt memory consolidation and affect brain connectivity between brain areas relevant for memory (Mouro et al., 2018 – J Neurochem 47:71-83). Importantly, adenosine A2A receptor (A2AR) antagonists attenuated the inhibitory action of CB1R agonists upon LTP and prevented memory consolidation impairment caused by acute (Mouro et al., 2017 – Neuropharmacology, 117:316-327) or chronic (Mouro et al., 2019 – Neuropharmacology 155:10–21) intake of CB1R agonists. A1Rs, though present in CB1R positive interneurons (Rombo et al., 2016 – Cereb Cortex 26:1081–1095), do not influence the inhibitory action of exogenous activation of CB1Rs on synaptic transmission

(Serpa et al., Eur J Pharmacol. 623:41–46) or plasticity (Silva-Cruz et al., 2017 – Front Pharmacol. 8:921.eCollection). In conclusion, interactions cannabinoid-adenosine interactions impact in synaptic plasticity and cognitive phenomena, which may prove relevant when mitigation of cognitive deficits during cannabinoid-based therapies is required.

S-03.1-2
Transcriptional regulation of acetylcholinesterase in different cell types: an enzyme in non-neuronal functions?

K. Tsim, Q. Wu, S. Mak, X.P. Kong, Y. Xia, W. Hu, R. Duan, T.T.X. Dong
Division of Life Science, The Hong Kong University of Science and Technology, Hong Kong, China

Acetylcholinesterase (AChE) hydrolyzes the neurotransmitter acetylcholine (ACh) in neurons. In vertebrate, the transcriptional regulation of AChE is happened during differentiation of various cell types, suggesting the non-neuronal roles of this enzyme. Here, we provide an example of AChE being regulated in skin epidermal cells, i.e. melanocyte and keratinocyte. Melanogenesis in skin is a process of melanin production in melanocyte, which is a physiological response to protect cells from DNA damage in exposing to sunlight. In melanocyte, G4 PRiMA-linked form is the major AChE isoform in this cell type. During melanogenesis of melanocyte, the level of AChE decreases markedly: the decrease is mediated by two transcription factors: CREB and MITF. On the other hand, in differentiating keratinocyte, the expression level of G4 AChE is upregulated. The transcription of ACHE gene in keratinocyte could be induced by exposure to sunlight, and the induction is triggered by intracellular Ca2+ mobilization. This light-induced AChE transcription is proposed to be mediated by the AP1 site located on the promoter. In skin, melanocyte and keratinocyte express all the cholinergic molecules, i.e. choline acetyltransferase (ChAT), acetylcholine receptors (AChRs) and AChE. The interplay of keratinocyte and melanocyte in regulating melanin production could be similar to a typical cholinergic synapse, i.e. forming a "skin synapse" by using ACh as a transmitter. ACh is being released, triggered by sunlight, from keratinocyte (pre-synaptic) binds to AChRs on melanocyte (post-synaptic), and ACh hydrolysis is triggered by AChE. This cholinergic signaling in skin plays roles in melanogenesis, particularly in regulating the effect of sunlight on our skin color.

ShT-03.1-1
Altered levels of cholinesterase splice variants in Parkinson's and Alzheimer's disease brains

M. Gok[1], N. Madrer[2], E. Bodur[1], D. Greenberg[2], H. Soreq[2]
[1]*Hacettepe University, Faculty of Medicine, Department of Medical Biochemistry, Ankara, Turkey,* [2]*The Silberman Institute of Life Sciences and The Edmond and Lily Safra Center for Brain Science, The Hebrew University of Jerusalem, Edmond J. Safra Campus, Jerusalem, Israel*

Alzheimer's and Parkinson's diseases (AD, PD) are the most common neurodegenerative disorders, and both are accompanied by imbalanced acetylcholine signaling. Here, we report that the cholinergic imbalance in AD and PD brains associates with post-transcriptional, but not inherited variations in the brain-expressed cholinesterase genes controlling acetylcholine hydrolysis. Briefly, we sought genetic variants and transcript levels of the butyrylcholinesterase (BChE) gene and quantified alternatively spliced acetylcholinesterase (AChE) transcripts in the Amygdala (AMG) and Substantia Nigra (SN) of PD brains and in the Superior temporalis gyrus (STG) of AD brains. Both the coding sequence BCHE-K allele (rs1803274) and the single nucleotide polymorphisms (SNPs) in the 5′UTR (rs1126680) and intron 2 (rs55781031) BCHE alleles showed similar incidence in diseased and healthy brains. However, compared with control brains, BChE levels increased in AD brains but were unchanged in the PD SN and AMG. Furthermore, quantitative qPCR tests revealed brain region-specific decline of the 'synaptic' membrane-bound AChE-S variant, the soluble stress and increases in the anxiety-related Readthrough AChE-R variant and the N-terminally extended AChE-Next variant. Specifically, the major AChE-S variant declined in the PD SN and the AD STG, but not in the PD AMG, indicating association with the loss of neurons in the affected brain regions. Inversely, the soluble AChE-R variant was elevated ($P < 0.05$) in the AD STG and the PD AMG, but not the SN, compatible with stress-related roles for this neuronal AChE splice variant in the deteriorating brains; whereas the AChE-Next variant showed elevated levels ($P < 0.05$) in the PD AMG, but not in the neurons-deprived PD SN and the AD STG. The AMG-specific AChE-R increases in AD and PD brains and the AChE-Next excess in the PD, but not AD brains may reflect disease-specific post-transcriptional modifications, compatible with patients' stress symptoms.

ShT-03.1-2
Activation of the membrane receptor RAGE by its fragment 60-76 stimulates calcium signal in neurons

A. Kamynina[1,2], N. Esteras[3], D. Koroev[1], P. Angelova[3], I. Okhrimenko[2], A. Zakharchev[2], D. Burkatovsky[2], O. Volpina[1], A. Abramov[3]
[1]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russia,* [2]*Research Center for Molecular Mechanisms of Aging and Age-Related Diseases, Moscow Institute of Physics and Technology, Dolgoprudny, Russia,* [3]*Department of Clinical and Movement Neurosciences, UCL Institute of Neurology, London, United Kingdom*

Receptor for advanced glycation end products (RAGE) is a membrane protein which plays an important role in Alzheimer's disease (AD), mediating mitochondrial damage and neuroinflammation. Previously we have found that peptide fragment 60–76 of the receptor is able to prevent memory impairments in AD mouse model, as well as to protect neuronal cells from β-amyloid toxicity. For elucidation of the protective mechanism, we investigated the effect of the synthetic RAGE peptide 60–76 on calcium signal in primary brain cells, revealing a pathway through which this signal occurs. We found that the peptide induces a peak-like calcium signal in neurons from primary co-culture of neurons and astrocytes derived from hippocampus and cortex of rat brains. The peptide-induced calcium signal could be completely blocked by pre-incubation of the co-cultures with high affinity RAGE antagonist – FPS-ZM1, while the removing of calcium from the media also blocks the signal. Using inhibitors of different neuronal receptors, we revealed that only antagonists of NMDA and AMPA/kainite receptors completely blocked the peptide-induced calcium signal, confirming that the signal is

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

associated with glutamate receptors. In addition, the peptide-induced calcium signal was prevented by depleting vesicular glutamate by incubating the co-cultures with concanamycin A, indicating that calcium signal in neurons induced by activation of RAGE is due to glutamate release. Moreover, we demonstrated that fluorescently labeled RAGE peptide does not penetrate into SH-SY5Y cells either in 15 minutes of incubation or 72 hours after it. Thus, our findings have revealed the molecular mechanism of the anti-AD protective effect of the RAGE peptide 60-76 associated with RAGE-activated glutamate release and calcium signal in neurons. Peptide synthesis was supported by Russian Science Foundation grant 20-64-46027; in cell experiments were supported by RFBR grants 20-015-00526 and 19-015-00064.

Monday 5 July
9:00–11:00, Marmorna Hall B

Imaging for life: From molecules to organisms

S-04.3-1
Super-resolution imaging of the nanoscale geometry and dynamics of the brain extracellular space in live tissue

J. Tonnesen[1,2]
[1]*Achucarro Basque Center for Neuroscience, Bilbao, Spain,* [2]*University of the Basque Country, Bilbao, Spain*

The brain extracellular space (ECS) is an extremely heterogeneous and structurally complex compartment. It facilitates fundamental functional processes, including neurotransmitter volume transmission and diffusional metabolite clearance. Yet, a detailed understanding of the ECS is lacking, and we routinely describe it in rudimentary terms. For example, the ECS volume fraction and diffusional resistance (tortuosity) are commonly stated as single representative values valid for the brain in general. This is to large extent based on a lack of experimental methods than can address it's convolved sub-micron geometrical structure in live tissue. To overcome this bottleneck, we have recently introduced super-resolution shadow imaging (SUSHI), which reconciles 3D-STED microscopy with fluorescent labeling of the interstitial fluid. It allows nanoscale imaging of the entire neuropil in the field of view, including all cellular structures and the ECS. SUSHI has allowed us to reveal the ECS sub-micron geometry and dynamics in live mouse brain slices, and relate this to the context of targeted visualized cells. In addition, we are currently establishing a computational diffusion model based on SUSHI images that allows us to make testable predictions about how ECS geometry shapes diffusion on the nanoscale, e.g. around individual synapses. Our findings supports the notion that ECS geometry by itself can impact neuronal excitability and signaling, thereby adding a new parametric variable to the active membrane properties that are conventional associated with signaling and plasticity.

S-04.3-2
Vesicle shrinking and enlargement play opposing roles in the release of exocytotic contents

L. Wu
National Institute of Neurological Disorders and Stroke, Bethesda, MD, United States of America

For several decades, two fusion modes were thought to control hormone and transmitter release essential to life: one facilitates release via fusion pore dilation and flattening (full-collapse), the other limits release by closing a narrow fusion pore (kiss-and-run). Using super-resolution STED microscopy to visualize fusion modes of dense-core vesicles in neuroendocrine cells, we found that facilitation of release was not mediated by full-collapse, but shrink-fusion, in which the W-profile generated by vesicle fusion shrank, but maintained a large non-dilating pore. We discovered that cells' physiological osmotic pressure squeezed, but not dilated the W-profile, explaining why shrink-fusion prevailed over full-collapse. Instead of kiss-and-run, enlarge-fusion, in which W-profiles grew while maintaining a narrow pore, slowed down release. Shrink- and enlarge-fusion may thus be the yin and yang of fusion modes that contribute to account for diverse hormone and transmitter release kinetics observed in secretory cells, previously interpreted within the full-collapse/kiss-and-run framework.

S-04.3-3
Imaging the length of a single vesicular SNARE protein and its role in fusion pore regulation

J. Jorgacevski[1,2], M. Kreft[1,2,3], M. Potokar[1], P. Singh[4], A. Guček[1], R. Zorec[1]
[1]*University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia,* [2]*Celica, BIOMEDICAL, Ljubljana, Slovenia,* [3]*University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia,* [4]*University of Ljubljana, Faculty of Medicine, Institute for Pathophysiology, Ljubljana, Slovenia*

Astrocytes, abundant and heterogeneous type of neuroglial cells, play many vital roles in the central nervous system (CNS). An important mode of intercellular communication with neurons and other cell types involves the exocytotic and endocytotic processes. Exocytotic transmitter release is considered to be preceded by the formation of the SNARE complex, which contains synaptobrevin2 (Sb2), a protein anchored in the vesicle membrane. Here, we used structured illumination microscopy (SIM) to study the prevalence and distribution of endogenous and exogenous Sb2 in single living astrocytes, as well as the Sb2 length and nanoarchitecture. Furthermore, we used stimulated emission depletion (STED) microscopy and SIM to examine the anatomy of single vesicles in astrocytes. Microscopy data was validated by electrophysiological approach, where the interaction of a single vesicle with the plasma membrane was monitored by high-resolution membrane capacitance measurements. Our results revealed two functional types of vesicles in astrocytes, containing distinct gliosignaling molecules, while the total number of endogenous Sb2 units per vesicle was 3–5 times smaller than the previously reported value for neurons.

ShT-04.3-1
Early fluorescent imaging of *de novo* synthesized proteins based on coiled-coils heterodimerization

M. Perfilov, A. Mishin
Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow 117997, Russia

The fluorescent proteins, which are covalently fused to target proteins, are commonly used for imaging in living cells. However, their use for imaging of de novo synthesized proteins is hindered by a slow maturation of fluorophore. Here we report a novel method of imaging the nascent proteins with transiently interacting artificial helices (K/E-coils). We have recently shown that K/E-coils despite their small size (21-28 amino acids) and low affinity are specific enough to be used for imaging intracellular proteins (Perfilov MM et al. (2020) Cell Mol Life Sci 77, 4429–4440). To achieve the labeling, one has to express in the cell simultaneously protein of interest tagged by one coil and a fluorescent protein tagged by the complementary coil. Furthermore, an excess of the pre-matured fluorescent protein is maintained in the cytoplasm, readily available to bind and therefore mark the location of the nascent protein of interest. We tested this idea by simultaneous labeling of caveolae with red fluorescent protein mCherry via K/E-coils and fused with green fluorescent protein EGFP (mCherry-K + caveolin-EGFP-E). After the appearance of the red signal in the cytoplasm, we triggered the synthesis of caveolin, using doxycycline-dependent system. Following the induction, we observed the translocation of mCherry-K to structures resembling caveolae, followed by a delayed appearance of a green signal from caveolin-EGFP-E, consistent with maturation time of EGFP. Also, our preliminary data shows the increased labeling contrast of nascent proteins when using multi-coils: for example, caveolin-EGFP-E-E-E recruits more mCherry-K than caveolin-EGFP-E, therefore increasing the structure/cytoplasm signal ratio. To conclude, we developed a simple approach for imaging of de novo synthesized proteins, bypassing the limit imposed by chromophore maturation of fluorescent proteins. Work was supported by Ministry of Science and Higher Education of Russian Federation (grant 075-15-2020-773).

ShT-04.3-2
Development of coumarin-based membrane probes for live-cell STED nanoscopy

S. Pajk[1], H. Kokot[2], B. Kokot[2], A. Pišlar[1], H. Esih[1], A. Gabrič[1], D. Urbančič[1], I. Urbančič[2], J. Štrancar[2]
[1]*University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia,* [2]*Jozef Stefan Institute, Ljubljana, Slovenia*

Plasma membranes are complex assemblies of lipids and proteins. Besides its barrier function, this dynamic structure is essential for numerous biological processes. Since the thickness of the membrane is well below diffraction limit of fluorescence microscopes, stimulated emission depletion (STED) nanoscopy is especially appropriate for studying plasma membrane. Even more so because STED is one of the fastest superresolution techniques, therefore ideal for live cell research. As most other fluorescent techniques, STED nanoscopy is strongly dependant on the performance of fluorescent labels. In our previous work we reported a small set of fluorescent membrane labels for live-cell imaging. Among them coumarin-based MePyr500 was the most promising, being highly photostable, bright and nontoxic.[1] Fast internalisation and redistribution to other membranes of the cell, however limited its use. Here we present second round of development of MePyr500 towards highly stable stains for labelling plasma membrane. This was achieved by addition of the second charge to the positively charged polar headgroup of MePyr500. New probes have eider zwiterion or two positive charges at the polar headgroup, probes SHE-2H and SHE-2N, respectively. Since no changes to the fluorescent coumarin core were made, novel probes retained advantageous characteristics of MePyr500. Contrary to the latter, SHE-2H in SHE-2N produce extremely stable labelling of plasma membrane and are especially suitable for tacking plasma membrane over longer periods (up to three days). Moreover, up to 20 μM concentrations of SHE-2H and SHE-2N did not produce any noticeable toxicity towards LA-4 cells. In addition, both probes exhibited substantial two-photon excitation and thus could be useful for two-photon microscopy of thicker samples. 1. Pajk S et al. (2019) Eur Biophys J 48, 485–490.

Monday 5 July
9:00–11:00, Gallery Hall

Membranes

S-03.3-1
Dissecting molecular membrane organization – a super-resolution fluorescence spectroscopy story

C. Eggeling[1,2,3]
[1]*Leibniz Institute of Photonic Technology (IPHT) Jena, Jena, Germany,* [2]*Institute of Applied Physics, Friedrich-Schiller-Universität Jena, Germany, Jena, Germany,* [3]*The MRC Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom, United Kingdom*

Molecular interactions are key in cellular signaling. They are usually ruled by the organization and mobility of the involved molecules. We present different fluorescence spectroscopic tools that are able to determine such organization mobility and potentially extract interaction dynamics. Specifically, the direct and non-invasive observation of the interactions in the living cell is often impeded by principle limitations of conventional far-field optical microscopes, for example with respect to limited spatio-temporal resolution. We depict how novel details of molecular membrane dynamics can be obtained by using advanced microscopy approaches such as the combination of super-resolution STED microscopy with fluorescence correlation spectroscopy (STED-FCS) or spectral detection, or ultrafast single-molecule tracking employing tools such as iSCAT or Minflux microscopy. We highlight how these tools can reveal novel aspects of membrane bioactivity such as of the existence and function of potential lipid rafts.

S-03.3-3
Lipid membranes modulate the activity of RNA through sequence-specific interactions

J.P. Saenz
B CUBE – Center for Molecular Bioengineering, TU Dresden, Dresden, Germany

RNA is a ubiquitous biomolecule that can serve as both catalyst and information carrier. Understanding how RNA activity is controlled and how it in turn regulates bioactivity is crucial for elucidating its physiological roles and potential applications in synthetic biology. Here we show that lipid membranes can act as RNA organization platforms, introducing a novel mechanism for ribo-regulation. The activity of R3C ribozyme can be modified by the presence of lipid membranes, with direct RNA-lipid interactions dependent on RNA sequence, structure and length. In particular, the presence of guanine in short RNAs is crucial for RNA-lipid interactions, while double-stranded RNAs further increase lipid-binding affinity. Lastly, by artificially modifying the R3C-substrate sequence to enhance membrane binding we unexpectedly generated a lipid-sensitive riboswitch. These findings introduce RNA-lipid interactions as a tool for developing riboswitches and novel RNA-based lipid biosensors, and bear significant implications for RNA World scenarios for the origin of life.

S-03.3-2
Membrane dynamics at cellular lipid storage sites

E. Ikonen
University of Helsinki, Helsinki, Finland

Cell organization and membrane-related functions depend on the correct compartmentalization of lipids. Our research aims at uncovering key mechanisms that govern the trafficking and storage of major lipid species in human cells and elucidating disturbances in these processes that can lead to human lipid storage diseases. Lysosomes and lipid droplets represent major cellular lipid storage sites that dynamically exchange lipids with other membrane compartments, with the co-operation of lipid transfer proteins and membrane trafficking. We are investigating machineries that are responsible for redistributing lipoprotein-derived cholesterol from endo-lysosomal compartments to other membranes and from the plasma membrane to the endoplasmic reticulum and lipid droplets for storage. We are also studying how lipid droplets are generated from the endoplasmic reticulum, for instance by re-localizing lipid storage sites in ER subdomains and by controlling specific proteins implicated in lipid droplet formation, regulation of ER morphology or lipid delivery. We are taking advantage of a system recently developed by us for rapid inducible degradation of endogenous proteins (Li et al., Nat Methods 16: 866-869, 2019), in combination with live cell imaging, correlative light electron microscopy, and lipid analyses. This strategy is powerful for studying rapidly adapting processes, such as lipid transport and metabolism, that typically harness compensatory routes.

ShT-03.3-2
Advanced microspectroscopy enlightens membrane organisation in activating T cells

I. Urbančič[1,2], E. Jenkins[2], A.M. Santos[2], E. Sezgin[2], C. Eggeling[2]
[1]*Jozef Stefan Institute, Ljubljana, Slovenia,* [2]*MRC Weatherall Institute of Molecular Medicine, Oxford, United Kingdom*

The quest for understanding of numerous vital membrane-associated cellular processes, such as signalling, has largely focussed on the spatiotemporal orchestration and reorganisation of the identified key proteins, including their binding and aggregation. Despite strong indications of the involvement of membrane lipid heterogeneities, historically often termed lipid rafts, their roles in many processes remain controversial and mechanisms elusive. To disentangle these intricate lipid-protein interactions on the example of the activation of T lymphocytes, we here investigate how externally imposed variations in mobility of the key membrane proteins – in particular the T-cell receptor (TCR), its main kinase Lck, and phosphatase CD45 – affect the properties of their lipid surrounding and hence their interactions. To this end, we examine their local lipid order and co-localisation in the most relevant passive model membranes (i.e. cell-derived giant plasma-membrane vesicles, GPMVs), and in live Jurkat T cells using spectral imaging with a polarity-sensitive membrane probe. We find that upon aggregation and partial immobilisation, the TCR changes its preference towards more ordered lipid environments, which can in turn passively recruit Lck. We observe similar aggregation-induced local membrane ordering and recruitment of Lck also by CD45, as well as by a membrane protein of antigen-presenting cells, CD86, which is not supposed to interact with Lck directly. This suggests that the cellular membrane is poised to modulate the frequency of protein encounters according to their aggregation state and alterations of their mobility, e.g. upon ligand binding, offering further mechanistic insight into the involvement of lipid-mediated interactions in membrane-hosted signalling events.

ShT-03.3-1
The unique story of *Pleurotus* aegerolysins: from specific lipid binding to potential biopesticides

A. Panevska[1], M. Mravinec[1], A. Trčak[1], M. Jotič[1], J. Razinger[2], Š. Modic[2], Z. Arsov[3], K. Sepčić[1]
[1]*Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia,* [2]*Agricultural Institute of Slovenia, Ljubljana, Slovenia, Ljubljana, Slovenia,* [3]*Jožef Stefan Institute, Ljubljana, Slovenia, Ljubljana, Slovenia*

Aegerolysins from the fungal genus *Pleurotus*, namely ostreolysin A (OlyA), pleurotolysin A2 (PlyA2) and erylysin A (EryA), were recently found to interact strongly with lipid vesicles that contained ceramide phosphoethanolamine (CPE), the major sphingolipid in invertebrate cell membranes and an analog of sphingomyelin, which dominates in vertebrates. Furthermore, OlyA6, PlyA2 and EryA were shown to combine with pleurotolysin B (PlyB) or erylysin (EryB), 59-kDa protein partners with a MACPF domain, to form multimeric bi-component, A2B-type, transmembrane cytolytic complexes. We provide insights into the molecular mechanisms of the interaction of *Pleurotus* aegerolysins with membranes containing CPE as well as their specificity to this sphingolipid. Moreover, we characterized their pore

formation in the presence of PlyB or EryB. Interestingly, spectral FRET analysis showed that monomers of fluorescently labeled OlyA pack closely together only on CPE-containing membranes. These aegerolysins bind to insect cells and artificial lipid membranes at physiologically relevant CPE concentrations. Moreover, aegerolysins permeabilize these membranes when combined with PlyB. OlyA/PlyB, PlyA2/PlyB and EryA/PlyB complexes have shown a selective toxic effect on Colorado potato beetle (CPB) larvae and Western corn rootworm (WCR), and not to other tested insect pests. Using brush border membrane vesicles (BBMVs) from WCR and CPB larvae, we evaluated the presence of other potential aegerolysin receptors. The current study highlights the unique binding of aegerolysins to CPE-containing membranes and their ability to form transmembrane pores with MACPF protein partner, thus suggesting their possible use as biopesticides for controlling selected insects.

Monday 5 July
9:00–11:00, Povodni mož Hall

Receptor–ligand interactions

S-03.2-2
Cyclotides: bioactive peptides from plants with applications in drug design and agriculture

S. Troeira Henriques[1], H. Peacock[2], A. Benfield[1], C. Wang[2], **D. Craik**[2]
[1]*Queensland University of Technology, Brisbane, Australia,* [2]*The University of Queensland, Brisbane, Australia*

Naturally occurring cyclic peptides offer great potential as leads for drug design. One class of cyclic peptides known as cyclotides [1] are topologically unique in that they have a head-to-tail cyclised peptide backbone and a cystine knotted arrangement of disulfide bonds. This makes them exceptionally stable to chemical, thermal or enzymatic treatments and, indeed, they are amongst nature's most stable proteins. Their stability and compact structure make them an attractive protein framework onto which bioactive peptide epitopes can be grafted to stabilise them. More than 30 examples have now been published where biologically active epitopes have been grafted onto cyclic peptide frameworks to produce lead molecules with potential in the treatment of cancer, cardiovascular disease, infectious disease, autoimmune disease (multiple sclerosis) and pain. Solid-phase peptide chemistry has been the primary method used to make cyclotides for laboratory evaluations of structure and function to underpin such drug design applications. The biological targets of naturally occurring cyclotides are generally thought to be membranes, although some studies have implicated GPCRs as targets. Mirror-image peptides are often used to discriminate between chirality driven binding (e.g. to protein receptors) and membrane-binding, generally assumed to be largely achiral. In the current study we synthesized all-L and all-D versions of key cyclotides as well as L and D lipids to probe membrane binding [2]. We show that the intrinsic chirality of the lipid headgroups can modulate cyclotide binding. Enantiomers of cyclotides have proven to be valuable synthetic tools for studying chirality-specific membrane binding interactions of cyclotides and have proved valuable for racemic crystallography to probe structures. References: 1. De Veer SJ et al. (2019) Chemical Reviews 119, 12375-12421. 2. Henriques ST et al (2019) JACS 141, 20460-20469.

S-03.2-1
How animal toxins contribute to explore the role of ASIC channels in pain

S. Diochot[1], C. Verkest[1], M. Salinas[1], E. Piquet[2], V. Friend[1], M. Dauvois[1], A. Alloui[3], A. Eschalier[3], G. Mourier[4], P. Kessler[4], M. Lanteri-Minet[5], D. Servent[4], E. Deval[1], E. Lingueglia[1], A. Baron[1]
[1]*Université Côte d'Azur, CNRS, Institut de Pharmacologie Moléculaire et Cellulaire, Valbonne, France,* [2]*Institut de Pharmacologie Moléculaire et Cellulaire du CNRS, Valbonne, France,* [3]*Université d'Auvergne, INSERM, NEURO-DOL, Clermond-Ferrand, France,* [4]*Université Paris Saclay, CEA, Service d'Ingénierie Moléculaire pour la Santé, Gif sur Yvette, France,* [5]*CHU-Nice, Hopital Cimiez, Département d'évaluation et de traitement de la douleur, Nice, France*

Acid-Sensing Ion Channels (ASICs) form a class of neuronal excitatory cation channels gated by extracellular protons. We have isolated several peptide toxins from sea anemone, spider and snake venoms which allowed to successfully explore the role of these channels in physiopathological processes such as pain. Mambalgin (Mamb-1), was used to reveal the role of subtypes of ASIC channels in pain, including inflammatory and neuropathic pain. It acts as a potent and specific inhibitor of ASIC1a-containing channels, which are the major channel subtypes expressed in the central nervous system, and of peripheral ASIC1b-containing channels that are specific of sensory neurons. Mamb-1 shows potent analgesic effects on thermal and mechanical nociception upon different route of injection in experimental animal models of inflammatory and neuropathic pain. The effects can be as strong as morphine but involve opioid-independent pathways, without producing apparent toxicity. Mamb-1 acts as a gating-modifier that binds to the extracellular domain of the channel to block ASICs by a pH sensor-trapping mechanism. We recently used this peptide to explore the involvement of the ASIC1 in a rodent model of acute and chronic migraine induced by systemic injections of isosorbide dinitrate (ISDN), a therapeutically used nitric oxide donor known to produce headache as side-effect in humans. We studied the effects of systemic injections of Mamb-1 on cutaneous allodynia, by testing cephalic and extra-cephalic mechanical sensitivity. A single ISDN injection induced migraine-like acute symptoms, whereas one daily ISDN injections during four days induced chronic effects. Intravenous injection of Mamb-1 or amiloride, inhibited both the acute and the chronic mechanical allodynia. These data using a venom-derived, peptide specific inhibitor support the involvement of central and peripheral ASIC1-containing channels in pain and highlight the therapeutic potential of blocking these channels.

S-03.2-3
Role of neuroendocrine signalling in early development of bivalve molluscs: interference by environmental stress

L. Canesi[1], A. Miglioli[1,*], T. Balbi[1,*], R. Dumollard[1,*]
[1]*University of Genoa, Genoa, Italy*

Neuroendocrine mechanisms play a vital role in endocrine homeostasis of invertebrates Molecular components of the

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

neuroendocrine system which share a similar molecular basis with their vertebrate counterparts have been characterized in different invertebrates, including ecologically important groups, such as bivalve molluscs. Increasing evidence supports pleiotropic roles for serotonergic, dopaminergic and adrenergic signaling in bivalve physiology. In different bivalve species, monoamine release by specific neurons modulate gill ciliary beating, which generates the currents that provide for respiration and feeding, as well as muscle contraction, including the ''catch'' state of adductor and byssus retractor muscles; moreover, these neurotransmitters are involved in immune responses. In particular, neuroendocrine mechanisms are known to play a vital role in reproduction and development, from oocyte maturation, sperm motility and spawning, as well as during early development. Available information on monoamine signaling in bivalves is summarized. Moreover, data obtained in neuroendocrine signaling during early larval development in model bivalve species, oysters and mussels are reported. The results indicate that exposure to chemical stressors, from ocean acidification to exposure to suspected endocrine disruptors, reveal key aspects of neuroendocrine signaling in early neurodevelopment and biomineralization. *The authors marked with an asterisk equally contributed to the work.

ShT-03.2-2
Translocation of protein antibiotics: How do Colicins B and D penetrate into *E. coli*?

R. Cohen Khait, P. Pham, M. Webby, N. Huosden, E. Lowe, S. Mohammed, D. Staunton, C. Kleanthous
University of Oxford, Oxford, United Kingdom

Bacteriocins are peptides or proteins with antimicrobial activity that contribute to the stability and dynamics of microbial communities. In an era of multidrug resistant bacteria, bacteriocins may have utility as natural antibiotic alternatives. However, little is known about the molecular mechanisms of their cellular import. Here, we follow the dynamic translocation process of two colicins, ColB, a pore-forming toxin, and ColD, a nuclease, which both parasitize the same *Escherichia coli* siderophore receptor (FepA) yet deliver toxic domains to different cellular compartments. Our multidisciplinary study combines photoactivatable crosslinking LC-MS/MS, Rosetta modeling and live cell fluorescence microscopy to understand how these potent protein antibiotics cross one or two membranes of the bacterium. Our structural studies imply that conformational changes are induced upon binding, which consequently allow the active colicin translocation into the cell. Using different colicin fragments we have developed fluorescent biomarkers of *E. coli* subcellular compartments including the outer membrane, periplasmic space, inner membrane and the cytoplasm. Furthermore, we were able to utilize colicin based constructs for complex molecule (ssDNA) delivery into different cellular compartments, emphasizing the robust nature of the active translocation process.

ShT-03.2-1
Functioning mechanisms of bitopic membrane proteins revealed by structural-dynamic studies of intra- and intermolecular interactions of their transmembrane domains

E. Bocharov[1,2], P. Volynsky[1,2], V. Britikov[3], A. Urban[1,2], Y. Bershacky[1,2], D. Lesovoy[2], E. Britikova[3], K. Pavlov[4], R. Efremov[1,2], S. Usanov[3], A. Arseniev[1,2]
[1]*Moscow Institute of Physics and Technology (State University), Dolgoprudny, Moscow Region, Russia,* [2]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russia,* [3]*Institute of Bioorganic Chemistry NASB, Minsk, Belarus,* [4]*Federal Clinical Center of Physical-Chemical Medicine of FMBA, Moscow, Russia*

Bitopic membrane proteins, having only one transmembrane (TM) segment, are directly involved in the development and maintenance of homeostasis of human body tissues, providing a variety of interactions on the cell membrane. Receptor tyrosine kinases (RTK) serve as convenient models of bitopic proteins to show how ligand-induced conformational rearrangements of extracellular and TM domains lead to allosteric activation of cytoplasmic domains during signal transmission through the cell membrane. Bitopic cytochrome b5 of membrane-associated P450 dependent monooxygenase system takes part in biosynthesis and metabolism of diverse physiologically active substances. Dysregulated functioning of these bitopic proteins play significant roles in promotion of number of human diseases, and their inhibitors have been among the most successful examples of targeted therapies to date. At the same time, Alzheimer's disease is an age-related pathology associated with the accumulation of β-amyloid peptides, – products of enzymatic cleavage by membrane sites of bitopic amyloid precursor protein (APP). Over several years, we determined diverse conformations and interactions of TM segments of microsomal cytochrome b5, APP and RTK-related proteins in membrane-like media. In agreement to recent biophysical and biochemical data, we shown that the functioning of these bitopic proteins is determined not only by specific protein-protein and protein-lipid interactions, but also by the physical state of the lipid environment, as one of the main components of self-consistent biological membrane system. This allowed us to propose new principles that underlie signal transmission through cell membrane and substrate recognition by membrane proteins, as well as the mechanisms of action of a number of TM pathogenic mutations in TM domains. Studies of bipopic cytochromes were funded by RFBR and BRFBR, projects 20-54-00041 and X20P-159. APP and RTK studies were supported by RSF, project 20-64-46027.

Monday 5 July
16:00–18:00, Kocka Hall

Bionanotechnology

S-05.2-3
Design and engineering nanopores for single-protein analysis and sequencing

G. Maglia
University of Groningen, Groningen, Netherlands

Biological nanopores are proteins that form nanoscale water conduits on biological membranes. In nanotechnology they can be used as nanoreactors to monitor chemical reactions, or as single-molecule integrated sensors in low-cost devices for DNA sequencing. Protein analysis, however, is challenging because of the heterogeneous structure and non-uniform charge of proteins, which complicates their electrophoretic capture and translocation by nanopores. Here, I will show how we can design new nanopores to control the unfolded translocation of proteins across a nanopore, and how to engineer nanopores to capture and characterize single proteins or peptides. These nanopores have application in the identification of rare protein isoforms, and will provide new tools for single-molecule proteomic analysis.

S-05.2-2
Designing membrane proteins using DNA: from ion channels to lipid scramblases

U. Keyser
Cavendish Laboratory, University of Cambridge, Cambridge, United Kingdom

DNA nanotechnology is transformative for experiments that require molecular control over the shape of nanometer-sized objects. One exciting application of DNA assembly is the design of functional structures that interact with lipid membranes and may provide building blocks for synthetic cells. One especially exciting example are DNA-origami nanopores [1] that can be integrated in lipide membranes and are used for a wide range of sensing applications. Beyond the use of nanopore sensors for molecules, DNA (origami) nanopores can be transformed into ion channels via hydrophobic modifications. Our artificial DNA systems span orders of magnitude in molecular weight from single helices to large porins [2,3] and show voltage-activated characteristics akin to ion channel gating. A combination of experiments and molecular simulations show that DNA ion channels act as enzymes that allow mixing of lipids between different leaflets of bilayers [4]. In an outlook, the incorporation of PNIPAM into DNA origami enables the temperature activated motion of DNA based structures [5] offering an attractive route towards molecular machines for controlling lipid membranes. [1] N. A. W. Bell and U. F. Keyser. Nanopores formed by DNA origami: a review. FEBS Letters, 588 (29):3564-3570, 2014 [2] K. Göpfrich, et al. Ion Channels Made from a Single Membrane-Spanning DNA Duplex. Nano Letters, 16(7):4665-4669, 2016. [3] K. Göpfrich, et al. Large-Conductance Transmembrane Porin Made from DNA Origami. ACS nano, 10 (9):8207-8214, 2016. [4] A. Ohmann, et al. A synthetic enzyme built from DNA flips 107 lipids per second in biological membranes. Nature Communications, 9:2426, 2018. [5] V. Turek, et al. Thermo-responsive Actuation of a DNA Origami Flexor. Adv. Funct. Mater., 28(25):1706410, 2018

S-05.2-1
Towards the buildup of magic bullet: multifunctional nanostructures for cancer diagnostics and treatment

V. Shipunova, V. Shipunova
Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russia

An intensively developing direction of modern biomedicine, theranostics, is aimed at the development of multifunctional agents with diagnostic and therapeutic functions. Organic and inorganic nano-objects are an excellent platform for creating such agents since they have non-standard properties that are not presented in macroscopic samples or individual atoms. Such features include a high fraction of surface atoms and the presence of surface-active chemical groups, the possibility of encapsulation of low-soluble compounds inside the structure, the heating under an external electromagnetic field, and the option of targeted delivery only to tumor cells with the realization of the concept of a "magic bullet," an ideal therapeutic agent, implies the selective targeting only a disease-causing object. Here we describe a series of works devoted to the design, modification and *in vivo* translation of nanoparticles of various nature, both organic and inorganic (magnetic, gold, silver, polymer-based, protein-based, silica-shelled, hybrid smart structures – biorobots, etc. (Zelepukin IV et al. (2019) Nanoscale 11 (4), 1636-1646; Shipunova VO et al. (2018) ACS applied materials & interfaces 10 (20), 17437-17447; Belova MM et al., (2019) Acta Naturae 11 (2 (41)). The developed supramolecular structures were successfully modified with different anti-cancer scaffold proteins (DARPins, affibody), and different methods of their successful administration for cancer theranostics were developed, including those affecting tumor-targeting efficiency and blood circulation half-lives of nanoagents. This study is a step towards the creation of a new generation of theranostic agents, which are capable of affecting only certain cell types under specific conditions and act as a therapeutic agent when necessary. The research was supported in part by the Russian Science Foundation (No. 17-74-20146, nanoparticle synthesis) and Russian Foundation for Basic Research (No. 20-34-70136, cell culture).

ShT-05.2-1
In situ rewiring of membrane receptors and signal transduction by light

M.F. Sánchez[1], S. Els-Heindl[2], A.G. Beck-Sickinger[2], R. Wieneke[1], R. Tampé[1]
[1]*Goethe University Frankfurt, Frankfurt am Main, Germany,* [2]*Leipzig University, Leipzig, Germany*

Cell-cell communication and signal transduction rely on the assembly of receptor-ligand complexes at the plasma membrane. The spatiotemporal receptor organization plays a pivotal role in evoking cellular responses. Nevertheless, the mechanism for cluster formation and how their localization within the plasma membrane influence cell responses is not comprehensively understood. Thus, traceless modifications with high spatiotemporal control that allow fine tuning of protein networks in a fast, reversible, and non-invasive manner are required. Here, by establishing a photo-instructive matrix with ultra-small lock-and-key interaction pairs, we rewired *in situ* the lateral membrane organization of the hormone neuropeptide Y_2 receptor in living cells by light. Within seconds,

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

receptor clustering can be modulated in size, location, and density. By *in situ* confinement, changes in cellular morphology, motility, and calcium signaling revealed a ligand-independent receptor activation. Overall, the matrix as well as the employed nanotool represents a versatile instrument for tracking of cellular processes and depict now the possibility to elucidate unexplored mechanisms in cell signaling and mechanotransduction.

ShT-05.2-2
Genetic code expansion: Enhancing biological nanopores with novel chemistry

A. Crnković, G. Šolinc, M. Srnko, M. Podobnik, G. Anderluh
National Institute of Chemistry, Ljubljana, Slovenia

Nanopore technology allows the detection of molecules at the femtomolar level. However, the use of biological pores may be limited by the finite number of available side-chain chemistries. Although at the forefront of synthetic biology, genetic code expansion (GCE) and co-translational insertion of non-canonical amino acids (ncAAs) remains relatively underutilized in biotechnology. To enable site-specific insertion of ncAAs, GCE relies on the process of nonsense suppression, in which a suppressor tRNA delivers the ncAA to the ribosome. Because the acylated suppressor tRNA must compete with a release factor for binding to the stop codons, multiple ncAA insertions can result in a comparatively low yield of target proteins. Homo-oligomeric actinoporins may be desirable nanopore targets for insertion of multiple ncAAs because they are expressed as monomers, and pore formation occurs *in vitro*. Therefore, incorporation of a single ncAA *in vivo* can yield pores with multiple ncAAs. Here we show that GCE can be readily used to incorporate 4-propargyloxy-L-phenylalanine (pPpa), an ncAA with an alkyne handle, into actinoporin monomers in *E. coli*. Because alkyne-bearing ncAAs may be suitable for sensing of azido group-containing compounds, we targeted positions in the α-helical transmembrane region of actinoporin whose side chains extend into the lumen of the pore. All four selected residues facing the lumen could be converted to pPpa; two of them also allowed the formation of functional pores carrying pPpa. Actinoporin variants generated to reduce noise in single-channel measurements were also amenable to reassignment. Importantly, incorporation of pPpa with competitive yields required minimal adjustments to the expression and purification processes. The facile incorporation of pPpa into nanopores could be very useful for the detection of common environmental pollutants, such as heavy metal azides.

Monday 5 July
16:00–18:00, Marmorna Hall A

Designed regulatory circuits and genome editing

S-05.3-2
Toward a world of ElectroGenetics

M. Fussenegger
ETH D-BSSE, Basel, Switzerland

With the advent of the internet of things, interconnected electronic devices are starting to dominate our daily lives and are reaching the control complexity of living systems, and yet work radically different: While human metabolism uses ion gradients across insulated membranes to simultaneously process slow analog chemical reactions and communicate information in multicellular systems via soluble or volatile molecular signals, electronic devices use multicore central processing units to control the flow of electrons through insulated metal wires with gigahertz frequency and communicate information across networks via wired or wireless connections. While analog biological systems and digital electronic devices efficiently work in their respective worlds there are no efficient interfaces between electronics and genetics. We will report our first attempts to design direct electro-genetic interfaces and our progress toward a world of ElectroGenetics and the internet of the body.

S-05.3-3
Unlocking CRISPR technology for precise and efficient genome editing

A. Cereseto
Department CIBIO - University of Trento, Trento, Italy

CRISPR technology is currently considered the state-of-the-art tool for genome editing in any field of life sciences. It is widely adopted in research and it is under development for a variety of uses ranging from the agro-alimentary to bio-medical sectors. The power of CRISPR technology is evident with the quick acceleration of its use in experimental clinic for the treatment of genetic diseases which generated early encouraging results. Yet, CRISPR technology is still under intense development towards enhanced target specificity and deliverability. We have recently developed a yeast-based evolution approach to generate more precise Cas9 variants; new data on the exploitation of this platform to expand the CRISPR toolbox will be presented.

S-05.3-1
Next-level riboswitch development – implementation of Capture-SELEX allows fast and easy identification of new synthetic riboswitches

B. Suess
TU Darmstadt, Darmstadt, Germany

RNA utilises many different mechanisms to control gene expression. Among the regulatory elements that respond to external stimuli, riboswitches are a prominent and elegant example. Riboswitches consist solely of RNA. They are characterised by binding of a small molecule ligand to the so-called aptamer domain, which results in a conformational change of the downstream expression platform that determines the output of the system. The modular organisation of riboswitches has resulted in the adoption of engineered riboswitches as artificial genetic control devices and a number of exciting proof-of-concept studies have been published. It should be noted that the majority of these studies were performed with the theophylline aptamer. Overall, there is no shortage of small molecule-binding aptamers. However, only a small fraction of them are suitable for RNA engineering since a classical SELEX protocol selects only for high-affinity binding but not for conformational switching. We now implemented RNA Capture-SELEX in our riboswitch developmental pipeline to integrate the required selection for high-affinity binding with the equally necessary RNA conformational switching. We consider this integrated approach a breakthrough

in riboswitch development, as suitable sensor domains for RNA-based devices can now be developed quickly and easily against any ligand of choice.

ShT-05.3-2
Timing the cell: towards a synthetic bio-clock

G. Lai
Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle upon Tyne, United Kingdom

A bio-clock can be described as a designer regulatory circuit that keeps track of time in biological systems and allows temporal control of gene expression in living organisms. Implications for synthetic biology, both in industry and in the clinic, would be tremendous. With a bio-clock, bacteria could be engineered to produce therapeutic molecules directly at the site of delivery, and at the desired time. In industry and bio-refineries, production pipelines could be streamlined and timed to match with more efficient workflows. We could even envisage microorganisms that are programmed to perform a complex series of tasks in a desired order, just like computers run algorithms, thus paving the way to developments in bio-computing. Despite many efforts, however, a bio-clock is still missing from the synthetic biology toolbox. Here, we report how we have hijacked the natural circadian system of photosynthetic cyanobacteria to engineer a bio-clock in *E. coli*. We have engineered a way to synchronize the clock using a chemical inducer rather than the light input, and after synchronization the designer circuit behaves remarkably similarly to the native system. Not only does it oscillate with the correct periodicity of 24 hours, it also exhibits a near-perfect amplitude of oscillations. Thus, we think we will soon be able to add our synthetic bio-clock to the toolkit available to synthetic biologists.

ShT-05.3-1
Modulation of long non-coding RNA Gas5 splicing using CRISPR/Cas9 editing of small nucleolar RNA genes

A. Matveeva[1,2], D. Vinogradov[1,2], E. Zhuravlev[1], D. Semenov[1], V. Vlassov[1], G. Stepanov[1]
[1]*Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences (ICBFM SB RAS), Novosibirsk, Russia,* [2]*Novosibirsk State University (NSU), Novosibirsk, Russia*

Small nucleolar RNAs (snoRNAs), specifically, box-C/D-snoRNAs, present an interesting and promising target for the regulation of multiple cell processes, such as ribosomal RNA maturation and splicing modulation in eukaryotic cells. These short regulatory RNAs are capable of participating in 2′-O-methylation of ribosomal RNA nucleotides due to the specific conserved elements in their structure. Current research is devoted to the CRISPR/Cas9-mediated functional analysis of box-C/D-snoRNAs encoded in *Gas5* (Growth Arrest Specific 5) gene introns. 293FT-derived single cell clones were obtained with single and several simultaneous point mutations in individual box-C/D-snoRNAs, which led to the decrease of target RNA levels as well as level of 2′-O-methylation of native target rRNA nucleotides. Multiple knockouts were performed through the simultaneous transfection of two constructs, thus creating large deletions in *Gas5* gene. Our results demonstrate the possibility of obtaining human cells with a suppressed level of several snoRNAs. The transcriptome of modified cell lines was examined using standard RNA-Seq of polyA fraction and small RNAs. The level of the host Gas5 transcript was shown to be decreased, in some cases the complete suppression was demonstrated. Further analysis of the host gene RNA structure demonstrated partial alterations in mature Gas5 RNA, namely, exon skipping and formation of novel isoforms. Mutation revealed in *SNORD75* and *SNORD81* led to changes in the sequence of the METTL3/METTL14-binding sites resulting in the deletion of the consensus motifs causing the formation of the alternative splicing products. Taken together, our data suggest that Gas5 splicing is m6A-dependent. (Previously published in: Filippova JA et al. (2019) Front. Pharmacol. 10, [01246].) This work was supported by the RFBR grant № 18-29-07073 and partially (in method development) by State Budget Program (0245-2019-0001).

Monday 5 July
16:00–18:00, Marmorna Hall B

Structures of nucleic acids

S-04.4-1
Conformational dynamics of unusual DNA structures: insights to G quadruplex folding pathways by real-time NMR-spectroscopy

H. Schwalbe
Goethe University, Frankfurt am Main, Germany

Guanine-rich DNA sequences can form four-stranded G-quadruplex structures. G-quadruplexes are especially enriched within oncogene promoter regions, where they regulate transcription and replication. The complex conformational landscape of DNA G-quadruplexes leads to a pronounced structural polymorphism that enables to target conformational specifics. The interconversion and coexistence of different conformations is a crucial feature for the functionality of G-quadruplex. The dynamic behaviour within a native ensemble of conformations are yet poorly understood. Herein, we present a combined strategy to investigate (re-)folding dynamics of different G-quadruplex topologies by transiently stabilizing single conformations. We prepare unfolded states by blocking hydrogen bonding with photo-labile protection groups. Upon irradiation, the block can be released and the kinetics of re-equilibration to the native conformational equilibrium can be determined by time-resolved NMR. With this approach, we are able to delineate individual steps that are involved in the folding and refolding dynamics of G-quadruplex ensembles found in the promoter region of the MYC-oncogene. References 1. J.T. Grün, A. Blümler, I. Burkhart, J. Wirmer-Bartoschek, A. Heckel, H. Schwalbe (2021) Unravelling the kinetics of spare-tire DNA G-quadruplex folding. J. Am. Chem. Soc., in press. 10.1021/jacs.1c01089 2. D. Müller, I. Bessi, C. Richter, H. Schwalbe (2021) The folding landscapes of human telomeric RNA and DNA G-quadruplexes are markedly different. Angew. Chem., in press. https://doi.org/10.1002/anie.202100280 3. J.T. Grün, C. Hennecker, D.-P. Klötzner, R.W. Harkness, I. Bessi, A. Heckel, A.K. Mittermaier, H. Schwalbe Conformational Dynamics of Strand Register Shifts in DNA G-Quadruplexes. JACS 2020, 142 (1), 264-273.

S-04.4-2
Quadruplexes are everywhere!

J. Mergny
Laboratoire d'Optique et Biosciences, Ecole Polytechnique, Palaiseau, France

G-quadruplexes and i-DNA are unusual nucleic acid structures which can find applications in biology, medicine, as well as biotech- and nano-technologies. We are developing tools to understand their folding and polymorphism and a new algorithm for prediction of G4 propensity, G4-Hunter. We also became interested in quadruplex-prone regions conserved in the genomes of a number of Archaea, pathogens such as bacteria, helminths, viruses (HIV, HCV, Ebola, SARS-CoV-2...) as and in "G4-poor" model organism such as *Dictyostelium discoideum* or *Plasmodium falciparum* to confirm the importance of G-quadruplexes in biology. In addition, we are investigating new families of G4 ligands (G4L), either as fluorescent light-up probes or as anti-viral or anti-parasitic drugs. Regarding cancer, our team developed various series of G4 ligands selective for G-quadruplexes. Using a wide set of analysis methods such as transcriptomic and proteomic analyses as well as imaging and biochemical studies, we showed that one of these compounds, the triarylpyridine 20A, elicits a significant activation of biological pathways related to DNA damage response (DDR), autophagy, lysosomal function and cell growth arrest. These observations prompted us to investigate the relationship between these processes and G-quadruplex ligands.

S-04.4-3
G-quadruplexes recruit transcription factors to promote gene expression in human cells and viruses

S. Richter, S. Lago, I. Frasson
Department of Molecular Medicine, Padua, Italy

Cell identity is maintained by activation of cell-specific gene programs, regulated by epigenetic marks, transcription factors and chromatin organization. Similarly, virus replication in infected cells is supported by expression of virus-specific genes. DNA G-quadruplexes (G4s) are non-canonical nucleic acid structures that may form in the presence of repeated stretches of guanines. G4s have been reported to be associated with either increased or decreased transcriptional activity in cells. By G4 ChIP-seq/RNA-seq analysis in a liposarcoma cell line we observed that G4s in promoters are invariably associated with high transcription levels in open chromatin. Comparing G4 presence, location and transcript levels in liposarcoma cells to available data on keratinocytes, we showed that the same promoter sequences of the same genes in the two cell lines had different G4-folding state: high transcript levels consistently associated with G4-folding. Transcription factors AP-1 and SP1, whose binding sites were the most significantly represented in G4-folded sequences, coimmunoprecipitated with their G4-folded promoters. Similarly, we found that G4s in the genome of the herpes simplex virus 1 recruited ICP4, the major viral transcription factor. Our data indicate that G4s and their associated transcription factors cooperate to determine cell- and virus-specific transcriptional programs, making G4s to strongly emerge as new epigenetic regulators of the transcription machinery.

ShT-04.4-1
Pyrene modified analogues of thrombin binding aptamer exhibit unique structural features

M. Kovačič[1,2], P. Podbevšek[1,2], H. Tateishi-Karimata[3], S. Takahashi[3], N. Sugimoto[3,4], J. Plavec[1,2,5]
[1]Slovenian NMR Centre, National Institute of Chemistry, Hajdrihova ulica 19, Ljubljana, Slovenia, [2]EN-FIST Centre of Excellence, Trg Osvobodilne fronte 13, Ljubljana, Slovenia, [3]Frontier Institute for Biomolecular Engineering Research (FIBER), Konan University, 7-1-20 Minatojima-minamimachi, Kobe 650-0047, Japan, [4]Graduate School of Frontiers of Innovative Research in Science and Technology (FIRST), Konan University, 7-1-20 Minatojima-minamimachi, Chuo-ku, Kobe 650-0047, Japan, [5]University of Ljubljana, Faculty of Chemistry and Chemical Technology, Vecna pot 113, Ljubljana, Slovenia

G-quadruplexes are secondary DNA structures formed by guanine-rich nucleic acid strands and play a role in various cellular functions. As independent aptamer molecules, G-quadruplexes are able to bind specific target proteins, which makes them potential drug candidates for the treatment of numerous diseases, most notably cancer. Small polyaromatic compounds can interact with solvent accessible DNA nitrogen bases through aromatic stacking, which leads to the stabilization of G-quadruplexes and subsequently regulation of their functions. Alternatively, various chemical moieties and nucleotide analogs can be incorporated into G-quadruplex structure to fine-tune desirable properties of aptamers including structural stability, resistance against nuclease degradation and binding of target proteins. Thrombin-binding aptamer (TBA) is a 15-mer 5′-d[GGTTGGTGTGGTTGG]-3′ DNA oligonucleotide, that folds into a chair-type G-quadruplex capable of binding and inhibiting thrombin protease in the presence of sodium and potassium cations [1,2]. In our study, TBA G-quadruplex served as a well-defined starting model which we modified by substitutions of individual thymines with fluorescent U^{py} (5-(pyrene-1-yl-ethynyl)-dUMP) nucleotides [3]. We showed that in the presence of potassium ions the individual replacements of T4, T9 and T13 with U^{py} nucleotides stabilize G-quadruplex and greatly increase its resistance against nuclease degradation while retaining the TBA fold. In the case of T9 substitution, a dynamic equilibrium between the unimolecular and bimolecular G-quadruplex structure was observed, the latter being comprised of two distinct G-quadruplex units. We believe that our approach could potentially be used for the optimization of various therapeutically important G-quadruplex based aptamers. Previously published in: 1. Bock L et al. (1992) Nature 355, 564-566. 2. Pica A et al. (2013) FEBS J 280, 6581–6588. 3. Kovačič M et al. (2020) Nucleic Acids Res, gkaa113.

ShT-04.4-2
Cryo-EM reconstruction of the *Candida albicans* 80S ribosome

Y. Zgadzay[1,2], O. Kolosova[1], A. Stetsenko[3], K. Usachev[2], S. Validov[2], A. Guskov[3,4], M. Yusupov[1]
[1]*IGBMC, Illkirch-Graffenstaden, France,* [2]*Laboratory of Structural Biology, Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia,* [3]*University of Groningen, Groningen, Netherlands,* [4]*Moscow institute of physics and technology, Dolgoprudny, Moscow Region, Russia*

Candida albicans is considered as a commensal organism, but it can become pathogenic in immunocompromised individuals under a variety of conditions. It is one of a few species of the Candida family that causes the fungal infection called candidiasis in humans. Currently there are several treatments available against fungal infections, including those caused by *C. albicans*. Unfortunately, these medications show various side effects due to the similarities between human eukaryotic cells and *C. albicans* cells and do not affect one of the most promising pathways – protein synthesis. A major target on this pathway is the ribosome. In accordance with this, it is important to obtain the spatial structure of the *C. albicans* ribosome for further studies of potential inhibitors. In this study, we obtained for the first time the high-resolution (2.4 Å) structure of the vacant *C. albicans* ribosome by cryogenic electron microscopy. This structure strongly suggests that the mutation of the L42 protein in the E-tRNA binding site of the *C. albicans* ribosome confers resistance to several compounds such as cycloheximide. In this study for the first time we proved the resistance by structural methods. Our model clearly shows binding volume reducing and charge changing in the E-site. We confirmed that the mutation of the 56th proline to glutamine of the L42 protein gives strong resistance to such type of compounds. This study will provide a global understanding of *C. albicans* ribosome inhibition. We believe that this knowledge will significantly improve candidiasis treatment and decrease mortality rates associated with it. This work was supported by Russian Science Foundation grant 20-65-47031.

Tuesday 6 July
9:00–11:00, Kocka Hall

Stem cells and regenerative medicine

S-06.4-3
Understanding human liver development using single cell analyses and organoids

L. Vallier
Wellcome and MRC Cambridge Stem Cell Institute, University of Cambridge, Cambridge, United Kingdom

The liver is a unique organ by the broad spectrum of its functions which include drugs detoxification and, glycogen storage, lipid metabolisms and secretion of protein such as Albumin. End-stage liver disease are systematically life threatening and orthoptic liver transplantation is the only treatment available. However, transplantation entails high risk of surgical complications, indefinite immunosuppression associated with severe side effects, and organ dysfunction. More importantly, the number of organ donors remains constant the past 10 years while the demand for liver transplantation has more than doubled. This situation will continue to worsen in the foreseeable future due increase in cirrhosis associated with obesity. Thus, alternative therapies are urgently needed to address this growing health care challenge. Understanding liver development could help to develop alternative therapies by providing the knowledge necessary to control regenerative process. However, the study of human liver development has been impaired by technical and ethical challenges. Consequently, little is known about the mechanisms directing the cellular composition of the human liver and how this cellular diversity result into tightly regulated hepatic activity. Here, we start to address this knowledge gap by performing single cell transcriptomic on human livers between 5 weeks post conception to adult. These analyses reveal the cellular diversity of the human foetal liver and the developmental trajectory of its different cell types including hepatocytes, cholangiocytes, Liver sinusoidal endothelial cells, Kupffer macrophages and hepatic stellate cells. Our study provides a transcriptomic map of the developing fetal human liver. The resulting atlas reveals new developmental mechanisms which can then inform process of differentiation for the production of cell types with a clinical interest.

S-06.4-2
Safe and allo-tolerated pluripotent stem cell line: a global source for off-the-shelf therapeutic cell products

A. Nagy
Lunenfeld-Tanenbaum Research Institute, Sinai Health Centre, University of Toronto, Toronto, Canada

Clinical trials, using *ex vivo* grown cells as therapeutic means, are currently on their way to treat devastating degenerative conditions. However, cell-safety is a major concern in most cases, which holds back the full utilisation of these promising new treatments. We introduced a concept and showed a genome editing strategy that addresses the safety issue and provides a solution. The quantification of safety is critical to make informed decisions by the regulators, doctors, and patients to advance the modern medicine-transforming cell therapies. Our thoughtfully designed suicide switch (SafeCell) and the calculated mutation rate of the switch, allowed defining mathematically the level of safety of batches of cells planned to be grafted to patients. Building on the SafeCell technology, we addressed the next hurdle faced by cell therapies – the allograft tolerance. We found that the transgenic expression of eight local-acting, immune-modulatory transgenes is sufficient to protect cells against rejection and achieve induced Allogeneic Cell Tolerance (iACT) in fully immune-competent recipients. Allografts survived long-term, in different MHC-mismatched recipients, and without the use of immunosuppressive drugs. The combination of the SafeCell and iACT genome editing allows the generation a "single" pluripotent cell line as a source of off-the-shelf available therapeutic cell products for all humankind.

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

S-06.4-1
Stem cell-based modelling of complex diseases

A. Apati
Research Center for Natural Sciences, Budapest, Hungary

Human pluripotent stem cells (hPSCs) can be differentiated towards all the three germ layers and theoretically can develop into all the cell types of the human body. hPSCs and their differentiated derivatives are new, promising models for studying disease-related phenotypes in vitro regarding cell types which cannot be investigated directly or in long-term cultures, or when appropriate animal models are not available. We use hiPSCs derived from patients and healthy controls to investigate the pathomechanism of complex diseases such as schizophrenia (SCZ) or DiGeorge syndrome (DGS) at cellular level. For investigation of SCZ we selected SCZ case-parent trios, where the affected patients carry potentially disease causing de novo mutations and for studying DGS we choose the members of a family where the disease is present in three generations. Peripheral blood mononuclear cells of patients and healthy relatives were reprogrammed to pluripotent state by Sendai virus (SeV) transduction. The established hiPSC lines were characterized by mRNA and protein expressions of pluripotency markers and were genetically analysed. iPSCs were then differentiated towards disease affected cell lineages (neural or/and cardiac) to carry out comparative analyses of cellular morphology, viability, transcriptomics, proteomics and functionality.

ShT-06.4-1
Single dose application IGF-1 and HGF induced bone marrow mesenchymal stem cells reduced proteinuria in focal segmental gomerulosclerosis model

Ö.B. Şahan[1], I. Onbasilar[2], K.S. Gucer[3], F.F. Kaymaz[4], E. Atayar[5], F. Ozaltin[5,6], A. Günel Özcan[1]
[1]*Department of Stem Cell Sciences, Graduate School of Health Sciences, Center for Stem Cell Research and Development, Hacettepe University, Ankara, Turkey,* [2]*Laboratory Animal Breeding and Experimental Medical Research Unit, Hacettepe University Faculty of Medicine, Ankara, Turkey,* [3]*Department of Pediatric Pathology, Hacettepe University Faculty of Medicine, Ankara, Turkey,* [4]*Department of Histology and Embryology, Hacettepe University Faculty of Medicine, Ankara, Turkey,* [5]*Nephrogenetics Laboratory, Department of Pediatric Nephrology, Hacettepe University Faculty of Medicine, Ankara, Turkey,* [6]*Department of Pediatric Nephrology, Hacettepe University Faculty of Medicine, Ankara, Turkey*

Mesenchymal stem cells (MSCs) are used in the treatment of various pathologic states with their trophic and immunomodulatory properties. Induction of MSCs in culture by particular growth factors increases their migration, immunomodulator and survival capacity. IGF-1 and HGF have been proposed as promising therapeutic agents especially in renal diseases with their renotrophic and nephroprotective effects. In our study, we hypothesized that IGF-1 and HGF induced MSCs (iMSCs) would better migrate to the site of damage and ameliorate proteinuria in Focal Segmental Glomerulosclerosis (FSGS) rat model with their immunomodulatory properties. iMSCs was performed by 10 ng/ml IGF-1 and 20 ng/ml HGF. FSGS model was technically induced by an intraperitoneal single-dose Puromycin Aminonucleoside (PAN) (150 mg/kg). The occurrence of FSGS was assessed by biochemistry (i.e. elevated urinary protein/creatinine (P/C) ratio), immunohistochemistry (i.e. decreased glomerular SYNPO protein level) and electron microscopy (i.e. presence of podocyte effacement). We applied 2×10^5 MSCs or iMSCs treatment intravenously into the rats on day 4 following PAN administration. The effect of treatment was assessed by urinary P/C ratios in 24-hour urine collected on the treatment day of 9. iMSC treatment significantly decreased proteinuria ($P < 0.0001$). However, proteinuria levels of the MSC treatment group were not different from the untreated control group ($P = 0.56$). This result was also supported by immunohistochemistry analysis, which showed lower glomerulosclerosis and by electron microscopy, which showed better podocyte integrity in the iMSC treatment group. However, double dose treatment of iMSC had no curative effect compared to the other groups. As a result of our study, we demonstrated that iMSCs can partially reverse the glomerular damage and reduce proteinuria with regenerating glomerular filtering apparatus in the PAN model of FSGS and therefore may offer a new therapeutic strategy for FSGS.

ShT-06.4-2
New model for the study of mouse embryo implantation

L. Izmaylova, A. Kosykh, E. Vorotelyak, A. Vasiliev, A. Gaidamaka
Institute of Developmental Biology, Moscow, Russia

The implantation process is an attachment of an embryo to the uterus wall. Only attached embryos continue to develop. For the implantation, response of luminal epithelium to the hormonal and embryo-derived signals is crucial. Implantation failure is the cause of most spontaneous abortions in humans. To study implantation, remodeling of this process close to the conditions in vivo is important. Development of a mouse embryo in vitro from the blastocyst stage to the formation of egg cylinder has been shown using various substrates for attachment, but none of them included primary mouse endometrial cells. The goal of this work is to create a new model of implantation of mouse embryo in vitro onto a 3D substrate containing primary cultures of mouse endometrial cells. First, we have developed protocols for isolating cultures of luminal epithelium and stromal cells. We characterized endometrium epithelium migratory potential using scratch test. Also we characterized both cultures by the level of expression of key endometrial markers and receptors for steroid hormones under different hormonal regimes using immunocytochemistry, flow cytometry and qPCR. Then a model substrate was constructed on the basis of collagen gel and primary cultures of mouse endometrial cells. We characterized model substrate histologically and by expression of stromal and epithelial markers. Then we demonstrated the attachment of mouse blastocysts to the model substrate and its development to the egg cylinder stage. The egg cylinder was characterized morphologically and by the localization of expression of markers specific for epiblast, extraembryonic ectoderm and primitive endoderm. Thus, a new model was created to study the development of mouse embryos during implantation. This model can be used to study the interaction of the embryo with endometrial cells, and pathological changes that interfere with implantation. The work was funded by the Russian Science Foundation, Project № 21-74-30015

Tuesday 6 July
9:00–11:00, Marmorna Hall A

Plant biotechnology

S-05.4-1
Plasticity and GXE interaction in grapes

S. Zenoni, S. Dal Santo, G.B. Tornielli, **M. Pezzotti**
Department of Biotechnology - University of Verona, Verona, Italy

Phenotypic plasticity is the ability of a single genotype to produce a range of phenotypes as a function of its environment. This represents a key strategy to maximize fitness when challenged by environmental heterogeneity. Moreover, sessile organisms such as plants rely on phenotypic plasticity to cope with the changing environment, so the phenomenon has a significant impact on evolution, ecology and agriculture as well as on plant responses and adaption in the context of rapid climate change. Although phenotypic plasticity is an important ecological phenomenon, the underlying genetic and molecular mechanisms remain still poorly characterized. Grapevine (*Vitis* spp., family Vitaceae) is the most widely-cultivated perennial fruit crop in the world, the berries are characterized by considerable phenotypic plasticity, with the same clone showing variability within individual berries, among berries within a cluster, between clusters on a vine, and among vines in the vineyard, according to both environmental factors and viticulture practices. In grapevine (*Vitis vinifera*), complex interactions between different genotypes and climate, soil and farming practices yield unique berry qualities. Changes in the performance of genotypes in different environments are defined as genotype × environment (G × E) interactions. Omics studies will be presented to unravel the extent to which phenotypic plasticity in grape berries reflects underlying changes in the transcriptome and to dissect the basis of grapevine G × E interactions.

S-05.4-3
Can in-field testing help us to better use crop protection products?

N. Boonham
University of Newcastle, Newcastle upon Tyne, United Kingdom

Septoria tritici blotch (STB) caused by *Zymoseptoria tritici* has long been the most damaging foliar disease of wheat in northern Europe, whilst black grass (*Alopecurus myosuroides*) is currently the most significant challenge to winter wheat production in the UK. Intensive use of crop protection products with the same modes of action (MOA) has selected for resistance and is eroding our ability to control these damaging pathogens/weeds. Traditionally, screening for the presence of resistance is done in culture using fungicide/herbicide challenge assays which are time consuming and expensive, they don't produce data sufficiently rapidly that growers can change management during the growing season. In the current work we developed methods for rapidly identifying mutations that lead to target site resistance (TSR). For mutations in the ALS and ACCase genes of blackgrass we developed a suite of Loop mediated Amplification (LAMP) assays for single nucleotide polymorphisms (SNPs) associated with resistance. The tests could be run easily in the field using the hand-held Genie III instrument. For the CYP51 gene of Septoria we developed a high throughput sequencing (HTS) based approach based on the MinIon sequencing platform (Oxford Nanopore) to enable the rapid elucidation of haplotypes present within populations. The LAMP method was able to detect SNPs associated with resistance in black grass and could be used to discriminate homozygous and heterozygous plants. The HTS approach could be used to detect the presence of resistant haplotypes and estimate their prevalence within a population down to a cut off of approximately 5%. We plan to explore the use of these tools to improve our ability to select effective MoA of crop-protection products in real-time during the growing season to enable better control of pathogens and weeds.

S-05.4-2
Design of plant biofactories using the *Nicotiana chassis*

D. Orzaez
Instituto de Biología Molecular y Celular de Plantas, CSIC-Universidad Politécnica de Valencia, Valencia, Spain

In modern biobased economies, industrial crops are likely to expand and diversify in response to the increasing demand for bioproducts, from biomedicines to pest control reagents or food additives, among others. Plant biofactories are a relatively new type of crops for which breeding programs have had very short time to operate. Furthermore, non-conventional traits associated with biofactories such tuneable metabolic composition or biocontainment are often different to those pursued in food crops. Among the different plant platforms proposed as biofactories, the solanaceous plant species within the genus *Nicotiana*, most specifically *Nicotiana tabacum* and *Nicotiana benthamiana*, are the most widely used because of their favourable attributes, which include high metabolic versatility, ease of cultivation and high yield, availability of genetic tools for trait manipulation, and the non-food status, which minimises the possibility of contamination of the food supply with industrial designated products. In this context, our lab aims to design improved biofactories of the *Nicotiana* genus by (i) improving the plant chassis via gene editing, (ii) designing new genetic tools for the control gene expression, and (iii) engineering new metabolic pathways for added value products. Here we will briefly report our advances in all three directions. We will present new tobacco lines with increased biofactory traits obtained by multiplexed gene editing and will discuss the ability to precisely control the metabolic flux in the phenylpropanoid pathway using CRISPR-based programmable transcriptional activators. Finally, we will describe the engineering of *Nicotiana benthamiana* plants producing insect pheromones, a new pest control approach that proposes the use plants as life bio-dispensers of volatile semiochemicals.

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

ShT-05.4-1
Developing insect sex pheromone production in plants with the support of transcriptomic data

M. Juteršek[1], M. Petek[1], E. Moreno[2], M. Vasquez-Vilar[2], A. Fernández[2], I. Gerasymenko[3], E. Haumann[3], K. Kallam[4], S. Vacas Gonzales[5], J. Marzo Bargues[6], K. Gruden[1], I. Navarro Fuertes[6], N. Patron[4], H. Warzecha[3], D. Orzaez[2], Š. Baebler[1]
[1]*National Institute of Biology, Ljubljana, Slovenia,* [2]*Instituto de Biología Molecular y Celular de Plantas, Valencia, Spain,* [3]*Technische Universität Darmstadt, Darmstadt, Germany,* [4]*Earlham Institute, Norwich, United Kingdom,* [5]*CEQA, Centro de ecología y protección agrícola, Valencia, Spain,* [6]*Ecologia y Proteción Agrícola, Valencia, Spain*

Use of insect sex pheromones has become an important part of integrated pest management in agriculture as they provide a species-specific control of insect pests and contribute to reduction in conventional insecticide use. Despite of the great potential, their widespread use is still limited due to unsustainable and not cost-effective manufacturing by chemical synthesis. A green alternative, biomanufacturing in plants, is a goal of the SUPSHIRE project, which aims to upgrade the current proof-of-concept pheromone producing plant lines, called the SexyPlants, which successfully synthetize moth (Lepidoptera) pheromones. Our goals are twofold: to improve the plant chassis by removing molecular bottlenecks that cause growth penalty in lines with high pheromone yields and to develop new biosynthetic pathways, enabling synthesis of unique and chemically complex monoterpenoid pheromones of insects from the Coccoidea family. So far, we have used transcriptomic data to identify differentially expressed genes between the high and low producing SexyPlants, which where visualised in the MapMan tool and used for gene set enrichments analysis. This enabled us to pinpoint the cellular processes that are most affected by higher pheromone production, e.g. gibberellin synthesis. We are now working on network analysis to more specifically identify key molecular pathways and genes that lead to growth arrest. To develop the new biosynthetic pathway for production of Coccoidea pheromones, we have generated RNA-seq expression data from citrus mealybug (*Planoccoccus citri*) and combined it with functional predictions of contigs in the prepared *de novo* transcriptome assembly to extract candidate genes responsible for key conversions in the sex pheromone synthesis. Genes with confirmed desired enzymatic activity are used as baits in coexpression network analysis to find the full synthetic pathway that could be implemented in the plant chassis.

ShT-05.4-2
Investigation of the antioxidant effects of glycyrrhizin-containing nanoemulsion formulations in streptozotocin-induced diabetic rats

L. Duysak[1], A.B. Ugur Kaplan[2], M. Gulaboglu[1], M. Cetin[2], Z. Kutlu[1]
[1]*Ataturk University, Faculty of Pharmacy, Department of Biochemistry, Erzurum, Turkey,* [2]*Ataturk University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Erzurum, Turkey*

Diabetes Mellitus (DM), which has a prevalence and high mortality rate worldwide, is a chronic and metabolic disease. Interest in natural antidiabetic compounds is increasing due to the adverse effects of oral hypoglycemic agents used in the treatment of type 2 diabetes. It is known that glycyrrhizin, one of the main components of licorice root, has a potential therapeutic effect in the treatment of diabetes. The aim of this study is to investigate the antioxidant effects of nanoemulsion formulations (NE) containing GLY (GLY-NE) in streptozotocin (STZ)-induced diabetic rats. Sixty rats were divided into 12 groups with 5 rats in each group (control groups and experimental groups). To induce the diabetic model in rats, 40 mg/kg (single dose) of STZ solution in freshly prepared citrate buffer (0.1 M, pH 4.5) kept on ice was administered intraperitoneally to fasted rats. 72 hours after STZ treatment, the blood glucose levels of the rats were measured, with a range of above 200 mg/dL considered diabetic. GLY-NE or pure GLY at different doses (10, 20 and 40 mg/kg b.w.) was administered orally to diabetic rats for 21 days. At the end of the experiment, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured in the serum samples of the rats. Superoxide dismutase (SOD) activity, glutathione (GSH) and malondialdehyde (MDA) levels were determined in liver tissues of the rats. It was determined a significant decrease in serum glucose, AST and ALT levels in diabetic rats treated with GLY-NE ($P < 0.05$). Besides, SOD activity and GSH levels increased, while MDA levels decreased in diabetic rats treated with GLY-NE compared to the control group ($P < 0.05$). As a result, GLY-NE has been effective in reducing oxidative stress seen in diabetes, thus the use of GLY-NE in the treatment of diabetes may be a potential and beneficial approach. This study was supported by Ataturk University (Project number: TDK-2019-7211).

Tuesday 6 July
9:00–11:00, Marmorna Hall B

Immunotherapy

S-06.1-1
The role of the gut microbiota in drug response and toxicity

M. Zimmermann
European Molecular Biology Laboratory, Heidelberg, Germany

Individuals vary widely in their drug responses, which can be dangerous and expensive due to significant treatment delays and adverse effects. Growing evidence implicates the gut microbiome in this variability, however the molecular mechanisms remain mostly unknown. Using antiviral nucleoside analogues and clonazepam as examples, we recently reported experimental and computational approaches to separate host and gut microbiota contributions to drug metabolism and toxicity. The resulting pharmacokinetic models identified measurable physiological, microbial and chemical parameters that dictate host and microbiome contributions to the metabolism of xenobiotics. To systematically map the drug metabolizing capacity of the gut microbiota and assess its potential contribution to drug metabolism, we further measured the ability of 76 diverse human gut bacteria to metabolize each of 271 oral drugs. We found that two thirds of these drugs are chemically modified by at least one of the tested microbes. Through combination of high-throughput bacterial genetics with mass spectrometry, we systematically

identified drug-metabolizing microbial gene products. These gene products better explain the drug-metabolizing capacity of bacterial strains than their phylogenetic classification. We further demonstrate that the abundance of homologs of these gene products predict the capacity of complete human gut communities to metabolize the targeted drugs. These causal links between microbiota gene content and metabolic activities connect inter-individual microbiome variability to interpersonal differences in drug metabolism and response, which has translatable potential on medical therapy and drug development across multiple disease indications.

S-06.1-2
Interleukin-6 and ADAM17 in inflammation and cancer

S. Rose-John
Biochemical Institute, University of Kiel, Kiel, Germany

Cytokines receptors exist in membrane bound and soluble form. The IL-6/soluble IL-6R complex binds to and activates the gp130 receptor on target cells devoid of the membrane bound IL-6R. We have named this process 'trans-signaling'. The soluble IL-6R is generated via ectodomain shedding by the membrane bound metalloprotease ADAM17. A soluble form of gp130 is the natural inhibitor of IL-6/soluble IL-6R complex responses. A dimerized recombinant soluble gp130Fc fusion protein is a molecular tool to discriminate between gp130 responses via membrane bound and soluble IL-6R responses. We found that colorectal cancer in mouse models was completely inhibited. in the absence of ADAM17. Because EGF-R on myeloid cells, but not on intestinal epithelial cells, is required for intestinal cancer and because IL-6 is induced via EGF-R stimulation, we analyzed the role of IL-6 signaling. Tumor formation was equally impaired in IL-$6^{-/-}$ mice and sgp130Fc transgenic mice, in which only trans-signaling via soluble IL-6R is abrogated. Our data reveal the possibility of a novel strategy for treatment of colorectal and lung cancer that could circumvent intrinsic and acquired resistance to EGF-R blockade. Depending on the animal model used, global blockade of IL-6 signaling by neutralizing monoclonal antibodies and selective blockade of IL-6 trans-signaling can lead to drastically different consequences. Inhibition of IL-6 trans-signaling but not global IL-6 blockade was beneficial in several inflammation and cancer models. The extent of inflammation is controlled by trans-signaling via the soluble IL-6R. Therefore, sgp130Fc is a novel therapeutic agent for the treatment of chronic inflammatory diseases and cancer and it underwent phase I clinical trials as an anti-inflammatory in 2013/2014. Phase II clinical trials in patients with autoimmune diseases such as inflammatory bowel disease have been successfully finished in Germany as well as in China, Taiwan and South Korea.

S-06.1-3
Neutrophils predict T-cell depletion and anti-PD1 treatment failure in NSCLC

J. Kargl
Medical University of Graz, Graz, Austria

Immune checkpoint inhibitor (ICI) treatment has recently become first-line therapy for many non-small cell lung cancer (NSCLC) patients. Unfortunately, most NSCLC patients are refractory to ICI monotherapy and initial attempts to address this issue with secondary therapeutics have proven unsuccessful. A strong foundational knowledge of the immune cell composition and function in NSCLC will likely prove prerequisite to realizing the full potential of ICI treatment. To evaluate the complexity of the immune landscape in NSCLC and to identify entities precluding T cell accumulation, we analyzed flow cytometry, gene expression and multiplexed immunohistochemical data from a NSCLC patient cohort. Further, we performed T cell receptor sequencing to delineate the frequency with which antigen-driven immune responses exist, allowing the comprehensive characterization of the immune microenvironment in NSCLC. The results revealed the presence of a myeloid-rich subgroup, which was devoid of CD^{4+} and CD8+ T cells. Of all myeloid cell types assessed, neutrophils were the most highly associated with the myeloid phenotype. Additionally, the ratio of CD8+ T cells to neutrophils within the tumor mass optimally distinguished between active and myeloid cases. This ratio was also capable of separating patients responsive to ICI therapy from those with stable or progressive disease. Tumor-bearing mice treated with a combination of anti-PD1 and CXCR1/2 inhibitor, which reduces neutrophil migration to the tumor, displayed relocation of lymphocytes from the tumor periphery into malignant part, which was associated with induction of IFNg responsive genes. These results suggest that neutrophil antagonism may represent a viable secondary therapeutic strategy to enhance ICI treatment outcomes.

ShT-06.1-2
Regulatory T cells for immunotherapy of autoimmune diseases

J. Pohar[1,2], R. O'Connor[3], L. Jouneau[4], P. Boudinot[4], M. Bunse[5], W. Uckert[5], S.M. Anderton[3], R. Liblau[6], S. Fillatreau[2,7,8]
[1]National Institute of Biology, Ljubljana, Slovenia, [2]Institut Necker-Enfants Malades (INEM) INSERM U1151-CNRS UMR 8253, Paris, France, [3]University of Edinburgh, Edinburgh, United Kingdom, [4]Université Paris-Saclay, INRAE, UVSQ, VIM, Jouy-en-Josas, France, [5]Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany, [6]Centre de Physiopathologie Toulouse-Purpan, Centre National de la Recherche Scientifique CNRS, Institut National de la Santé et de la Recherche Médicale (INSERM), Université Paul Sabatier (UPS), Toulouse, France, [7]AP-HP, Hôpital Necker Enfants Malades, Paris, France, [8]Université de Paris, Paris Descartes, Faculté de Médecine, Paris, France

Autoimmune diseases affect people of all ages and often significantly impair their quality of life. Regulatory T cells (Treg) are critical for maintaining homeostasis and preventing immune responses against self-antigens. They can utilize direct and indirect mechanisms such as the expression of anti-inflammatory cytokines or via co-inhibitory receptors. Clinical studies have shown that polyclonal Tregs can control autoimmune responses after adoptive transfer. In preclinical models, antigen-specific Tregs were superior to polyclonal ones, which has spurred the development of antigen-specific Tregs for cell-based therapies. However, such cells are rare and difficult to isolate. This could be overcome by engineering the polyclonal Tregs into antigen-specific ones by T cell receptor (TCR) gene transfer. We used a preclinical model of experimental autoimmune encephalomyelitis (EAE), which is an animal model of demyelinating diseases of the central nervous system (CNS) including multiple sclerosis.

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

EAE is induced by immunization with encephalitogenic peptides such as myelin oligodendrocyte glycoprotein (MOG) peptide. Tregs engineered to express TCRs that recognize MOG protect mice from EAE. Genetically engineered antigen-specific Tregs employ IL-10, LAG-3, and CTLA-4 to inhibit disease in recipient mice, whereas expression of tissue repair factor AREG has no effect. Antigen-specific Tregs can intercept EAE when administered at the onset but not at the peak of the disease. The engineered cells persist in the animals and show an activated phenotype compared to endogenous Tregs. This study contributes significantly to our understanding of the properties that drive the efficacy of Treg cell therapy. Engineered autoreactive Tregs protect against CNS autoimmunity via multiple immune mechanisms and block disease progression when administered early in the disease, highlighting their immense potential for intercepting autoimmune responses and developing cell-based therapies.

ShT-06.1-1
IgE epitope-like peptides and their potential in immunotherapy of life-threatening allergies

M. Lunder[1], A. Zahirović[1], J. Luzar[1], A. Koren[2], B. Štrukelj[1], P. Korošec[1]
[1]*University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia,* [2]*University Clinic of Respiratory and Allergic Diseases Golnik, Golnik, Slovenia*

Type I allergies are considered a significant public health concern and are characterized by production of immunoglobulin E (IgE) antibodies against by itself harmless antigens – allergens. Two most life-threatening IgE-mediated allergies are allergy to peanuts and allergy to hymenoptera venom. We have focused our research on new therapeutic constructs (epitope-based preparations) for improved treatment options of these two allergies. IgE epitope-like peptides, mimetics of epitopes of major peanut allergen Ara h 2 and major bee venom allergen Api m 1, which have the ability to bind to allergen specific IgE paratopes were identified by screening biological peptide libraries. The sequences of peptides enabled mapping and identification of epitopes on the two allergens. In order to consider peptides for active immunization we coupled selected epitope-like peptides to different immunogenic carriers and evaluated their immunotherapeutic potential by *ex vivo* basophil activation test and measurements of cytokines released from peripheral blood mononuclear cells (Results for Api m 1 were previously published in: Zahirović A et al. (2019) J Allergy Clin Immunol 143, 791-794.e5). Synthetic epitope-like peptides were additionally tested for their ability to prevent effector cell activation in the presence of allergen. This is a completely new concept in allergy immunotherapy. Synthetic Api m 1 IgE epitope-like peptides were not able to prevent degranulation of patients' effector cells. However, we have identified Ara h 2 IgE epitope-like peptides that successfully prevented biding of causative allergen, and thereby prevented degranulation and secretion of allergic inflammation mediators from effector cells, after contact with the causative allergen (Previously published as PCT application WO2019/228612). Such peptides represent new preventive treatment option in accidental exposures to allergens or intentional exposures during immunotherapy.

Tuesday 6 July
9:00–11:00, Gallery Hall

Pharmacogenomics and biomarkers

S-06.3-2
Pharmacogenomic biomarkers for prediction of drug response: Recent method development and clinical importance

M. Ingelman-Sundberg
Karolinska Institutet, Stockholm, Sweden

The genomic inter-individual heterogeneity remains a significant challenge for both clinical decision-making and the design of clinical trials. Major reasons are the paucity of sufficiently powered trials that can quantify the added value of pharmacogenetic testing and the considerable pharmacogenetic complexity with millions of rare variants with unclear functional consequences. However recent studies encompassing a very large number of patients have revealed that genotyping of genes encoding enzymes active in the metabolism of drugs can provide useful information regarding the dosing of antidepressant and antipsychotic drugs for optimization of efficacy. Recent data indicate that also genetic variation outside the gene in question contributes to specific inter-individual variation in drug response that should be considered for personalized drug therapy. Recent examples are the finding of a common new CYP2C haplotype in linkage disequilibrium to CYP2C19*1 causing ultrarapid metabolism of e.g. escitalopram and a polymorphism in the NFIB gene of importance for expression of genes encoding drug metabolism. This lecture will provide an update of the field and show examples on useful pre-emptive genotyping for more effective drug treatment. It is concluded that improvement of clinical applications of pharmacogenomics to reach a higher resolution for personalized medicine would require (i) very large studies using well-characterized patients, (ii) more consensus in the definition of clinically used pharmacogenomic biomarkers, (iii) evaluation of genetic variants on a global scale e.g. by GWAS based on 15 million SNPs, (iv) more focus on rare genetic variants, (v) more focus on haplotypes as compared to genotypes and (vi) the further development of AI-based interpretations of the functionality of novel mutations detected.

S-06.3-1
Diagnostic proteomic biomarkers to detect kidney diseases

T. Ozben[1], E. Bellei[2], E. Monari[2], S. Bergamini[2], L.A. Pini[3], A. Tomasi[2]
[1]*Akdeniz University, Antalya, Turkey,* [2]*Department of Diagnostic Medicine, Clinic and Public Health, University of Modena and Reggio Emilia, Modena, Italy,* [3]*Headache and Drug Abuse Study Center, University Hospital of Modena and Reggio Emilia, Modena, Italy*

Urinary proteomics is primarily applied to the study of renal and urogenital tract disorders. Here are reported two distinct successful examples of this approach for the discovery of early urinary biomarkers of kidney-related dysfunctions: diabetic nephropathy (DN), a well-known complication of diabetes frequently leading

to dialysis, and drug-induced nephrotoxicity, a possible condition caused by medication-overuse headache (MOH). Early detection of kidney disorders based on selective biomarkers could permit to diagnose patients at the initial stage of the disease, where the therapy is still possible to stop or prevent occurrence of advance disease. Urine samples were first concentrated and desalted. Subsequently, they were subjected to two-dimensional gel electrophoresis (2-DE) coupled to mass spectrometry (MS) for protein identification. Furthermore, some proteins were verified by Western blot and ELISA test. In diabetes-related study, 11 differentially expressed proteins were detected (8 up-regulated and 3 down-regulated) in type 2 diabetic (T2D) and T2DN patients compared to the healthy control subjects. In MOH study, a total of 21 over-excreted proteins was revealed in urine of non-steroidal anti-inflammatory drugs (NSAIDs) and mixtures abusers vs controls. Particularly, 4 proteins were positively validated by immunoblotting and ELISA. Urinary proteomics allows non-invasive assessment of renal diseases at an early stage by the identification of characteristic protein pattern.

S-06.3-3
Mode of action and therapeutic potential of the ER stress regulating protein CDNF

M. Saarma
Institute of Biotechnology, University of Helsinki, Helsinki, Finland

Parkinson's disease (PD) affects about10 million people and no treatment exists that can slow down or stop the disease progression. In PD midbrain dopamine neurons degenerate and die causing in addition to major motor symptoms also non-motor symptoms. Current drugs can only temporarily alleviate the motor symptoms, but non-motor symptoms remain untreated. Our group has discovered an endoplasmic reticulum (ER) located protein with neurotrophic factor (NTF) activities – cerebral dopamine neurotrophic factors (CDNF). We have solved the three -dimensional structure of CDNF and its homologous protein MANF and found that their structure and mode of action radically differs from other known NTFs. We have demonstrated that CDNF can protect and repair midbrain dopamine neurons in rodent neurotoxin models of PD at least as efficiently as other known NTFs. However, differently from other NTFs, CDNF and MANF are mainly located in the ER, where they regulate ER stress and unfolded protein response pathways. We developed CDNF knockout (KO) mice that develop an age-dependent loss of enteric neurons resulting in pathological changes of gastrointestinal function. The deficiencies of CDNF KO mice are similar to those seen in early stages of Parkinson's disease. CDNF was more efficient than GDNF in rhesus monkey neurotoxin model of PD. Herantis Pharma Plc. has tested CDNF in phase I-II clinical trials in PD patients and CDNF achieved its primary endpoint of safety and tolerability. Moreover, significant increases in DAT PET signaling and improved UPDRS scores were observed in some, but not all, CDNF-treated patients Since CDNF cannot pass through the blood–brain barrier (BBB), it is delivered directly into the patient's brain via catheters that are installed during invasive surgery. We have recently discovered a fragment of CDNF (ngCDNF) that can pass through the BBB after subcutaneous administration. Use of ngCDNF may allow peripheral delivery avoiding intracranial surgery.

ShT-06.3-2
Far upstream element binding protein 3 expression is associated with bone remodeling

P. Malavašič[1], A. Ogrin[1], M. Zrimšek[2], E. Wircer[3], I. Khrystoforova[3], C. Schochat[3], V. Prijatelj[2], J. Marc[1], B. van der Eerden[2], F. Rivadeneira[1], D. Karasik[3], **N. Lovsin**[1]
[1]*University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia,* [2]*Erasmus MC, Rotterdam, Netherlands,* [3]*The Faculty of Medicine in the Galilee, Bar Ilan Univesity, Safed, Israel*

Genome-wide association studies (GWAS) are one of the most powerful approaches to identify genetic loci that are associated with bone mineral density (BMD). GWAS have identified hundreds of associations with BMD; however, only few have been functionally evaluated, and functional characterization remains a challenge. One of the loci significantly associated with femoral neck BMD at genome-wide level ($P = 3.4 \times 10^{-22}$) is SNP rs7851693 from the intron of far upstream element binding protein 3 (FUBP3) gene. To date, nothing is known about this gene's function. Here, we investigated the functional role of FUBP3 in bone biology across multiple *in-silico*, *in-vitro* and *in-vivo* functional layers in human cell and model organisms. Variants mapping to FUBP3 were prioritized using GCTA and FINEMAP. Expression of FUBP3 in 43 osteoporotic and osteoarthritic human bone tissue samples was compared to healthy controls. The expression of FUBP3 was significantly decreased ($P = 0.004$) in bone tissues from osteoporotic patients as compared to controls. Furthermore, we examined FUBP3 expression in whole fish during zebrafish development and adulthood, and fin regeneration, by *in situ* hybridisation and Q-PCR. Two fold increase in FUBP3 expression ($P = 0.003$) in the newly formed zebrafish fins suggests that FUBP3 is involved in tissue regeneration and formation of bone tissues. Moreover, we also investigated expression of FUBP3 during osteogenic, adipogenic and myogenic differentiation of human mesenchymal stem cells. Indeed, silencing of FUBP3 inhibited osteogenic differentiation confirming the involvement of FUBP3 in the formation of osteoblasts. Altogether, our results suggest that FUBP3 plays an important role in bone biology and osteoporosis susceptibility in humans. 1 Estrada, K. et al. Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. Nat Genet 44, 491-501, https://doi.org/10.1038/ng.2249 (2012).

ShT-06.3-1
Insights into the biological role of thiopurine S-methyltransferase

D. Urbančič[1], A. Šmid[1], M. Kandušer[1], A. Kotar[2], J. Plavec[2], I. Mlinarič-Raščan[1]
[1]*University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia,* [2]*Slovenian NMR Center, National Institute of Chemistry, Ljubljana, Slovenia*

Thiopurine *S*-methyltransferase (TPMT) is an important enzyme involved in deactivation of thiopurine drugs. Its activity represents a major determinant of thiopurine-related toxicities. However, the endogenous role of TPMT is still unknown. Our genome-wide expression population studies suggested a close relation of TPMT to genes involved in oxidoreductive processes. Since selenium is a representative micronutrient involved in oxidoreductive reactions in biological systems and certain selenium-containing organic compounds have chemical properties similar

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

to thiopurines, we investigated the role of this enzyme in selenium metabolism. By STD NMR spectroscopy and tryptophan fluorescence measurements, we showed that selenocysteine (Sec) binds *in-vitro* to human recombinant TPMT. After the incubation of Sec with TPMT and methyl donor, *S*-adenosylmethionine, a methylated product, *Se*-methlyselenocysteine, was formed. To explore the biological relevance of this finding, we performed experiments on lymphoblastoid cell lines (LCLs) from different individuals. LCLs were genotyped for TPMT genetic variants and TPMT activity was measured in all cells. We observed genotype-activity correlation – LCLs with at least one variant TPMT allele had TPMT activity significantly decreased. When evaluating the sensitivity of the cells to selenium compounds with proliferation assay, we found that LCLs with wild-type TPMT were less sensitive to Sec and sodium selenite compared to LCLs with heterozygous or double-variant genotype. Applying similar approach, results were further confirmed on knock-out Hap1 cell model and Hek293T cells overexpressing TPMT. When inducing oxidative stress, the production of reactive oxygen species was lower, if cells were pre-treated with Sec2 and had low TPMT activity. This study revealed Sec as the first known biological compound acting as a substrate for TPMT. Further studies are needed to elucidate the exact role of TPMT in selenium-mediated oxidative reactions.

Tuesday 6 July
9:00–11:00, Povodni mož Hall

Biochemistry of toxins

S-07.1-1
The challenge of investigating the toxicity of mycotoxins

I. Oswald
INRA TOXALIM, Toulouse, France

Food is contaminated by multiple contaminants, mycotoxins being the most frequently occurring natural ones. Mycotoxins are secondary fungal metabolites produced mainly by Aspergillus, Penicillium and Fusarium. Mycotoxins co-contamination is confirmed by the co-occurrence of these toxins in food and feed stuff and by co-exposure monitoring survey. The co-occurrence of mycotoxins in food is explained by three different reasons: (i) most fungi are able to simultaneously produce several mycotoxins, (ii) commodities can be contaminated by several fungi simultaneously or in quick succession, and (iii) the complete diet comprised different commodities. In practice, the co-occurrence of mycotoxins represents the rule and not the exception. Besides mycotoxins, food can be contaminated with other contaminant such as heavy metals. Unfortunately, the toxicity of combinations of contaminant cannot always be predicted based upon their individual toxicities. The data on the combined toxic effects of mycotoxins are limited and therefore, the health risk from exposure to a combination of mycotoxins is incomplete. Most of the studies concerning the toxicological effect of contaminant have been carried out taking into account only one compound. A synergistic effect between trichothecenes mycotoxins was observed both for intestinal cytotoxicity and inflammatory response and the synergy was already seen at low doses. The combined exposure to DON and Cadmium was also studied in several human cell lines and interactions were specific to the target organ. The interaction between DON and microbial toxin was also investigated. We demonstrated that DON exacerbated the intestinal DNA damages induced by Escherichia coli stains producing colibactin. Altogether, these data demonstrated that mycotoxin cocktails can lead to synergistic interaction and that mycotoxin contamination should be taken in the global context of all food contaminants and the host intestinal microbiota.

S-07.1-3
Cyanotoxins: adverse effects beyond acute toxicity

B. Žegura, K. Hercog, A. Štern, M. Filipič
National Institute of Biology, Ljubljana, Slovenia

Cyanobacteria produce a wide variety of bioactive compounds including highly toxic cyanobacterial toxins that can affect human and animal health as well as the ecosystem. In humans, acute intoxication with cyanotoxins is not very likely; however, they are of high human health concern due to their potential long-term adverse effects (genotoxic, carcinogenic, reproductive) that can occur with a delay of several years. Humans can be chronically exposed to low concentrations of these toxins through consumption of contaminated water and food, dermal exposure and/or inhalation. The mechanisms behind the toxic effects of cyanotoxins are different as they are structurally diverse chemicals. Defined by their toxicological activity they fall into different groups including hepatotoxins, neurotoxins, cytotoxins, dermatotoxins, irritants, gastrointestinal toxins and many yet unknown biologically active metabolites. To identify the threat posed by cyanotoxins to human and animal health, it is important to evaluate their potential toxicological profile. Of particular concern is their possible genotoxic activity. Genotoxic compounds interfere with the function of genetic material resulting in cancer development and/or other chronic diseases, reproductive effects, heritable diseases, and as shown by more recent studies also neurodegenerative disorders. Therefore, genotoxicity assessment represents an important component of cyanotoxin risk assessment, which is relevant for the protection of human health and the environment. The presentation will discuss the adverse genotoxic effects of cyanotoxins with emphasis on the emerging cyanotoxin cylindrospermopsin.

S-07.1-2
The booming field of Botulinum neurotoxins

O. Rossetto, M. Pirazzini, C. Montecucco
Department of Biomedical Sciences, University of Padova, Padova, Italy

Botulinum neurotoxins (BoNTs) are a growing family of bacterial protein toxins that cause a generalized flaccid paralysis of botulism. They are the most potent toxins known with a MLD50 as low as 20 picogram/kg and such a potency is due to their marvel of protein design, which underlines their capability to inactivate the molecular machinery responsible for neurotransmitter release at peripheral nerve terminals. Extreme potency and exquisite neurospecificity make BoNT a "double face" molecule: a potential bioweapon and a highly effective and successful therapeutic agent for the treatment of a variety of human syndromes at the same time. Traditionally, BoNTs are classified in seven serotypes according to their immunological properties, yet many

novel toxins are now being discovered thanks to the use of NGS, bioinformatics and metagenomics studies. Presently, more than 40 BoNT variants have been isolated from clostridia, and, interestingly, BoNT-like genes and toxins have been found in non-clostridial species. These novelties are now reviving the interest for BoNT basic and applied research. Large efforts are being made worldwide to discover novel antitoxins to counteract botulism and for engineering BoNTs both to improve their clinical utilization and to expand the landscape of their therapeutic application. This booming field will be discussed in relation to the recent advances. Key words: botulism, botulinum neurotoxins, neuromuscular junction, peripheral neuroparalysis

ShT-07.1-1
Molecular characterization of the ΦX174-E mediated cell lysis pathway

J. Mezhyrova[1], J. Martin[2], O. Peetz[2], S. Toews[1], V. Doetsch[1], Y. Ma[3], N. Morgner[2], F. Bernhard[1]
[1]*Institute for Biophysical Chemistry & Centre for Biomolecular Magnetic Resonance, Goethe-University Frankfurt, Frankfurt am Main, Germany,* [2]*Institute of Physical and Theoretical Chemistry, Goethe University Frankfurt, Frankfurt am Main, Germany,* [3]*South China University of Technology, Guangzhou, China*

The rapidly emerging microbial resistances become a serious threat to global public health and the discovery and development of new antibiotics experiences a new peak of interest. In particular, drugs addressing enzymes located in the inner cell membrane are rare and highly desired. Bacteriophages such as ΦX174 efficiently lyse bacterial cells by small membrane bound toxins. The ΦX174-E toxin requires activation by the chaperone SlyD and inhibits cell wall precursor forming enzyme MraY. However, the final cell lysis by large pore formation may require close interaction with additional targets. Functional and structural details of the lysis mechanism could not be studied so far due to its high toxicity in conventional cell-based expression systems. We combine cell-free expression systems with nanoparticle technologies to synthesize the functionally folded full-length ΦX174-E toxin and engineered derivatives. By this new approach, the toxins are co-translationally inserted into preformed nanodiscs containing defined membrane compositions. This strategy allowed studying the role of SlyD in ΦX174-E activation as well as subsequent mechanisms of membrane insertion and cell lysis at the molecular level. We could show for the first time that SlyD keeps the hydrophobic toxin in solution and catalyzes its membrane insertion. Furthermore, by implementing native mass spectrometry techniques, we could show that ΦX174-E forms higher oligomeric assemblies in the membrane. By co-insertion of MraY enzymes together with ΦX174-E toxins into the same nanodisc particles, we could demonstrate the inhibition of the cell-wall precursor formation. The established fully defined synthetic system allows to address key questions of bacteriophage toxin mediated lysis of bacterial cells. Detailed functional and structural studies including NMR spectroscopy and electron microscopy will reveal underlying mechanisms of toxin function such as target selectivity and pore forming mechanisms.

ShT-07.1-2
Novel insights into pore-forming process of listeriolysin O using solution and solid-state NMR

M. Kozorog[1], M. Sani[2], M. Lenarčič Živković[1], G. Ilc[1], J. Plavec[1], F. Separovic[2], G. Anderluh[1]
[1]*National Institute of Chemistry, Ljubljana, Slovenia,* [2]*School of Chemistry, Bio21 Institute, The University of Melbourne, Melbourne, Australia*

Cholesterol-dependent cytolysins (CDCs) are a large group of pore-forming toxins, produced by diverse, mainly Gram-negative bacteria. CDCs are secreted as soluble monomeric proteins that bind to lipid membranes, where they oligomerize and form biggest known biological pores. As their name suggests high cholesterol content in target membranes is crucial for efficient pore formation, however the membrane-binding and pore-forming process has not been explained at the molecular level yet. Since tryptophan amino acid residues are highly represented in cholesterol-binding domain of CDCs we studied their role in binding and pore-forming process of CDC representative listeriolysin O (LLO) from pathogenic bacteria *Listeria monocytogenes*. Using solution and solid state NMR techniques we showed that several tryptophan residues are involved in either pore-forming or in membrane-binding processes as published in Kozorog M et al. (2018) Sci Rep 8, 6894. We also showed that the dynamics of phospholipid acyl chains was significantly affected in more liquid-disordered cholesterol-rich bilayers upon LLO binding without lipid bilayer organization being affected. We also observed changed holesterol dynamics in the presence of the protein what serves as a proof of direct interaction of LLO with membrane cholesterol, what was published in Kozorog, M et al. (2018) Chem Eur J 24, 14220–14225.

Tuesday 6 July
16:00–18:00, Kocka Hall

New frontiers in medicinal chemistry

S-06.2-1
Addressing Alzheimer's disease complexity with multi-target-directed ligands

M.L. Bolognesi
Department of Pharmacy and Biotechnology, Alma Mater Studiorum, University of Bologna, via Belmeloro 6, 40 126 Bologna, Italy

Alzheimer's disease (AD) is recognized as an international public health crisis as well as a titanic challenge for drug development. Currently AD has no cure, no new medication has entered the global market since 2003, and no ground-breaking treatment is on the horizon. Across the pharmaceutical community, there has been an ever-increasing awareness that addressing the enormous challenges AD poses might require a shift from the prevailing 'single-target' paradigms towards polypharmacology. Polypharmacology is defined as "the design or use of pharmaceutical agents that act on multiple targets or disease pathways". Hence, by accounting for the actual AD etiological complexity, it may provide concrete means to identify therapies with maximum impact on the diseased brain network. Polypharmacology can involve both combinations

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

and so-called multi-target-directed ligands (MTDLs). Although they are not mutually exclusive, MTDLs possess the intrinsic features of a single molecular entity, when compared to combinations. Accordingly, for the past 10 years we have embarked on a program focused on the development of different classes of MTDLs, namely fragments, hybrids and conjugates. In this talk, we will discuss pro and cons of each class through examples from our own research and provide medicinal chemistry considerations that may impact their future clinical translation.

S-06.2-3
Novel tools and drugs for G proteins and G protein-coupled receptors

C.E. Müller
University of Bonn, Pharmaceutical Institute, Pharmaceutical & Medicinal Chemistry, Bonn, Germany

Integral cell membrane proteins such as membrane receptors, in particular G protein-coupled receptors (GPCRs), represent the most important classes of drug targets. With more than 800 members GPCRs constitute the largest protein family in humans. However, only a small percentage of membrane proteins is targeted by current therapies. For about 100 (non-olfactory) GPCRs the endogenous agonist still remains unknown or has been postulated but could not be confirmed (yet). These so-called orphan receptors belong to the most enigmatic members of the GPCR superfamily. In order to study their roles and functions, and for validating them as (potential) novel drug targets, selective agonists and antagonists are required as tool compounds. Our group has been interested in purinergic and lipid-activated GPCRs, including adenine, adenosine, nucleotide (P2Y), and cannabinoid receptors. Recently, we have extended our focus of interest to related orphan GPCRs belonging to the delta-branch of rhodopsin-like GPCRs, e.g. the nucleotide receptor-like GPCRs GPR17 and GPR35, and the lipid-activated GPCRs GPR18, GPR55 and GPR84. Our goal has been to develop potent and selective ligands as biological tools that will allow pharmacological studies of these scarcely investigated orphan receptors. GPCRs transduce their signals across cell membranes by activation of guanine nucleotide-binding proteins (G proteins). The mechanisms of signal transduction are currently not completely understood. Direct blockade of G protein subtypes, e.g. Gq proteins, has been proposed as a novel strategy for the treatment of complex diseases. We have recently developed the first radioligands for the sensitive detection of Gq proteins, which enable a variety of studies including the screening for novel G protein inhibitors.

S-06.2-2
Geometric isomers of 4-styrylpiperidine discriminate between monoamine oxidase isoforms A and B

D. Knez[1,*], N. Colettis[2,*], L.G. Iacovino[3,*], M. Sova[1], A. Pišlar[1], J. Kos[1], C. Binda[3], M. Marder[2], **S. Gobec**[1]
[1]*University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia,* [2]*Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina,* [3]*University of Pavia, Department of Biology and Biotechnology, Pavia, Italy*

Monoamine oxidase A (MAO-A) and monoamine oxidase B (MAO-B) are important targets in the therapy of several neurological disorders such as depression, Parkinson's disease and Alzheimer's disease. Fueled by the recent biological findings, MAOs are also gaining interest in other areas such as cardiovascular disorders and cancer. This further extends potential therapeutic applicability of MAO inhibitors to a wider spectrum of diseases. Selective inhibitors of either MAO isoform are valuable chemical tools to study the role of MAOs in disease pathogenesis, and further on to achieve the desired therapeutic effect while minimizing the undesired side effects caused by nonselective inhibition of both isoforms. Biological receptors differ greatly in shape, polarity and conformational dynamics. Achieving selectivity between structurally related targets such as isoenzymes, however, represents a challenging task, which requires different ligand-design approaches. This study demonstrates a unique case study how configuration of double bond in stilbene-like derivatives can define the selectivity for either MAO-A or MAO-B. Starting from an identified pair of cis-trans isomers, which selectively inhibit human MAO-A and human MAO-B, respectively, a series of analogues was synthesized. The inhibition was studied exhaustively by enzyme kinetics analysis and X-ray crystallography. *Ex vivo* and *in vivo* results showed that compounds were able to inhibit MAO-A and MAO-B activities in mice brain homogenates. Importantly, selective hMAO-A inhibitor showed antidepressant-like activity in mice in chronic 10-day treatment regime. In addition, we provide the first, thorough kinetic and structural characterization of compounds containing 1-propargylpiperidine as a new fragment that can be used in the design of pharmaceuticals targeting MAO. *The authors marked with an asterisk equally contributed to the work.

ShT-06.2-1
Novel potent PSMA ligands and their docetaxel conjugates: *in vitro* and *in vivo* evaluation

A.E. Machulkin[1,2,3], A.A. Uspenskaya[1], A.P. Ber[1], S.A. Petrov[1], E.A. Nimenko[1], N.U. Zyk[1], Y.A. Ivanenkov[1,2,4], A.V. Aladinskaya[4], D.A. Skvortsov[5], Y.A. Borisova[6], G.B. Smirnova[6], V.S. Pokrovsky[6], A.N. Vaneev[1,3], A.S. Erofeev[1,3], P.V. Gorelkin[1], E.K. Beloglazkina[1], N.V. Zyk[1], A.G. Majouga[1,3,7]
[1]*Lomonosov Moscow State University, Chemistry Department, Moscow, Russia,* [2]*Institute of Biochemistry and Genetics Ufa Science Centre Russian Academy of Sciences (IBG RAS), Ufa, Russia,* [3]*National University of Science and Technology MISiS, Moscow, Russia,* [4]*Moscow Institute of Physics and Technology (State University), Moscow, Russia,* [5]*A.N. Belozersky Institute of Physico-Chemical Biology MSU, Moscow, Russia,* [6]*N.N. Blokhin Cancer Research Center, Moscow, Russia,* [7]*Dmitry Mendeleev University of Chemical Technology of Russia, Moscow, Russia*

Prostate specific membrane antigen (PSMA), also known as glutamate carboxypeptidase II (GCPII), is an effective target for specific delivery of antitumor drugs because it is highly overexpressed in prostate cancer cells. The most promising group of PSMA inhibitors is low-molecular urea-based ligands with a flexible linker. In the current work we describe the design, synthesis, and biological evaluation of novel low-molecular PSMA ligands and their conjugates with Docetaxel. A series of more than 20 PSMA-targeting ligands were synthesized and evaluated using prostate GCPII inhibition assay. The most promising ligands demonstrated good affinity less than 25 nM, in comparison with standard PSMA ligand DCL (Glu-urea-Lys) with IC_{50}= 1470 nM in the same test assay. The best ligands were selected for

synthesis of docetaxel conjugates via azide-alkyne cycloaddition. The most active compound showed an CC_{50} value of 2.7 nM vs PSMA positive 22Rv1 cells. This conjugate was tested *in vivo*, on 22Rv1 xenograft mice model and demonstrated dramatic tumor growth inhibition. Also, we evaluated pharmacokinetic parameters such as: C_0, $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, K_{el}, $T_{1/2}$, V_d, Cl; and investigated intracellular ROS concentration under the influence of docetaxel and conjugates based on them. Additionally, acute toxicity, chronic and subchronic toxicities were explored. The results of this experiments would be minutely discussed in the report. Acknowledgments: The reported study was funded by RFBR, project number 20-33-70089 (ligands screening). This work was done mainly in MSU and kindly supported by Ministry of Education and Science of the Russian Federation (No. 03.G25.31.0219) (full scope of *in vivo* evaluation).

ShT-06.2-2
Safety and efficacy of a promising cationic nucleolipid formulation for the nanodelivery *in vivo* of an anticancer ruthenium(III) complex in human preclinical models of breast cancer

M. Piccolo[1], M.G. Ferraro[1], F. Maione[1], G. Misso[2], D. Montesarchio[3], L. Paduano[3], R. Santamaria[1], C. Irace[1]
[1]*Department of Pharmacy, School of Medicine, University of Naples "Federico II", Naples, Italy,* [2]*Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy,* [3]*Department of Chemical Sciences, University of Naples "Federico II", Naples, Italy*

New anticancer drugs based on ruthenium complexes are among the most investigated and advanced non-platinum metallodrugs. Behaving as prodrugs capable of generating *in situ* active cytotoxic species, Ru(III) complexes exhibit a strong antiproliferative activity in addition to a lower toxicity. In this context, our current efforts have been focused on a novel approach for the *in vivo* delivery of a Ru(III) pyridin NAMI-A analog – nicknamed AziRu – caged into nucleolipidic nanocarriers, preparing a suite of stable Ru-based formulations endowed with significant anticancer activity, previously published in: Irace C et al. (2017) SciRep 7, 45236 and Piccolo M et al. (2019) SciRep 9(1), 7006. Here, we report about both safety and efficacy, and cellular response to a cationic DOTAP-based nanosystem loaded with AziRu, by focusing on its ability to inhibit breast cancer *in vivo*. To evaluate biological responses to systemic administration and effects on the progression of breast cancer, we performed an antitumour study using athymic nude mice bearing human BCC xenografts. Results showed a significant inhibition of tumor proliferation in treated mice. In addition, to investigate prospective toxicity in treated groups, we collected mice intracardiac blood samples to carry out blood count test, kidney and liver toxicity tests, leukocyte formula and coagulation factors dosage. Blood diagnostics, as well as biological responses analysis in both acute and chronic treated mice, demonstrate an excellent tolerability profile, with 100% of survival and no sign of toxicity. Furthermore, pharmacokinetic parameters such as plasma concentration curves, distribution and bioaccumulation will be determined by using ICP-MS spectrometry. Alongside a promising biological response to the therapeutic regimens, these outcomes – coupled to future investigations about the multi-target mode of action – validate the efficacy of our Ru nanosystem for a forthcoming use in clinical trials.

Tuesday 6 July
16:00–18:00, Marmorna Hall A

Molecular machines

S-04.2-1
The structure of human thyroglobulin

F. Coscia[1], A. Taler-Verčič[2], V.T. Chang[1], L. Sinn[3], F. O'Reilly[3], T. Izoré[1], M. Renko[2], I. Berger[4], J. Rappsilber[5], D. Turk[2], **J. Löwe**[1]
[1]*MRC Laboratory of Molecular Biology, Cambridge, United Kingdom,* [2]*Jozef Stefan Institute, Ljubljana, Slovenia,* [3]*Technische Universität Berlin, Berlin, Germany,* [4]*University of Bristol, Bristol, United Kingdom,* [5]*Wellcome Centre for Cell Biology, University of Edinburgh, Edinburgh, United Kingdom*

Thyroglobulin (TG) is the protein precursor of thyroid hormones, which are essential for growth, development and the control of metabolism in vertebrates. Hormone synthesis from TG occurs in the thyroid gland via the iodination and coupling of pairs of tyrosines, and is completed by TG proteolysis3. Tyrosine proximity within TG is thought to enable the coupling reaction but hormonogenic tyrosines have not been clearly identified, and the lack of a three-dimensional structure of TG has prevented mechanistic understanding. Here we present the structure of full-length human thyroglobulin at a resolution of approximately 3.5 Å, determined by cryo-electron microscopy. We identified all of the hormonogenic tyrosine pairs in the structure, and verified them using site-directed mutagenesis and *in vitro* hormone-production assays using human TG expressed in HEK293T cells. Our analysis revealed that the proximity, flexibility and solvent exposure of the tyrosines are the key characteristics of hormonogenic sites. We transferred the reaction sites from TG to an engineered tyrosine donor–acceptor pair in the unrelated bacterial maltose-binding protein (MBP), which yielded hormone production with an efficiency comparable to that of TG. Our study provides a framework to further understand the production and regulation of thyroid hormones.

S-04.2-3
Unveiling transcriptional and extra-transcriptional roles of the RNA Polymerase III machinery

A. Vannini[1,2]
[1]*The Institute of Cancer Research, London, United Kingdom,* [2]*Human Technopole, Milan, Italy*

RNA Polymerase III transcribes essential non-coding short RNAs, such as the entire pool of tRNAs. However, the RNA Polymerase III transcription machinery is additionally involved in fundamental nuclear processes that goes beyond gene trasncription and strongly impacts eukaryotic genome folding and organization. Harnessing an integrated structural biology approach we unveil transcriptional and extra-transcriptional roles of the RNA Polymerase III transcription apparatus.

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

S-04.2-2
Towards molecular architecture of the muscle Z-disk assembly

K. Djinovic Carugo
Department of Structural and Computational Biology, Max Perutz Labs, University of Vienna, Vienna, Austria

The sarcomere is the minimal contractile unit in the cardiac and skeletal muscle, where actin and myosin filaments slide past each other to generate tension. This molecular machinery is supported by a subset of highly organised cytoskeletal proteins that fulfil architectural, mechanical and signalling functions. The ultrastructure of the sarcomere is highly organized and delimited by Z-disks, which play a central role in the mechanical stabilization and force transmission. In the Z-disks – the lateral boundaries of the sarcomere machinery – the protein α-actinin-2 cross-links antiparallel actin filaments from adjacent sarcomeres and additionally serves as a binding platform for a number of other Z-disk proteins. In striated muscle cells, the Z-disk represents a highly organized three-dimensional assembly containing a large directory of proteins orchestrated in a multi-protein complex centred on its major component α-actinin, with still poorly understood the hierarchy and three-dimensional interaction map. On the way to elucidate the molecular structural architecture of the Z-disk, the hierarchy of its assembly and structure-function relationships, we are studying binary and higher order sub-complexes of α-actinin using biophysical, structural and cell biological approaches. I will present our recent data on the interaction of muscle α-actinin with titin and adaptor proteins FATZ-1 and myotilin, forming dynamic fuzzy complexes studied by an integrative structural approach, and discuss findings in view of muscle Z-disk architecture and assembly.

ShT-04.2-2
Gating movements and ion permeation in HCN4 pacemaker channels

A. Chaves Sanjuan[1], **A. Saponaro**[1], D. Bauer[2], H. Giese[3], P. Swuec[1], A. Porro[1], F. Gasparri[1], A. Sharifzadeh[1], L. Alberio[1], O. Clarke[3], F. Mancia[3], W. Hendrickson[3], S. Siegelbaum[3], D. Dario[1], M. Bolognesi[1], G. Thiel[2], B. Santoro[3], A. Moroni[1]
[1]University of Milan, Milan, Italy, [2]TU-Darmstadt, Darmstadt, Germany, [3]Columbia University, New York, United States of America

HCN channels underlie hyperpolarization-activated cation current If/Ih that controls automaticity in cardiac and neuronal pacemaker cells. We present cryo-EM structures of HCN4 in the presence/absence of bound cAMP, displaying the pore domain in closed and open conformations. Analysis of cAMP-bound and unbound structures sheds light on how ligand-induced transitions in the channel cytosolic portion mediate the effect of cAMP on channel gating, and highlights the regulatory role of a Mg^{2+} coordination site formed between the C-linker and the S4-S5 linker. Comparison of open/closed pore states shows that the cytosolic gate opens through concerted movements of the S5 and S6 transmembrane helices. In combination with molecular dynamics, the open pore structures provide insights into the mechanisms of K^+ /Na^+ permeation, revealing distinctive ion-binding dependent adaptation in the selectivity filter. Our results contribute new mechanistic understanding on HCN channel gating, cyclic nucleotide-dependent modulation, and ion permeation.

ShT-04.2-1
Oligomeric self-assembly of a coiled-coil-based bipyramidal protein cage

F. Lapenta[1,2], J. Aupič[1], M. Vezzoli[3], Ž. Strmšek[1], S. Da Vela[4], D.I. Svergun[4], J.M. Carazo[5], R. Melero[5], R. Jerala[1]
[1]Department of Synthetic Biology and Immunology, National Institute of Chemistry, Hajdrihova 19,1000, Ljubljana, Slovenia, [2]University of Nova Gorica, Laboratory for Environmental and Life Sciences, Nova Gorica, Slovenia, [3]Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco Area delle Scienze 11/a 43124, Parma, Italy, [4]EMBL DESY, Notkestr. 85, 22607, Hamburg, Germany, [5]CNB-CSIC, Madrid, Spain

Coiled-coils (CC) are one of the most widespread type of protein super-secondary structure in nature and, due to their properties, have been widely used in protein engineering. Specific arrangements of CC dimers in a polypeptide chain can be used to design *de novo* polyhedral protein cages. However, the design of these modular nanostructures, named Coiled-Coil Protein Origami (CCPO), is intrinsically limited by the number of available orthogonal CC elements. This limitation can be surpassed by using an oligomeric bottom-up approach to cage self-assembly, thus facilitating the design of larger cages. Here, we describe the design and characterization of oligomeric CCPO assemblies with the case study of a bipyramidal CCPO cage, designed both as single-chain and as heterodimeric complex, showing a way to build oligomeric CC-based protein cages that undergo controlled self-assembly. Biophysical characterization and small angle X-ray scattering (SAXS) confirmed the proteins assumed the intended conformation in solution in all cases but one, indicating how the implementation of favorable topologies was crucial for the correct self-assembly of the protein cage. Additionally, we showed how, with the introduction of a protease recognition site in the heterodimeric CCPO bipyramid, we obtained a protein able to undergo an irreversible structural rearrangement and transition from an open conformation to a closed bipyramidal conformation, paving the way for designing dynamic and more complex protein cages.

Tuesday 6 July
16:00–18:00, Marmorna Hall B

Mechanisms of microbiome–host interactions

S-07.2-2
Exploring microbiome function using single-cell isotope probing

D. Berry
University of Vienna, Vienna, Austria

Microbial communities are essential for human nutrition and health as well as for the function of virtually all ecosystems. It remains challenging, however, to identify the activity and function of microbial cells under natural conditions. In recent years, sequencing-based approaches such as metagenomics and metatranscriptomics have greatly expanded our understanding of the composition and metabolic potential of the gut microbiota. Now, new tools are needed to determine the actual activities of the microbes in these communities. Stable isotope-based approaches

are powerful tools to reveal microbial function in situ. This talk will focus on how microbes can be studied at the single cell level using molecular methods combined with two powerful chemical imaging tools: nano-scale secondary ion mass spectrometry (NanoSIMS) and Raman microspectroscopy. I will discuss how isotope probing can be used to identify microbes that are utilizers of specific compounds using foraging of secreted mucus by the gut microbiota as an example. In order to identify intestinal mucin- and mucosal-sugar degrading bacteria *in situ*, we developed stable isotope probing and single-cell analysis approaches using NanoSIMS and Raman microspectroscopy coupled to automated microfluidic sorting and post-measurement sequencing. We find that a diverse consortium of bacteria is involved in mucin degradation, including several members of the underexplored "Candidatus Homeothermaceae" family, highlighting both the complexity of this niche and the potential of Raman-based sorting for identifying key players in targeted processes. We then constructed a 5-species cocktail of commensals that reduce colonization levels of the enteropathogen *Clostridioides difficile* by competing for mucosal sugars, suggesting that this may be a promising approach for rational design of bacteriotherapy.

S-07.2-3
Intestinal antibodies in the context of the gut ecosystem

M. Diard[1,*], E. Bakkeren[2], W. Hardt[2], **E. Slack**[2,*]
[1]*Biozentrum, University of Basel, Basel, Switzerland,* [2]*ETH Zurich, Zurich, Switzerland*

Secretory IgA ("SIgA") is the only component of the adaptive immune system present in the gut lumen, i.e. in the same physical space as the intestinal microbiota. Whilst the specificity of endogenous SIgA for the microbiota remains a hotly debated topic, it is clear that oral vaccines can induce high-affinity, T-cell dependent SIgA responses specific for vaccine-antigens. These can protect the intestine, predominantly by driving enchained growth and agglutination of bacteria deep in the gut lumen. Large bacterial clumps generated cannot interact with the epithelium and are rapidly cleared in the fecal stream. However, the majority of protective SIgA in fact recognizes bacterial surface glycans such as the O-antigens of lipopolysaccharide. As these are highly repetitive and enzymatically generated, single point mutations are sufficient to generate huge changes in glycan structure, and thus vaccine escape. Given the very large population size of most gut bacterial species, within-host evolution is rapid and inevitable. This has long been seen as the Achilles heel of vaccines targeting bacterial surfaces, but in fact the very inevitability of this evolution can be turned to our advantage: Oral vaccines can be specifically designed to direct bacterial evolution in the gut lumen. We have recently demonstrated this concept to force the evolution of attenuation in *Salmonella typhimurium*. "Evolutionary trap" vaccines therefore have the potential to generate an overlooked form of non-sterilizing herd immunity with major implications in clearing livestock reservoirs of zoonotic and animal pathogens. We could also begin to imagine more subtle applications of this technique for directed evolution of bacteria in the gut lumen. *The authors marked with an asterisk equally contributed to the work.

S-07.2-1
Health effects of microbiota mediated food transformations

M. Rajilić-Stojanović
University of Belgrade, Belgrade, Serbia

Gut microbiota responds to several environmental factors of which nutrition is certainly among the most relevant. Health effects of nutrients, both beneficial and hazardous, can be modified by microbiota, while specific food components or dietary patterns have the ability to shape microbiota and thereby impact health. Traditionally, food–microbiota interactions are the most thoroughly studied for specific food components, such as probiotics and prebiotics, while systemic health effects of the predominant, beneficial carbohydrates' metabolites – short-chain fatty acids (SCFAs) – have been widely reported. Given the complexity of both microbiota and food, it is clear that the interplay between these two by far exceeds the effects of the predominant metabolites. In contrast to carbohydrates fermentation, protein fermentation yields numerous, diverse, both health-promoting and health-compromising compounds. In that respect, recently two protein metabolites have been assigned to one pathology – type 2 diabetes (T2D), in the opposite direction. In one study an inverse correlation between the risk of T2D development and the abundance of a microbial metabolite of the amino acid tryptophan (indolepropionic acid) was established. Another microbiota metabolite, that is produced starting from another amino acid – histidine (imidazole propionate) – was found in increased levels in subjects suffering from T2D. This metabolite impairs insulin signaling and is produced only in individuals with specific gut microbiota composition. It has been widely shown that individual microbiota signature has a tremendous impact on response to particular foods and, therefore, provides the basis for a personalized approach to nutrition. Today, when technological developments have enabled detailed microbiota assessment, this should be utilized to define nutritional strategies with a beneficial effect on both *Homo sapiens* and microbiota as only such nutrition can confer long term health.

ShT-07.2-2
Microbiota-derived short-chain fatty acids as modulators of intestinal serotonin transporter

E. Latorre[1,2,3], A. Forcén[1], B. Buey[4], L. Grasa[2,3,4], J.E. Mesonero[2,3,4]
[1]*Department of Biochemistry and Molecular and Cellular Biology, Science Faculty, University of Zaragoza, Zaragoza, Spain,* [2]*Instituto de Agroalimentartio de Aragón (IA2), Zaragoza, Spain,* [3]*Instituto de Investigación Sanitaria de Aragón, Zaragoza, Spain,* [4]*Department of Pharmacology and Physiology. University of Zaragoza, Zaragoza, Spain*

Serotonin is a key neuromodulator of intestinal physiology. Serotonin transporter (SERT) is responsible for serotonin uptake, modulating its availability and consequently, serotonergic signalling. Recently, microbiota has been described to affect intestinal homeostasis through microbiota recognition receptors (TLRs). In fact, TLRs activation seems to regulate intestinal serotonergic system. However, whether intestinal microbiota can modulate SERT by short-chain fatty acids (SCFA) is unknown. Microbiota-derived short-chain fatty acids such as acetate, propionate and butyrate, are important metabolites from non-digestible dietary fibers bacterial fermentation. These metabolites have been shown to maintain intestinal homeostasis through protecting epithelial barrier

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

integrity, promoting IgA production and regulating T-cell differentiation. In this study human enterocyte-like Caco-2/TC7 cells were used as intestinal epithelial cells model, which expresses serotonin transporter. Caco-2/TC7 cells were treated for 24 h with different concentrations of acetate, propionate and butyrate and then, and 5-HT uptake was measured. SERT molecular expression was analysed by measuring both, mRNA levels by real-time PCR and protein expression by western blotting. Our results show that the treatment with SCFA modulates SERT function and expression, in a different way for each fatty acid. Consequently, a different production of SCFA by microbiota could differently modulate SERT and affect to serotonergic signalling and intestinal physiology. Our study contributes to growing evidence about the key role of microbiota on host physiology regulation, and it opens a cutting-edge opportunity of microbiota modulation to balance serotonergic signalling alterations.

ShT-07.2-1
Effect of per oral administration of the ŽP strain, a new potential probiotic, on intestinal microbiota and immune status of rat and chicken

M.V. Kuznetsova[1,2,*], I.L. Maslennikova[1,*], E.G. Orlova[1,*], J.S. Gizatullina[1,*], N.P. Loginova[2,*], Y.N. Troinich[2,*], **M. Starčič Erjavec**[3]

[1]*Institute of Ecology and Genetics of Microorganisms Ural Branch Russian Academy of Sciences, Perm, Russia,* [2]*Perm State Medical University named after Academician E. A. Wagner, Perm, Russia,* [3]*Biotechnical Faculty, Ljubljana, Slovenia*

The spread of pathogenic antibiotic resistant bacteria in poultry farms triggered the development of novel methods for maintaining poultry health during its industrial production. The use of probiotics in this field is promising. A new antimicrobial agent, the ŽP strain, was constructed: the bacteriocin ColE7 immunity gene was inserted into the chromosome of the *Escherichia coli* probiotic strain Nissle 1917 and a conjugative plasmid carrying the colicin ColE7 activity gene was introduced into the strain (Starčič Erjavec et al., 2015). The effect of ŽP strain on 4 weeks old rat (Wistar line), as well as on one-day old chicken (cross ROSS 308) animal model systems after oral administration in the drinking water at a dose of 5×10^8 and 5×10^{10} cell/ml/head was studied. Intestinal microbiota, histology of the intestine, spleen and Peyer's patches, blood biochemistry, hematology, microbicidal activity of leukocytes and macrophages, as well as body weight were analyzed. Our results showed that the ŽP strain effectively colonized rat and chicken intestinal tract and remained in the host animals at ~$1–5 \times 10^7$ cell /g feces for 21 days. Further, our results showed that in the intestinal microbiota, the number of beneficial bacteria (lactic acid bacteria and bifidobacteria) increased, while the number of pathogenic microbes (staphylococci, pseudomonas, clostridia and fungi) decreased, compared to controls with no ŽP strain in the diet. In addition, evidence was obtained that the ŽP strain administered in the drinking water was safe, exhibited a positive effect on local and non-specific immune response of animals and led to weight gain of rats. Thus, due to these beneficial effects revealed by the used rat and chicken animal models the ŽP strain may be considered an efficient potential probiotic for farm animals. The study was carried out in the framework of the project No. C-26/792 supported financially by the Government of Perm Krai. *The authors marked with an asterisk equally contributed to the work.

Tuesday 6 July
16:00–18:00, Povodni mož Hall

Bioinformatics and computational biology

S-06.5-1
Evolution of small RNA pathways in rodents

P. Svoboda

Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic

Small RNA pathways are found in most eukaryotes and serve multitutude roles. Mammals utilize three small RNA pathways: PIWI-associated small RNA (piRNA) pathway, RNA interference (RNAi) pathway and microRNA (miRNA) pathway. All three pathways co-exist in mouse oocytes, which offer a unique model for understanding their function and evolution. The piRNA pathway functions in the germline where it recognizes and suppresses mobile elements. It employs entirely different protein factors than the other two pathways and is non-essential in the mouse female germline. RNAi is an ancient eukaryotic defense mechanism against viruses and mobile elements. In mammals, RNAi became a vestigial pathway, being surpassed during vertebrate evolution by other innate and acquired immunity mechanisms. The molecular mechanism for RNAi remains essentially intact, but it used by the microRNA pathway, which regulates endogenous gene expression. In mouse oocytes, RNAi evolved into an essential mechanism thanks to a long terminal repeat (LTR) insertion in a gene encoding Dicer, a factor producing small RNAs acting in RNAi. This insertion occured in the common ancestor of mice and hamsters and represents a great example how LTRs remodeled gene expression and its control in the mouse germline during evolution. In fact, evolution of the RNAi pathway in mice accommodated effects of dozens of such insertions. The last pathway, the miRNA pathway, is involved in control of gene expression. Genetic analysis showed that maternal miRNAs are non-essential for oocyte growth and development. This is apparently because unique constraints existing in the oocyte. My presentation will summarize our latest results concerning mechanistical and functional overlaps of the three pathways and will provide a revised insight into their (in) significance in the female germline and beyond it.

S-06.5-3
Exascale biology: from genome to climate with a few stops along the way

D. Jacobson

Oak Ridge National Laboratory, Oak Ridge, United States of America

The cost of generating biological data is dropping exponentially, resulting in increased data that has far outstripped the predictive growth in computational power from Moore's Law. This flood of data has opened a new era of systems biology in which there are unprecedented opportunities to gain insights into complex biological systems. Integrated biological models need to capture the higher order complexity of the interactions among cellular components. Solving such complex combinatorial problems will give us extraordinary levels of understanding of biological systems. These exponentially increasing volumes of data, combined with the desire

to model more and more sophisticated sets of relationships within a cell, across an organism and up to ecosystems and, in fact, climatological scales, have led to a need for computational resources and sophisticated algorithms that can make use of such datasets. The traits or phenotypes of an organism, including a its adaptation to its surrounding environment and the interactions with its microbiome, are the result of orchestrated, hierarchical, heterogeneous collections of expressed genomic variants regulated by and related to biotic and abiotic signals. However, the effects of these variants can be viewed as the result of historic selective pressure and current environmental as well as epigenetic interactions, and, as such, their co-occurrence can be seen as genome-wide associations in a number of different manners. We have developed supercomputing and explainable-AI approaches to find complex epistatic architectures responsible for all measurable phenotypes as well as an organism's ability to adapt to its environment and detect and modulate its microbiome. The result is a comprehensive systems biology model of an organism and how it has adapted to and responds to its abiotic and biotic environment which has applications in bioenergy, precision agriculture, and ecosystem studies among other disciplines.

S-06.5-2
Computational modeling of RNA and RNP 3D structures, with the use of experimental data

J.M. Bujnicki, M.J. Boniecki, C. Nithin, S. Mukherjee, I.P.N. Pandaranadar Jeyeram, M. Amiri Farsani, P. Ghosh, B. de Aquino
International Institute of Molecular and Cell Biology, Warsaw, Poland

Ribonucleic acid (RNA) molecules are master regulators of cells. They are involved in many molecular processes: they transmit genetic information, sense cellular signals and communicate responses, and even catalyze chemical reactions. RNA function and in particular its ability to interact with other molecules such as proteins, is encoded in the sequence. Understanding how RNAs and RNA-protein complexes carry out their biological roles requires detailed knowledge of the RNA structure. Due to limitations in experimental structure determination, complete high-resolution structures are available for a tiny fraction of all the known RNA molecules crucial for numerous fundamental cellular processes. < 1% of RCSB entries represent RNA structures, and only around 3% of RNA families available in the Rfam database have at least one experimentally determined structure. This relative paucity of information compared to what is available for proteins also makes purely computational RNA 3D structure prediction much less successful. I will present strategies for computational modeling of RNA and RNA-protein complex structures that utilize SimRNA, a suite of methods developed in my laboratory, which use coarse-grained representations of molecules, rely on the Monte Carlo method for sampling the conformational space, and employ statistical potentials to approximate the energy and identify conformations that correspond to biologically relevant structures. In particular, I will discuss the use of computational approaches for RNA structure determination based on low-resolution experimental data. References 1 Ponce-Salvatierra, A. et al. Biosci. Rep. 39, BSR20180430 (2019) 2 Boniecki, M. J. et al. Nucleic Acids Res. 44, e63 (2016)

ShT-06.5-2
The abundance of intrinsic disorder in proteins, associated with aging

V. Manuylov[1], S. Nesterov[1,2], G. Dayhoff[3], V. Gordeliy[1,4,5], N. Ilyinsky[1], V. Uversky[1,3]
[1]*Research Center for Molecular Mechanisms of Aging and Age-Related Diseases, Moscow Institute of Physics and Technology, Dolgoprudny, Moscow Region, 141701, Russia,* [2]*National Research Center "Kurchatov Institute", Moscow, Russia,* [3]*Department of Molecular Medicine and USF Health Byrd Alzheimer's Research Institute, Morsani College of Medicine, University of South Florida, Tampa, Florida, United States of America,* [4]*Forschungszentrum Juelich GmbH, Juelich, Germany,* [5]*Institut de Biologie Structurale, Grenoble, France*

The development of aging is associated with distortion of several cellular processes. These include genome instability, epigenetic changes, loss of proteostasis, mitochondrial dysfunction, and impaired cellular metabolism (López-Otín C. et al (2013) Cell 153, 1194–1217). Loss of proteostasis defines the pathogenesis of many age-related diseases. Neurodegenerative diseases develop due to the accumulation of toxic aggregates often formed by intrinsically disordered proteins (Uversky VN and Fink AL (2004) Biochim Biophys Acta 1698, 131–153). Such proteins play a number of crucial roles in various cellular processes. To better understand the molecular mechanisms of aging, it is important to find out how abundant the intrinsic disorder in proteins associated with aging is, and in which cellular processes it is most pronounced. Previously, similar analyses were performed for proteins involved in Alzheimer's disease, Parkinson's disease, frontotemporal lobe degeneration, and amyotrophic lateral sclerosis (Uversky VN (2014) Front Biosci (Landmark Ed) 19, 181-258). In this work, we analyzed ~600 proteins associated with aging for their propensity for the intrinsic disorder. To this end, their percentage of predicted intrinsic disorder (PPID, based on 6 algorithms) was evaluated, and the prevalence of MoRFs (Molecular recognition features, which are disorder-based protein-protein interaction sites that undergo binding-induced folding at interaction with their partners) in these proteins was studied. We show that for a group of intracellular proteins that do not perform catalytic functions, the level of intrinsic disorder and abundance of MoRFs are comparable to those found in signaling proteins. This may indicate a role for intrinsic disorder in aging, suggesting that problems of aging are related, at least in part, to dysfunction of signaling proteins, which are often disordered.

ShT-06.5-1
A rational approach for structure design based on a new architectural level

C. Périn[1], Y. Ghouzam[2], J.C. Gelly[1]
[1]*Team DSIMB, Paris, France,* [2]*Paris Diderot University, Paris, France*

Computational Protein Design is a challenging and emerging field of computational biology. Its goal is to create new proteins with new functions. While most work are focused on the discovery of the appropriate sequence to fold in a given architecture, some aim to define original and new structures. But despite recent breakthroughs in the field, designed proteins are small or display simple conformations, thus limiting the possibilities. A simple way to build new complex objects consists in the combination of some more simple parts. Identify and use such parts of

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

protein architecture could ease and rationalise the creation of new proteins. Well-known protein structure organisation levels are secondary structures and domains, respectively too simple or too complex to be efficiently reassembled in new ensembles. Since the birth of structural biology field, the existence of an intermediate level has been discussed and recurrent structural motifs called supersecondary structures have been identified. These intermediate-sized motifs are mandatory to explain protein evolution and folding. Still, only a few have been discovered so far. We thus systematically and exhaustively characterized suitable sub-regions of proteins with complexity and size between secondary structures and domains that we denominated Protein Units. We demonstrated that these 3.000 recurrent structural motifs identified without a priori correspond to a new level of structural organisation and would be useful objects to build artificial proteins. We also studied their implication in protein evolution, genesis and folding.

Wednesday 7 July
9:00–11:00, Kocka Hall

Cancer immunology and immunotherapy

S-08.1-3
Prognostic and predictive value of tumor-associated B cells and tertiary lymphoid structures in human cancers

C. Sautès-Fridman[1], F. Petitprez[2], J. Calderaro[3], M. Meylan[1], W.H. Fridman[1]
[1]*Centre de Recherche des Cordeliers, INSERMU1138, Paris, France,* [2]*Programme Cartes d'Identité des Tumeurs, Ligue Nationale contre le Cancer, F-75013 Paris, France,* [3]*Département de Pathologie, Assistance Publique Hôpitaux de Paris, Groupe Hospitalier Henri Mondor, Créteil, Paris, France*

While much has been elucidated regarding the importance of T-cells in the therapeutic response to immune checkpoint blockade (ICB) in cancer, data regarding the characterization and contribution of B-cells in patient's survival and ICB response are scarce. Tertiary lymphoid structures (TLS) are B-cell rich ectopic lymphoid organs that develop at sites of chronic inflammation and in tumors. TLS are associated with clinical benefit in most solid cancer types. Soft-tissue sarcomas (STS) represent a heterogenous group of tumors with low response to ICB. We established an immune-based transcriptomic classification identifying five distinct phenotypes called SIC (sarcoma immune classes) A to E. We show that the immune high SIC E is characterized by the presence of B cell-rich TLS. B cells are the strongest prognostic factor even in the context of high or low $CD8^+$ and cytotoxic contents. SIC E patients had improved survival and were associated with response to PD-1 blockade by pembrolizumab in a phase 2 clinical trial in advanced STS. We also show a role for B-cells within TLS in the response to ICB in patients with melanoma and kidney cancer, hinting towards a broader applicability. TLS undergo maturation from lymphoid aggregates to B cell rich secondary follicles. In hepatocellular carcinoma (HCC), an inflammation-driven cancer characterized by a series of adjacent preneoplasic lesions driving to full blown HCC, we show that the presence of intratumoral TLS and their degree of maturation are linked with lower rates of early relapse. The early lesions are characterized by immature lymphoid agregates in an immune activated microenvironment, along with immune inhibitory and immunosuppressive characteristics, suggesting that immunity may not be fully efficient, and paradoxically favor immune evasion and cancer progression. These results highlight the impact of the maturation and localization of TLS in tumors, from the early stages to the late stage of cancer development.

S-08.1-1
The role of MDSC in regulation of immune response

V. Umansky[1,2], R. Weber[1,2], X. Hu[1,2], I. Arkhypov[1,2], P. Altevogt[1,2], J. Utikal[1,2]
[1]*Clinical Cooperation Unit Dermato-Oncology, German Cancer Research Center (DKFZ), Heidelberg, Germany,* [2]*Department of Dermatology, Venereology and Allergology, University Medical Center Mannheim, Ruprecht-Karl University of Heidelberg, Mannheim, Germany*

Despite the recent progress in melanoma immunotherapy, a number of patients show a poor response to current treatments. Insufficient anti-tumor reactivity could be induced by the chronic inflammation that results in immunosuppression and cancer progression. Myeloid-derived suppressor cells (MDSC) induced by chronic inflammation play a major role in immunosuppressive tumor microenvironment. Using the RET transgenic murine melanoma model that mimics human melanoma and samples from melanoma patients, we found a significant enrichment and stimulation of MDSC that strongly correlated with melanoma progression and worse clinical outcome. We found that MDSC recruitment to the tumor was mediated by the CCR5-CCR5 ligand interaction. CCR5 expression during MDSC differentiation in vitro was induced by IL-6 via the STAT3 dependent mechanism. IL-6 levels in mouse tumors correlated with the frequency of tumor-infiltrating CCR5+ MDSC. CCR5+ MDSC showed increased phosphorylated STAT3 levels. In addition, an accumulation of activated MDSC could be mediated not only by soluble inflammatory factors but also by extracellular vesicles (EV) secreted by tumor cells. EV contain different biologically active molecules such as proteins, mRNA, microRNA and lipids and are considered as mediators of intercellular communication. We demonstrated that EV isolated from Ret mouse melanoma cells induced an upregulation of programmed cell death ligand 1 (PD-L1) on murine immature myeloid cells (IMC) via TLR signaling that resulted in the inhibition of T cell functions. Similarly, EV from human melanoma cells or plasma of melanoma patients induced PD-L1 upregulation on normal monocytes, resulting in their conversion into immunosuppressive cells. Our results suggest that the generation of highly immunosuppressive MDSC in melanoma could be mediated both by soluble inflammatory factors and by tumor-derived EV PD-L1, indicating promising targets for cancer immunotherapy.

S-08.1-2
Multiple defects of natural killer cells in cancer patients: anarchy, dysregulated systemic immunity, and immunosuppression in metastatic cancer

A. Jewett
UCLA, 10833 Le Conte Ave, Los Angeles, CA 90095, United States of America

We have previously demonstrated that natural killer (NK) cells are the main immune effectors that can mediate selection and differentiation of different cancer stem cells and poorly- differentiated tumors via lysis and secreted or membrane-bound interferon-γ and tumor necrosis factor–α, respectively. This leads to growth inhibition and tumor metastasis curtailment. In this report, we present an overview of our findings on NK cell biology and its significance in selection and differentiation of stem-like tumors using in vitro and in vivo studies conducted in non-obese diabetic/severe combined immunodeficiency/interlukin-Rγ-/-, humanized–bone-marrow/liver/thymus mice (Hu-BLT), and those of human cancer patients. Moreover, we present recent advances in NK cell expansion and therapeutic delivery and discuss the superiority of allogeneic supercharged NK cells over their autologous counterparts for cancer treatment. We also review potential loss of NK cell numbers and function at neoplastic and pre-neoplastic stages of tumorigenesis as a potential mechanism for pancreatic cancer induction and progression. We believe that NK cells should be placed highly in the armamentarium of tumor immunotherapy due to their indispensable role in targeting cancer stem-like undifferentiated tumors and a variety of other key NK cell functions that are discussed in this report, including their role in CD8+ T-cell expansion and targeting gene knockout or dedifferentiated tumors. The combination of allogeneic supercharged NK cells and other immunotherapeutic strategies such as oncolytic viruses, antibody-dependent cellular cytotoxicity–inducing antibodies, checkpoint in-hibitors, chimeric antigen receptor (CAR)-T cells and CAR-NK cells, chemotherapeutics, and radiotherapeutic strategies can be used for optimal eradication of tumors.

ShT-08.1-1
Super-charged NK cells preferentially expand $CD8^+$ T cells; Results from pancreatic cancer patients and tumor-bearing humanized-BLT mice

M. Ko[1], K. Kaur[1], A. Jewett[1,2,3]
[1]*Division of Oral Biology and Oral Medicine, School of Dentistry, UCLA, Los Angeles, California, United States of America,* [2]*The Jane and Jerry Weintraub Center for Reconstructive Biotechnology, UCLA School of Dentistry, Los Angeles, California, United States of America,* [3]*The Jonsson Comprehensive Cancer Center, UCLA, Los Angeles, California, United States of America*

Natural killer (NK) cells are known to play a crucial role in halting the progression of aggressive tumors by selecting and differentiating cancer stem cells through direct cell lysis and IFN-γ secretion, respectively. In this report, we present evidence for the role of super-charged NK cells in expanding preferentially large numbers of $CD8^+$ T cells. Osteoclast (OC) expanded super-charged NK cells from healthy donors expand for a longer period of time (day 30–60) before the small fraction of contaminating T cells start expanding resulting in the decline of NK cells and expansion of $CD8^+$ T cells. Kinetics of expansion of $CD8^+$ T cells (day 12-24) with OC expanded super-charged NK cells from cancer patients is faster when compared to healthy controls. In addition, dendritic cells (DCs) are found to promote faster kinetics of T cell expansion than OCs. Moreover, NK cells expanded by DCs expand $CD4^+$ T cells from patient and healthy donors. Addition of αCD3 monoclonal antibody inhibits T cell proliferation while enhancing NK cell expansion, however, expanding NK cells have lower cytotoxicity but higher secretion of IFN-γ. Expansion and functional activation of super-charged NK cells by OCs is dependent on IL-12 and IL-15. Super-charged NK cells showed the ability to lyse $CD4^+$ T cells but not $CD8^+$ T cells in target cell visualization assay. In cell death assays, both $CD4^+$ T cells and $CD8^+$ T cells exhibited no significant differences in cell death when either cultured alone or with cisplatin. When comparing primary NK cells, $CD8^+$ T cells expanded by super-charged NK cells or $CD8^+$ T cells expanded by OCs alone, CD8+ T cells expanded by super-charged NK cells exhibited the highest activation and secretion of IFN-γ. In vivo, oral tumor-bearing humanized-BLT mice injected with super-charged NK cells demonstrated an increase in numbers and function of $CD8^+$ T cells. Thus, NK cells are important in the selection and expansion of $CD8^+$ T cells by increased targeting of $CD4^+$ T cells.

ShT-08.1-2
Antigen-specific stimulation and expansion of CAR-T cells using membrane vesicles as target cell surrogates

V. Ukrainskaya
Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaya 16/10, 117997 Moscow V-437, Russia

Development of CAR-T therapy led to immediate success in the treatment of B cell leukemia and lymphoma. It also raised an opportunity to design new protocols to target solid tumors. Manufacturing of therapy-competent functional CAR-T cells needs robust protocols for ex vivo/in vitro expansion of modified T-cells. This step is challenging, especially if non-viral low efficiency delivery protocols are used to generate CAR-T cells. Modern protocols for CAR-T cell expansion are based on incubation with high doses of recombinant cytokines to support proliferation, non-specific stimulation with surface-bound antibodies to induce TCR cross-linking, or co-cultivation with antigen-expressing feeder cell lines. These approaches are imperfect since non-specific stimulation results in rapid outgrowth of CAR-negative T cells, and removal of feeder cells from mixed cultures necessitates additional purification steps. In an effort to develop a specific and improved protocol for CAR-T cell expansion, we took advantage of cell-derived membrane vesicles, and the simple structural demands of the CAR-antigen interaction. Our approach was to make antigenic microcytospheres from common cell lines stably expressing surface-bound CAR antigens (antigenic vesicles, AVs), and then use them for stimulation and expansion of CAR-T cells. We developed a rapid, simple, efficient, and inexpensive protocol to generate, stabilize and purify AVs. As proof-of-concept we tested the efficacy of our AV constructs on several CAR-antigen pairs. The data presented in this article clearly demonstrate that our protocol produced AVs with the capacity to induce stronger stimulation, proliferation and functional activity of CAR-T cells

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

than is possible with existing protocols. We predict that this new methodology will significantly improve the ability to obtain improved populations of functional CAR-T cells for therapy. This study was supported by the Russian Foundation for Basic Research grant № 19-29-04087_mk.

Wednesday 7 July
9:00–11:00, Marmorna Hall A

Cellular organization

S-07.3-1
Autophagy as a key cellular stress response mechanism

D. Gozuacik
Koç University School of Medicine, Istanbul, Turkey

Autophagy is an evolutionarily conserved biological process that allows lysosome-dependent degradation and recycling of various components in cells. In addition to a basal activity, autophagy is rapidly upregulated under cellular stress conditions, including starvation, exposure to drugs and toxins, mutant protein accumulation and damage to mitochondria. Acting as one of the major stress response mechanisms in cells, autophagy contributes to the preservation of cellular and organismal health. Consequently, abnormalities of autophagy are linked to several diseases, including cancer, neurodegenerative diseases and genetic rare diseases. In our laboratory in Koç University, we focus on the discovery of novel regulators of autophagy and their characterization at a molecular, cellular and organismal level. Using these novel basic findings, we then investigate how abnormalities of autophagy and related pathways impact pathogenesis and progression of human diseases. Based on our discoveries, we are also interested in finding and developing new diagnosis, treatment and follow-up techniques. Results from our recently published and unpublished studies will be presented, and physiological and pathological implications of our results will be discussed.

S-07.3-2
Architecture and dynamics of the mitotic spindle

I. Tolic
Ruder Boškovic Institute,Division of Molecular Biology, Zagreb, Croatia

The mitotic spindle is a fascinating and complex micro-machine made of microtubules and the accompanying proteins. Spindle microtubules attach to chromosomes via specialized protein complexes called kinetochores. We have recently shown that a bundle of antiparallel microtubules, termed "bridging fiber", connects sister kinetochore fibers. Bridging microtubules are linked together by the protein regulator of cytokinesis 1 (PRC1). To explore the role of bridging fibers in chromosome alignment, we developed an optogenetic approach to remove PRC1 from the spindle to the plasma membrane in a fast and reversible manner by using light. PRC1 removal resulted in reduction of bridging fibers and straightening of outermost kinetochore fibers. The inter-kinetochore distance decreased, the metaphase plate widened, and lagging kinetochores appeared, suggesting that PRC1, by mechanically coupling bridging and kinetochore fibers, regulates spindle mechanics and buffers kinetochore movements in metaphase. During anaphase, bridging microtubules slide apart driven by the motor activity of kinesin-4 and kinesin-5, thereby pushing the attached kinetochore fibers poleward to segregate chromosomes. In addition to pushing and pulling forces, rotational forces (torques) may also exist in the spindle. We showed that the spindle is chiral, which is evident from our finding that bridging fibers follow a left-handed helical path, dependent on kinesin-5. This result cannot be explained by forces but rather by torques. Our theoretical model predicts that bending and twisting moments generate curved shapes of microtubule bundles. We conclude that torques, in addition to linear forces, exist in the spindle and determine its chiral architecture.

S-07.3-3
Multiparametric imaging approaches to dissect the role of ROS and eNOS signaling pathways using chemogenetic tools and genetically encoded biosensors

E. Eroglu
Sabanci University Faculty of Engineering and Natural Sciences, Istanbul, Turkey

Hydrogen peroxide (H_2O_2) is a versatile signaling molecule that belongs to the most studied reactive oxygen species (ROS) in biology. At higher concentrations, it causes oxidative stress while lower concentrations of H_2O_2 modulate intracellular signaling pathways. However, for many decades H_2O_2 signaling pathways have been examined by the administration of physiologically irrelevant levels of H_2O_2 to living cells and tissues. Owing to the lack of suitable tools, the role of endogenous H_2O_2 remained a mystery and led to contradictory results. Here we exploit a yeast-derived D-amino acid oxidase (DAAO) as a chemogenetic tool to generate on-demand intracellular H_2O_2 production with high spatial and temporal resolution. Chemogenetics refers to experimental systems that dynamically regulate the activity of a recombinant protein by providing or withholding the protein's specific biochemical stimulus. D-amino acid oxidases (DAAO) are flavoenzymes that have been exploited as potent H_2O_2-producing chemogenetic tools in life sciences and cancer therapy. These powerful tools enable experimenters to precisely control the spatial and temporal production of specific signaling molecules on command and to trigger distinct signaling pathways, metabolic activities, or to evoke cytotoxicity and cell death. We exploited multiparametric imaging approaches to visualize different intracellular ROS related parameters such as nitric oxide (NO) and H_2O_2 to define the relationships between ROS and RNS in cultured endothelial cells.

ShT-07.3-1
The SNARE proteins vti1a and vti1b affect myogenesis

N. Heitzig, C. Wiegand, J. Grosse, G. Fischer von Mollard
Department of Biochemistry III, Bielefeld University, Bielefeld, Germany

Myogenesis is the process of embryonic muscle development, or takes place during regeneration after severe muscle injury. Intracellular trafficking seems to control muscle differentiation and maturation, e.g. by providing the plasma membrane of myoblasts

with myogenesis-specific fusion proteins. Such transport routes require soluble N-ethylmaleimide-sensitive-factor attachment receptor (SNARE) proteins for proper fusion of transport vesicles with the target membrane. Little is known about the influence of SNARE-dependent intracellular vesicular trafficking on myogenesis. Here we show, that the loss of the SNARE proteins vti1a and vti1b (double-knockout DKO), which is perinatal lethal, leads to reduced skeletal muscle mass and development without distinct muscle groups in the limbs of mouse embryos (E18.5). Biochemical and immunohistological analyses suggest an influence of vti1a/vti1b on myogenic regulatory factors (MRFs). Moreover, we observe an altered distribution of these SNARE proteins during differentiation of C2C12 myoblasts to multinucleated myotubes. Taken together, vti1a/vti1b-assoziated intracellular transport has a pivotal role in proper embryonic muscle development.

ShT-07.3-2
An ATM-Chk2-INCENP signaling pathway delays abscission to prevent chromatin bridge breakage in cytokinesis

E. Petsalaki, G. Zachos
Department of Biology, University of Crete, Heraklion, Crete, Greece

Chromatin bridges are strands of missegregated chromatin connecting the anaphase poles or daughter nuclei and have been linked to tumorigenesis. In response to chromatin bridges in cytokinesis, cells delay abscission, the severing of the narrow cytoplasmic canal that connects the two daughter cells, to prevent chromatin breakage or tetraploidization by regression of the cleavage furrow that are associated with genomic instability and cancer predisposition. In mammalian cells, this abscission-delay is called "the abscission checkpoint" and is dependent on the localization of the Chromosomal Passenger Complex (CPC) at the midbody. The CPC comprises the catalytic subunit Aurora B kinase, the scaffolding protein INCENP and the non-enzymatic subunits Survivin and Borealin; however, the molecular mechanisms that signal chromatin bridges to the CPC are incompletely understood. In the present study, we show that inhibition of the DNA damage kinases ATM or Chk2 impairs CPC-localization to the midbody and correlates with premature abscission and chromatin breakage in cytokinesis with trapped chromatin in human carcinoma cell lines. ATM phosphorylates Chk2-threonine 68 (T68) to activate Chk2 at the midbody. In turn, active Chk2 phosphorylates INCENP at the newly identified site serine 91 (S91) to promote CPC-localization to the midbody, to delay abscission. Expression of siRNA-resistant phosphomimetic mutant INCENP-S91D, but not the wild-type protein, rescues CPC-midbody-localization and prevents chromatin breakage in Chk2-deficient or ATM-deficient cells. In contrast, in the absence of the endogenous INCENP, the non-phosphorylatable mutant INCENP-S91A does not localize to the midbody and its expression promotes chromatin breakage. These results identify an ATM-Chk2-INCENP pathway that prevents chromosome breakage in cytokinesis with chromatin bridges, by promoting CPC-midbody localization through Chk2-mediated INCENP-S91 phosphorylation.

Wednesday 7 July
9:00–11:00, Marmorna Hall B

Molecular interactions of plants with the environment

S-07.4-2
Germplasm meets systems biology – the next green revolution in agroecology

W. Weckwerth
University of Vienna, Vienna, Austria

Genomics, transcriptomics, proteomics, phosphoproteomics, metabolomics and mathematical and statistical computer modelling (PANOMICS) are revolutionizing biology and life sciences (1). However, agroecological questions addressed with systems biology approaches are rather underrepresented. Here an integrated PANOMICS platform and the Vienna Metabolomics Center (VIME) (http://metabolomics.univie.ac.at/) are introduced. We apply these PANOMICS platforms to biomedical up to environmental sciences. A special focus is on the investigation of environmental adaptation of crop plant germplasm collections as well as plant model systems to changing climates and ecosystem analysis (2). Germplasm collections in combination with a PANOMICS platform allow for the systematic investigation of intra- and interspecific genetic and epigenetic variation and consequences for stress physiology, plant productivity and quality. We apply the PANOMICS platform to crop plants potato, tomato, legumes, grapevine, lotus, cacao, millet, wheat, barley, model systems for third generation biofuels *Chlamydomonas reinhardtii*, and other model systems tobacco and *Arabidopsis thaliana*. Further novel algorithms for data driven inverse modelling from untargeted GC-MS and LC-MS based metabolomics data are presented. I will have a specific focus on stress signaling networks in plants and algae which cannot be predicted with any genomic tools. I will review our studies on these signaling pathways and further discuss consequences for plant and algae stress physiology and systems biology. References 1. W. Weckwerth, Green systems biology - From single genomes, proteomes and metabolomes to ecosystems research and biotechnology. J Proteomics 75, 284-305 (2011). 2. W. Weckwerth, A. Ghatak, A. Bellaire, P. Chaturvedi, R. K. Varshney, PANOMICS meets germplasm. Plant Biotechnol J 18, 1507-1525 (2020).

S-07.4-3
Hormone-induced gene regulatory networks in plant defense

S. Van Wees
Utrecht University, Utrecht, Netherlands

To survive attack by pathogens and insects, plants have evolved sophisticated immune signaling networks that enable them to mount an effective defense response upon recognition of the invader. Plant hormones, especially jasmonic acid (JA), salicylic acid (SA), abscisic acid (ABA) and ethylene, emerged as core players in the orchestration of the signaling networks that underlie activation of defense responses. The individual hormone pathways can cross-communicate, providing plants with a highly flexible regulatory capacity to finely tune their defense responses against the attacker at hand. To advance our understanding of

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

the different modes of regulation of hormone-induced defense responses, we performed high-resolution RNA-seq time series analyses of hormone-treated Arabidopsis plants. Together with input of publicly available DAP-seq data and other RNA-seq data, we built dynamic gene regulatory network (GRN) models and inferred novel regulators of the individual and combinatorial hormone-induced responses. The significance of these novel regulatory components was validated by molecular and genetic analyses and by bioassays using pathogens and insects.

S-07.4-1
Study of spatial responses in combination with network analysis reveals mechanisms of plant resistance to viruses

Š. Baebler, T. Lukan, A. Coll, M. Križnik, Ž. Ramšak, M. Zagorščak, M. Pompe Novak, M. Petek, A. Blejec, **K. Gruden**
National Institute of Biology, Ljubljana, Slovenia

Infection of a plant by a pathogen initiates a complex interaction between both players involved, leading to changes in the complex signalling network, which result in gene activity changes and reprogramming of the cell metabolism. A systems biology approach was adopted to understand the mechanisms and dynamics involved in potato plant defense following the infection with potato virus Y. We collected multi-layered omics datasets with spatiotemporal resolution. A qualitative model of potato plant immune signalling network was constructed describing the biosynthesis and signal transduction pathways for three crucial phytohormones involved in plant defence: salicylic acid, jasmonic acid and ethylene. The prior knowledge from literature was expanded with information on the viral and plant component interactions, protein-protein interactions and protein-DNA interactions in plant Arabidopsis and translated to potato. Additionally, potato smallRNA regulatory network and gene regulatory networks were constructed from experimental data and overlayed with prior knowledge network. The resulting robust qualitative model offers new insights into the plant-virus interaction by expanding the knowledge on critical properties of plant defence signalling, thus producing novel hypotheses to be tested in the wet lab. Examples of two tested and confirmed hypothesis will be given.

ShT-07.4-2
Omic insights into the molecular response of *Sargassum vulgare* to acidification at volcanic CO_2 vents

S. Nonnis[1,2], A. Kumar[3], I. Castellano[4], H. AbdElgawad[5], G.T. Beemster[6], M.C. Buia[3], G. Tedeschi[1,2], A. Palumbo[4]
[1]*Dipartimento di Medicina Veterinaria, DIMEVET, Università degli Studi di Milano, Lodi, Italy,* [2]*CRC "Innovation for wellbeeing and environment" (I-WE), Università degli Studi di Milano, Milan, Italy,* [3]*Stazione Zoologica Anton Dohrn, Department of Integrative Marine Ecology, Marine Research Center, Ischia, Naples, Italy,* [4]*Stazione Zoologica Anton Dohrn, Department of Biology and Evolution of Marine Organisms, Naples, Italy,* [5]*Department of Botany, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt,* [6]*University of Antwerp, Department of Biology, Integrated Molecular Plant Physiology Research Group, Antwerp, Belgium*

Ocean acidification is impacting marine life all over the world. Understanding how species are able to cope with the changes of seawater carbonate chemistry represents a challenging issue. We addressed this topic using underwater CO_2 vents that naturally acidify some marine areas occurring off the island of Ischia. In the most acidified area of the vents, the biomass is dominated by the brown alga *Sargassum vulgare*. In the present study, we performed the characterization of the *S. vulgare* proteome, by a label-free quantification MS-based shot-gun proteomic approach, together with metabolites analyses to identify the key proteins, metabolites and pathways affected by ocean acidification. Several differentially expressed proteins were involved in a wide range of cellular and metabolic processes such as protein synthesis and folding, transport, photosynthesis and oxidation-reduction. Analyses of other metabolites revealed variations in the levels of some fatty acids and phenols. Overall, the results obtained by proteins and metabolites analysis, integrated with previous transcriptomic (1), physiological and biochemical studies, have allowed to delineate the molecular strategies adopted by S. vulgare to grow in future acidified environments. 1. Previously published in: Kumar A et al. (2017) Molecular Ecology 26

ShT-07.4-1
How is salicylic acid perceived in potato?

Š. Tomaž[1,2], A. Coll[1], T. Stare[1], T. Lukan[1], K. Pogačar[1], R. Vollmeier[1], K. Gruden[1]
[1]*National Institute of Biology, Ljubljana, Slovenia,* [2]*Jozef Stefan International Postgraduate School, Ljubljana, Slovenia*

Potato (*Solanum tuberosum*) is one of the most important crops of our time, produced, processed and consumed by countries all around the globe. At the same time, it is exposed to a number of plant pathogens, causing high loss in yield and lower product quality. Plants respond to pathogen infections with a complex network of signalling cascades, which are orchestrated by hormones and ultimately affect the expression of defence genes. One of the main hormonal pathways in plant immunity is the salicylic acid pathway, which is also a key component of potato defence response to potato virus Y (PVY), the most dangerous virus infecting potato. The salicylic acid pathway has been extensively studied in *Arabidopsis thaliana*, where it regulates the expression of *PR-1* defence gene through NPR transcription cofactors and TGA transcription factors. Although this pathway has also been investigated in some economically important crops, the transcription regulation during potato PVY infection is mostly unknown and data on similar mechanisms in potato are scarce. We have selected three potato TGA transcription factors involved in potato defence response, investigated the chromosomal arrangement of their genes, classified them phylogenetically and analysed their protein sequences and domains *in silico*. We determined the localization of all three TGAs in plant cells and studied the protein–protein interactions among them, their interactions with potato NPR cofactors and proteins from other signalling pathways using the yeast two-hybrid assay. As we found several positive interactions between investigated proteins in yeast, we continued with their confirmation *in planta* with co-immunoprecipitation experiments, currently in progress. The results of our study provide new insights into salicylic acid mediated transcription regulation of defence genes and will help us to better understand the potato immune response on a molecular basis.

Wednesday 7 July
9:00–11:00, Gallery Hall

Life on the edge – extremophilic/ extremotolerant organisms

S-07.5-2
New thermophilic prokaryotes with unusual metabolic pathways

E. Bonch-Osmolovskaya[1,2], E. Frolov[1], A. Slobodkin[1], T. Kochetkova[1], A. Lebedinsky[1], I. Kublanov[1]
[1]*Federal Research Center of Biotechnology RAS, Moscow, Russia,* [2]*Lomonosov State University, Moscow, Russia*

Thermophilic prokaryotes inhabiting natural thermal environments are phylogenetically and metabolically diverse, often representing deep phylogenetic lineages and specific metabolic groups. The latter comprise lithoautotrophic thermophiles utilizing inorganic energy substrates of volcanic origin and assimilating inorganic carbon for cell material synthesis. Thermoacidophilic hydrogenotrophic sulfate-reducing bacterium *Thermodesulfobium acidiphilum* represents a deep phylogenetic lineage of the phylum level. It was found to assimilate inorganic carbon via a novel version of the Calvin cycle – its transaldolase variant with RubisCO Form III playing the pivotal role in carbon assimilation; previously published in: Frolov et al. (2019) Proc Natl Acad Sci USA. 116, 18638-18646. The genome of *Deferribacter autotrophicus* (phylum Deferribacteres, lithoautotrophic growth by hydrogen-dependent iron reduction) was found to contain no genes of the classic autotrophic pathways; previously published in: Slobodkin et al. (2019) Genes 10, 849. Thus, we assumed that this organism assimilates CO_2 via the recently proposed reversible tricarboxylic acids cycle. New thermophilic bacterium *Tepidiforma bonchosmolovskayae* is capable of lithoautotrophic growth by aerobic iron respiration and represents a novel class Tepidiformia in phylum Chloroflexi; previously published in: Kochetkova et al. (2020) Intern J System Evol Microbiol 70,1192-1202. Its genome contains some but not all the genes encoding enzymes of the hydroxypropionate pathway of CO_2 assimilation, which suggests that this organism might possess a new version of this cycle. These and other examples demonstrate the diversity of inorganic carbon assimilation pathways in thermophilic prokaryotes, being analogs of early Earth inhabitants, and provide material for hypotheses regarding their evolution. This work was supported by RFBR grant # 20-54-20001.

S-07.5-1
How archaea swim: the archaellum

S. Albers
University of Freiburg, Freiburg, Germany

The archaeal motility structure, the archaellum, is a unique structure, which is evolutionary not related to bacterial flagella, but shows structural homology to type IV pili. The archaellum is a rotary motility structure which achieves forward propulsion of the cells by ATP hydrolysis. We have analysed the subunit interaction of archaellum components from different archaeal species and performed detailed studies on archaellum assembly in the crenarchaeon *Sulfolobus acidocaldarius* and euryarchaeon *Haloferax volcanii*. In the presentation our current understanding of archaellum assembly and how rotation of the structure is achieved, will be discussed.

S-07.5-3
Functional metagenomics of microbial communities in desert soils

D. Cowan
University of Pretoria, Pretoria, South Africa

Desert soils represent some of the most extreme environments on Earth. Their exposure to extremes of temperature, desiccation and physical instability, together with oligotrophy, elevated salinity and oxidative stress, have led researchers to assume that such systems would support low diversity and highly homogenized microbial communities with limited capacity to adapt to changes in environmental conditions. Our studies of the microbial ecology of open soil and niche habitats in the Namib Desert of southwestern Africa have provided some understanding of the structures, functions, responses and evolution of microbial communities in these extreme hyper-arid edaphic environments. The applications of modern metagenomic and meta-transcriptomic methods, together with physicochemical and micro-environmental data, have demonstrated that these microbial communities are more diverse, responsive and adaptive than previously thought, and that the drivers of community structure and function are extremely complex. References 1. Armstrong A, Valverde A, Ramond J-B, Makhalanyane TP, Janssen J, Hopkins DW, Aspray TJ, Seely M, Trindade MI, Cowan DA (2016) Temporal dynamics of hot desert microbial communities reveal structural and functional responses to water input. Scient Rep. 6: 34434 2. Gunnigle E, Frossard A, Ramond J-B, Guerrero L, Seely M, Cowan DA. (2017) Diel-scale temporal dynamics recorded for bacterial groups in Namib Desert soil. Scient Rep. 7: 40189 3. León-Sobrino C, Ramond J-B, Maggs-Kölling G, Cowan DA (2019) Nutrient acquisition, rather than stress response over diel cycles, drives microbial transcription in a hyper-arid Namib Desert soil. Frontiers Microbiol. 14 May https://doi.org/10.3389/fmicb.2019.01054

ShT-07.5-1
Autocatalytic activation process of thermostable protease from the extremophilic archaeon *Aeropyrum pernix*

M. Bahun[1], M. Šnajder[1], D. Turk[2,3], N. Poklar Ulrih[1,3]
[1]*University of Ljubljana, Biotechnical Faculty, Chair of Biochemistry and Food Chemistry, Ljubljana, Slovenia,* [2]*Jožef Stefan Institute, Department of Biochemistry and Molecular and Structural Biology, Ljubljana, Slovenia,* [3]*Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins (CIPKeBiP), Ljubljana, Slovenia*

Hyperthermophilic microorganisms are a source of extremely stable enzymes that are desired in industrial applications. Pernisine is a subtilisin-like protease from the archaeon *Aeropyrum pernix* and is remarkably stable at 90–100°C. Under such conditions, it degrades the infective prion proteins, which makes this protease interesting for use as a cleaning agent. In nature, pernisine is synthesized as an inactive precursor composed of the propeptide and the catalytic domain and undergoes the autocatalytic processing into the mature, active protease. Therefore, understanding the maturation process is crucial for preparation

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

of the active pernisine *in vitro*. Here, we addressed the mechanism of pro-pernisine maturation. We showed that the recombinant unprocessed pro-pernisine produced in *Escherichia coli* is disordered and unable to undergo the autocatalytic activation. Using spectrofluorimetry, circular dichroism spectroscopy and electrophoresis we demonstrated that Ca^{2+} ions induce a two-state transition of pro-pernisine, from disordered Ca^{2+}-free state into the folded Ca^{2+}-bound state. This transition is a prerequisite for efficient pro-pernisine maturation, a process that is completed by dissociation and degradation of the propeptide by the catalytic domain. Besides the conserved Ca^{2+}-binding sites from the other thermostable subtilisins, pernisine contains a unique insertion sequence, which presumably forms an additional Ca^{2+}-binding site. Indeed, this insertion proved to be crucial for the mature pernisine stabilisation. Furthermore, we demonstrated that the propeptide forms a tight inhibitory complex with the catalytic domain only at high temperatures. Such temperatures are in turn required for the propeptide destabilisation upon its dissociation, making it susceptible for degradation by the catalytic domain. These results provide a novel insight into the maturation of thermophilic subtilisins.

ShT-07.5-2
The interaction between the F55 virus-encoded transcription regulator and the RadA host recombinase reveals a common strategy in Archaea and Bacteria to sense the UV-induced damage to the host DNA

I. Iacobucci[1,2], S. Fusco[3], M. Aulitto[3], G. Crocamo[3], P. Pucci[4], S. Bartolucci[3], M. Monti[2,5], P. Contursi[3]
[1]*CEINGE-Advanced Biotechnologies, NAPOLI, Italy,* [2]*Department of Chemical Sciences, University of Naples "Federico II", NAPOLI, Italy,* [3]*Department of Biology, University of Naples Federico II, NAPOLI, Italy,* [4]*CEINGE Advanced Biotechnologies, Napoli, Italy,* [5]*Department of Chemical Sciences, University of Naples Federico II, Napoli, Italy*

Sulfolobus spindle-shaped virus 1 is the only UV-inducible member of the virus family Fuselloviridae. Originally isolated from *Saccharolobus shibatae* B12, it can also infect *Saccharolobus solfataricus*. Like the CI repressor of the bacteriophage λ, the SSV1-encoded F55 transcription repressor acts as a key regulator for the maintenance of the SSV1 carrier state. In particular, F55 binds to tandem repeat sequences located within the promoters of the early and UV-inducible transcripts. Upon exposure to UV light, a temporally coordinated pattern of gene expression is triggered. In the case of the better characterized bacteriophage λ, the switch from lysogenic to lytic development is regulated by a crosstalk between the virus encoded CI repressor and the host RecA, which regulates also the SOS response. For SSV1, instead, the regulatory mechanisms governing the switch from the carrier to the induced state have not been completely unravelled. Therefore, in this study we have applied an integrated biochemical approach based on a variant of the EMSA assay coupled to mass spectrometry analyses to identify the proteins associated with F55 when bound its specific DNA promoter sequences. Here, we show that the archaeal molecular components (F55 and RadA), which sense the host DNA damage, are functional homologs of λ (CI) and *Escherichia coli* (RecA) system.

Wednesday 7 July
9:00–11:00, Povodni mož Hall

Immune and inflammatory disorders

S-08.3-2
Investigating the timing of NKT cell effector subset differentiation

S. Bortoluzzi, N. Dashtsoodol, **M. Schmidt-Supprian**
TranslaTUM, School of Medicine, Technical University Munich, Munich, Germany

Natural killer T (NKT) cells are a small subset of T cells which acquire unconventional innate/memory-like cellular states during their development. To date, the molecular mechanisms that control the acquisition of their peculiar phenotypes are only incompletely understood. This particularly applies to the earliest developmental phases, which are hard to characterize due to the rarity of the cells. We established a genetic model to induce a timed wave of synchronous NKT cell generation in order to elucidate early developmental phases and functional differentiation of NKT cell subsets in the mouse thymus. The analysis of several known markers confirmed that the NKT cells generated in our genetic system undergo developmental processes which closely resemble the ones physiologically occurring in NKT cells. We therefore thoroughly monitored with high temporal precision the kinetics of RAR-related orphan receptor gamma and promyelocytic leukaemia zinc finger protein during NKT cell development and defined their relation with transcription factors and other markers, as well as TCR signaling, proliferation, cytokine production and gene expression.

S-08.3-1
The T cell/phagocyte interface in inflammation and immunopathology

B. Becher
Inst. exp. Immunology, University of Zurich, Zurich, Switzerland

Whereas T cells are generally thought of as mediators of tissue damage in chronic tissue inflammation, the cellular infiltrate is always dominated by myeloid cells. The granulocyte-macrophage colony-stimulating factor (GM-CSF) was initially classified as a hematopoietic growth factor. However, unlike its close relatives macrophage CSF (M-CSF) and granulocyte CSF (G-CSF), the majority of myeloid cells do not require GM-CSF for steady-state myelopoiesis. Instead, in inflammation, GM-CSF serves as a communication conduit between tissue-invading lymphocytes and myeloid cells. Even though lymphocytes are in all likelihood the instigators of chronic inflammatory disease, GM-CSF-activated phagocytes are well equipped to cause tissue damage. The pivotal role of GM-CSF at the T cell:myeloid cell interface might shift our attention toward studying the function of the myeloid compartment in immunopathology and targeting specifically the crosstalk between T cells and myeloid cells through GM-CSF holds promise for the development of therapeutics to combat chronic tissue inflammation. I will discuss how GM-CSF licenses phagocytes to initiate tissue damage in chronic inflammatory diseases and present new tools for tracing and fate-mapping of GM-CSF expressing cells and their role in tissue inflammation *in vivo*.

S-08.3-3
Clone wars: failure in self-antigen presentation leading to the autoimmune neurodegeneration

A. Belogurov Jr
Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russia

Human leukocyte antigen (HLA) genes encode proteins presenting thousands of self and foreign peptides and, therefore, play a critical role in the immune responses against pathogens and tumor cells as well as those resulting in autoimmunity. Here we report how antigen presentation either by HLA class I or class II may modulate the pathogenesis of autoimmune neurodegeneration including multiple sclerosis (MS). Our data revealed that HLA-DRB1*01:01 may kinetically discriminate between antigenic exogenous peptides and endogenous myelin-derived peptides. Analysis of distribution of HLA-DRB1 locus in more than one thousand relapsing-remitting MS patients and healthy individuals of Russian ethnicity confirmed that carriage of HLA-DRB1*01 is a protective factor. We showed that myelin peptides encapsulated into the mannosylated liposomes, referred as Xemys, suppress development of experimental autoimmune encephalomyelitis (EAE), and furthermore successfully passed the Phase II of clinical trials. Effect of the Xemys is a fine tuning of the immune system rather than simplified Th1/Th2 shift. We demonstrated that exposure to the Epstein-Barr viral antigen LMP1 induces myelin-reactive antibodies *in vivo*. We thus reasoned that antibodies induced against LMP1 during EBV infection might act as inflammatory trigger by reacting with myelin, suggesting molecular mimicry in the mechanism of MS pathogenesis. Here we observed the novel third class of ubiquitin-independent degrons, which promote efficient engagement of myelin by REGα or REGϒ-capped immunoproteasomes. These immunoproteasomes generate significantly enhanced amounts of myelin-derived immunodominant peptides, which being presented by MHC class I on the surface of oligodendrocytes attract cytotoxic lymphocytes, thus suggesting that immunoproteasomes equipped by REGαβ heptamers became deadly machines coordinating autoimmune attack on the myelin sheaths ab intra. Study was supported by Russian Science Foundation 19-14-00262.

ShT-08.3-2
Mice lacking DNA repair factor XLF and MRI show leaky severe combined immunodeficiency (SCID)

S. Castaneda-Zegarra[1,2], Q. Zhang[1,3], A. Alirezaylavasani[1], M. Fernandez-Berrocal[1], V. Oksenych[1,4,5]
[1]*Department of Clinical and Molecular Medicine (IKOM), Norwegian University of Science and Technology (NTNU), Trondheim, Norway,* [2]*Science for Life Laboratory, Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden,* [3]*Department of Cancer Immunology, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway,* [4]*Department of Biosciences and Nutrition (BioNut), Karolinska Institutet, Stockholm, Sweden,* [5]*Institute of Clinical Medicine, University of Oslo, Oslo, Norway*

Non-homologous end-joining (NHEJ) is a DNA repair pathway required to detect, process, and ligate DNA double-stranded breaks (DSBs) throughout the cell cycle. The NHEJ pathway is necessary for V(D)J recombination in developing B and T lymphocytes. During NHEJ, Ku70 and Ku80 form a heterodimer that recognizes DSBs and promotes recruitment and function of downstream factors PAXX, MRI, DNA-PKcs, Artemis, XLF, XRCC4, and LIG4. Inactivation of *Ku70* or *Ku80* genes in mice results in severe combined immunodeficiency phenotype (SCID) and high levels of genomic instability. Deletion of the *Dna-pkcs* gene results in SCID, while inactivation of *Xlf*, *Paxx*, or *Mri* results in viable mice with no or modest phenotypes. A combined deficiency of XLF and PAXX, or XLF and MRI, results in embryonic lethality in mice, which correlates with extensive apoptosis in the central nervous system. These findings indicate important overlapping functions between PAXX, MRI and XLF. To determine if the embryonic lethality is *Trp53*-dependent, we intercrossed mice homozygous for *Xlf* null allele and heterozygous for both *Mri* and *Trp53* ($Xlf^{-/-}Mri^{+/-}Trp53^{+/-}$). We demonstrated that inactivation of pro-apoptotic factor p53 rescues embryonic lethality of $Xlf^{-/-}Mri^{-/-}$ double knockout mice. Similarly, we rescued the embryonic lethality of $Xlf^{-/-}Paxx^{-/-}$ mice. We demonstrated that the triple-deficient mice possessed reduced body weight, size of spleens and thymi, lack of mature B cells in the spleen, and dramatically reduced numbers of T cells in both spleen and thymus. Moreover, there was an accumulation of progenitor B cells in the bone marrow of triple deficient mice. Overall, we concluded that $Xlf^{-/-}Mri^{-/-}Trp53^{+/-}$ and $Xlf^{-/-}Paxx^{-/-}Trp53^{+/-}$ mice possess leaky SCID phenotype.

ShT-08.3-1
Molecular mechanisms of NLRP3 inflammasome activation and its regulation

I. Hafner Bratkovič[1], P. Sušjan[1], D. Lainšček[1], A. Tapia-Abellan[2], P. Pelegrin[2], R. Jerala[1]
[1]*National Institute of Chemistry, Ljubljana, Slovenia,* [2]*Biomedical Research Institute of Murcia, Murcia, Spain*

NLRP3 inflammasome is a multiprotein complex mediating inflammatory response in a variety of inflammatory, metabolic and degenerative diseases. Upon activation with diverse triggers NLRP3 oligomerizes, recruits adaptor protein ASC and pro-caspase-1. Active caspase-1 processes IL-1β and IL-18 cytokines to their mature forms and gasdermin D to a pore-forming protein that induces pyroptotic cell death. Although the role of NLRP3 in various pathologies has been described, not much is known about the molecular mechanism of NLRP3 inflammasome activation. In order to define the role of particular domains of NLRP3 in inflammasome trigger sensing, assembly and autoregulation systematic truncation of NLRP3 and reconstitution of NLRP3 variants in NLRP3-deficient macrophages was performed. We demonstrate that LRR domain is dispensable for NLRP3 activation and self-regulation. A minimal NLRP3 truncation variant was found fully responsive to various canonical NLRP3 activators. Substitution of the pyrin domain of NLRP3 with the CARD domain of NLRC4 or ASC led to a constitutive activation, demonstrating that the pyrin domain restricts NLRP3 in an inactive conformation. NLRC4 inflammasome is formed by self-catalytic polymerization of NLRC4 initiated with bacterial ligand/NAIP complex. We were interested whether similar process is involved in NLRP3 activation. We show that pathological mutations of NLRP3 failed to engage wild-type NLRP3 in a self-catalytic oligomerization, demonstrating that the activating signal is not enhanced at the level of NLRP3 oligomerization, representing an additional level of NLRP3 regulation. These results contribute to the understanding of the molecular basis of NLRP3 inflammasome activation and demonstrate the versatility of

recognition and regulation mechanisms of the innate immune receptors. Previously published in: Hafner-Bratkovic et al. (2018) Nature Communications 9, article no. 5182.

Wednesday 7 July
16:30–18:30, Kocka Hall

Cancer initiation and progression

S-08.2-3
Breast cancer-initiating cells: what we know and what we expect from them

M.G. Daidone[1], E. Mosca[2,*], N. Di Nanni[2,*], V. Appierto[1], C. De Marco[1], L. Milanesi[2]
[1]*Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy,* [2]*Institute of Biomedical Technologies, National Research Council, Segrate (MI), Italy*

A growing body of evidence showed that a small population of breast cancer (BC) initiating cells (BCICs) has a crucial role in tumor development and progression. We performed studies by whole exome sequencing: (1) to investigate whether the genetic heterogeneity detectable in clinical tumors is also represented in the subpopulation of BCICs propagated as tumor-spheres; (2) to identify in BCICs molecular pathways clinically relevant for patient outcome on a series of BCs (bulk tumors) and corresponding BCICs. First study: from a metastatic lesion we derived BCICs, and from tumor tissue and BCICs obtained xenografts. We followed tumor evolution by (i) transition from clinical tumor to in vitro culture, (ii) subsequent passages in the mouse, (iii) metastatic lesions from the 2nd passage xenografts. The intratumoral heterogeneity observed in the clinical sample is maintained in BCICs, suggesting that distinct populations of BCICs sustain distinct subclones, and changes in CNAs after different *in vivo*/*in vitro* passages provide a genomic explanation for the increased aggressiveness experimentally observed. In the transition from cultured cells to tumor in mice, changes due to selection/evolution were observed although all tumors presented the same profile, while comparison of metastatic specimens from the same organs in different mice showed subclonal differences in CNA profile, indicating a different clonal composition in metastatic sites Second study: the genetic similarity between BCICs and the corresponding bulk tumors points towards a dynamic switch during tumor development. Our screening indicates, mostly in the BCICs fraction, mutations in molecular pathways regulating mechanisms of BCIC maintenance, proliferation, treatment resistance which are involved in stem cell pluripotency regulation (i.e., ABC transporters, Notch and signaling pathways), and the presence of actionable targets (within hedgehog and phospholipase-D signaling pathways) suitable for therapeutic approaches. *The authors marked with an asterisk equally contributed to the work.

S-08.2-2
Future pre-clinical drug testing in GBM: 3D, all-human, multiple cell type, *in vitro* systems accurately reflect *in situ* brain microenvironment and can assess differing cytotoxic pathways

G. Pilkington
University of Portsmouth, Portsmouth, United Kingdom

Five decades of treating the most frequent and most malignant brain tumour in adults, glioblastoma multiforme (GBM), with a combination of radiotherapy and DNA alkylating agents has led to little clinical benefit. The failure of virtually all new therapeutic approaches is due to the poor animal models and *in vitro* (2D/monolayer and cellularly homogeneous spheroid) pre-clinical testing systems which are unreflective of the human brain and its multicellular non-neoplastic cell microenvironment. We have, therefore, engineered 3D, all-human, multiple cell type, *in vitro* systems which better reflect the brain microenvironment. Here, these systems incorporate non-neoplastic microglia and astrocytes as well as induced pluripotent stem cells (iPSCs), under differing differentiation pathways, along with biopsy-derived GBM cells under a range of oxygen conditions. These sophisticated *in vitro* systems are then used to assess standard GBM cytotoxics along with re-purposed drugs which act via different pathways. Notably, we previously showed that mitochondria in GBM cells demonstrated numerous mito-DNA mutations, including one which may reflect sensitivity to the tricyclic, clomipramine. We now demonstrate that mitochondria from non-neoplastic astrocytes can be delivered via tunnelling nanotubules (TNTs) to GBM cells thus conferring resistance to clomipramine (which induces GBM cell specific apoptosis/autophagy via a mitochondrial pathway). Thus, we have demonstrated through these sophisticated, *in vitro* human models that micro-environmental non-neoplastic cells can play a major role in drug sensitivity in GBM via both cellular solutes and TNT-mediated organelle transfer. We are now engineering human iPSCs with silencing of gene pathway underlying GBM drug resistance to further develop these systems.

S-08.2-1
Cancer stem cells (CSC): genetic drivers and therapeutic targeting via CCR5

R. Pestell
Baruch S. Blumberg Institute, Pennsylvania Cancer and Regenerative Medicine Center, 3805 Old Easton Rd, Doylestown, Philadelphia, PA 18902, United States of America

Tumor heterogeneity, new driver mutations, aberrant tumor immune responses and the development of cancer stem cells (CSCs) contribute to cancer recurrence and death. CSCs convey phenotypic plasticity, have distinct metabolic needs and contribute resistance to cancer therapies. Our prior studies using genetically engineered mouse models, defined genes (p21CIP1, p27Kip1, p16INK4a, NFkB, Akt1, Cav1, and cyclin D1) governing mammary tumor progression. These, and our subsequent studies, mapped the importance of these pathways in the expansion of interrelated programs governing CSC. Our recent studies show the G protein coupled receptor (GPCR), CCR5, drives BCa stem cell expansion and therapy resistance. CCR5, is normally expressed on a subset of immune cells. We showed CCR5

is expressed in >50% of human breast cancer (BCa), and in >95% of triple negative BCa, correlating with poor outcome. Subsequent studies have confirmed that CCR5 expression is increased in cancers of the breast, prostate, colon and melanoma. We showed that CCR5+ BCa epithelial cells have characteristics of cancer stem cells, forming mammospheres and initiating tumors with >60-fold greater efficiency in mice. Reintroduction of CCR5 expression into CCR5 negative BCa cells promotes tumor metastases and induced DNA repair gene expression and activity. Single cell sequencing identified completely distinct gene expression profiles in CCR5+ BCa vs. CCR5- BCa cells. CCR5 inhibitors dramatically reduced metastasis and augmented cell killing by DNA damage inducing agents. As CCR5 augments DNA repair (DDR) and is expressed selectively on cancerous, CCR5 inhibitors may enhance the tumor-specific activities of DDR-based treatments, allowing a reduction in dose of standard chemotherapy and radiation. CCR5-targeted cancer clinical trials using small molecular inhibitors and biologics have opened to accrual. Jiao, X., Patel,T., Jaeger, D., Pestell, RG. Cancer Research, 2019, Oct 1;79(19):4801-4807.PMID:31292161

ShT-08.2-1
Phenotype switching in melanoma cells resistant to targeted therapy

M. Radić, A. Dekanić, M. Jazvinšćak Jembrek, I. Vlašić, P. Ozretić, M. Herak Bosnar, N. Slade
Ruder Boškovic Institute, Division of Molecular Medicine, Zagreb, Croatia

Melanoma is an aggressive malignancy that is, despite being a rare type of cancer, responsible for the vast majority of skin cancer-related deaths. Recent advances in melanoma therapy, like targeted therapy and immunotherapy, contributed profoundly to the increased overall survival of patients. Nevertheless, the development of resistance to therapy remains a major clinical issue. Targeted therapy, like the BRAF inhibitor (BRAFi) therapy for melanoma patients harboring the V600E mutation, is initially highly effective, but the majority of patients develop resistance and relapse within a few months. To better understand the mechanisms of resistance to the BRAFi targeted therapy, we generated cell lines resistant to vemurafenib, a BRAF inhibitor used for the treatment of late-stage melanoma with the common BRAFV600E mutation. Vemurafenib-resistant human melanoma cell lines were generated by growing primary melanoma, WM793B cell line, and metastatic melanoma, A375M cell line, both harboring the BRAFV600E mutation, in the vemurafenib-enriched medium. The occurrence of resistance was confirmed by MTT assay. Newly generated resistant cell lines showed immense phenotype changes in terms of cell migration and proliferation. Our results indicated partial EM transition, which is known to increase invasive cell properties, promoting resistance to anti-cancer drugs. We performed mass-based parallel cell mRNA sequencing (RNA-seq) and found that the mechanism of resistance differs between the two cell lines. Furthermore, we have demonstrated a significant downregulation of metastasis suppressor genes, NME1 and NME2 and the p53 protein isoform Δ133p53β, which was shown to promote cancer cell invasion. A number of previous studies suggested several mechanisms of resistance and phenotype switching in targeted therapy. Our results set a new direction for further research in therapy resistance that needs to be elucidated.

ShT-08.2-2
Adipocyte-derived extracellular vesicles as new modulators of prostate cancer progression

F. Fontana[1], M. Marzagalli[1], M. Raimondi[1], E. Carollo[2], P. Sartori[1], P. Procacci[1], D.R.F. Carter[2], P. Limonta[1]
[1]*University of Milan, Milan, Italy,* [2]*Oxford Brookes University, Oxford, United Kingdom*

It is known that an association exists between obesity and risk of prostate cancer (PCa). A crosstalk between adipose tissue and PCa has been demonstrated; however, the study of this dialog has been limited to metabolites and adipokines, although emerging evidence points to a key role of extracellular vesicles (EVs) in the control of tumor progression. Herein, we demonstrated that 3T3-L1 adipocyte conditioned media can affect PC3 and DU145 PCa cell features, inducing increased proliferation, associated with AKT phosphorylation, and invasion, correlated with MMP2 and 9 activation, E/N-cadherin switch and Snail upregulation. Moreover, after exposure to adipocyte conditioned media both PCa cell lines were found to accumulate lipid droplets and, more importantly, to undergo a neuroendocrine differentiation, accompanied by CD44 enhanced expression and docetaxel resistance. Notably, these results were confirmed in 3T3-L1 EV-treated PCa cells, where an increase in glucose consumption, mitochondrial activity, ATP production and ROS generation was also observed, suggesting that adipocyte EVs can reprogram PCa metabolism and drive its aggressiveness. Further studies will be performed to identify the adipocyte EV molecular cargo responsible for the modulation of this dialog.

Wednesday 7 July
16:30–18:30, Marmorna Hall A

Aging stress and neurodegeneration

S-08.4-2
Failures in protein clearance partly underlie late onset neurodegenerative diseases and link pathology to genetic risk

J. Hardy
UCL Queen Square Institute of Neurology, London, United Kingdom

As we identify the loci involved in late onset neurodegenerative disease, we are finding that the majority of them are involved in damage response processes. I propose that many of them represent a failure in these damage response processes which underlie late onset disease and that the resultant pathology is a marker of the type of failing damage response: microglial clearance of damaged neuronal membranes in Alzheimer's disease, ubiquitin proteasome clearance in the tauopathies, and lysosomal clearance in Parkinson's disease. I will outline these relationships and contrast these findings with what we learn from the genetics of the early onset forms of the diseases. I will argue that this concept of failing damage response fits squarely with what we know about other late onset conditions and more closely aligns the genetics of these diseases with what we learn from their epidemiology

S-08.4-1
NFYB-1 regulates mitochondrial function and longevity via lysosomal prosaposin

A. Antebi
Max Planck Institute for Biology of Ageing, Cologne, Germany

Mitochondria are multidimensional organelles whose activities are essential to cellular vitality and organismal longevity, yet underlying regulatory mechanisms spanning these different levels of organization remain elusive. From RNAi screens in *C. elegans*, we discovered NFYB-1, a subunit of the NF-Y transcriptional complex, as a crucial regulator of mitochondrial function. NFYB-1 loss leads to perturbed mitochondrial gene expression, reduced oxygen consumption, mitochondrial fragmentation, disruption of mitochondrial stress pathways, decreased mitochondrial cardiolipin levels, and abolition of organismal longevity triggered by mitochondrial impairment. Multi-omics analysis reveals that NFYB-1 is a potent repressor of lysosomal prosaposin, a regulator of glycosphingolipid metabolism. Limiting prosaposin expression unexpectedly restores cardiolipin production, mitochondrial function, and longevity in the nfyb-1 background. Similarly, cardiolipin supplementation rescues nfyb-1 phenotypes. These findings suggest that the NFYB-1/prosaposin axis coordinates lysosomal to mitochondria signaling via cardiolipin to enhance cellular mitochondrial function and organismal health.

S-08.4-3
Mechanisms controlling aberrant phase separation of neurodegeneration-linked RNA-binding proteins

D. Dormann[1,2]
[1]*Johannes Gutenberg University, 55131 Mainz, Germany,* [2]*Institute of Molecular Biology (IMB), Mainz, Germany*

Pathological protein aggregates are a central hallmark of all neurodegenerative diseases. In the related disorders ALS (amyotrophic lateral sclerosis) and FTD (frontotemporal dementia), the pathological aggregates consist mostly of the ubiquitous RNA-binding proteins (RBPs) TDP-43 or FUS. Both proteins are usually located in the nucleus, where they are involved in splicing regulation and DNA damage repair, whereas in neurons and glial cells of ALS/FTD patients, they are lost from the nucleus and accumulate in large cytoplasmic inclusions. Which molecular defects cause TDP-43 or FUS mislocalization and aggregation in ALS/FTD patients and how we can possibly prevent or revert them are central questions we address in the lab. Research over the last few years has suggested that aberrant liquid liquid phase separation (LLPS) of RBPs plays an important role in the formation of pathological RBP aggregates in ALS and FTD. We found that LLPS and stress granule accumulation of FUS can be suppressed by its nuclear import receptor Transportin and by post-translational arginine methylation and that these control mechanisms can be disrupted in ALS and FTD. Moreover, we found that nuclear import receptors can be protective in the most frequent genetic form of ALS/FTD caused by a long hexanucleotide repeat expansion in the C9ORF72 gene. This supports the concept that increasing cellular concentrations of nuclear import receptors could be broadly protective against RBP aggregation in different forms of ALS and FTD. More recently, we found that hyperphosphorylation of TDP-43, a pathological hallmark of pathological TDP-43 inclusions in ALS/FTD patients, suppresses TDP-43 phase separation, renders TDP-43 condensates more liquid-like and dynamic and suppresses accumulation of TDP-43 in cellular RNP granules as well as TDP-43 insolubility in neurons. We propose that TDP-43 hyperphosphorylation is a protective cellular response to counteract TDP-43 aggregation.

ShT-08.4-1
Influence of the BCHE gene polymorphism on the inhibition of butyrylcholinesterase by biscarbamates, a potential Alzheimer's disease drugs

A. Matošević[1], A. Knežević[2], A. Zandona[1], M. Katalinić[1], Z. Kovarik[1], A. Bosak[1]
[1]*Institute for Medical Research and Occupational Health, Ksaverska cesta 2, Zagreb, Croatia,* [2]*Ruder Boškovic Institute, Bijenicka cesta 54, Zagreb, Croatia*

In the middle and late stages of Alzheimer's disease (AD), the activity of butyrylcholinesterase (BChE), a co-regulator of acetylcholine levels in brain and muscles increases, indicating that selective inhibition of BChE can represent a new pathway in treating AD. The *BCHE* gene is highly polymorphic and thus far, 66 isoforms of BChE have been discovered. Clinically, the most interesting is atypical BChE because people with it are not able to hydrolyse succinylcholine, a positively charged muscle relaxant and can experience prolonged apnea if it is administered. The efficiacy of any drug that targets BChE activity can be affected by human *BCHE* gene polymorphisms. We designed and synthesized six biscarbamates with a modified carbamoyl and amine part of the molecule with the aim of determining their BChE inhibition potency toward usual and atypical BChE. The order of magnitude of the carbamylation rate constants k_i of all six biscarbamates for usual BChE was 10^3 $M^{-1}min^{-1}$, which makes these carbamates fast inhibitors for usual BChE. The carbamylation rates of atypical BChE were 400 to 1,500 time slower compred to usual BChE, proving to the fact that new carbamates are very discriminative to usual BChE. The most discriminative were bisdiethylcarbamates indicating that the carbamylation rate of atypical BChE depends on the substituents on the carbamoyl part of the molecule. In order to evaluate synthesized compounds as potential drugs, their cytotoxicity on human hepatic and neuronal cell was determined. The determined IC_{50} values showed that adamantyl in the amine part of the molecule decreases carbamate drugability since these carbamates are toxic in concentration ranges used for the inhibition of both types of BChE. Supported by the CFS grants IP-01-2018-7683, UIP-2017-05-7260 and IMI-IP-2017-2.

ShT-08.4-2
Inhibition of cathepsin X as a novel strategy for the treatment of neuroinflammation-associated diseases

A. Pišlar[1], L. Tratnjek[2], B. Božić[3], G. Glavan[4], N. Zidar[1], S. Peković[5], M. Živin[2], J. Kos[1]
[1]*University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia,* [2]*University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia,* [3]*University of Belgrade, Faculty of Biology, Belgrade, Serbia,* [4]*University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia,* [5]*University of Belgrade, Institute for Biological Research "Siniša Stankovic", Belgrade, Serbia*

Inflammation is closely implicated in the pathogenesis of several neurodegenerative disorders, including Parkinson's disease (PD)

and multiple sclerosis (MS), where the hallmark of neuroinflammation is activated microglia. Microglia-derived lysosomal cathepsins have been increasingly recognized as important inflammatory mediators that trigger signalling pathways that aggravate neuroinflammation. In the past, a contribution to neuroinflammation processes has been shown for cathepsin X *in vitro*, however; the expression patterns and functional roles of cathepsin X in neuroinflammatory brain pathology remained elusive. Our recent studies revealed a strong neuroinflammation-induced upregulation of cathepsin X expression and activity using *in vivo* models that mimic the pathology of PD and MS, respectively. Unilateral injection of lipopolysaccharide into the rat striatum induced strong upregulation of cathepsin X expression and its activity in the ipsilateral striatum and in other brain areas such as cerebral cortex, corpus callosum, subventricular zone and external globus pallidus, whereas the upregulation was mainly restricted to activated microglia and reactive astrocytes. Similarly, a marked increase in expression and activity of cathepsin X was observed in spinal cord in rat model of experimental autoimmune encephalomyelitis. Additionally, cathepsin X upregulation was observed in injured peripheral nerve, localized in the inflammatory cell type, M1 macrophages. Nevertheless, continuous administration of the cathepsin X inhibitor showed moderate protective effects against neuroinflammation-induced degeneration; further indicating that cathepsin X plays a role as a pathogenic factor in neuroinflammation-induced neurodegeneration and represents a potential therapeutic target for neurodegenerative diseases associated with inflammation.

Wednesday 7 July
16:30–18:30, Marmorna Hall B

Redox biology – oxidative stress signalling

S-08.5-3
Network pharmacology approach for stroke therapy: towards the end of medicine as we know it

A.I. Casas
Department of Pharmacology & Personalised Medicine. Faculty of Health, Medicine & Life Science, Maastricht University, Maastricht, Netherlands

Existing drugs fail to provide benefit for most patients. In fact, the efficacy of drug discovery is in a constant decline. This poor translational success of biomedical research is due to false incentives, lack of quality/reproducibility and publication bias. In fact, drug discovery faces an efficacy crisis to which ineffective mainly single-target and symptom-based rather than mechanistic approaches have contributed. Systems medicine opened a new concept of therapeutic treatment focused on the so-called network pharmacology, where several targets modulated at the same time lead to the first effective therapy for high unmet medical need indications with no treatment so far. Therefore, in stroke, we here validated three single ROS-related enzymes, i.e. NADPH oxidase, NO synthase and soluble guanylate ciclase as promising therapeutic targets for brain ischemia within a network pharmacology strategy focused on reducing ROS-dependent detrimental effects in stroke. Pharmacological modulation of these targets leads to less infarct size, reduced blood-brain barrier leakage, improved neuro-motor functioning, reduced ROS biomarkers and therefore direct neuroprotection. Our therapeutic approach is now in the last pre-clinical step (large animal validation) towards a Phase II safety clinical trial, currently being designed.

S-08.5-2
NADPH oxidases in infection and inflammation

U.G. Knaus
University College Dublin, UCD School of Medicine, Dublin, Ireland

A number of diseases and conditions have been associated with prolonged exposure to increased levels of reactive oxygen species (ROS). Similarly, ROS underproduction due to permanently reduced superoxide or hydrogen peroxide (H_2O_2) generation is a risk factor or causative for certain diseases. It is now widely accepted that physiological ROS levels are required for basic cellular functions; in particular the diffusible second messenger H_2O_2 maintains redox signaling, interkingdom communication and antimicrobial host defense. This chemical mediator role is vital at complex eukaryote-prokaryote interfaces such as mucosal barriers. NADPH oxidases, key oxidant generating enzymes in mammalian cells, transmit incoming signals while also actively changing the external environment. Here we present examples of H_2O_2 dependent processes taking place at limited oxygen availability in the gastrointestinal tract that affect the host, the microbiota, pathogens and their interactions, and effective therapeutic approaches. The integration of chemical signals in space and time will be necessary for understanding and therapeutically modifying the mucosal environment in infections and inflammatory conditions.

S-08.5-1
Targeting NRF2 for brain protection in Alzheimer's disease

A. Cuadrado[1,2], A.I. Rojo[2], G. Manda[1]
[1]*Victor Babes National Institute of Pathology, Bucharest, Romania,* [2]*Department of Biochemistry, Medical School, Autonomous University of Madrid, Madrid, Spain*

There is an urgent need to find a neuroprotective therapy for Alzheimer's disease. We are studying the relevance of homeostatic deviations that result from loss of activity of transcription factor NRF2, a crucial regulator of multiple stress responses. Methods: we generated a mouse model that combines amyloidopathy and tauopathy with either wild type (AT-NRF2-WT) or NRF2-deficiency (AT-NRF2-KO). We are also analyzing redox and inflammatory biomarkers in blood of AD patients. AT-NRF2-KO mice exhibit exacerbated deficits in spatial learning and memory and increased markers of proteinopathy, oxidative stress and neuroinflammation compared to AT-NRF2-WT mice. The NRF2 activator dimethyl fumarate (DMF), which is the only approved drug targeting NRF2, and is being used for the treatment of remitting relapsing multiple sclerosis reduced glial and inflammatory markers and improved cognition and motor complications in the AT-NRF2-WT mice. Regarding biomarkers in the blood of AD patients we analyzed the gene expression of 168 genes related to oxidative stress and inflammation in peripheral blood in a cohort of 40 non-demented controls vs. 40 matched early ADs. We found a positive correlation between several NRF2-regulated and several NFκB-regulated

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

genes. This study demonstrates the relevance of normal homeostatic responses that decline with ageing, such as NRF2 activity, in the protection and biomarker monitoring of AD. Acknowledgements: This study was funded by the European Regional Development Fund, Competitiveness Operational Program 2014-2020, through the grant P_37_732/2016 REDBRAIN.

ShT-08.5-1
Oxidative stress-induced DNA damage signaling: the role of poly(ADP-ribose) polymerase 1

Z. Regdon[1], M. Demény[2], C. Hegedűs[1], A. Kiss[1], É. Szabó[1], **L. Virag**[1]
[1]Department of Medical Chemistry, University of Debrecen, Debrecen, Hungary, [2]MTA-DE Cell Biology and Signaling Research Group, Debrecen, Hungary

When the carefully balanced equilibrium between the production and elimination of reactive oxygen and nitrogen species (ROS and RNS, respectively) is disrupted, ROS and RNS can cause cell dysfunction and cell death. In the most severe cases of oxidative stress, necrotic cell death modalities become predominant and contribute to aggravated inflammation. Parthanatos is one such form of regulated necrotic cell death mediated by the NAD-dependent enzyme poly(ADP-ribose) polymerase-1 (PARP1). It is important to mention here that PARP1 is also involved in inflammatory signaling (e.g. NFkappaB and AP1). Based on the role of PARP1 in the DNA damage response, PARP1 inhibitors (PARPi) have been developed and are used for the treatment of DNA repair deficient cancers. It is plausible to hypothesize that these inhibitors can be repurposed for the treatment of oxidative stress-related and PARP1 dependent pathologies such as stroke, neurodegenerative diseases, myocardial ischemia reperfusion injury, various forms of shock and inflammation. Moreover, our recent data also suggest that PARP1 activity can also be indirectly controlled. These novel routes of interventions include NAD availability and regulation of PARP1 expression. Development of cell-based functional assays in our laboratory also identified novel compounds targeting the PARP1-mediated cell death pathway both at proximal and at distal steps. The talk will discuss these novel routes of controlling oxidative stress-induced PARP1-mediated cell death as well as its potential in vivo consequences. Funding: This work was supported by grants from the National Research Development and Innovation Office (GINOP-2.3.2-15-2016-00020 TUMORDNS", GINOP-2.3.2-15-2016-00048-STAYALIVE, OTKA K132193 to LV, PD 116845 to CH), from the Hungarian Academy of Sciences (BO/00468/17/8 to CH) and from the Ministry of Human Capacities (ÚNKP-19-4-DE-299 to CH).

ShT-08.5-2
Hydroxytyrosol treatment ameliorates reserpine-induced fibromyalgia: evaluation of pain, oxidative stress and inflammation

R. Di Paola[1], M.L. Ontario[2], R. Fusco[1], M. Scuto[2], R. Siracusa[1], A. Trovato[2], V. Calabrese[2], S. Cuzzocrea[1]
[1]University of Messina, Messina, Italy, [2]University of Catania, Catania, Italy

Fibromyalgia is a chronic condition characterized by increased sensory perception of pain, neuropathic/neurodegenerative modifications, oxidative and nitrosative stress. An appropriate therapy is hard to find, and the currently used treatments are able to target only one of these aspects. The aim of this study was to investigate the beneficial effects of hydroxytyrosol administration in a rat model of reserpine-induced fibromyalgia. Sprague–Dawley male rats were injected with 1 mg/kg of reserpine for three consecutive days and later administered with hydroxytyrosol. Administration of reserpine led to a significant decrease in nociceptive threshold as well as significant increase in depressive-like symptoms. These behavioral changes were accompanied with increased oxidative and nitrosative stress. Lipid peroxidation was significantly increased, as well as nitrotyrosine and PARP expression, while superoxide dismutase, nonprotein thiols and catalase were significantly decreased. Endogenously produced oxidants species are responsible of mast cells infiltration, increased expression pro-inflammatory mediators and microglia activation. Hydroxytyrosol administration was able to ameliorate the behavioral defects, oxidative and nitrosative stress, mast cells infiltration, inflammatory mediators overexpression and microglia activation induced by reserpine injection with more efficacy than them separate administration.

Wednesday 7 July
16:30–18:30, Gallery Hall

Metabolic engineering: emerging technologies for industrial process development

S-05.1-1
Computational enzyme design for metabolic engineering

J. Damborsky, D. Bednar, Z. Prokop, M. Marek, S. Mazurenko, J. Stourac, S. Marques, G. Pinto, J. Planas-Iglesias, M. Musil, J. Hon, O. Vavra, R. Khan, J. Filipovic, B. Kozlikova
Masaryk University, Brno, Czech Republic

Metabolic engineers strive for optimal enzymes to assemble into highly efficient metabolic pathways. Improvements in the catalytic activity, substrate specificity, or enantioselectivity of enzymes are traditionally achieved by modification of the active sites of enzymes. We have proposed that enzyme engineering endeavours should target both active sites and access tunnels/channels [1,2]. Using haloalkane dehalogenases as model enzymes, we have demonstrated that engineering the access tunnels can significantly improve their catalytic properties [3] and stability [4]. User-friendly software tools such as Caver [5], Caver Analyst [6], CaverDock [7] and Caver Web [8] have been developed for the computational design of protein tunnels/channels; or as FireProt [9] and HotSpot Wizard [10] for the automated design of stabilizing mutations and smart libraries. Using these tools, we were able to introduce a new tunnel to a protein structure and modify its conformational dynamics [11]. We envisage that the next generation of computational enzyme design tools will rely on big data analysis using machine learning [12]. See: https://loschmidt.chemi.muni.cz/peg/software/. 1. Damborsky, J., et al., 2009: Current Opinion in Chemical Biology 13: 26-34. 2. Prokop, Z., et al., 2012: Protein Engineering Handbook, Wiley-VCH, 421-464. 3. Brezovsky, J., et al., 2016: ACS Catalysis 6: 7597-7610. 4. Koudelakova, T., et al., 2013: Angewandte Chemie 52: 1959-1963. 5. Chovancova, E., et al., 2012: PLOS Computational Biology 8: e1002708. 6. Jurcik, A., et al., 2018:

Bioinformatics 34: 3586-3588. 7. Vavra, O., et al., 2019: Bioinformatics 1-8: btz386. 8. Stourac, J., et al. 2019: Nucleic Acids Research W1: W414–W422. 9. Musil, M., et al., 2017: Nucleic Acids Research 45: W393–W399. 10. Sumbalova, L. et al., 2018: Nucleic Acids Research 46: W356–W362. 11. Kokkonen, P., et al., 2018: Journal of the American Chemical Society 140: 17999–18008. 12. Mazurenko, S., et al., 2019: ACS Catalysis 10: 1210–1223.

S-05.1-2
Approaches towards innovative antibiotics from microbes

R. Mueller
Helmholtz-Institute for Pharmaceutical Research Saarland, Saarbrucken, Germany

Amongst the well-established bacterial producers, myxobacteria have a great track record for the discovery of entirely new natural product scaffolds exhibiting promising bioactivities. This is at least in part because they have been much less studied as compared to other traditional sources such as actinomycetes and bacilli. Comparisons of myxobacterial metabolite profiles with the number of underlying biosynthetic gene clusters encoded in their very large genomes show, that many compounds still remain unknown. Further, recent studies indicate that the order of myxobacteria likely comprises many more biodiverse representatives than previously assumed. According to metagenomics analyses, myxobacteria (including many underexplored representatives) are highly abundant in the soil microbiome, where they play a crucial role in soil nutrient and carbon cycling. Taken together with our recent genomic analyses, these findings suggest that the biosynthetic potential of myxobacteria is a long way from being exhausted. Nevertheless, the issue of rediscovery is a major hurdle for myxobacterial extracts as well. In an attempt to tackle this issue, we recently demonstrated that chemical diversity correlates with taxonomic distance in myxobacteria. Accordingly, we are more likely to isolate novel compound classes from strains which are phylogenetically distant from previously characterized strains as compared to closely related strains. This knowledge can be applied to prioritize strains for natural product discovery, thus increasing the chance of discovering compound classes with yet unknown chemical structures and biological activities. I will discuss recent results from our laboratory regarding the identification, structure elucidation, biosynthesis and mode of action of bioactive natural products from (myxo)bacteria based on different approaches, and will show our recent advances in their preclinical development.

S-05.1-3
An engineered Calvin cycle enables the yeast *Komagataella phaffii* to grow and produce organic acids from CO_2

D. Mattanovich
University of Natural Resources and Life Sciences, Muthgasse 18, A-1190 Vienna, Austria

Global warming, caused by rapidly increasing atmospheric carbon dioxide levels, calls for novel measures to sequester CO_2. Assimilation of CO_2 into biomass and bulk chemicals is an attractive option. Besides the use of photosynthesizing organisms, synthetic biology offers the change to develop strains for a novel CO_2 based biotechnology. The methylotrophic yeast *Komagataella phaffii* (formerly known as *Pichia pastoris*) is widely used in the manufacture of industrial enzymes and pharmaceuticals. Like most biotechnological production hosts, *K. phaffii* is heterotrophic, growing on organic feedstocks. By addition of eight heterologous genes and deletion of three native genes, we engineered the peroxisomal methanol-assimilation pathway of *K. phaffii* into a CO_2 fixation pathway resembling the Calvin-Benson-Bassham (CBB) cycle, the predominant natural CO_2 fixation pathway. Due to the modular metabolic design which separates carbon assimilation from energy production, any NADH-yielding energy source can be used. At present methanol oxidation is employed. The resulting strain can grow continuously with CO_2 as a sole carbon source, demonstrating a chemoorganotrophic lifestyle. Intergration of heterologous genes encoding enzymes for the synthesis of short organic acids led to the production of these chemical building blocks at the g/L level. This engineered *K. phaffii* strain may promote sustainability by sequestering the greenhouse gas CO_2 and converting it into yeast biomass and chemicals.

ShT-05.1-2
Improving the use of CRISPR-Cas12a (Cpf1) in cyanobacteria

F.G. Guevara Acosta, S. Baldanta, J. Navarro-Llorens
Universidad Complutense de Madrid, Madrid, Spain

Nowadays, prokaryotes are broadly used in the production of valuable compounds for industrial and pharmacological purpose. One of the most interesting candidates, as microbial cell factories, are the cyanobacteria owing to their capability of carbon fixation of atmospheric CO_2 through photosynthesis, eliminating the need for expensive feedstocks. Therefore, this ability has been coupled to production of a wide range of value products by metabolic engineering such as biofuels, sugars, pigments, bioactive compounds and bioplastics. However, the principal constraint in their full exploitation is the sparse availability of genetic modification tools. In recent years, CRISPR technology for genome editing has made far-reaching changes in the field of genetic modification by enabling precise and highly efficient modification of DNA sequences. This technology enables a tailored engineer for genome markerless modifications such as knock-ins, knock-outs and point mutation in numerous species. The most common tool used has been CRISPR-Cas9 nuclease. Unfortunately, Cas9 has been proved to be toxic in cyanobacteria, to overcome this setback CRISPR-Cas12a (Cpf1) technology has been developed, but the plasmid pSL2680 with Cpf1 has problems of stability and large quantities are needed to work with it. With the objective of improve such a useful technology, the CRISPR-Cpf1 cassette has been tested in a new and more stable vector from the pSEVA collection. This collection is particularly interesting owing to the modular and interchangeable structure of this plasmids, opening a new set of CRISPR tools. In this work, as a probe of concept, the deletion of the gen *nbl*A in *Synechocystis* 6803 has been obtained by the use of the new designed pSEVA-Cpf1 plasmid.

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

ShT-05.1-1
Dye-decolorizing peroxidases from *Streptomyces coelicolor* show organosolv lignin remodeling activity

H. Pupart, E. Ojangu, K. Zovo, T. Lukk
Institute of Chemistry and Biotechnology, Tallinn University of Technology, Tallinn, Estonia

Lignin is the second most abundant biopolymer on earth and makes up about 15–30% of plant biomass. Due to its complex and highly phenolic and heterogeneous nature, lignin is by many seen as a green alternative to petrochemicals. The chemically recalcitrant nature of lignin provides plant biomass with structural rigidity and resistance against degradation by the elements. Several groups of organisms have been identified that can use the lignin portion of lignocellulose as a carbon source. Almost four decades ago some soil bacteria in the actinomycete phylum were identified as lignin degraders by Crawford and coworkers. Thanks to availability of full genome sequences, we now know that these organisms lack a type of the redox enzymes typically associated with fungal biochemical pathways that lead to lignin mineralization – manganese peroxidases (MnPs). Although streptomycetes and other soil bacteria are devoid of MnPs, they encode a different class of heme-containing peroxidases – dye-decolorizing peroxidases (DyPs). We have cloned and heterologously expressed the three DyP-type peroxidases from *Streptomyces coelicolor* – SCO2276, SCO3963 and SCO7193. All three enzymes are able to oxidize the classical peroxidase substrates, such as 2,6-dichlorophenol. However, using HPLC techniques, we also show that all three DyP-type peroxidases can use organosolv lignin as a substrate. We thus hypothesize that next to the small laccase of *S. coelicolor*, the DyP-type peroxidases fulfill the second oxidative enzyme class category required by that organism to break down lignin.

Thursday 8 July
9:00–11:00, Kocka Hall

Structural and functional glycobiology

S-09.1-1
The multiscale architecture of complex carbohydrates

S. Perez[1], F. Spinozzi[2]
[1]*University of Grenoble Alpes, CNRS, Centre de Recherche sur les Macromolecules Vegetales, Grenoble, France,* [2]*Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy*

As with other scientific fields, glycoscience benefits from significant advances in biochemistry and structural biology; detailed knowledge accumulates about the components' nature and the architecture of glycan's complex structures. For the time being, the description of these structures is at length scale ranging from nano- to micrometres. Nevertheless, from these nanostructural components, sophisticated engineering behaviours yield highly complex construction materials. The hierarchical structure of biological materials arises from the generation of systems resulting from subsystems' accretion during self-assembly and growth. Nature retains hierarchical material structures at all levels since most (bio)chemical processes occur over a limited distance. One has to understand the underlying physics and the principles governing the formation of such developments, both in plants and animals, over several orders of magnitude. In plants, the systems are more straightforward and more accessible to a kind of structural analysis. The starch granule structure is an example: the presentation will describe how these complex biological materials' hierarchical structures are assembled over six orders of magnitude. A particular emphasis will be given to applying phyllotactic principles at this level of organisation. The results offer a new model for the 3D structure of amylopectin. Reference. The Architecture of Starch Bloclets follows Phyllotaxic Rules, F. Spinozzi, C. Ferrero & S. Perez, Scientific Reports, (2020) 10, 20093

S-09.1-3
Glycans as biomarkers and functional effectors in diabetes

O. Gornik Kljaić
University of Zagreb Faculty of Pharmacy and Biochemistry, HR-10000 Zagreb, Croatia

Accumulating evidence is demonstrating that N-glycosylation participates in various processes involved in the regulation and maintenance of glucose homeostasis and is tightly connected to nutrient sensing through the hexosamine biosynthetic pathway. Distinctive N-glycosylation profiles are reported for different diabetes subtypes. Pro-inflammatory-like changes of plasma N-glycome were noticed in cross-sectional studies of type 2 diabetes mellitus (T2DM) and N-glycans showed the ability in identifying individuals at high risk of disease development. Individuals with a higher risk due to recorded hyperglycemia through acute illness had increased complexity of plasma N-glycome, possibly reflecting the altered flux of glucose through the hexosamine pathway. This was subsequently confirmed in two prospective studies with incident T2DM showing that N-glycome could predict diabetes years before clinical manifestation. N-glycome also showed great discriminative power in maturity-onset diabetes of the young where triantennary sialylated plasma N-glycan with antennary fucose was successful in extracting individuals with damaging HNF1A mutations. As this phenomenon is also present on alpha-1 acid glycoprotein, targeted analysis of specific glycopeptides could easily be incorporated in diagnostic procedures due to the increasing availability of LC-MS/MS in hospital laboratories. Recently, we also analyzed glycosylation in children with type 1 diabetes mellitus (T1DM). Associations between glycan structures and genetic polymorphisms previously associated with T1DM risk were found. Differences in N-glycomes between children with T1DM and their healthy siblings possess great discriminative potential, which exceeds other predictive models used and is comparable to the most recently established genetic risk score.

S-09.1-2
Functional role of the glycan shield in the activation of the SARS-CoV-2 S protein

E. Fadda
Department of Chemistry and Hamilton Institute, Maynooth University, Maynooth, Co., Kildare, Ireland

Protein glycosylation plays a crucial role in viral pathogenesis, as suggested by the extensive N-glycosylation coat on viral fusion

proteins. Recent structural and glycoanalytic studies have shown that the SARS-CoV-2 spike (S) protein is not shielded as effectively as the envelope glycoproteins of "evasion strong" viruses, with the receptor binding domain (RBD) exposed to potential antibody recognition. Also, experimental evidence indicates important differences in the type of glycosylation, where complex, rather than oligomannose N-glycans, constitute the majority of the SARS-CoV-2 S shield[1]. Understanding the specific functions of this unique glycosylation pattern is particularly tricky because of the glycans' intrinsic conformational disorder prevents them from being easily characterised with standard structural biology techniques. In this talk I will present how high-performance computing (HPC) molecular simulations have contributed to advance our knowledge on the role of glycosylation in the SARS-CoV-2 infection mechanisms. I will focus in particular on how we identified a unique functional role of the glycan shield in the activation of the S glycoprotein[2] and how specific glycoforms intertwined with the S glycan shield's evolution[3] may modulate its binding to ACE2. 1. Watanabe, Y.; Allen, J. D.; Wrapp, D.; McLellan, J. S.; Crispin, M., Science 2020. 2. Casalino, L.; Gaieb, Z.; Goldsmith J.; Hjorth C.; Dommer, A.; Harbison, A.; Fogarty, C.; Barros, E.; Taylor, B.; McLellan J.; Fadda, E.; Amaro, R., ACS Central Sci (2020). 3. Harbison, A.; Fogarty, C.; Phung, T.; Satheesan A.; Schulz B.; Fadda E., bioRxiv (2021)

ShT-09.1-2
The plasticity of the beta-trefoil-type lectins from higher fungi

J. Sabotič[1], J. Kos[1,2]
[1]*Jozef Stefan Institute, Department of Biotechnology, Ljubljana, Slovenia,* [2]*University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia*

Lectins are a diverse group of proteins that specifically bind carbohydrates and act as recognition molecules through binding to glycosylated ligands playing essential cellular and biological functions. We have described lectins isolated from fruiting bodies of different higher fungi, including clouded agaric (*Clitocybe nebularis*) and parasol mushroom (*Macrolepiota procera*). These exceptionally stable proteins share low sequence similarity but similar biochemical properties, which are summarized in the common 3D structure, the beta-trefoil fold. It is formed by the core six-stranded beta-barrel which supports 11 loops of different shapes and composition that provide a versatile surface for different types of interactions. These lectins display extraordinary versatility of carbohydrate-binding specificity, the position of glycan-binding sites and dimerization plasticity. Their unique features are reflected also in their biological activity showing immunomodulatory properties and high specificity and selective toxicity against different cancer cell lines. Lectins from higher fungi offer a wide variety of possible applications in the fields of biotechnology and medicine. Previously published in: Sabotič J and Kos J. (2019) Molecules 24, 4204.

ShT-09.1-1
Individuals at increased risk of type 2 diabetes have increased branching and lower sialylation of alpha-1-acid glycoprotein N-glycans

T. Keser[1], M. Tijardović[1], G. Lauc[1,2], O. Gornik Kljaić[1], M. Novokmet[2]
[1]*University of Zagreb Faculty of Pharmacy and Biochemistry, Zagreb, Croatia,* [2]*Genos Glycoscience Research Laboratory, Zagreb, Croatia*

Glycosylation, the addition of oligosaccharide chains is one of the most abundant co- and post-translational modifications. Changes in human plasma N-glycome are associated with many diseases and represent promising diagnostic and prognostic biomarkers. Recently, we showed that the increased branching of plasma N-glycan structures is associated with higher risk of developing type 2 diabetes. There we measured the whole plasma protein N-glycome, which is comprised of different glycans originating from many different glycoproteins. The most likely candidate for the origin of those glycan changes is alpha-1-acid glycoprotein (AGP), since it is the source of the most branched glycan structures present in the whole plasma protein N-glycome. Using a recently developed high-throughput and site-specific AGP N-glycosylation LC-MS analysis method, we analyzed N-linked glycans on a glycopeptide level from plasma of 59 patients who developed hyperglycemia during ICU hospitalization due to an acute illness (a known predictor of type 2 diabetes) and compared them with glycans from 49 similar ICU patients who remained normoglycemic. Samples were taken after the cessation of inflammatory process was confirmed (based on blood count and CRP). Individuals at higher risk of diabetes presented increased N-glycan branching on AGP's second glycosylation site and lower sialylation of N-glycans on AGP's third and AGP1's fourth glycosylation site. Even though this should be additionally confirmed in larger longitudinal prospective studies, it indicates that site-specific AGP N-glycan profile could help to develop stratification methods which could reliably distinguish individuals who are at risk of type 2 diabetes.

Thursday 8 July
9:00–11:00, Marmorna Hall A

The circadian clock and disease

S-08.6-3
Human clocks, sleep and metabolism

D.J. Skene
Faculty of Health and Medical Sciences, University of Surrey, Guildford, United Kingdom

Biological clocks regulate most physiological processes (e.g. cell cycle, immune function, sleep/wake regulation, metabolism, cardiovascular function). This has implications for time of day sampling, disease diagnosis (biomarker discovery) and therapeutics. Modern life with rapid travel across time zones or working rotating shifts causes a mismatch between the circadian timing system and the sleep/wake, feeding/fasting cycle with adverse health consequences. There is overwhelming epidemiological evidence linking shift work with multiple pathologies including metabolic

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

disorder, cardiovascular disease and cancer, resulting in great cost, both to individuals and society. A key feature of night shift work involves a 3-way mismatch between behavioural rhythms (e.g. sleep, physical activity, and meal timing), the central circadian clock in the hypothalamic suprachiasmatic nuclei (SCN), and peripheral tissue clocks. Studying circadian regulation of metabolism requires reliable markers of human peripheral clocks as well as measuring the SCN-driven circadian rhythms (melatonin, cortisol). Metabolic profiling (metabolomics) provides a novel, powerful tool to investigate the underlying mechanisms linking metabolic disease, circadian misalignment and sleep restriction. Our research has characterised time of day and circadian variation in the human metabolome as well as the effect of sleep deprivation on metabolite rhythms, providing a baseline for future metabolomics studies in shift workers. Following simulated shift work, our recent metabolomics study showed profound misalignment (12 h) of metabolite rhythms. For >90% of the metabolites, 24-h rhythmicity was not locked to the central SCN circadian clock, rather, their rhythms aligned with the behavioural timing of the prior 3-day simulated shift schedule. These findings provide a window onto metabolic pathways potentially involved in the elevated risk of metabolic disorders in shift work.

S-08.6-2
Intercellular coupling between peripheral circadian oscillators by TGF-β signaling

A. Kramer
Charité Universitätsmedizin, 10117 Berlin, Germany

Background: Coupling among single cells allows noisy circadian oscillators to form cohesive and robust rhythm generating networks. While in the SCN, coupling between individual neurons is established, it remains controversial whether coupling exists among single cell oscillators within peripheral tissues. It has been shown by *ex vivo* and *in vivo* imaging that peripheral clocks sustain synchronized rhythms independently of the SCN, suggesting coupling among individual cells. However, the underlying mechanisms are still elusive. Here, we show that peripheral circadian oscillators indeed exchange time information among each other and provide a molecular mechanism driving this intercellular coupling. Results: Rhythmicity of human osteosarcoma cells (U-2 OS) was found to depend on culture density and functional secretory pathway. Co-cultures of U-2 OS cells under 2-D and 3-D conditions displayed characteristics of weak intercellular coupling such as amplitude expansion, decreased damping, as well as phase- and frequency-pulling. This coupling appeared to be achieved via the exchange of paracrine signals since (i) disruption of secretory pathway attenuated intercellular coupling, and (ii) secreted proteins were found to phase shift peripheral oscillators time-dependently and via the rapid, cAMP response elements (CRE) driven transcription of Per2. Ultimately, TGF-β was identified as potential peripheral coupling factor using RNA sequencing and mass spectrometry. Not only did TGF-β induce CRE driven transcription and Per2 expression, but genetic and pharmacological perturbation of TGF-β signaling pathway also weakened circadian rhythms and attenuated intercellular coupling among peripheral circadian oscillators. Conclusion: This data suggests that peripheral circadian oscillators communicate via the exchange of secreted TGF-β, which may act as coupling factor promoting partial synchronization, as well as robustness of peripheral circadian clock networks. From a physiological perspective, intercellular coupling in the periphery likely is of integral importance for the temporal coordination of rhythmic organ functions in response to Zeitgeber signals.

S-08.6-1
Circadian regulation of glucose and lipid homeostasis upon type 2 diabetes

C. Dibner
Department of Endocrinology, Diabetes and Nutrition, Faculty of Medicine, University of Geneva, Geneva, Switzerland

Molecular clocks operative in the peripheral organs play a critical role in orchestrating the respective organ function. Indeed, the pancreatic islet oscillators impact on insulin and glucagon secretion, whereas skeletal muscle clocks regulate glucose uptake, myokine secretion and lipid homeostasis. The worldwide increase in obesity and type 2 diabetes (T2D) represents a major health challenge. Such epidemic growth in metabolic diseases is partly attributed to chronic circadian misalignments and sleep deprivation imposed by the modern 24/7 lifestyle. At the molecular level, circadian oscillators operative in human pancreatic islets and skeletal muscle are attenuated upon T2D. Concomitant with the clock perturbation, temporal regulation of the islet hormone secretion, as well as glucose and lipid metabolism in the skeletal muscle are disturbed. A targeted lipidomic approach, covering a broad range of lipid metabolites involved in energy homeostasis, membrane function, and signaling proved an efficient tool allowing to identify lipids associated with the development of obesity and T2D. In turn, chronically altered lipids induced by obesity may further promote the development of T2D and the accumulation of toxic lipid metabolites in serum and peripheral organs may contribute to the diabetic phenotype. Thus, exploring changes in temporal regulation of lipid species under metabolic diseases is of utmost importance for understanding the disease etiology. Moreover, lipid metabolites altered at the T2D onset, or even at pre-diabetes stage, may serve as novel biomarkers for this disease along with hyperglycemia. Uncovering a novel link between human molecular clockwork and T2D highlights the importance of clock modulators as putative pharmacological intervention to combat this disorder.

ShT-08.6-1
Deletion of the clock gene period 2 from glial cells alters mood-related behavior in mice

T. Martini, J.A. Ripperger, J. Stalin, M. Stumpe, A. Kores, U. Albrecht
University of Fribourg, Fribourg, Switzerland

The major depressive disorder is a common debilitating condition that has major effects both on the individual as well as on their family, and which carries with it a large economic burden due to medical costs, absence from work and lower productivity. According to the WHO, around 800,000 people commit suicide each year as a consequence of depression. Our laboratory has previously shown that a whole-body mutation in the molecular clock gene period 2 (*Per2*) results in depressive-resistant-like mice. This opened opportunities to study signalling that underlies mood-related behaviour via *Per2* manipulation. Our results show that the depressive-resistant-like phenotype can be reproduced by glial *Per2* knockout (KO) alone. Such mice have both reduced despair as well as reduced anxiety, two components of depression, but they have no other defects that are associated with

total-body *Per2* deletion or mutation. The reduced despair and anxiety are paralleled by an upregulation of the GABA transporter 2 (*Gat2*) and dopamine receptor D3 (*Drd3*), as well as a reduction of glutamate in the nucleus accumbens (NAc). We achieved glial-specific *Per2* KO with both cross-breeding of animals, as well as by means of intravenous injections of a novel engineered adeno-associated virus with blood-brain barrier permeability, which allowed genetic manipulation in adult mice, circumventing potential developmental effects. Stereotactic injections of a virus expressing *Cre* recombinase under a glial driver directly into the nucleus accumbens revealed that the phenotype of the despair-resistant mice could be reproduced by knocking out *Per2* only from glia of this brain region. Our results characterise a valuable new mouse model for studying manic-depressive-like behaviour in mice, as well as reveal candidates for studying the signalling pathways of mood regulation.

ShT-08.6-2
Genetic variations in clock controlled genes and sleep phase disorders

L. Plavc[1], C. Skubic[2], L. Dolenc Grošelj[3], D. Rozman[2]
[1]*University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia,* [2]*Centre for Functional Genomics and Bio-Chips, Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia,* [3]*Institute of Clinical Neurophysiology, University Medical Centre Ljubljana, Ljubljana, Slovenia*

Circadian sleep disorders, such as Delayed sleep phase disorder (DSPD) and Advanced sleep phase syndrome (ASPS), cause disruption of the circadian clock and present with extreme morning/evening chronotype. Diseases linked to circadian disruption include cancer, metabolic and neurodegenerative diseases. We conducted an initial study researching genetic etiology of sleep phase disorders in patients treated in the sleep clinic of University Medical Centre Ljubljana. Based on the Morning-evening questionnaire, we selected 15 patients where 13 presented with extreme evening chronotype (indicating DSPD) and two with extreme morning chronotype (indicating ASPS). Sanger sequencing was used to determine the presence of six candidate single nucleotide polymorphisms (SNP) in four circadian genes; rs397514693 and rs104894561 in CSNK1D, rs121908635 in PER2, rs150812083 and rs139315125 in PER3 and rs184039278 in CRY1. All but the CRY1 variant which is linked to susceptibility to DSPD were previously linked to Familial Advanced sleep phase syndromes (FASP). PER3 rs150812083 and rs139315125 heterozygous genotypes were significantly more likely to present with ASPS (OR 55.0, 95% CI 1.51-2008.78, $P = 0.029$). Both variants in PER3 were found in a patient with extreme morning chronotype and family history suggesting FASP. Additionally, a rare SNP was found in the PER2 gene in a patient with extreme evening chronotype. Even though the correlation of PER2 rs1029124354 heterozygous genotype and DSPD was statistically insignificant (OR 6.84, 95% CI 0.26-179.79, $P = 0.249$), the positive family history and rarity of the variant (frequency in European population estimated at 0.0003) suggest further investigation of the SNP is sensible to assess the causality of the variant. The variant is yet to be linked to any disease. The finding of two rare PER3 variants in our small sample is indicative of circadian gene variants being a likely player in etiology of ASPS.

Thursday 8 July
9:00–11:00, Marmorna Hall B

Biomembranes and lipid mediators

S-09.2-1
Malfunctions in lipid rafts during menopause and Alzheimer's disease: a window to develop new biomarkers

R. Marin[1], F. Mesa-Herrera[1,*], A. Canerina-Amaro[1,*]
[1]*Universidad de La Laguna, Tenerife, Spain*

Estrogens play a rapid neuroprotective effect against Alzheimer's disease (AD) by interacting with membrane estrogen receptors (mERs). This neuroprotection is mediated by mERs integrated in lipid rafts (LR). LR are functional membrane microstructures that allow the anchoring of numerous signalling proteins including ER. In LR, ERs are complexed with other proteins that confer neuroprotection, such as voltage-dependent anion channel (VDAC1) and insulin-like growth factor 1 receptor beta (IGF-1RB). During menopause, decreased levels of estrogens lead to LR disruption, altering the functionality of mER protein complexes integrated into these microdomains, thereby contributing to AD development. In the aim of characterising the potential impact of estrogen detriment occurring during menopause in neuronal LR integrity, we have analysed LR-related mER multicomplex in the frontal cortex from pre-menopausal and menopausal women as compared to female AD brains. Furthermore, we analysed whether the molecular raft changes may be reflected peripherally in cerebrospinal fluids (CSF) as potential early biomarkers of neurodegeneration. Therefore, we analysed by ELISA different protein markers in CSF from AD women and men and their correlated age-matched healthy controls. We observed that the proteomic profiles from CSF of both healthy men and women were not altered with aging. However, decreased levels of cavelin-1, ER, IGF and prion protein were observed in CSF from AD women as compared to healthy women. Overall, these data suggest that LR disruption observed during menopause may be assessed by characterization of CSF, thus enabling the development of novel and specific diagnostic and prognostic biomarkers in the clinical practice. Supported by SAF2017-84454-R. ACA holds a fellowship from ACIISI and F.M.H from La Caixa Fundation. References: Marin, R. (2011). Front. Physiol. 2, 23. Marin, R., et al. (2012). Front. Biosci. E4 (1), 1420. Canerina-Amaro, A., et al (2017). Front. Biosci. *The authors marked with an asterisk equally contributed to the work.

S-09.2-2
Plasma membrane and nanoparticles: love, hate and immune regulation

R. Bilyy[1], G. Bila[2]
[1]*Danylo Halytsky Lviv National Medical University, Lviv, Ukraine,* [2]*Danylo Halytsky Lviv National Medical University, Institute of Animal Biology NAAS, Lviv, Ukraine*

Nano- and microparticles have become a normal part of our life, starting from medical drugs and cosmetics and ending in television screens. Many of the nanoparticles are quite dangerous since the materials they use are not easily compatible with

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

human tissue and provoke inflammation and immune response, mainly those that (a) cannot be oxidized, (b) with superhydrophobic surface resulting in direct interaction with membrane lipids, (c) those composed of single atoms for which cellular receptors are not existing. However, our body has developed a response on how to cope with those nanoparticles which are causing damaging effects to cells and tissue. This response is mediated by neutrophilic granulocytes producing neutrophil extracellular traps (NETs) – a weapon used to isolate and sequester particular matter in the safe deposits in the body. The size and chemistry of such nanoparticles is critical for mediating interaction with plasma membrane (Munoz, Bilyy, PNAS, 2016) and causing the leakage through membrane followed by membrane recycling and subsequent leakage of lysosomal content. The letter causes death in many cell types and initiates the formation of NETs. The current report will focus on mechanisms of how nanoparticles interact with neutrophils. Three main groups of nanoparticles will be discussed naturally occurring in the body (e.g. cholesterol), those formed during pathological conditions (oxalate) as well as artificial pollutants (soot, nanodiamonds). Different junk-food diets, like those containing high cholesterol or high fructose content, also stimulate formation of endogenous nanoparticles, stimulation on neutrophils activation in the gastrointestinal tract and subsequent low-grade inflammation. NETs were recently demonstrated to initiate gallstone formation under some dietary conditions as well a cause other tissue damage in the gastrointestinal tract. Examples of the beneficial use of nanoparticle-induced inflammation are described for novel nanoadjuvants.

S-09.2-3
Brain glycomics by novel mass spectrometry approaches based on ion mobility and chip-nanoelectrospray ionization

A.D. Zamfir[1], M. Sarbu[1], R. Ica[2], D. Clemmer[3], Z. Vukelić[4]
[1]*National Institute for Research and Development in Electrochemistry and Condensed Matter, Timisoara, Romania,* [2]*Faculty of Physics, West University of Timisoara, Timisoara, Romania,* [3]*Department of Chemistry, Indiana University, Bloomington, United States of America,* [4]*Department of Chemistry and Biochemistry, Faculty of Medicine, University of Zagreb, Zagreb, Croatia*

Ion mobility separation (IMS) mass spectrometry (MS) has recently emerged as one of the most resourceful techniques in targeted and untargeted omics-related workflows since, based on the properties of the ion transport driven by the electric field, it is able to separate isomers, isobars and conformers and provide detailed information on the stoichiometry, topology and structures with biomarker value in highly complex mixtures. On the other hand, modern chip-based systems for electrospray (ESI) MS have been shown to offer an excellent ionization, sensitivity and reproducibility, hence contributing to the discovery and characterization of minor, albeit relevant species in various biological matrices. In this context, we report here on the first implementation of IMS and chip-based MS in glycomics of central nervous system (CNS) for a comprehensive mapping and detailed structural investigation of sialylated glycosphingolipids, known as gangliosides, expressed in healthy and pathological human brain. The option for platform development and introduction in human brain ganglioside research was guided by the major role played by gangliosides in CNS not only as molecular markers, but also as potential therapeutic targets. Due to the combined high sensitivity, ionization and separation efficiency, the IMS and chip-based MS methodologies alone, or in combination with collision induced dissociation (CID) for ion fragmentation, provided a better understanding of human brain gangliosidome and a deeper insight into the complexity and variability of structures expressed in normal vs. pathological brain than ever reported. Most importantly, IMS CID MS/MS enhanced the discovery and structural elucidation of new, previously not even detected, gangliosides species associated to the highly aggressive glioblastoma multiforme, which was found characterized by no less than 160 distinct glycoforms, representing 3 folds the number of structures identified in this tumor type before.

ShT-09.2-1
Skeletal muscle metabolomics post liraglutide and metformin treatments

A. Rončević[1], Ž. Debeljak[2,3], D. Mandić[2], R. Gaspar[4], S.G. Vari[5], **M. Heffer**[6]
[1]*Faculty of Medicine Osijek, Department for medical biology and genetics, Osijek, Croatia, Osijek, Croatia,* [2]*University Hospital Osijek, Clinical Institute of Laboratory Diagnostics, Croatia, Osijek, Croatia,* [3]*Faculty of Medicine Osijek, Department of Pharmacology, Croatia, Osijek, Croatia,* [4]*University of Szeged, School of Medicine, Hungary, Szeged, Hungary,* [5]*International Research and Innovation in Medicine Program, Cedars-Sinai Medical Center, Los Angeles, CA, USA, Los Angeles, United States of America,* [6]*Faculty of Medicine Osijek, Croatia; Department of Medical Biology and Genetics, Osijek, Croatia*

Introduction: Type 2 diabetes (T2D) is a metabolic disease affecting many tissues and altering their cellular metabolism. Skeletal muscle plays a major role in glucose homeostasis and often displays insulin resistance in T2D patients. We investigated effects of high fat-high sugar diet (HFHSD) and diabetes drugs, metformin and liraglutide on skeletal muscle metabolism of male and female rats. Methods: 24 Sprague-Dawley male and 24 female rats were fed standard diet for 44 weeks. When they reached 45 weeks, rats were randomly separated into 3 groups per sex of 8 rats each and were fed HFHSD till the end of experiment. After 5 weeks of HFHSD only feeding, two groups per sex underwent 13 week-long treatments with metformin (HFHSD-M) or liraglutide (HFHSD-L). Skeletal muscle biopsies of 64 weeks-old rats were performed, and tissues were analyzed using imaging mass spectrometry instrument iMScope TRIO (Shimadzu, Japan). Data analysis was performed using R software for Statistical Computing (R Foundation for Statistical Computing, Vienna, Austria) using packages FELLA, resample and KEGGREST, and custom script. Results: For HFHSD-L compared to HFHSD, in females we discovered 5 altered metabolites of which only 1 was identified, whereas for males there were 16 altered compounds of which 5 were identified. When comparing HFHSD-M to HFHSD, there were none detected differences in females. However, in males there were 83 altered compounds of which we identified 14. Based on identified metabolites, metabolic pathway analysis elucidated 11 altered pathways in HFHSD-L males and 18 in HFHSD-M males, both compared to HFHSD males. Discussion: Our study suggests that metformin and liraglutide disproportionately affect male rats compared to females. This isn't surprising, given the fact that drugs are usually developed and tested in males. However, this

common practice might produce drugs that aren't as efficacious in females and therefore not provide same benefits as seen in males.

ShT-09.2-2
Nutrient sensing pathways and cytosolic pH regulate a transcriptional circuit for lipid droplet formation

V. Teixeira[1,2,3], T.S. Martins[1,2,3], W.A. Prinz[4], **V. Costa**[1,2,3]
[1]IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal, [2]i3S - Instituto de Investigação e Inovação em Saúde, Universidade of Porto, Porto, Portugal, [3]ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal, [4]Laboratory of Cell and Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, Maryland, United States of America

Lipid droplets (LDs) are ubiquitous organelles that fulfill essential roles in response to metabolic cues. The identification of several neutral lipid synthesizing protein complexes have propelled significant advance on the mechanisms of LD biogenesis in the endoplasmic reticulum (ER). However, our understanding of signaling networks, especially transcriptional mechanisms, regulating membrane biogenesis is very limited. Here, we show that nutrient sensing Target of rapamycin complex 1 (TORC1) regulate LD formation at a transcriptional level, by targeting *DGA1* expression, in a Sit4 and Sfp1-dependent manner. We show that cytosolic pH (pHc), co-regulated by the plasma membrane H^+-ATPase Pma1 and the vacuolar ATPase (V-ATPase), and dependent on carbon availability, acts as a second messenger, upstream of protein kinase A (PKA) signaling, to adjust the activity and localization of the major transcription factor Opi1, which controls the metabolic switch between phospholipid metabolism and lipid storage. Together, this work delineates hitherto unknown molecular mechanisms that couple nutrient availability and pHc to LD formation through a transcriptional circuit regulated by major signaling transduction pathways. VT (CEECIND/00724/2017 and CEECIND/00724/2017/CP1386/CT0006) and TM (SFRH/BD/136996/2018) were supported by FCT - Fundação para a Ciência e a Tecnologia, I.P.. This work was funded by national funds through FCT, under the project UIDB/04293/2020. W.A.P. is supported by the Intramural Research Program of The National Institute of Diabetes and Digestive and Kidney Diseases.

Thursday 8 July
9:00–11:00, Gallery Hall

Lipidomics

S-09.3-2
The International Lipidomics Society

K. Ekroos[1], G. Liebisch[2], M. Holčapek[3], R. Ahrends[4], H. Köfeler[5], X. Han[6], M. Wenk[7]
[1]Lipidomics Consulting Ltd., Esbo, Finland, [2]University of Regensburg, Regensburg, Germany, [3]University of Pardubice, Pardubice, Czech Republic, [4]University of Vienna, Vienna, Austria, [5]Medical University of Graz, Graz, Austria, [6]University of Texas Health Science Center at San Antonio, San Antonio, Texas, United States of America, [7]National University of Singapore, Singapore, Singapore

Lipid metabolism is tightly regulated to maintain homeostasis. Loss of control can result in unwanted cascades of biological events triggering deleterious pathophysiological conditions. The evolution of lipidomics technologies is set to tackle this problem, driven by high expectation in its ability to afford new opportunities for studying lipids in health and disease, and put forward new biomarker candidates. However, many studies differ vastly in methodologies, workflows and data presentation and do not report absolute lipid concentrations. The discrepancies in published data and broader issues of irreproducibility attenuate lipidomics research and hinder successful inter-laboratory studies and transitions into the clinical landscape. We introduce the International Lipidomics Society (ILS) flagship, fostering international community-wide coordination and communication for the creation of lipidomics specific guidelines for good scientific practice and future development. A central focus lies, among others, in the engagements of interest groups actively developing lipidomics standards guidelines, reference materials, clinical lipidomics, instrumental and methodological developments, applied bioinformatics, ontology, lipid function, and a close crosstalk to lipid biology and medicine. ILS is the point of contact for lipidomics research, development and commercialization, and actively guiding the developments towards unlocking the full potential of lipidomics and adoption in the clinical arena.

S-09.3-1
Lipidomics of phospholipase A2 allosteric regulation by membranes generating mediators of inflammation

E.A. Dennis
University of California, San Diego, La Jolla, CA, United States of America

From the cell signaling and inflammation perspective, the omics revolution began with genomics, proteomics and metabolomics, but lipidomics now dominates as the largest number of cellular metabolites are lipids, many playing critical roles in cell signaling, and over 44,000 distinct molecular species have been identified by the LIPID MAPS Consortium (www.lipidmaps.org). We[1] have also employed lipidomics analysis to characterize the *in vivo* role of specific phospholipase A2 (PLA2) enzymes in initiating the inflammatory response and cellular lipid signaling in

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

stimulated macrophages as models of bacterial infection and inflammation[2]. We can now explain *in vitro* enzyme specificity with *in vivo* specificity, inhibition and pro-inflammatory and pro-resolution lipid mediator formation pathways and inhibition[3]. We will also discuss how membranes interact allosterically with enzymes to regulate cell signaling and metabolic pathways leading to inflammation[1]. We have recently used substrate lipidomics coupled with molecular dynamics to reveal enzyme specificity linked to hydrophobic binding sites for membrane phospholipid substrates[4]. We discovered that each PLA2 releases a specific fatty acid after the enzyme associates allosterically with membranes and extracts a single phospholipid substrate into its catalytic site. (NIHGM20501-44) 1. Dennis EA (2016) REFLECTIONS: Liberating chiral lipid mediators, inflammatory enzymes and LIPID MAPS from biological grease, J Biol Chem, 291, 24431–48. 2. Dennis EA, Norris PA (2015) Eicosanoid storm in infection and inflammation. Nature Immunology Reviews, 15, 511-523. 3. Navratil AR, Shchepinov MS, Dennis EA (2018) Lipidomics reveals dramatic physiological kinetic isotope effects during the enzymatic oxygenation of polyunsaturated fatty acids ex vivo. J Am Chem Soc, 140, 235-43. 4. Mouchlis VC, Chen Y, McCammon JA, Dennis EA (2018) Membrane allostery and unique hydrophobic sites promote enzyme substrate specificity, J Am Chem Soc, 140, 3285-91.

S-09.3-3
Zooming in on the curious case of a key cholesterol synthesis enzyme

H. Coates, **A. Brown**
UNSW Sydney, Sydney, Australia

Squalene monooxygenase (SM, also known as squalene epoxidase) is a rate-limiting enzyme of cholesterol synthesis that converts squalene to monooxidosqualene and is oncogenic in numerous cancer types. SM is subject to feedback regulation via cholesterol-induced proteasomal degradation, which depends on its lipid-sensing N-terminal regulatory domain. Here, we characterize an endogenous truncated form of SM with a similar abundance to full-length SM in a range of human cell types, and show that this truncated SM is cholesterol-resistant and therefore constitutively active. Truncation of SM occurs during its endoplasmic reticulum-associated degradation and requires the proteasome, which partially degrades the SM N-terminus and disrupts cholesterol-sensing elements within the regulatory domain. Furthermore, truncation relies on a ubiquitin signal that is distinct from that required for cholesterol-induced degradation. Using mutagenesis studies, we demonstrate that partial proteasomal degradation of SM depends on both an intrinsically disordered region near the truncation site and the stability of the adjacent catalytic domain, which escapes degradation. Finally, truncation converts SM from an integral to a peripheral ER membrane protein. These findings uncover an additional layer of complexity in the post-translational control of cholesterol synthesis and establish SM as the first eukaryotic enzyme known to undergo proteasomal truncation.

ShT-09.3-1
Unusual membrane lipids with highly asymmetric acyl chains in a fission yeast: identification, synthesis and evolutionary consequences

G. Balogh[1,*], M. Makarova[2,3], M. Peter[1,*], A. Glatz[1], J.I. MacRae[2], N.L. Mora[3], P. Booth[3], E. Makeyev[3], L. Vigh[1], S. Oliferenko[2,3]
[1]*Biological Research Centre, Institute of Biochemistry, Eötvös Loránd Research Network, Szeged, Hungary,* [2]*The Francis Crick Institute, London, United Kingdom,* [3]*King's College London, London, United Kingdom*

The fission yeasts *Schizosaccharomyces pombe* (*S. pombe*) and *Schizosaccharomyces japonicus* (*S. japonicus*) show striking differences in fundamental membrane-associated properties, such as cell size, growth in anaerobic conditions, and nuclear envelope behavior during mitosis. To investigate membrane lipid compositions of the two sister species, we performed comprehensive mass spectrometry-based shotgun lipidomic analysis. We observed remarkable differences in the fatty acyl (FA) chain composition of glycerophospholipids (GPLs) between *S. pombe* and *S. japonicus*. The most abundant molecular species in *S. pombe* were 36:2 and 34:1, while in *S. japonicus* the shorter and saturated 26:0 and 28:0 species dominated. Fragmentation analysis and phospholipase A2 digestion revealed that S. japonicus synthesizes structurally asymmetrical GPLs; the medium-chain 10:0 is linked to the *sn-2* position, whereas the *sn-1* position is occupied by a long-chain saturated FA. FA supplementation experiments showed that both 10:0 and 16:0/18:0 are critical for the fitness of *S. japonicus*. In addition, gene deletion studies, metabolic labeling experiments and *in vitro* fatty acid synthase (FAS) assays revealed that 10:0 is produced *de novo* by the cytosolic FAS. Retroengineered *S. pombe* synthesizing *S. japonicus*-type phospholipids exhibited unfolded protein response. Protein sequence comparisons and domain swap experiments of the two sister species suggested that transmembrane helices fit with surrounding lipids to maintain membrane protein functions. We propose that the adaptation to an anaerobic lifestyle could be the selection pressure that led to the acquisition of shorter FAs to maintain membrane fluidity in *S. japonicus* under conditions when oxygen demanding unsaturation is not functional. Previously published in: Makarova M et al. (2020) *Curr Biol* 30, 367–380. Funding: OTKA ANN 112372 *The authors marked with an asterisk equally contributed to the work.

ShT-09.3-2
Lipid droplets drive inflammatory lipid mediator production in cancer cells

E. Jarc Jovičić[1,2], A. Pucer Janež[1], V. Brglez[1], J. Gerstmeier[3], O. Werz[3], T. O. Eichmann[4,5], R. Zimmermann[4,6], **T. Petan**[1]
[1]*Department of Molecular and Biomedical Sciences, Jožef Stefan Institute, Ljubljana, Slovenia,* [2]*Jožef Stefan International Postgraduate School, Ljubljana, Slovenia, Ljubljana, Slovenia,* [3]*Friedrich-Schiller Institute, University of Jena, Jena, Germany,* [4]*Institute of Molecular Biosciences, University of Graz, Graz, Austria,* [5]*Center for Explorative Lipidomics, BioTechMed-Graz, Graz, Austria,* [6]*BioTechMed-Graz, University of Graz, Graz, Austria*

Lipid droplets (LDs) are cytosolic fat storage organelles that have long been regarded merely as inert fat depots. They are composed

of a neutral lipid core containing triacylglycerols (TAGs) and sterol esters, covered by a phospholipid monolayer embedded with numerous proteins. In cancer cells, LDs contribute to metabolic stress resistance, invasiveness and resistance to therapy via poorly defined mechanisms. Emerging evidence suggests that LDs are an integral part of the cellular stress response, including various aspects of inflammation and immunity. We have previously shown that LDs regulate the trafficking of unsaturated and polyunsaturated fatty acids (PUFAs), thereby protecting breast cancer cells from nutrient and oxidative stress. Here we show that LDs in metastatic breast cancer cells are enriched with PUFA-containing TAGs. During cell starvation these LDs undergo lipolytic breakdown, whereby ω-3 and ω-6 PUFAs are released from the LD core by adipose triglyceride lipase (ATGL). Importantly, ATGL-derived PUFAs are then used by cyclooxygenases (COXs) and lipoxygenases to produce pro- and anti-inflammatory lipid mediators, including eicosanoids and specialized pro-resolving mediators, which are released into the extracellular space. Notably, cancer cells depleted of LDs are not capable of producing prostaglandin E_2 (PGE_2), suggesting that transient storage of PUFAs in LDs is required for their delivery to COX enzymes and eicosanoid production. Finally, we found that cancer cell proliferation depends on functional LD turnover driving the production of mitogenic lipid mediators, such as PGE_2. Thus, LDs regulate the availability of fatty acids for the production of inflammatory lipid mediators in cancer cells, thereby potentially affecting both cancer and immune cell function in the tumour microenvironment. Targeting LD turnover may thus represent a novel strategy for reducing inflammation and inflammation-related tumorigenesis.

Thursday 8 July
10:00–11:00, Povodni mož Hall

Biochemistry: a success story

S-10.1-2
Multiple faces of the potato virus Y coat protein

M. Podobnik
National Institute of Chemistry, Ljubljana, Slovenia

Potyviruses are the largest genus of plant viruses. Their viral particles contain single-stranded positive-sense RNA enveloped by thousands of copies of the coat protein (CP) thereby forming flexuous filaments, 680–900 nm long and 11–13 nm wide. The RNA genome of approximately 10 kb encodes at least 10 proteins, most of them playing multiple roles. Potato virus Y (PVY) is the type species of the genus and one of the most important plant viruses affecting potato production. Expression of PVY CP in bacteria leads to autoassembly of filamentous flexuous virus-like particles (VLPs) devoid of viral RNA. Recently, we determined the near-atomic structures of PVY virion and respective VLP using cryo-electron microscopy (cryo-EM) (Kežar et al. (2019), Sci Adv, 5 (7), eaaw3808). The structures showed that each CP unit contains a central region with defined secondary structure elements, and extended regions at N- and C-termini enabling structural differences between the two types of filaments. Namely, in PVY virion, CP units assemble in helical symmetry around the viral RNA, while in RNA-free VLP, CPs uniquely arrange into octameric rings, longitudinally connected to form filaments. Pivotal roles of the extended, structurally plastic regions of CP for PVY or VLP assembly and viral infection in plant was additionally shown by mutational analysis. Based on these results we now further explore the potyviral infection mechanism as well as the extraordinary modularity of the potyviral CP to design VLPs of various architectures and physicochemical properties for potential use in medicine or biotechnology. Moreover, PVY/VLP structures were the first cryo-EM structures determined by the Slovenian researches based in Slovenia. A high demand for cryo-EM led to the establishment of the cryo-EM facility in Slovenia in 2019, which has been since then continuously active in addressing academic or industrial questions and providing education to new generations of cryo-EM scientists.

S-10.1-1
Structural insights into tRNA modification enzymes

S. Glatt
Max Planck Laboratory, Malopolska Centre of Biotechnology, Jagiellonian University, Krakow, Poland

All types of RNA molecules are post-transcriptionally modified, constituting the so-called cellular "epitranscriptome". In particular, tRNAs and their anticodon stem and loop regions represent major modification hotspots. The attachment of small chemical groups at the heart of the ribosomal decoding machinery can directly affect translational rates, reading frame maintenance, co-translational folding dynamics and overall proteome stability. The variety of tRNA modification patterns is driven by the activity of specialized tRNA modifiers and large modification complexes. Notably, the absence or dysfunction of these cellular machines is correlated with severe human pathophysiologies, like cancer or neurodegenerative diseases. I will present our structural and biochemical work comparing the enzymatic core of the highly conserved Elongator complex in eukaryotes, bacteria and archaea. In particular, I aim to focus on our most recent electron microscopy analyses that have allowed us to understand its active site and modification chemistry at atomic resolution. In addition, I will present our latest data on Elongator's unfortunate link to human diseases and show how the regulatory network surrounding this large macromolecular machine is organized in eukaryotic cells. Last but not least, I will introduce our most recent work on other tRNA modification pathways and highlight how single particle Cryo-EM facilitates the structural characterization of these relatively small and highly dynamic enzymes. Dauden MI et al. (2019) Science Adv. 5(7) Krutyhołowa R, Zakrzewski K and Glatt S (2019) Curr Opin Struct Biol. 55 Krutyhołowa R et al. (2019) Nucleic Acids Res. 47(9) Lin TY et al. (2019) Nat Commun. 10(1)

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

FEBS Special Sessions

Sunday 4 July
16:00–18:00, Povodni mož Hall

Research and Career Skills: assessment of research and researchers

SS-RCS-3
Scientific impact and the quest for visibility

R. Madsen
UCL Cancer Institute, London, United Kingdom

Numerous reports have emphasised the damaging consequences of our current research culture, for individuals as well as for knowledge advance in general. As an early career researcher (ECR) experiencing this damage first-hand, I set out to understand the underlying drivers, with a hope that this understanding would lead me down a solutions path. In the first part of this talk, I will discuss how inadequate research metrics and intrinsic human fallacies drive a deteriorating research culture, forcing individuals to choose between "doing what's right vs what's necessary for maintaining an academic career". The second part will cover emerging solutions, all of which are entrenched in the "Open Science" movement. I will highlight how each one of us – irrespective of career stage – can help amplify the positive ripples started by this movement. Many of the points raised during this talk are also covered in my FEBS Journal 2019 commentary of the same title (Madsen, R.R. (2019) FEBS J, 286: 3968-3974. https://doi.org/10.1111/febs.15043).

SS-RCS-2
Current academic reward systems in relation to research integrity

L. Fésüs
Department of Biochemistry and Molecular Biology, University of Debrecen, Debrecen, Hungary

The direct relationship between research assessment practices and research integrity has been increasingly recognized by all stakeholders, realizing that one of the most critical factors that determines the way researchers behave is how they are assessed. The current research culture pays much attention to outputs, metrics, competition and prestige instead of process and quality content. Agreeing on and implementing newly defined principles can be the basis for establishing best practices and redesigning the current approaches to assessing scientists. Research institutions and organisations should reward open and reproducible research practices in hiring and promotion of researchers taking into account the value and impact of all research outputs. Researchers are expected to meet the responsibility to review and evaluate submissions for publication, funding, appointment, promotion or reward in a transparent and justifiable manner, and to support best assessment practices catalyzing progressive changes in their institutional and research environment.

SS-RCS-1
Assessment of scientists for hiring, promotion and tenure at German universities and research institutes

M. Brunner
FEBS, BZH, Heidelberg, Germany

German universities and research institutions generally hire PIs at the level of junior research group leaders and permanent professors. More and more institutions are also hiring young scientists for tenure track positions. Assessment and selection procedures for scientists applying for these positions and strategies that lead to successful recruitment are discussed.

SS-RCS-4
Assessing research outputs: alternatives to the Journal Impact Factor

M. Purton
FEBS, Cambridge, United Kingdom

The Journal Impact Factor (JIF) is widely (mis)used as a measure of research output. Originally devised as a method to rank journals, the JIF does not provide a satisfactory method to judge the quality of an individual research paper. As research institutes and funders redesign their approaches to assessing scientists, I will look at other alternative measures that might be used in place of the JIF.

Sunday 4 July
18:15–19:15, Gallery Hall

European research infrastructure and initiatives

SS-ERI-2
EU-PEARL: changing the paradigm of clinical trials in Europe

L. Cash-Gibson
Vall d'Hebron Research Institute, VHIR, Barcelona, Spain

Recently, innovations clinical trial designs have been proposed, which have the potential to revolutionize the way clinical research is conducted. An adaptive platform trial, for example, embeds various trials under a shared master protocol to enable the perpetual evaluation of multiple interventions in a disease or condition, by allowing sequential inclusion and exclusion of arms due to interim analyses mostly based on Bayesian statistical approaches. This allows for potentially cost saving and enabling more rapid and efficient drug and health technology development through multi-source, multi-stakeholder collaboration, as well as improvements in societal access to timely and affordable medicines. Given this context, "EU-PEARL", an H2020 European Commission and Innovative Medicines Initiative 2 funded project (2019-2023), has been developed and built around a strategic alliance between public and private sectors. EU-PEARL will stage the clinical trials of the future, which will be more patient friendly by design and patient focused by outcome. The intention is to transform the current siloed approach to single-compound clinical trials into a cross-company collaborative, multi-compound platform that will

centre the clinical study around patients, not molecules. EU-PEARL will establish this approach as a sustainable and scalable global solution, able to meet complex regulatory, ethnical, legal, statistical and data requirements. This endeavor is centred around the concept of an integrated research platform (IRP), which consists of an infrastructure and set of workflows, and will be built on five key pillars: (i) Patients and their representatives; (ii) Hospital hubs; (iii) Operational framework; (iv) Data Governance Ecosystem; and (v) Regulatory framework. In addition, EU-PEARL is creating trial ready (collaborative) clinical (research) networks in four disease areas which still face high unmet clinical needs, and both late and early stage trials will be deployed.

SS-ERI-1
ELIXIR Europe

A. Smith
ELIXIR Hub, Hinxton, Cambridge, United Kingdom

In this talk, Andrew Smith will introduce ELIXIR Europe, the life science data infrastructure. He will present the services and activities carried out within ELIXIR that can be used by researchers across the life sciences. These include databases, software tools, training materials, cloud storage and supercomputers. These resources can be used for free by researchers across a range of life science domains, from genomics to metabolomics, and across application areas from rare diseases through to marine metagenomic. He will also introduce the general support services that ELIXIR, as a research infrastructure, can provide for data management. He will also briefly introduce some of the other services that are provided by other ESFRI research infrastructures at the European level, such as biobanks and samples, clinical trials, translational medicine expertise, and facilities for structural biology and imaging. These services can be accessed through projects such as EOSC-life, which Andrew will introduce.

Sunday 4 July
18:15–19:15, Povodni mož Hall

Structural Biology Publications: how to improve results presentation and write a better paper

SS-HT-1
How to publish and how not to publish structural results

A. Wlodawer
National Cancer Institute, Frederick, MD, United States of America

High-resolution macromolecular structures determined using crystallography, NMR, and cryo-EM provide a gold standard for evaluation of important properties of biomolecules, but the quality of some structures, as well of their presentation, is not always fully acceptable. Whereas quality checking tools provided by the PDB during deposition process may flag some common problems, the resulting red flags are not always addressed by deposition authors. Some journals require that manuscripts be accompanied by the validation reports in order to assist reviewers in evaluation of the validity of presented structures, whereas other journals do not have such requirements. Additionally, validation reports are more helpful in identifying global problems, while some local problems may not be apparent. Utilization of additional tools and addition of interactive software such as Proteopedia might assist readers in making the best use of published structural data. Using examples extracted from the Protein Data Bank, as well as from journal publications, some common problems will be identified and suggestions will be made on how to avoid their reoccurrence.

SS-HT-2
Application of the Mol* 3D molecular viewer to Proteopedia

D. Sehnal[1], J. Prilusky[2], **J. Sussman**[2]
[1]*Masaryk University, CEITEC - Central European Institute of Technology, Brno, Czech Republic, Brno, Czech Republic,* [2]*The Weizmann Institute of Science, Rehovot, Israel*

Proteopedia [https://proteopedia.org], a web-based Wiki encyclopedia of biomacromolecular structures, aids in understanding the relationship of 3D structure to function. Mol* is a new generation web-based WebGL 3D molecular viewer that provides near-instant access to a wide range of model and volumetric (experimental) data, including macromolecules with millions of atoms and large Cryo-EM data sets. Mol* has been incorporated into Proteopedia making it possible to visualize, in an instant, fascinating structure/function relations of virtually any structure from the smallest protein to the largest macromolecular complexes, including being able to see how well the models fit to experimental data, e.g. density maps from X-rays or EM, e.g. see the full Cryo-EM 3D structure of the Zika virus at https://proteopedia.org/w/5ire. With Proteopedia integrating the Mol* Viewer, the wealth of crowd-sourced content in Proteopedia, accumulated over the past eleven years, is now directly available on devices ranging from smartphones through computers, without the need to download any additional applet. More information about Mol* is available at https://molstar.org.

Monday 5 July
16:00–18:00, Gallery Hall

Science and Society – plastics: revolution, pollution and substitution

SS-S&S-1
From plastics to microplastics and organisms

O. Bajt
National Institute of Biology, Marine Biology Station, Piran, Slovenia

Marine litter has been shown to accumulate in different compartments of marine environment. Its major part is composed from different plastic materials. Plastic is a very practical material, long-lasting, resistant to degradation, inert and easy to shape, with a rather low production price. As such it became an important material for everyday use. The annual world production of plastic materials is about 300 million tons and it increased in last 60 years more than 20 times. In Europe only about 30% of plastic material is recycled. About 80% of the total amount of plastic is carried into

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

the seas from land-based sources, mostly by rivers. Once there, it endangers wildlife and enters the marine food chain. In this way, it might be also harmful for human health. Microplastics are very small pieces of plastic, less than 5 mm in length. It is usually classified in primary and secondary. The primary enters natural ecosystems directly from different sources, including cosmetics and clothing. Secondary microplastics are created from larger particles through natural weathering processes. Both types of microplastics have been recognized to accumulate and persist in natural aquatic ecosystems. Microplastic particles can be harmful to marine life. Ingested plastic fragments can cause alteration in the feeding behavior, reproduction and mortality. Their toxic effect is mostly connected to the release of different harmful compounds from the plastic material and pollutants adsorbed on surface of floating particles. The results of many studies show the bioaccumulation and harmful effect on different groups of marine organisms, e.g. fish, bivalves, crabs, sea birds, phytoplankton, corals, and meiofauna. Plastic surfaces are also good substrates for the adsorption of different non-indigenous species and their transport to remote areas worldwide. In this paper results of different studies about the distribution and effects of microplastics on marine life will be presented and discussed.

SS-S&S-3
The role of biotechnology in the transition from plastics to bioplastics

P. Fabbri, F. Fava, D. Morselli, M. Degli Esposti, L. Bertin, F. Cavani, D. Viaggi
Alma Mater Studiorum University of Bologna, Bologna, Italy

Building new value chains through the valorization of biomass components for the development of innovative bio-based products (BBPs) aimed at specific market sectors, will accelerate the transition from traditional production technologies to the concept of biorefineries. BIOSPRI, a recent Tender Study on support to R&I policy in the field of bio-based products, commissioned by the European Commission DG RTD, selected the twenty most promising BBPs currently under development, and in the framework of this selection, many products were directly related to the plastics sector. Findings put in evidence the strategic role of biotechnology in driving and boosting the transition from fossil-based plastics to bioplastics obtained from renewable resources. Three relevant examples of innovative bioplastics obtained through biotechnological processing can be discussed: (1) PA12, which represents an innovative polyamide mainly for engineering applications; (2) fungal chitosan, which offers relevant features for advanced applications in the biomedical field and for decontamination of waters and soils; and (3) PHA derived from vegetable oils and animal fats, which represent an example of bioplastic fulfilling the complete set of requirements of the circular bioeconomy. Biotechnology results the key enabling technology for the development of all these innovative biomaterials, and clearly supports their transition to the full technological maturity and commercial accessibility. Valorization of wastes and forward-looking management of critical raw materials are main drivers for the further development of the bioplastics obtained by biotechnological methods, offering increasing opportunities for the whole plastic segment.

SS-S&S-2
Active biopackaging produced from by-products and wastes from food and marine industries

F. Debeaufort
University of Bourgogne, Bourgogne

The problem of wastes from fishery and seafood industries has increased in the last decade, becoming a global concern. Excluding aquatic plants, global production of fish, crustaceans, molluscs and other aquatic animals reached almost 175 million tons in 2017, among which 25% are wasted. "Fish wastes" include fish species or by-catch products having no or low commercial value, undersized or damaged as well as species of commercial value but not caught in sufficient amounts to warrant sale. Fish and seafood industry waste is related to the wastes and by-products resulting from the processing and storage of fish and seaproducts. It has been estimated that more than 66% of fish, crustaceans and cephalopods tissues among the 120 million tons processed in 2017 including fins, heads, skin and viscera are discarded as they are considered "wastes". By-products and wastes from the fish and seafood industry contain many components of key interest to be valorized, such as polyosides (chitosans), proteins (gelatins, peptides), ether extracts and fats (e.g. fatty acids, DHA), minerals and oligoelements (e.g. phosphorus, nitrogen, magnesium, calcium), pigments, etc. Among these, many could be envisaged for packaging applications. Indeed, chitosan, obtained from crustacean chitin deacetylation, and gelatin mainly extracted from fish and cephalopod skins as well as the fish myoproteins have great film-forming properties, able to be crosslinked by both physical or chemical in aim to produce biopolymers and providing structural properties of packaging films. Peptides and some enzymes extracted from fish and cephalopod have antioxidant properties that could be introduce in biopolymer matrix either to delay the film oxidative degradation, and also able to migrate into food to prevent oxidation when considering active packaging films. These have also antimicrobial and healing efficacies for the development of dressings and active packaging systems for the pharmaceutical industry.

Monday 5 July
16:00–18:00, Povodni mož Hall

Gender issues in science

SS-GIS-3
Women in science across Europe – North/West, South/East: different backgrounds, same challenges

J. Dumic
University of Zagreb Faculty of Pharmacy and Biochemistry, Zagreb, Croatia

Being a woman in science is always challenging and demanding, no matter if you come from the north, west, south, or east of Europe. Despite many initiatives undertaken to tackle women's under-representation in science and technology, the problems are still persisting in all European countries. Although in many of them the majority of graduate students are women (54% of all tertiary students in the EU-27 in 2018), the majority of the

students following doctoral studies are men (52%). The situation is getting much worse if women's participation in scientific research and technological development, especially holding senior positions in universities and research institutes (<25%) is considered. "Hitting the glass ceiling" i.e. women's under-representation in higher academic life highlights the persisting issue of gender inequality across Europe. In 2019, 29.3% of the world's researchers were women; in Western Europe 32.7% but 39.3% in Central and Eastern Europe. Among countries of FEBS interest, those where women accounted for 50% of researchers were Tunisia, Georgia, (Republic of North Macedonia), Latvia, Armenia, Lithuania, and Serbia, followed by Bulgaria, Moldova, Croatia, Montenegro, (Iceland), Bosnia & Herzegovina, Romania, Ukraine, (Albania), Estonia, Portugal, Slovakia, and Spain (40-50%). Interestingly, among these "top-twenty" countries, only Tunisia, Iceland, Portugal, and Spain are not post-socialistic countries. Therefore, is that a legacy of socialistic paradigm of women and men equality or in post-transition times women seek a path to financial freedom, which often implies higher education and leads them into the science? Or does the reason lie in higher salaries in business and industry, so the positions in science are "left" for women? Yet, what about Hungary and Czech Republic, also former socialistic countries, in which the percentage of women in research is < 30%? These and some other questions on equal opportunities for all across the Europe will be discussed.

SS-GIS-1
From the bench to the boardroom: lessons learned

M. Swanson
University of Michigan, Medical School, Ann Arbor, MI, United States of America

Michele Swanson was raised in a large traditional family in small towns in the American midwest. She was recruited to play field hockey at Yale, where she earned a biology degree. Michele was introduced to the world of experimental science as a research technician at Rockefeller University with Samuel C. Silverstein, who conducted seminal studies of *Legionella pneumophila* growth in macrophages. At Silverstein's insistence, Swanson applied to PhD programs where she developed her love of genetics studying with Marian Carlson at Columbia and Fred Winston at Harvard. After a one-year hiatus devoted to her daughter and son who were born during graduate school, Swanson trained as a postdoctoral fellow with Ralph Isberg at Tufts and HHMI where she developed cell biological methods to analyze the fate of *L. pneumophila* in macrophages. In 1996 Dr. Swanson joined the University of Michigan Medical School faculty where she is now a Professor in the Department of Microbiology & Immunology and Director of the Office of Postdoctoral Studies. After analyzing regulatory pathways that govern the *L. pneumophila* life cycle, her lab now investigates this pathogen's resilience in water and aerosols. Their interdisciplinary collaboration on the 2014–15 Legionnaires' disease outbreaks aimed to contribute scientific evidence to address the social, political, and legal upheaval of the Flint Water Crisis. Subsequently she served on Committee on Management of Legionella in Water Systems for the National Academies of Sciences, Engineering and Medicine, a body that advises the federal government on pressing research and policy matters. An active member of the American Society of Microbiology, Swanson is past-President, co-host of the podcast This Week in Microbiology, and co-author of the textbook Microbe. To promote the advancement of a new generation of diverse scientists, Swanson will share observations and lessons learned from her own life as well as the current literature.

SS-GIS-2
Women in science: is positive discrimination the right decision?

C. Gallego
Institut de Biologia Molecular de Barcelona (IBMB-CSIC), Barcelona, Spain

Despite the fact that women are well represented at the doctoral and postdoctoral levels, trajectories for women then diverge, with a lower proportion of women achieving tenured, chair or leadership positions. Across countries and disciplines, studies show that disparities are maintained in funding and recognition in sciences. For instance, while female representation as first authors of articles has increased significantly in the past two decades, their representation as senior authors has not shown the same degree of advancement. Such disparities are greatest in high-impact journals. Different actions have been proposed to tackle the diverse aspects of gender bias. Some solutions are at individual level involving family, education and religion. These are pivotal aspects to change the male dominant culture but although there is a rise in social awareness, improvements in this direction are very slow. Other actions belong to the institutional and organizational level, where universities, funding agencies, and scientific journals have an important role to play. One strategy in this respect is to introduce some degree of positive discrimination, giving preferential treatment to minority groups that have been prejudiced against in the past. Gender quotas have elicited many reactions, and have been considered unfair because women may be favoured independently of skill levels. However, studies from institutions taking this strategy have shown that qualified men were not negatively affected. Another criticism is specifically raised by women scientists, and states that positive discrimination might question the value of their work and attribute their success to gender. However, the outcome of all evaluation procedures is always subject to some degree of uncertainty, and final prioritized lists always suffer from stochastic effects in a range where, most often, a threshold is placed. In my view, here is where positive discrimination could be readily applied.

Tuesday 6 July
16:00–18:00, Gallery Hall

FEBS/IUBMB Special Sessions on Education 1 – learning and the brain: translating the science of learning to educational practice

SS-Edu1-1
Exploring new horizons in education: using multiple pathways to enhance and energize science instruction

J. Zadina
Brain Research and Instruction, Indian Shores, United States of America

This presentation bridges neuroscience and education, offering both science and strategies for applying new understandings

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

about the science of learning to classroom practices. When instructors understand how the brain learns, what is required for learning, and the most important factor in learning, they can optimize instruction. I will dismantle a major "neuromyth" and show how multiple brain pathways are involved in learning. Use of multiple pathways strengthens the learning while making it more engaging. This presentation will reveal brain processes that may not be visible to the instructor, but powerfully impact learning, such as working memory, cognitive load, and the effects of stress. Be prepared to experience, engage, and enjoy.

SS-Edu1-2
Implementing cognitive science research in the undergraduate science classroom

I. Davidesco
New York University, New York, United States of America

Cognitive science research on learning and instruction is often not directly connected to how we teach biology in university classrooms. In an effort to narrow this gap, this talk will review cognitive science research on five key learning and instruction strategies: active retrieval, distributed (spaced) learning, dual coding, concrete examples, and feedback and assessment. These strategies can significantly enhance the effectiveness of science instruction, but they typically do not find their way into the classroom. The implementation of these strategies will be illustrated through an undergraduate science course for nonmajors called "Science in Our Lives." This course provides students with opportunities to use scientific information to solve real-world problems and view science as part of their everyday life.

Wednesday 7 July
16:30–18:30, Povodni mož Hall

FEBS/IUBMB Special Sessions on Education 2 – you should. . . I should. . . – Let's clarify our roles and responsibilities in PhD education

SS-Edu2-2
Expectations of professionalism in doctoral education in an ever-changing academic world

R. Harris
Karolinska Institutet, Stockholm, Sweden

A doctoral education is a collaborative venture in which a doctoral student, one or more supervisors and an academic department work together to enable the academic development of an individual towards becoming an independent researcher. It is implicit that all these stakeholders are not only aware of their professional responsibilities, but that they also conduct themselves according to mutual expectations. A presentation of the RESPECT framework (Reflection; Expectations; Successful supervision; Professionalism; Engagement; Critical friends; Time) of best professional practices as a basis for life learning will be presented.

SS-Edu2-1
The postdoc experience – hopes, fears, expectations and reality

G. Hanin
University of Cambridge, Department of Genetics, Cambridge, United Kingdom

The transition from PhD to Postdoc is an exciting career development step, but it can also be a little overwhelming and challenging. It is an essential and natural scientific career progression for those interested in pursuing an academic career and it is crucial to make good choices and have realistic expectations. Numerous professional and personal factors must be taken into account, including interactions with the PI and the other members of the lab, scientific infrastructure, feasibility and novelty of the project, financial considerations and cultural differences. As a postdoc you are not only expected to conduct independent research with minimal supervision, but also to think about research broadly and train other scientists. Some people choose to enter a new field, move to a new model organism or switch from theoretical to experimental fields. Adjusting to a new discipline can be challenging and requires learning new research approaches, techniques and sometimes even jargon. The differences between disciplines can be surprising even when the science seems to overlap. This talk is designed to assist PhD students in their transition to a postdoc position, and to highlight important considerations and strategies for planning the next stage of their career.

Speed Talks

Sunday 4 July
18:15–19:15, Marmorna Hall A

Molecular Genetics, Education

SpT-01-01
Rearrangement of nuclear lamina complexes during stress – identification and quantification of lamin- associated proteome after heat shock induction

M. Pałka, A. Tomczak, J. Jabłońska, R. Rzepecki
Faculty of Biotechnology, University of Wroclaw, Wroclaw, Poland

One of the most important structural components of the nuclear envelope in the cell nucleus is nuclear lamina. The major component of the nuclear lamina are lamins (type V intermediate filaments). They play a key role in nuclear assembly, chromatin organization and regulation of cytoskeleton organization. Two types of lamins are distinguished due to their structural features: A- and B-type. In this study, we focused on the identification of potential lamin protein partners and the rearrangement of those complexes after stress induction. The best-known stress is heat shock response which is based on activation of a single transcription factor – HSF. For studies, we chose the *Drosophila melanogaster* model system, since it has only one isoform of each lamin (C and Dm, which corresponds to A- and B-type, respectively).

Experiments were performed on Drosophila Kc cell line. To investigate the lamin- associated proteome the co-immunoprecipitation of cross-linked cell extracts against specific antibodies against lamin was performed followed by tandem mass spectrometry analysis. We observed several differences in the quantitative and qualitative analysis of the protein composition after stress induction. A significant increase in the number of interacting proteins has been noticed after heat-shock. Functional analysis of those identifications showed that proteins that occurred only after heat shock induction are mainly responsible for RNA binding, nucleic acid binding and ATP activity. Moreover, we demonstrated that heat shock increases the solubility of lamin Dm and associated proteins such as topoisomerase II. This finding led us to a suggestion that heat shock indicates changes in the lamin phosphorylation rate. In conclusion, we believe that lamins may play a key role in the regulation of transcription after stress induction either by changes in interaction with chromatin or changes in lamin-associated composition.

SpT-01-03
Zinc-finger associated domain of pita architectural protein forms tetramers

A. Bonchuk[1], K. Boyko[1,2], A. Nikolaeva[1,3], A. Fedotova[1], V. Popov[2,3], P. Georgiev[1]
[1]*Institute of Gene Biology, Moscow, Russia,* [2]*Bach Institute of Biochemistry, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russia,* [3]*National Research Center «Kurchatov Institute», Moscow, Russia*

Pita is Drosophila architectural protein that organizes open chromatin in promoters, forms chromatin boundaries, and supports long-range interactions between regulatory elements in chromatin. Pita contains Zinc-finger-Associated multimerization Domain (ZAD) which is widespread in arthropodan transcription factors with arrays of C2H2 zinc-finger domains. Presumably ZAD plays a key role in architectural function of Pita. We determined crystal structure of Pita ZAD at 1.9Å resolution. While overall fold is similar to other known ZADs it has two-times larger dimerization surface. Unlike other dimeric ZADs Pita ZAD exists as tetramer in crystal. Tetramer formation occurs mainly through hydrophobic interaction between two dimers and is further stabilized with a number of coordinated water molecules. However, axis of rotational symmetry matches to the symmetry axis of tetramer and tetramer formation could be result of crystal packing. ΔG of tetramer formation is relatively high (−17.4 kcal/mol). We tested oligomeric state of Pita ZAD in solution using small-angle X-ray scattering (SAXS). The molecular weight of particles calculated from the extrapolated I0 scattering intensity was in the range of 52.0–68.2 kDa corresponding to the tetramer (Mw of monomer is 13.6 kDa). Approximation of scattering profile with theoretical scattering calculated from structures of dimer and tetramer clearly shows that scattering profile fits well with tetramer structure. Two point mutations were designed to break tetramer formation: M48A resulted in unstable protein with poor solubility, whereas ZAD^{L45A} in SAXS experiment demonstrated scattering intensity corresponding to the dimer (25.6–33.1 kDa) and scattering profile which is better aligned with calculated scattering curve for dimer. Tetramer formation could have implications in establishing distant interactions between multiple cooperatively bound sites on the DNA. The study is supported by Russian Science Foundation grant №19-74-10099.

SpT-01-05
DDIT4 gene expression is switched on by a new HDAC4 function in ataxia teleangiectasia

A. Ricci[1], L. Galluzzi[1,*], M. Magnani[1,*], M. Menotta[1,*]
[1]*University of Urbino 'Carlo Bo', Urbino, Italy*

Ataxia telangiectasia (AT) is a rare, severe, neurodegenerative disease caused by biallelic mutations in the ATM gene. Unfortunately, no effective disease-modifying treatment is available. Observational studies and clinical trials (1) have shown that treatment with glucocorticoids analogues improves symptoms and neurologic functions in AT patients. Since HDAC4 nuclear accumulation has been related to neurodegeneration in AT (2), the effects of dexamethasone (dex) on HDAC4 on AT cell lines were investigated. Dexamethasone was able to further increase HDAC4 nuclear accumulation in AT cells, by cysteine reduction and not by phospho-signaling. Nuclear HDAC4 had the ability to stabilize HIF-1a transcription activity on the DDIT4 promoter after dex induction, bypassing ATM activity, selectively in AT cells. DDIT4 transcription dependence on HIF-1a/HDAC4 represents a very important pathway, since it can lead to the inhibition of mTORC1 activity and promotes the autophagy process, one of the compromised biological pathways in AT cells (3). The results for LC3B, p62 and VPS18 support the positive dex-induced effects on autophagic flux in AT cells, while mTORC1 targets were inexplicably unaffected. Only in AT HIF-1a silenced dex stimulated cells there was a large amount of p-p70S6K. This behavior could be due to mTORC1 activation in AT cells after dex treatment, since its upstream pathway was found to be activated: the AKT signaling in AT cells should promote mTORC1 activation, but the simultaneous DDIT4 expression counteracts the mTORC1 activity. Here we describe a novel molecular mechanism for the non-epigenetic role of HDAC4, acting as a direct transcription regulator in AT cells and leading to a positive effect on autophagic flux. This study was partially supported by FanoAteneo and by EU H2020 IEDAT (Grant n°: 667946). 1.Chessa L et al. (2014) Orphanet J Rare Dis 9, 5. 2. Li J et al. (2012) Nat Med 18, 783-790. 3. D'Assante R et al. (2017) Clin Immunol 175, 16-25. *The authors marked with an asterisk equally contributed to the work.

SpT-01-04
tRNA 2′-O-methylation by TRM7/FTSJ1 proteins modulates small RNA silencing and tRNA fragmentation

D. Gospodinova Dimitrova[1], M. Todorova Angelova[1], B. Da Silva[1], M. Brazane[1], T. Ye[2], J. Gecz[3], A. Piton[4], I. Motorin[5], C. Carré[1]
[1]*Transgenerational Epigenetics & small RNA Biology, IBPS, UMR7622, UPMC, Sorbonne Université, Paris, France,* [2]*GenomEast Plateform, IGBMC, CNRS - UMR7104, Inserm U1258, Illkirch, France,* [3]*The University of Adelaide, Adelaide, Australia,* [4]*Laboratoire de diagnostic génétique, Hôpital Civil, CHRU Strasbourg, Strasbourg, France,* [5]*IMoPA, UMR7365 CNRS, BioPole Lorraine University, Nancy, France*

2′-O-methylation (Nm) can affect RNAs in multiple ways and Nm-modifying enzymes are highly conserved and often linked to the development of cancers and brain diseases. An excellent example is the human FTSJ1 – a tRNA Nm-methyltransferase (Nm-MTase) that is conserved in yeast and is associated with Intellectual Disability (ID) in humans and mice. We have

recently extended the evolutionary portfolio of this enzyme by demonstrating the molecular function of its predicted Drosophila homologs (Trm7-32 and Trm7-34) by using the cutting edge RiboMethSeq and MALDI-TOF RNA detection techniques. Curiously, in the mutant flies, our genetic sensors detected a deregulation of Ago2-dependent small non-coding (snc)RNA silencing pathways and northern blot analysis showed an accumulation of specific tRNA fragments (tRFs) derived from tRNA Phe (a major target of the enzymes). Today, we are considering the biomarker potential of these tRFs and testing their conservation in human cell lines coming from ID patients carrying various mutations in FTSJ1. In addition to the already known targets of FTSJ1, we are also testing new ones that we just discovered in humans by RiboMethSeq. Finally, we used Next Generation Sequencing (NGS) methods to evaluate any deregulation in the small RNA populations as well as mRNAs in FTSJ1 mutant context in order to gain further insights in the molecular pathways that govern FTSJ1-dependant ID pathogenesis.

SpT-01-06
A novel method for reliable detection of somatic retroelement insertions

S. Urazbakhtin, M. Saliutina, G. Nugmanov, Y. Lebedev, A. Komkov, I. Mamedov
Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia

Retroelements (RE) are interspersed repeats that occupy more than 50% of the human genome. The majority of them are transpositionally inactive, although several dozens are still functional and can be a source of genetic instability associated with some types of cancer. Investigation of these somatic RE is limited because it's difficult to distinguish newly acquired copies from the known ones due to their very similar sequence. In this study we developed a new method for novel RE insertions detection. The method is based on selective amplification and subsequent high-throughput sequencing of genomic regions flanking young (AluYa5 and L1HS) RE insertions at both 5' and 3' ends in parallel. Original computational pipeline is used to map these sequences to the human genome and filter out various artifacts. This approach allows to identify Target Site Duplication for each insertion which is a hallmark of true RE transposition event. Moreover, our approach allows to utilize the genomic DNA used for library preparation to verify new insertions by independent method – locus-specific PCR and sequencing. Unique molecular identifiers are ligated to each RE containing molecule before the amplification and are used to count the number of cells bearing each new insertion. To test the developed method, we prepared a mixture of cells from 4 healthy donors, with different cell concentrations. Polymorphic RE insertions, distinguishing donors from each other, were used to determine method sensitivity. We were able to detect 95% of insertions characteristic for the donor, whose cells comprised as little as 1% of the mixture at sequencing depth of 2,000,000 reads per Alu library and 300,000 per L1 library. Using our method and pipeline we detected several tumor-specific Alu insertions in 6 colorectal cancer paired tumor/normal samples. This work was supported by GACR 19-11299S grant. Previously published in: Komkov, AY et al. (2020) Mob DNA 11(1), 33.

SpT-01-07
DNA hypomethylating drug induces oxidative stress in the mammalian embryo and placenta during gestation

S. Nikola[1,2,*], M. Himelreich-Peric[1,3,*], A. Katusic-Bojanac[1,3], N. Sincic[1,3], J. Krasic[1,3], Z. Majic[3], G. Juric-Lekic[1,4], S. Ljiljana[1,3], A. Maric[5], D. Jezek[1,6], **F. Bulic-Jakus**[1,3,*]
[1]Centre of Excellence for Reproductive and Regenerative Medicine, University of Zagreb School of Medicine, Zagreb, Croatia, Zagreb, Croatia, [2]Deartment of Gastroenterology, Clinical Hospital Merkur, School of Medicine University of Zagreb, Zagreb, Croatia, [3]University of Zagreb, School of Medicine. Department of Medical Biology, Zagreb, Croatia, [4]University of Zagreb, School of Medicine, Department of Histology and Embryology, Zagreb, Croatia, [5]Department of Internal Medicine, County Hospital Cakovec, Cakovec, Cakovec, Croatia, [6]University of Zagreb, School of Medicine, Department of Histology and Embryology, Zagreb, Croatia

A large body of evidence from our previous experimental work has shown that the DNA hypomethylating drug 5-azacytidine (5azaC) is a teratogen that causes intrauterine growth restriction (IUGR), malformations in the embryos of treated dams, and disruption of placental development. Such effects were ameliorated by the antioxidant acetylsalicylic acid, recently proposed as a prophylactic agent for the adverse perinatal outcome in humans. To investigate in more detail the possible impact of 5azaC on the induction of oxidative stress markers, we also used a free-radical scavenger N-tert-butyl-a-phenylnitron (PBN) as a pretreatment. On 12-13 GD Fisher rat dams were pretreated by PBN (40 mg/kg, i.v.) and one hour later by 5azaC (5 mg/kg, i.p.) or only with 5azaC or PBN. Embryonic, fetal (Sobocan et al. Stem Cells Dev 28, 717–733), and placental samples were evaluated compared to controls on 15 and 20 GD by classical histology, stereological quantification by numerical density (Nv) of proliferating cell nuclear antigen and oxidative markers 8-oxoDG and nitrotyrosine. Apoptotic index was calculated, and global DNA-methylation was assessed by pyrosequencing. 5azaC significantly lowered the global DNA methylation that was partly rescued by PBN-pretreatment in limb buds. The impact of 5azaC on the PCNA level was tissue-dependent (from lower to higher compared to controls). PBN-pretreatment was able to significantly ameliorate survival and growth of embryos and placentas, diminish the level of severe malformations, markers of oxidative/nitrosative processes, and apoptosis in embryos and placentas of treated dams. We may conclude that a DNA hypomethylating agent caused the oxidative stress during mammalian development that was partly prevented by a free radical scavenger's prophylactic impact. This supplements hypothesis that ROS is the primary cause of global DNA hypomethylation as based on cancer and aging research. *The authors marked with an asterisk equally contributed to the work.

SpT-01-02
Human chromatin reconstitution by cell-free translation for epigenetic modifier enzyme assays

S.K. Nagy[1], P. Bankó[2], A. Tar[1], K. Bendali[1], T. Mészáros[1], T.E. Takasuka[2]
[1]Semmelweis University, Institute of Biochemistry and Molecular Biology, Department of Molecular Biology, Budapest, Hungary,

[2]*Hokkaido University, Research Faculty of Agriculture, Sapporo, Japan*

Structural changes of histone proteins caused by a diverse array of epigenetic modifications. Their dynamic, temporal, and spatial regulation provides access to the genetic information, moreover, their role was found in carcinogenesis, mental and metabolic diseases. To date, a complex understanding of the epigenetic network is limited, partially due to a lack of methodology for functional enzyme screening. We established a novel, straightforward in vitro chromatin assembly assay that could serve as a universal tool for epigenetic studies. Firstly, we selected and cloned a set of human histone modifiers: writers (methyltransferases, acyltransferases, kinases, ubiquitin ligases) and erasers (demethylases, deacetylases, phosphatases, deubiquitinases). Secondly, the wheat germ based, cell-free translation system enabled us to reconstitute functional, unmodified human chromatin (composed of 4 core histones H2A, H2B, H3.1, H4, and pBSK plasmid), that is used as a substrate for modifier enzyme assays, which are also prepared by *in vitro* translation. Finally, we analyze the modified histone samples with state-of-art mass spectrometry (Q Exactive Plus Orbitrap) at Hokkaido University, Japan. We aim to demonstrate the proof of concept by confirming known modifications of histones, then to determine novel histone marks and histone modifiers. The effect of various histones chaperons and assembly factors (NapI, ISWI/Acf1, NLP) along with histone H1 was also tested. Recently, we focused on the lesser-known ubiquitination and phosphorylation event on the human chromatin by testing histone modifier ubiquitin ligases and kinases. Ultimately, our method can be automatized, and used for high-throughput screening to test epigenetic drugs. (This project is supported by 129083 grant of the National Research, Development and Innovation Office, Hungary.)

SpT-01-09
Riboregulation of human serine hydroxymethyltransferase activity in cellular metabolism

F.R. Liberati[1], A. Bouzidi[2], S. Spizzichino[2], F. Di Fonzo[2], G. Boumis[2], S. Rinaldo[2], G. Giardina[2], A. Paone[2], F. Cutruzzolà[2]
[1]*Department of Biochemical Sciences "A. Rossi Fanelli", University of Sapienza Rome, Rome, Italy,* [2]*Department of Biochemical Sciences "A.Rossi Fanelli" University of Sapienza Rome, Rome, Italy*

Serine hydroxymethyltransferase (SHMT) is an enzyme that catalyses the reversible conversion of serine and tetrahydrofolate into glycine and 5,10-methylenetetrahydrofolate. SHMT plays a pivotal role in the one-carbon metabolism, a complex network fuelling the biosynthesis of nucleotide precursors, NADPH and methylation factors fundamental for highly proliferating cells. SHMT1 and SHMT2, respectively cytosolic and mitochondrial isoforms, are overexpressed in tumours: in fact, SHMT is considered as a good chemotherapeutic target candidate (Paone,A. et al. (2014) Cell Death Dis, 5, 1–11). Although several molecules have been tested in the past years at the moment there aren't inhibitors that can be successfully used *in vivo* and, for this reason a completely new approach is needed. We have previously demonstrated that SHMT1 is a moonlighting protein (Guiducci G. et al. (2019) Nucleic Acids Res, 47, 4240–4254): beyond its enzymatic function it can bind RNA, regulating the expression of specific targets. In detail, we revealed that SHMT1 has the capability to bind 5′ UTR of SHMT2 isoform regulating its concentration. We also observed that this RNA binding selectively inhibits the SHMT1 enzymatic activity: the conversion of serine to glycine is significantly affected in presence of inhibitory RNA sequences. These results suggest that the RNA-mediated inhibition may contribute to the control of serine consumption by SHMT1. To better understand what happens when SHMT binds RNA now we are using plasmids controlled by the Tetracycline-inducible system to finely regulate the expression of the shmt2-derived UTR sequences and studying energy metabolism as well as survival and proliferation rate of cancer cells. Our data suggest that the moonlighting function of SHMT1 could be used as a Trojan horse to control cancer cell one carbon metabolism and inhibit cancer growth. Now we are focusing on this alternative approach based on inhibitory RNA that it seems to be a promising strategy.

SpT-01-08
Bringing a well-run lab book back into focus: experience from the education workshop

N. Sincic[1,2], M. Dulic[3], I. Stuparevic[4], J. Dumic[1,5]
[1]*Centre of Excellence for Reproductive and Regenerative Medicine, Zagreb, Croatia,* [2]*Department of Medical Biology, School of Medicine, University of Zagreb, Zagreb, Croatia,* [3]*Department of Chemistry, Faculty of Science, University of Zagreb, Zagreb, Croatia,* [4]*Laboratory for Biochemistry, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia,* [5]*Department of Biochemistry and Molecular Biology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia*

In search for new topics for education workshops, the Croatian Society of Biochemistry and Molecular Biology realized that most mentors do not pay much attention to developing basic laboratory skills, such as running laboratory notebook and its importance. Therefore, we launched the new education workshop under the title 'Excellent lab book for an excellent career'. It was formatted as two afternoon sessions on 17th and 18th of December 2020. The workshop was supported by the FEBS Education Committee and the Croatian Ministry of Science and Education. The presentations were designed to address the following questions: "Why is a well-run lab book a prerequisite for well-performed scientific work? How does a well-run lab book drive our carreers? What are the benefits of a well-run lab book at all?" Considerations of a well-run lab book in various fields were presented: e.g. the importance of well-run lab book in publishing, academia, industry, translational and clinical research as well as big data world. It all culminated in a discussion about the pros and cons of a digital lab book which is already seen as a future of scientific data logging (or not?). The first exercise was about Designing a well-structured Lab Book where general rules for a well-run lab book were discussed. The second exercise was all about logging specific laboratory information, discussing the concept of logging important information about experiments performed by different methods. It was a quite challenge to translate the exercises into an online format. Therefore, we were quite surprised when the quality control survey revealed that many participants suggested keeping it as an online event. Indeed, the quality control survey confirmed a lack of training on lab book running, highlighting the need to put this basic laboratory skill back in the focus of young scientist education.

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

Sunday 4 July
18:15–19:15, Marmorna Hall B

Protein turnover, organisms–molecules interactions

SpT-02-03
Post-transcriptional transfer RNA modifications as modulators of heterologous protein production

H. Hotti, L.P. Sarin
University of Helsinki, Helsinki, Finland

Heterologous protein production strains the host cell's translation system, causing biotic stresses that impact negatively on protein quality and yield. Translation is modulated by post-transcriptional transfer RNA (tRNA) modifications (PTMs) that alter tRNA isoacceptor recognition and codon-anticodon base pairing interactions, affecting the processivity and fidelity of translation. As PTMs are dynamic and readily respond to biotic stresses, I hypothesize that careful editing of PTM levels will prime translation towards enhanced heterologous protein production. This reasoning is further supported by preliminary results that show significant host cell PTM adaptation following a long-term expression of a heterologous protein. In this project, I will assess which PTM changes are drivers of translation and devise a PTM-based strategy for enhancing the yield and quality of heterologous protein production. To this end, I have generated differently tagged reporter constructs by which translation is monitored in real-time using Saccharomyces cerevisiae as the model system. By means of quantitative mass spectrometry and ribosome profiling, I will characterize PTM levels and study translational changes to obtain a consolidated dataset from which specific translational events are assigned to key PTMs. The effect of these translational drivers will be independently validated in a simplified cell-free translation system. Finally, I will employ the above results to generate codon-optimized reporter constructs that, in a long-term competition study, will compete against native and codon-impaired versions, to unravel the real-life advantage of PTM-based codon optimization. Consequently, this project will further our insight into translational regulation and provide a proof-of-concept for PTM-based stress mitigation and enhancement of heterologous protein production in cell factories.

SpT-02-09
Reconstruction of multifactor human translation termination complex

T. Egorova, E. Shuvalova, N. Biziaev, S. Mukba, A. Shuvalov, E. Sokolova, E. Alkalaeva
Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia

Translation termination is one of the critical stages of protein biosynthesis. Regulation of the release of synthesized new peptides allows the cell to react very quickly to changes in the environment, changing the protein composition of the cytoplasm. It is now becoming clear that the role of termination of translation has been greatly underestimated. The publications of recent years demonstrate the participation of many additional regulators in this process and its connection with other stages of translation. It was believed that only two proteins, eRF1 and eRF3, were involved in eukaryotic translation termination, but data from different groups in recent years show that the termination complex consists of a much larger number of proteins. In addition to the eRF1 and eRF3, such proteins as PABP, DDX19, Gle1, eIF5A and ABCE1 significantly affect translation termination. Using a reconstituted mammalian translation system, we reconstructed more difficult termination complexes in vitro and studied their properties. We have discovered interesting patterns in the formation of pretermination complexes. It was found that some additional proteins, including translation initiation factors, and even conglomerates of some proteins, co-purify with pretermination complexes, which can subsequently affect the efficiency of translation termination and ribosomal recycling. Also, it was found that on different model mRNAs containing short and long coding sequences, the pretermination complex is formed in different ways and depends on various additional protein factors. This work is supported by the Russian Science Foundation grant no. 19-14-00349.

SpT-02-02
Altered autophagy-dependent c-Src/Fyn degradation in Huntington's disease – impact on NMDAR activity

L. Fão[1,2], S. Mota[1,2], A.C. Rego[1,2]
[1]CNC - Center for Neuroscience and Cell Biology and IBILI - Institute for Biomedical Imaging and Life Sciences, Coimbra, Portugal, [2]CIMAGO/iCBR/CIBB, Faculdade de Medicina da Universidade de Coimbra, Coimbra, Portugal

Huntington's Disease (HD) is an autosomal dominant progressive neurodegenerative disorder that affects the striatum and later the cortex, with no effective therapies. Mutant huntingtin (mHTT), the main HD hallmark, participates in reactive oxygen species (ROS) formation, mitochondrial dysfunction and modified NMDAR activity. Importantly, c-Src and Fyn, two members of the Src Kinase Family activated by ROS, are enriched in striatal neurons, and are implicated in brain neuronal transmission, synaptic regulation of NMDAR activity and mitochondrial function. These observations favor a common inter-player between mHTT and HD-related neuronal dysfunction, suggesting a relevant role for c-Src/Fyn-regulated pathways in HD pathogenesis, which has been largely unexplored. Thus, in this study, we analyzed the levels/activation of c-Src/Fyn in different HD models as well as the influence of autophagy on c-Src/Fyn regulation. We also investigated the role of these tyrosine kinases on NMDAR regulation in HD context. Our data showed consistent decreased c-Src/Fyn levels and activation in several models, namely in human postmortem brain samples, brain tissue and primary neurons derived from YAC128 transgenic mice and STHdhQ111/Q111cells, along with augmented c-Src/Fyn degradation by autophagy in HD, which can partially explain c-Src/Fyn decreased levels. Moreover, YAC128 mouse primary striatal neurons evidenced decreased c-Src/Fyn levels in distal neurites and postsynaptic density, as well as diminished PSD-95 levels and puncta, suggesting a role of c-Src/Fyn on synapse number in HD neurons. Concordantly, decreased c-Src/Fyn in YAC128 mice was accompanied by decreased Tyr1472 phosphorylation of GluN2B-composed NMDAR and by decreased NMDAR-mediated intracellular Ca2+levels. Thus, we demonstrate for the first

time a direct involvement of c-Src/Fyn tyrosine kinases in HD pathogenesis, supporting that c-Src/Fyn-related pathways may constitute potential neuroprotective targets in HD.

SpT-02-05
The role of N and M domains of Sup35 yeast protein in biomolecular condensate formation

N. Gorsheneva[1], A. Grizel[1], K. Kulichikhin[1], A. Maitova[1], A. Zobnina[1], A. Rubel[1,2,3], Y. Chernoff[1,4]
[1]*Laboratory of Amyloid Biology, St. Petersburg State University, St. Petersburg, Russia,* [2]*Department of Genetics and Biotechnology, St. Petersburg State University, St. Petersburg, Russia,* [3]*Sirius University of Science and Technology, Sochi, Russia,* [4]*School of Biological Sciences, Georgia Institute of Technology, Atlanta, Georgia, United States of America*

Biomolecular condensates are membraneless microcompartments in eukaryotic cells, accumulating proteins and nucleic acids. The processes underlying their formation are liquid-liquid phase separation and gelation. Biomolecular condensates participate in RNA metabolism, ribosome biogenesis, DNA damage response. The condensate formation is frequently driven by intrinsically disordered regions (IDRs). IDRs may also be involved in the formation of solid fibrous aggregates (amyloids). Sup35 protein, a yeast counterpart of the eukaryotic translation termination factor eRF3, is shown to form both biocondensates and amyloids. Data implicated the same or overlapping regions of Sup35 being responsible for the formation of biocondensates (during pH stress) and amyloids; however, the relationship between these processes remained elusive. The goal of this study is to investigate the role of environmental factors and dissect the input of particular sequences within the N-proximal domain of Sup35 into biocondensate and amyloid formation. Our data identify osmotic stress as one of the factors causing reversible biocondensate formation by Sup35-derived constructs. We show that the ability of osmotic stress to drive phase separation is conserved among distantly related yeast species. Sequences of Sup35N region, involved in the control of phase separation, are identified, and analysis of their effects on amyloid formation is undertaken. This work is supported by the Russian Science Foundation under grant №18-74-00041. This work is supported by the Russian Foundation for Basic Research under grant №19-34-51054, and by the St. Petersburg State University, grant №51140332. Authors acknowledge the St. Petersburg State University Resource Centers «Biobank», «CHROMAS» and «Molecular and Cell Technologies» for technical support.

SpT-02-08
Development of a bio-active fluorescent inhibitor probe targeting the Rho kinase ROCK for monitoring inhibition action

J. Corret[1], S. Hammoud[2], F. Buron[2], P. Daligaux[3], C. Chesne[4], K. Ple[2], H. Benedetti[1], S. Routier[2], B. Vallee[1]
[1]*Centre de Biophysique Moléculaire, CBM, Orleans, France,* [2]*Institut de Chimie Organique et Analytique, ICOA, Orleans, France,* [3]*Laboratoire de Biochimie Stalight, Orleans, France,* [4]*Laboratoire Biopredic International, Rennes, France*

Our knowledge of cell signal transduction pathways has deeply increased in recent decades. However, for some processes, molecular links are still lacking to fully describe signalisation cascades. One possible approach to identify new elements is to study the effects of an inhibitor targeting a specific protein or kinase in order to perform Omic studies, such as transcriptomic, proteomic or metabolomic. Furthermore, with single-cell analysis, the understanding of transduction pathways is even more accurate. For these dedicated studies, cells that have actually been subjected to a specific inhibitor must be detected and selected. To this end, we have developed fluorescent inhibitor probes targeting ROCK kinase. ROCK kinase was selected as it plays a crucial role in the dynamics of the actin cytoskeleton, which is responsible for many biological processes and dysfunctions. We tested our probes on HeLa model cell lines as well as a more specialized HepaRG cell line, a unique and well-established hepatic cell system capable of producing early hepatic progenitor cells as well as fully mature human hepatocytes. This model is a tool for *in vitro* absorption, distribution, metabolism, and excretion (ADME) studies, and it has the advantage of directly linking ROCK activity to the contraction of the bile canaliculi. We could monitor the penetration of our probes into these different cell lines and showed they are still bioactive. The probes we have developed are powerful tools to study the pathways involving ROCK kinase.

SpT-02-10
Investigating the function of the anaphase promoting complex ubiquitin ligase in the regulation of mitochondrial ATP synthase beta subunit

A.C. Leite[1,2,3], T. Martins[1,2,3], V. Costa[1,2,3], C. Pereira[1,2]
[1]*i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal,* [2]*IBMC – Instituto de Biologia Celular e Molecular, Universidade do Porto, Porto, Portugal,* [3]*Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal*

Mitochondrial ATP synthase is an enzyme complex that generates ATP and is a key determinant of mitochondrial function. Previously, we showed that the 2A-like protein phosphatase Sit4 promotes the dephosphorylation of ATP synthase catalytic beta subunit (Atp2 in yeast) at Thr124/317, which downregulates Atp2 levels, impacting on ATP synthase activity and mitochondrial function [1]. Remarkably, the two Atp2 phosphosites lie on typical anaphase-promoting complex/cyclosome (APC/C) recognition motifs. APC/C is an E3 ubiquitin ligase that plays a key role in cell cycle progression by targeting cell cycle regulators for degradation. Here, we set out to investigate whether APC/C targets Atp2 for degradation, in a phosphorylation regulated manner. We found Atp2 levels vary in accordance with cell cycle phases, as expected from an APC/C substrate. We also confirmed, using co-immunoprecipitation assays, that Atp2 physically interacts with both APC/C co-activators Cdc20 and Cdh1. Moreover, we found that Atp2 levels decrease after Cdh1 and Cdc20 overexpression and increase upon ablation of Cdh1 and Cdc16 (a core APC/C subunit), in agreement with Atp2 being an APC/CCdh1 substrate. Confirming a role of Cdh1 in degrading Atp2, we found an increase in mitochondrial respiration in cells lacking Cdh1. Furthermore, preventing Atp2-Thr124/317 phosphorylation decreased overall mitochondrial respiration during cell cycle progression. These results indicate that Atp2 regulation by APC/CCdh1 may contribute to coordinate mitochondrial activity with cell cycle progression. [1] Pereira C et al. (2018) Biochim Biophys Acta Bioenerg 1859, 591-601 This work was

supported by FCT - Fundação para a Ciência e Tecnologia, I.P., under the project UIDB/04293/2020, and through PhD grants attributed to ACL (SFRH/BD/135921/2018) and TM (SFRH/BD/136996/2018) and FCT investigator grant attributed to CP (IF/00889/2015).

SpT-02-01
Conformational flexibility and allosteric modulation in a heme-binding resurrected ancestral glycosidase

L.I. Gutierrez-Rus[1], G. Gamiz-Arco[1], V.A. Risso[1], B. Ibarra-Molero[1], Y. Hoshino[2], D. Petrović[3,4], J. Justicia[5], J.M. Cuerva[5], A. Romero-Rivera[4], B. Seelig[6], J.A. Gavira[7], S.C.L. Kamerlin[4], E.A. Gaucher[2], J.M. Sanchez-Ruiz[1]

[1]Departamento de Quimica Fisica. Facultad de Ciencias, Unidad de Excelencia de Quimica Aplicada a Biomedicina y Medioambiente (UEQ), Universidad de Granada, 18071, Granada, Spain, [2]Department of Biology, Georgia State University, GA, 30303, Atlanta, United States of America, [3]Present address: Hit Discovery, Discovery Sciences, Biopharmaceutical R&D, AstraZeneca, 431 50, Gothenburg, Sweden, [4]Science for Life Laboratory, Department of Chemistry-BMC, Uppsala University, BMC Box 576, S-751 23, Uppsala, Sweden, [5]Departamento de Quimica Organica. Facultad de Ciencias, Unidad de Excelencia de Quimica Aplicada a Biomedicina y Medioambiente (UEQ), Universidad de Granada, 18071, Granada, Spain, [6]Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, Minnesota, United States of America, & BioTechnology Institute, University of Minnesota, Saint Paul, United States of America, [7]Laboratorio de Estudios Cristalograficos, Instituto Andaluz de Ciencias de la Tierra, CSIC, Unidad de Excelencia de Quimica Aplicada a Biomedicina y Medioambiente (UEQ), Universidad de Granada, Avenida de las Palmeras 4, 18100, Granada, Spain

Resurrected ancestral proteins generally display unusual properties which reflect ancestral adaptations to conditions that differed from the conditions in which extant proteins operate. In this work, we present the remarkable biophysical and biochemical properties of a putative resurrected ancestor of bacterial and eukaryotic family GH1 glycosidases. The ancestral protein conserves the typical TIM-barrel fold of GH1 glycosidases, with a rigid core barrel that enables an optimum enzymatic activity temperature within the experimental range of thermophilic GH1 glycosidases. However, the rest of the structure displays large regions with greatly enhanced conformational flexibility as determined by experimental and computational approaches. Remarkably, the ancestral protein binds a heme molecule tightly and stoichiometrically in a well-defined buried site. Heme binding triggers an increase in the catalytic power through an allosteric modulation of enzymatic activity likely linked to the rigidification of the protein structure. These results suggest a complex molecular evolutionary history for family GH1 glycosidases, and demonstrate the capability of ancestral protein resurrection to reveal novel and valuable biomolecular features. The potential of the ancestral glycosidase as a flexible scaffold for custom catalysis and biosensor engineering is discussed.

SpT-02-07
Intercellular communication: new method to estimate the transnodal exchange of photometabolites in green algae

A. Alova[1], A. Eremin[2], N. Krupenina[1], A. Bulychev[1]

[1]Lomonosov Moscow State University, Moscow, Russia, [2]Otto-von-Guericke-Universität, Magdeburg, Germany

Cellular communication involving fluidic active transport play an essential role in functioning of living organisms. Transcellular permeation and long-distance transport of solutes are important in plants because they deliver the photosynthetic assimilates to growing cells. Cytoplasmic streaming has long been conjectured to aid in overcoming the slowness of diffusion, but its precise role in metabolic function remains still unclear. The cells of characean algae represent an excellent example of an interlinked living microfluidic system to study the relation between cytoplasmic streaming, long-distance intercellular communication and their physiological function. A new method employing modulated chlorophyll fluorometry was developed to study plasmodesmal permeation by naturally produced photometabolites and to elucidate physiological means for modulation of cell-to-cell conductance. The method is based on cyclosis-dependent distribution of metabolites produced in brightly illuminated chloroplasts and exported to the cytoplasmic flow. The products released from chloroplasts travel with the streaming liquid and enter the chloroplasts in shaded areas. The entry of reducing substrates into the chloroplast stroma modulates photosynthetic electron flows, thus causing a transient increase in chlorophyll fluorescence due to a temporal reduction of the primary quinone acceptor Q_A in photosystem II. The results show that the plasmodesmata are permeable to low-molecular photometabolites. The time required for crossing the intercellular barrier was determined and used to estimate the coefficient of metabolite diffusion inside plasmodesmata. The intercellular transport was highly sensitive to moderate changes in external osmolarity and cell turgor. The inhibition of cell-to-cell transport under osmotic treatments was reversible and specific. This study was supported by Russian Foundation for Basic Research within the framework of Russian–German cooperation, project № 20-54-12015.

SpT-02-04
Characterization of recombinant protein components of the photoprotective system of marine cyanobacterium *Crocosphaera watsonii*

Y. Slonimskiy[1,2], **V. Botnarevskii**[1,2], E. Maksimov[3], T. Friedrich[4], N. Sluchanko[1,3]

[1]A.N. Bach Institute of Biochemistry, Federal Research Center of Biotechnology of the Russian Academy of Sciences, 119071 Moscow, Russia, [2]M.V. Lomonosov Moscow State University, Department of Biochemistry, Faculty of Biology, 119991 Moscow, Russia, [3]M.V. Lomonosov Moscow State University, Department of Biophysics, Faculty of Biology, 119991 Moscow, Russia, [4]Technical University of Berlin, Institute of Chemistry, PC 14, Straße des 17. Juni 135, D-10623 Berlin, Germany

Cyanobacteria use a unique photoprotection mechanism based on the quenching of the light-harvesting antenna by the interaction with the water-soluble Orange Carotenoid Protein (OCP). OCP is composed of the N- and C-terminal domains (NTD, CTD) sharing a single ketocarotenoid. OCP photoactivation is

accompanied by the domain separation and ketocarotenoid migration into the NTD. The process can be reversed by the Fluorescence Recovery Protein (FRP), which inactivates OCP by reassembling its domains. While most cyanobacteria have genes of full-sized OCP, some active photosynthesizers (such as the unicellular marine diazotroph *Crocosphaera watsonii*) do not, instead having only genes of FRP and of OCP domain homologs – HCP (NTD homolog) and CTDH (CTD homolog). The presence of only these three uncharacterized proteins in *C. watsonii* suggests an alternative photoprotection mechanism based on a putative non-covalent OCP-like species formed by HCP and CTDH with the participation of FRP. By using recombinant proteins, here we show that *C. watsonii* CTDH (CrCTDH) can dimerize upon extracting ketocarotenoids echinenone and canthaxanthin from membranes and efficiently transfers them to the apoform of OCP from *Synechocystis* (SynOCP). *C. watsonii* FRP (CrFRP) inactivates SynOCP, but interacts with it with a lower affinity than SynFRP, suggesting the significant role of SynFRP residues that are not conserved in CrFRP. *C. watsonii* HCP (CrHCP) could not be obtained in a soluble form, likely due to splitting of the coding sequence. The intact gene was reconstructed by homology using one from a closely related cyanobacterium. Its functionality as a carotenoid donor to the apoform of CrCTDH was also shown. Thus, three novel OCP-related proteins from *C. watsonii* were successfully obtained and characterized, and their functional activity in vitro was demonstrated. Partly supported by RFBR-DFG (20-54-12018; FR-1276/6-1).

SpT-02-06
OCRL controls actin reorganization in spreading and activation of human platelets

A. Bura, A. Jurak Begonja
Department of Biotechnology, University of Rijeka, Rijeka, Croatia

Phosphatidylinositol-4,5-bisphosphate [$PI(4,5)P_2$] is one of seven different phosphoinositides mostly localized at the plasma membrane. Its main functions are the regulation of endocytosis and actin reorganization. OCRL is a phosphatase that dephosphorylates $PI(4,5)P_2$ resulting in the formation of PI4P. Mutations in the OCRL cause Lowe syndrome which is characterized by congenital cataracts, central hypotonia, and renal proximal tubular dysfunction. Furthermore, an increased risk of bleedings was shown in some patients with Lowe syndrome. Platelets (PLTs) have a major role in hemostasis by detecting vessel wall injury. They recognize extracellular matrix proteins, become activated, aggregate, and adhere to the injury to prevent bleeding. We hypothesize that the loss of OCRL function leads to the changes in actin dynamics resulting in PLT dysfunction. Here, we show that pharmacological inhibition of OCRL in human PLTs spread on fibrinogen leads to an inhibition of full PLT activation which was shown by the reduced PLT spread area and the formation of actin-rich structures, called actin nodules. The characterization of actin nodules revealed that they partially colocalize with vinculin and SNX9 and substantially with ARP2/3 complex, as well as with phosphotyrosine suggesting the impairment of the cytoskeletal reorganization during PLT activation. To investigate the role of OCRL-inhibition on signaling pathways we inhibited OCRL in PLTs and then activated them with thrombin. The Western blot analysis revealed reduced phosphorylation levels of myosin light chain (MLC), but not p38 or ERK. Furthermore, flow cytometry analysis showed that P-selectin expression was not affected in thrombin-stimulated platelets if OCRL was inhibited. Our data suggest that OCRL modulates actin cytoskeleton rearrangements, but not granule release, during platelet activation and thus contributes to their proper function.

Monday 5 July
18:15–19:15, Marmorna Hall A

Signals, membranes, glycans and lipids

SpT-03-08
Structure and substrate specificity determinants of the taurine biosynthetic enzyme cysteine sulphinic acid decarboxylase

E. Mahootchi, A. Raasakka, W. Luan, G. Muruganandam, R. Loris, J. Haavik, P. Kursula
University of Bergen, Bergen, Norway

Pyridoxal 5′-phosphate (PLP) is an important cofactor for amino acid decarboxylases with many biological functions, including the synthesis of signalling molecules, such as serotonin, dopamine, histamine, and taurine. Taurine is an abundant amino acid with multiple physiological functions, including osmoregulation, pH regulation, antioxidative protection, and neuromodulation (Lourenco R et al. Nutr Hosp, 17 (2002), pp. 262-270). In mammalian tissues, taurine is mainly produced by decarboxylation of cysteine sulphinic acid to hypotaurine, catalysed by the PLP-dependent cysteine sulphinic acid decarboxylase (CSAD) (E.C. 4.1.1.29), followed by oxidation of the product to taurine. Using X-ray crystallography, we determined the crystal structure of mouse CSAD and compared it to other PLP-dependent decarboxylases in order to identify determinants of substrate specificity and catalytic activity. Recognition of the substrate involves distinct side chains forming the substrate-binding cavity. In addition, the backbone conformation of a buried active-site loop appears to be a critical determinant for substrate side chain binding in PLP-dependent decarboxylases. Phe94 was predicted to affect substrate specificity, and its mutation to serine altered both the catalytic properties of CSAD and its stability testing by different methods such as circular dichroism and differential surface fluorimetry. Using small-angle X-ray scattering (SAXS), we further showed that CSAD presents open/close motions in solution. The structure of apo-CSAD indicates that the active site gets more ordered upon internal aldimine formation. Taken together, the results highlight details of substrate recognition in PLP-dependent decarboxylases and provide starting points for structure-based inhibitor design with the aim of affecting the biosynthesis of taurine and other abundant amino acid metabolites.

SpT-03-10
VEGF and VEGFR2 binding to fibronectin

M. Usuelli, T. Meyer, R. Mezzenga, **M. Mitsi**
ETH Zurich, Zurich, Switzerland

Physical interactions between Vascular Endothelial Growth Factor (VEGF), a central player in blood endothelial cell biology, and fibronectin, a major fibrillar protein of the extracellular matrix, are important determinants of angiogenic activity in health and disease. Conditions signaling the need for new blood

vessel growth, such as hypoxia and low extracellular pH, increase VEGF-fibronectin interactions. These interactions can be further fine-tuned through changes in the availability of the VEGF binding sites on fibronectin, regulated by conformational changes induced by heparin and heparan sulfate chains within the extracellular matrix. These interactions may alter VEGF bioavailability, generate gradients, or alter the way VEGF is recognized by and activates its cell-surface receptors. In this study, we discovered by equilibrium and kinetic studies that fibronectin can also interact with the extracellular domain of the major VEGF receptor (VEGFR2). The VEGFR2 binding sites were very similar to the VEGF binding sites, as they were also exposed upon heparin-induced conformational changes in fibronectin, and the interaction was enhanced at acidic pH. Kinetic parameters and affinities for VEGF and VEGFR2 binding to fibronectin were determined by surface plasmon resonance measurements, revealing two populations of fibronectin binding sites for each molecule. Our study also suggests that a VEGF/VEGFR2/fibronectin triple complex may form by either VEGF or VEGFR2 first binding to fibronectin and subsequently recruiting the third binding partner. The formation of such a complex may lead to the activation of distinct angiogenic signaling pathways, offering new possibilities for clinical applications that target angiogenesis

SpT-03-01
Cholesterol stimulates pore forming activity of Bordetella adenylate cyclase toxin promoting oligomerization

J. Amuategi[1,2], D. González-Bullón[1,2,*], K. B. Uribe[1,2,*], X. Fernández-Martínez[1,2,*], C. Martín[1,2], H. Ostolaza[1,2]
[1]*Biofisika Institute (CSIC-UPV/EHU), Leioa, Spain,*
[2]*Department of Biochemistry and Molecular Biology. University of the Basque Country (UPV/EHU), Leioa, Spain*

Adenylate cyclase toxin (ACT or CyaA), a single polypeptide chain of 1706 amino acids, is a member of the RTX (Repeats in ToXin) family of pore-forming toxins, and like other members in this family, it may bind mammalian cell membranes and cause disruption of the permeability barrier, leading to efflux of cell contents and death. By analogy with known pore-forming toxins it may be assumed that the process that ultimately leads to osmotic cell lysis by ACT pores consists of several stages, namely, binding to the membrane, insertion into the lipid bilayer and assembly into a lytic pore structure. In a previous work we had found that cholesterol increases the susceptibility for membrane permeabilization by ACT. Here we show that ACT binds cholesterol, which promotes toxin oligomerization and increases the toxin lytic potency. Moreover, our data reinforce recent findings showing that ACT "holes" are not fixed-sized proteinaceous small pores as currently assumed, but rather are tunable size pores that can achieve several nanometers of diameter. *The authors marked with an asterisk equally contributed to the work.

SpT-03-06
Identification of the physiological roles of the surface-exposed loops of chitin-uptake channel from the marine bacterium *Vibrio campbellii* type strain BAA 1116

W. Suginta, A. Aunkham
Vidyasirimedhi Institute of Science and Technology (VISTEC), Rayong, Thailand

VhChiP is chitoporin found in the outer membrane of *Vibrio campbellii* (formerly known as *V. harveyi*), and is responsible for the molecular uptake of chitooligosaccharides, which are the main carbon source for the Vibrio bacterium living in oceans. VhChiP is composed of three identical subunits, each containing 16-stranded β-barrels connected by 8 extracellular loops. However, the functions of these loops are unknown. The structural analysis shows that VhChiP contains three exceptionally long loops, L2, L3 and L8. The VhChiPΔL2 mutant was highly susceptible to thermal and chemical denaturation, due to the complete disruption of the L2-L2 interactions within the three subunits. The VhChiPΔL3 mutant completely lacked binding affinity for the preferred substrate of VhChiP, chitohexaose, due to the loss of the key sugar-binding residues locating on L3. The VhChiPΔL8 mutant became voltage-sensitive, exhibiting heavily gating behavior at all applied potentials due to the loss of L3-L8 interactions. In conclusion, our results suggested the unique roles of the three prominent loops in channel properties, with L2 maintaining structural integrity, L3 facilitating sugar transport and L8 retaining the open state of the channel. These data shed further light on the molecular basis of chitin transport through chitoporin in the Vibrio bacteria.

SpT-03-02
Functional characterization of seven novel APOE variants found in patients with primary hypercholesterolemia

A. Larrea[1], A. M Bea[2,*], U. Galicia[1,*], A. Benito-Vicente[1,*], I. Lamiquiz-Moneo[2,*], K. B Uribe[1,*], S. Jebari[1,*], A. Cenarro[2,*], F. Civeira[2,*], C. Martin[1,*]
[1]*Biofisika Institute (CSIC, UPV/EHU), Leioa, Spain,* [2]*Hospital Universitario Miguel Servet, IIS Aragon, CIBERCV, Universidad de Zaragoza, Zaragoza, Spain*

Autosomal dominant hypercholesterolemia (ADH) is characterized by a marked increase in LDL cholesterol and premature cardiovascular complications. ADH represents one of the most common monogenic disorders, which is mainly caused by mutations within LDLR, APOB, PCSK9 and APOE genes. The majority (60–80%) of the patients with ADH harbor mutations in the LDLR gene, while mutations in the remaining genes account for a smaller percentage of ADH. The use of targeted next-generation sequencing techniques allows identifying new mutations in the genes known to be implicated in ADH. However, understanding the mechanism of action of new pathogenic variants is important to understand the severity of the disease and to provide a more personalized treatment. In a previous work we have shown that p.(Leu167del) is a 'gain of function' (GOF) variant for the lipoprotein uptake by LDLR or other receptor of LDLR family involved VLDL in (very low-density density lipoprotein) catabolism which produces a phenotype indistinguishable from classical familial hypercholesterolemia. Therefore, the aim of the present work has been to functionally characterize 7 new APOE variants in order to

determine their pathogenicity and involvement in hypercholesterolemia development. The affinity between VLDL and LDLR was determined by ELISA technique and the effect of ApoE variants on VLDL uptake was determined by flow cytometry, where HepG2 cells were incubated with DiI labelled VLDL particles, so that the uptake of lipoproteins could be quantified. Among characterized variants, 5 of them show less affinity to LDLR than controls, as well as less VLDL uptake, meaning that those variants decrease the binding between VLDL and LDLR and, therefore, cholesterol accumulate in blood. Meanwhile, the other two variants increase the affinity to the receptor and VLDL uptake but we discovered that the increase on the affinity affects the LDL uptake by competition, accumulation LDL instead of VLDL in blood. *The authors marked with an asterisk equally contributed to the work.

SpT-03-09
Role of ceramide and ER stress in the antiproliferative effects of thymoquinone in MCF-7 and HepG2 cancer cells

M. Aslan[1], E. Afşar[1], E. Kirimlioglu[2], T. Ceker[1], C. Yilmaz[1]
[1]*Akdeniz University Faculty of Medicine, Department of Medical Biochemistry, Antalya, Turkey,* [2]*Akdeniz University Faculty of Medicine, Department of Histology, Antalya, Turkey*

We aimed to investigate the impact of thymoquinone (TQ), on sphingolipid metabolites, ER stress and apoptotic pathways in MCF-7 and HepG2 cancer cells. Antiproliferative effect was exerted in cancer cells via TQ incubation at different doses and durations. Cell viability was measured by MTT assay. Levels of sphingosine-1-phosphate (S1P), C16-C24 sphingomyelins (SM) and C16-C24 ceramides (CER) were determined by LC-MS/MS. Neutral sphingomyelinase (N-SMase) enzyme activity was measured by colorimetric assay and ceramide-1-phosphate (C1P) levels were determined by immunoassay. Nuclear factor kappa-k subunit 1 (NFkB1) and glucose-regulated protein 78-kd (GRP78) gene expressions were evaluated by quantitative PCR analysis, while NF-kB p65, GRP 78 and cleaved caspase-3 protein levels were assessed by immunofluorescence and western blot analysis. Incubation with TQ significantly decreased cell viability, S1P, C1P, NF-kB1 mRNA and NF-kB p65 protein levels in cancer cells compared to controls. A significant increase was observed in N-SMase activity, cellular levels of C16-C24 CERs and cleaved caspase-3 levels in cancer cells treated with TQ. GRP78 mRNA and protein levels also increased in cancer cells treated with TQ. In conclusion, TQ-induced ceramide accumulation and ER stress in conjunction with decreased S1P, C1P and NF-kB mediated cell survival may promote cancer cell death by triggering apoptosis.

SpT-03-07
Fungal extracellular particles and their relevance in adaptation to osmotic stress

T. Lavrin[1], S. Hudoklin[2], P. Veranič[2], A. Plemenitaš[1], M. Lenassi[1]
[1]*Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Ljubljana, Slovenia,* [2]*Institute of Cell Biology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia*

Introduction: Extracellular vesicles are potential mediators of intercellular communication that are released by organisms of all kingdoms of life. In fungi, they are enriched in proteins of adaptive mechanisms, pigments, cell wall components and other virulent factors. We aimed to isolate, characterize and determine the role of extracellular particles (EPs; they include ex. vesicles) of extremotolerant *Hortaea werneckii* that grows w/o or with up to 5.5M NaCl. Methods: Hortaea was cultured in defined media with or w/o melanin biosynthesis inhibitor and 3M NaCl. EPs were isolated from conditioned media by sequential centrifugation followed by separation on sucrose density gradient or size exclusion chromatography. EPs morphology was characterized by TEM, concentration and size by Nanoparticle Tracking Analysis, and molecular content by immunoblotting and spectrophotometry. The role of EPs was evaluated by *in vitro* functional assay in different conditions. Results: We successfully optimized isolation of EPs from Hortaea cultures. TEM micrographs showed heterogeneous nature of EPs, which also included ex. vesicles with typical cup-shaped morphology. Hortaea EPs had the av. mode diameter of 97 nm and concentration of 1.7×10^9 particles per mL of media. They carried typical ex. vesicle marker proteins α-tubulin and GAPDH, as shown by immunoblotting, but additionally packed Hog1, the main kinase in osmotic stress response. Further separation of EPs (density or size) showed heterogeneous population that differed depending on the external stimulus: depleted media, high osmolarity and/or absence of melanin. Hortea EPs can be stratified into 3 subgroups: ex. vesicles, ex. vesicles with bound melanin and melanin ghosts. Functional assays showed that the presence of melanin in EPs improved growth of Hortaea culture exposed to high osmolarity. Conclusion: Extremotolerant *H. werneckii* releases EPs enriched in melanin and protein Hog1, which contribute to osmotic stress adaptation.

SpT-03-03
Tebuconazole induces apoptosis through ROS-mediated endoplasmic reticulum stress pathway

Y. Ben Othmène[1], C. Lemaire[2], K. Moncaux[1], S. Abid-Essefi[3]
[1]*Faculty of dentel medecine of monastir, Monastir, Tunisia,* [2]*INSERM UMR-S 1180, LabEx LERMIT, Faculty of Pharmacy, Université Paris-Sud, Faculté de Pharmacie, 92296 Châtenay-Malabry, France and Université de Versailles Saint Quentin en Yvelines, 78035 Versailles, France, Paris, Gabon,* [3]*Laboratory for Research on Biologically Compatible Compounds, Faculty of Dentistry, Rue Avicenne, 5019 Monastir, Tunisia, Monastir, Tunisia*

Tebuconazole (TEB) is an effective systemic fungicide that belongs to the triazoles family. It has been widely used in both agricultural and medical sectors for the control of fungal diseases. This study investigated the mechanism of TEB-induced toxicity in human embryonic kidney cells (HEK293). Our results showed that TEB activated endoplasmic reticulum (ER) and unfolded protein response as evidenced by up-regulation of GRP78, GRP94 and GADD34, splicing of XBP1 mRNA, and expression of the proapoptotic factor CHOP. This ER stress response was accompanied by the induction of the mitochondrial apoptotic pathway. Apoptosis occurred with ROS production, drop in mitochondrial membrane potential and caspase activation. Pretreatment of cells with the chemical chaperone 4-phenylbutyrate (PBA), known to alleviate ER stress, prevented significantly the apoptotic process triggered by TEB. The treatment of cells with the ROS scavenger N-acetyl cysteine inhibits

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

the ER stress response and prevents mitochondrial apoptosis. Taken together, these results suggest that TEB induces cytotoxicity through a ROS-dependent mechanism involving ER stress and activation of mitochondrial apoptotic pathway in human kidney cells.

SpT-03-04
Assessment of a common food additive E407a toxicity

A. Tkachenko, V. Myasoedov*, G. Gubina-Vakulyck*, A. Onishchenko*, V. Tkachenko*, O. Nakonechna*
Kharkiv National Medical University, Kharkiv, Ukraine

A food additive E407a, whose major component is a marine heteropolysaccharide carrageenan, is the subject of controversy due to some reports about its ability to induce intestinal inflammation despite the fact that it is generally recognized as safe. The aim of our research was to assess the toxicity of a thickener E407a upon oral exposure by rats. Samples of the small intestine of 8 rats orally exposed to E407a solutions at a dose of 140 mg/kg of weight daily for two weeks and 8 control animals were stained with hematoxylin and eosin. Furthermore, expression of CD68 and CD3 was analyzed in the small intestinal mucosa in both groups of animals by immunostaining. To evaluate the ability of leukocytes to generate reactive oxygen species (ROS), leukocyte suspensions were stained with 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA, InvitrogenTM), APC-CyTM 7 mouse anti-rat CD45 (BD Pharmingen, USA) and 7-aminoactinomycin D (7-AAD, BD Pharmingen, USA) with the subsequent analysis using a BD FACSCanto™ II flow cytometer (Becton Dickinson, USA). Mann-Whitney U test was performed to statistically process numerical data (Graph Pad Prism 5.0). Oral exposure to E407a resulted in deformation of villi, desquamation of epithelia, leukocyte infiltration in the lamina propria, as well as a higher number of CD68+ and CD3+ cells in the small intestinal stroma compared with the control group. Thus, E407a promoted the development of intestinal inflammation. Moreover, the percentage of ROS^{high} viable leukocytes (CD45+, 7-AAD-) analyzed by flow cytometry was statistically significantly ($P < 0.001$) over 40% higher in rats treated with E407a compared with controls, indicating a more intense ROS generation. Thus, the common food additive E407a promotes ROS generation by leukocytes and induces intestinal inflammation associated with macrophage and T-lymphocyte infiltration in upon oral exposure during 2 weeks. *The authors marked with an asterisk equally contributed to the work.

SpT-03-05
Glucose metabolism in cultured rat astrocytes

K. Fink[1], N. Vardjan[1,2], R. Zorec[1,2], M. Kreft[1,2,3]
[1]*University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology, Laboratory of neuroendocrinology Molecular Cell Physiology, Ljubljana, Slovenia,* [2]*Celica Biomedical, Tehnoloski Park 24, Ljubljana, Slovenia,* [3]*University of Ljubljana, Biotechnical faculty, Department of Biology, Ljubljana, Slovenia*

Astrocytes are neuroglial cells of the central nervous system positioned between neurons and vasculature, which enables them to provide glucose for neurons. Neuronal networks activation and energy consumption is mediated by chemical messengers including noradrenaline (NA). NA targets astroglial aerobic glycolysis, the process of which the end-product is L-lactate. Biochemical studies revealed that astrocytes exhibit a prominent glycogen shunt, where a part of glucose molecules entering the cytoplasm are transiently incorporated into glycogen. We used FRET nanosensor Laconic to study single astrocyte L-lactate metabolism and tested how the noradrenaline-induced cytosolic L-lactate ($[lactate]_i$) increase is influenced by: (i) inhibiting glycolysis by 2-deoxy-D-glucose (2-DG, 3 mM), a molecule that enters cytosol but inhibits the glycolytic pathway; by (ii) inhibiting glycogen degradation by 1,4-dideoxy-1,4-imino-D-arabinitol (DAB, 300 μM), a potent inhibitor of glycogen phosphorylase; and by iii) the application of 3-nitro-propionic-acid (3-NPA, 1 mM), and inhibitor of the Krebs cycle. The results of these studies revealed that D-glucose uptake is essential for the NA-induced increase in [lactate]i, and that it exclusively arises from the glycogen degradation, indicating that most, if not all, D-glucose molecules entering the cytosol transit the glycogen shunt. Moreover, at these experimental conditions of defined transmembrane D-glucose gradient, the glycolytic intermediates are not only used to produce L-lactate, but also significantly support oxidative phosphorylation as revealed by the elevation in ($[lactate]_i$) while the Krebs cycle was inhibited. We conclude that glycogen turnover is a target of noradrenergic stimulation. We showed that all lactate after NA stimulation derives from glucose originating from glycogen shunt. We found that oxidative metabolism is equally important since Krebs cycle blockage results in increased lactate formation.

Monday 5 July
18:15–19:15, Marmorna Hall B

Structural biology, imaging, new drugs

SpT-04-06
Biosynthesis-assisted structural study of the gausemycins, novel lipoglycopeptide antibiotics

V. Alferova[1], A. Paramonov[2], O. Lapchinskaya[1], M. Shuvalov[1,3], T. Kravchenko[1], G. Kudryakova[1], A. Baranova[1], M. Biryukov[1,4], A. Chistov[2,5], I. Ivanov[2], Z. Shenkarev[2], N. Grammatikova[1], A. Mardanov[6], A. Beletsky[6], N. Ravin[6], S. Terekhov[2,3], I. Osterman[3,7], A. Tyurin[1], V. Korshun[1,2]
[1]*Gause Institute of New Antibiotics, Moscow, Russia,* [2]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia,* [3]*Chemical Department of Moscow State University, Moscow, Russia,* [4]*Biological Department, M.V. Lomonosov Moscow State University, Moscow, Russia,* [5]*V.N. Orekhovich Institute of Biomedical Chemistry, Moscow, Russia,* [6]*Institute of Bioengineering, Research Center of Biotechnology, Russian Academy of Sciences, Moscow, Russia,* [7]*Skolkovo Institute of Science and Technology (Skoltech), Moscow, Russia*

The spreading antibiotic resistance of microbial pathogens dictates urgent need for novel antimicrobial agents and scaffolds. Here we report the discovery of gausemycins – antibacterial cyclic lipoglycopeptides isolated from Streptomyces sp. INA Ac 5812. Two individual components, named gausemycins A and B, are produced along with a large number of minor components of similar structure. Isolation technology included several chromatographic (reverse-phase, normal-phase, ion-exchange and size-

exclusion chromatography) and crystallization steps, conducted under LCMS and bioassay-guided control. There is a number of unique structural features in molecules of gausemycins, including rare amino acids (e.g. 4-chlorokunurenine, 2-amino-4-hydroxy-4-phenylbutanoic acid), tyrosine glycosylation with arabinose, absence of a DXDG motif, a long exocyclic fragment (5 amino acids) and a distinct peptide core. The structure of gausemycins significantly differs from naturally abundant glycopeptides (e.g. vancomycin, ristocetin), lipopeptides (e.g. daptomycin, surfactins), and lipoglycopeptides (ramoplanin, teicoplanin, hassalidins). Therefore, we studied the biosynthetic gene cluster putatively responsible for biosynthesis of gausemycins. Characterization of NRPS and tailoring enzymes by protein homology allowed us to propose the mechanism of biosynthesis of gausemycins, including a biosynthetic route to nonproteinogenic amino acids, not previously reported or rarely found in natural peptides. These assumptions were supported by feeding experiments, which led to incorporation of fluorinated amino acids into produced gausemycins. Gausemycins exhibit pronounced antibacterial activity against Gram-positive strains, targeting the bacterial membrane. This work was supported in part by the Russian Foundation for Basic Research (project No. 20-33-70215) and Russian Science Foundation (project No. 20-15-00361).

SpT-04-04
CryoEM structure of PSII assembly intermediate: insights into the role of auxiliary proteins

J. Zabret[1,*], S. Bohn[2,*], S.K. Schuller[3,4], M. Möller[1], J. Meier-Credo[5], P. Liauw[1], A. Chan[6], E. Tajkhorshid[6], J.D. Langer[5,7], A. Krieger-Liszkay[8], B. D. Engel[9,10], T. Rudack[11,12], J. M. Schuller[3,4], M.M. Nowaczyk[1]

[1]*Department of Plant Biochemistry, Faculty of Biology & Biotechnology, Ruhr University Bochum, Bochum, Germany,* [2]*Department of Molecular Structural Biology, Max Planck Institute of Biochemistry, Martinsried, Germany,* [3]*Department of Structural Cell Biology, Max Planck Institute of Biochemistry, Martinsried, Germany,* [4]*SYNMIKRO Research Center, Philipps-University Marburg, Marburg, Germany,* [5]*Department of Molecular Membrane Biology, Max Planck Institute of Biophysics, Frankfurt am Main, Germany,* [6]*NIH Center for Macromolecular Modeling and Bioinformatics, Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, United States of America,* [7]*Max Planck Institute for Brain Research, Frankfurt am Main, Germany,* [8]*Institute for Integrative Biology of the Cell, Commissariat a' l'E'nergie Atomique, CNRS, Saclay, France,* [9]*Department of Molecular Structural Biology, Max Planck Institute of Biochemistry, Munich, Germany,* [10]*Helmholtz Pioneer Campus, Helmholtz Zentrum München, Munich, Germany,* [11]*Biospectroscopy, Center for Protein Diagnostics (ProDi), Ruhr University Bochum, Bochum, Germany,* [12]*Department of Biophysics, Faculty of Biology & Biotechnology, Ruhr University Bochum, Bochum, Germany*

Oxygenic photosynthesis, the conversion of light energy into biologically useful chemical energy, is the basic reaction needed to sustain aerobic life. Not only does it form the basis of our food chain, but it dictates the balance of our atmosphere by splitting water and thereby releasing molecular oxygen. The oxygen-evolving reactions are carried out by photosystem two (PSII), nature's water-splitting catalyst. The biogenesis of PSII is facilitated by numerous auxiliary protein factors. These bind to PSII and with it form transient complexes to facilitate a series of stepwise assembly events. Using cryo-electron microscopy, we were able to solve the first structure of such a PSII assembly intermediate, with a 2.94 Å resolution. Our model contains almost all subunits and redox cofactors, except for the water oxidizing cluster and the corresponding extrinsic subunits (PsbO, PsbU, and PsbV). Most importantly it contains the assembly factors Psb27, Psb28 and Psb34, a novel PSII auxiliary protein. Independent pulldown experiments on Psb34, in combination with our structural data, allowed us to determine the sequence of events leading to the attachment of the PsbC antenna subunit. Furthermore, we were able to observe that the binding of auxiliary proteins induces large conformational changes to PSII′s luminal side. Combining this data with spectroscopic characterization of the PSII assembly intermediate allowed us to construct, for the first time, a mechanistic model of the role auxiliary proteins play PSII assembly. *The authors marked with an asterisk equally contributed to the work.

SpT-04-01
Development of nanobody-based probes as non-invasive tools for molecular imaging theragnosis of GBM

E. Ruiz-López[1], I. Jovčevska[2], R. González-Gómez[1], N. Mendoza Calvo[1], R. Komel[2], A. J. Schuhmacher[1]

[1]*Aragón Health Research Institute, Molecular Oncology Group, Zaragoza, Spain,* [2]*Medical Centre for Molecular Biology, Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia*

The high prevalence and aggressiveness of glioblastoma (GBM) urge non-invasive, quantitative and real-time technologies capable of predicting and monitoring in vivo tumoral development. Overexpressed tumor biomarkers have allowed the desing of antibody-based probes for molecular imaging of cancer. Immuno-Positron Emission Tomography (iPET) is an innovative approach which merges the target selectivity and specificity of antibodies with the spatial resolution, sensitivity, and quantitative capabilities of PET. iPET allows a non-invasive diagnosis, treatment stratification and monitoring of patients by using in vivo, integrated, quantifiable, 3D, full body "immunohistochemistry". We have identified molecular targets for the development of iPET agents for the theragnosis of GBM. Nevertheless, targeting of GBM with highly specific antibody-based probes is limited by the blood-brain barrier (BBB). To overcome the high size-selectivity of BBB, small protein scaffold-based probes such as nanobodies, affibodies, knottins, anticalins, adnectins and DARPins targeting GBM are being developed. Their lower MW enables better tumor uptake and faster clearance compared with monoclonal antibodies. We are currently developing nanobody-based probes targeting GBM biomarkers. To this end we have designed peptides with potential immunogenicity to be used for the isolation of specific nanobodies from a previously constructed phage display library of nanobodies, against glioma stem cells. Upon antigen validation, we will conduct in vivo studies in xenograft and tumorgraft mouse models. For clinical development, we will fuse our nanobodies with molecular BBB shuttles to favor BBB permeability. Our goal is to develop a library of multifunctional nanobodies which allow not only non-invasive diagnosis but also treatment stratification and monitoring GBM patients.

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

SpT-04-03
Miscoding properties of abasic site adducts with methoxyamine, an antitumor base excision repair inhibitor

A. Yudkina[1], E. Shilkin[2], E. Boldinova[2], A. Makarova[2], D. Zharkov[1,3]
[1]*Novosibirsk State University, Novosibirsk, Russia,* [2]*Institute of Molecular Genetics, Moscow, Russia,* [3]*Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia*

Cancer is among the main mortality factors. Chemotherapy and radiotherapy are still the most widely used ways of cancer treatment. However, due to the high toxicity and eventually developing cancer cell resistance to these types of treatment, new strategies to cure cancer are of great interest. Cancer cells resistance to chemotherapy and radiotherapy is often due to DNA repair mechanisms, including base excision repair (BER). Methoxyamine (MOX) is the first inhibitor of the general BER pathway, which effectively sensitizes cancer cells to a wide spectrum of DNA-damaging agents. It is a small compound that forms conjugates with apurinic/apyrimidinic (AP) sites and prevents their cleavage by APEX1, the first universal enzyme of the BER pathway. Although MOX is currently investigated in several clinical trials, the mechanisms of interactions of MOX adducts with DNA metabolism enzymes are still unclear. We have investigated the ability of DNA polymerases belonging to different families to bypass MOX-AP site in comparison with the natural aldehydic AP site. We have obtained kinetic parameters of the nucleotide incorporation opposite these lesions under the steady-state conditions for the Klenow fragment of *E. coli* DNA polymerase I, DNA polymerase of RB69 phage, and human DNA polymerases β, λ, η, ι, κ, ξ, and PrimPol. We have also studied the preferences of these DNA polymerases for incorporation of various nucleotides under the running start conditions. Finally, we have addressed the effect of MOX-AP site conjugates on DNA glycosylases with the associated lyase activity. According to our data, MOX adducts with AP sites, being resistant to BER, would not have enhanced mutagenic properties compared to the initial damage, the AP site. This lack of pro-mutagenic properties suggests the relative safety of methoxyamine as a potential antitumor drug with a synergistic effect with DNA damaging agents. The work was supported by RFBR (project 20-04-00554)

SpT-04-05
Ru(III)-complexes as novel therapeutical approach in breast cancer: evaluation of triggered cell death pathways

M.G. Ferraro[1], M. Piccolo[1], G. Misso[2], M. Bocchetti[2], D. Montesarchio[3], L. Paduano[3], R. Santamaria[1], C. Irace[1]
[1]*Department of Pharmacy, School of Medicine, University of Naples "Federico II", Naples, Italy,* [2]*Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy,* [3]*Department of Chemical Sciences, University of Naples "Federico II", Naples, Italy*

Currently tumors are the second cause of mortality worldwide and are expected to be the first soon (WHO). Therefore, the discovery of novel therapeutical approaches is crucial. Moving in this direction, ruthenium complexes – in metal-based chemotherapy field – are showing promising antitumor activity both in vitro and in vivo, some of them are even being tested in clinical trials. Our research group is focusing on a newly synthesized Ru(III) complex – named AziRu – incorporated in different nanostructures used to enhance its stability and *in vivo* delivery. Antiproliferative effects of these formulations have been tested on a selected human tumor cells panel, showing the most promising results on breast cancer cell lines (BCC) such as ER-positive human adenocarcinoma MCF-7 and triple-negative (TNBC) human adenocarcinoma MDA-MB-231, previously published in: Irace C et al. (2017) SciRep 7,45236; Piccolo M et al. (2019) SciRep 9(1),7006. However, the mode of action of Ru(III)-complexes is still unknown. In this context, we focused on the analysis of cell death pathways potentially triggered by our Ru compounds on BCC. In particular, after nanosystems administration *in vitro*, we observed the inversion of Bax/Bcl-2 ratio. Bax up-regulation and Bcl-2 down-regulation may predispose cancer cells to apoptosis. We then found evidences of intrinsic apoptotic activation in both cell lines, and extrinsic apoptotic activation in MDA-MB-231. Moreover, LC-3 and Beclin-1 protein expression suggested an activation of autophagic pathway. Finally, after *in vivo* evaluation of antiproliferative effects of Ru(III)-complexes in breast cancer xenograft models, we performed *ex vivo* analysis to support *in vivo* the activation of specific cell death by both proteomic and transcriptomic analysis. By also limiting chemoresistance onset, the activation of different cell death pathways by multi-target Ru-based formulations could represent a new chemotherapeutic option for breast cancer treatment.

SpT-04-07
The development of new low-molecular-weight allosteric antagonists of thyroid-stimulating hormone receptor and their effect on the basal and thyroliberin-stimulated production of thyroid hormones

A. Bakhtyukov[1], K. Derkach[1], V. Sorokoumov[2], E. Fokina[1], A. Shpakov[1]
[1]*I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, Saint Petersburg, Russia,* [2]*Saint Petersburg State University, Department of Organic Chemistry, Saint Petersburg, Russia*

Hyperthyroidism, including Graves' disease, is a widespread thyroid disorder and is mainly caused by hyperactivation of thyroid-stimulating hormone receptor (TSHR). A promising approach to treat hyperthyroidism is the use of drugs with TSHR antagonistic activity, including low-molecular-weight compounds interacting with the transmembrane allosteric site of TSHR. The aim of this work was to develop new allosteric regulators of TSHR based on thieno[2,3-d]pyrimidine structure and to study their inhibitory effect on the basal and thyroliberin-stimulated production of thyroid hormones (THs) and the expression of thyroid genes encoding thyroglobulin (Tg), thyroperoxidase (TPO), D2-deiodinase (Dio2) and sodium-iodide symporter (Nis) in male Wistar rats. We have synthesized 5-amino-N-(tert-butyl)-4-(4-iodophenyl)-2-(methylthio)thieno[2,3-d]pyrimidine-6-carboxamide (TPY) and its derivatives TPY1 and TPY3, which reduced TSH-stimulated adenylyl cyclase activity in rat thyroid membranes. Pretreatment of rats with TPY and TPY1 (20 and 40 mg/kg, i.p.) resulted in a decrease of the stimulatory effect of thyroliberin (300 μg/kg, i.n.) on the blood levels of THs and the expression of thyroid genes. TPY3 was a less effective inhibitor of thyroliberin-stimulated thyroidogenesis, but to a greater extent than TPY and TPY1, it

reduced the basal TH levels and thyroid genes expression. TPY3 increased the expression of Dio2 gene, and increased the expression of Tshr gene, which can be considered as a compensatory mechanism in TH deficiency. Thus, we have developed thienopyrimidine derivatives that inhibit thyroliberin-induced stimulation of TH production (TPY1≥TPY>TPY3) and reduce the basal levels of THs (TPY3>TPY1≈TPY), functioning as allosteric antagonists or inverse agonists of TSHR. These compounds can become prototypes of drugs to treat hyperthyroidism and TSH-dependent tumors. This work was supported by the Russian Science Foundation (No. 19-75-20122).

SpT-04-02
ATP-competitive dual DNA gyrase and topoisomerase IV inhibitors with broad spectrum antibacterial activity and low potential for resistance acquisition

M. Durcik[1], A. Nyerges[2], Ž. Skok[1], N. Zidar[1], J. Ilaš[1], A. Zega[1], P. Tammela[3], G. Draskovitz[2], T. Révész[2], D. Kikelj[1], C. Pál[2], T. Tomašič[1], L. Peterlin Mašič[1]
[1]*University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia,* [2]*Synthetic and Systems Biology Unit, Institute of Biochemistry, Hungarian academy of sciences, Biological Research Centre, Szeged, Hungary,* [3]*Faculty of Pharmacy, Centrefor Drug Research, University of Helsinki, Helsinki, Finland*

Bacterial topoisomerases, DNA gyrase and topoisomerase IV are validated targets for the discovery of new antibacterials. They are heterotetrameric enzymes that catalyze changes in DNA topology and are composed of two catalytic GyrA/ParC subunits and two GyrB/ParE subunits that provide energy necessary for the catalytic reaction through the hydrolysis of two ATP molecules. Due to their structural homology, DNA gyrase and topoisomerase IV offer an opportunity for dual targeting to reduce the rate of resistance acquisition. The ATP-binding site became an attractive target especially after successful introduction of novobiocin, the only marketed GyrB/ParE inhibitor until now, into the therapy. However, novobiocin, was later withdrawn from the clinic due to toxicity, low effectivity and resistance development. Using structure-based design, we have discovered and optimized several structural classes of potent ATP-competitive inhibitors (1-3). Our new benzothiazole-based inhibitors display low nanomolar inhibitory activities against GyrB and ParE. The most potent compounds possess low μg/mL antibacterial activity against the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) as well as potent activity with MIC values lower than 0.5 μg/mL against many Gram-positive strains (e.g. *Streptococcus pneumoniae*, *Clostridium difficile*, *Neisseria gonorrhoeae*, and several MRSA strains). Moreover, they show no cross-resistance with fluoroquinolones. Our most promising compound, based on its potent anti-MRSA activity, *in vivo* efficacy, and low frequency-of-resistance, represents new possibilities for the discovery of antibacterials that overcome resistance to currently applied antibiotic drugs. References: (1) Tomašič T et al. (2015) J Med Chem 58, 5501-5521. (2) Gjorgjieva M et al. (2016) J Med Chem 59, 8941-8954. (3) Durcik M et al. (2018) Eur J Med Chem 154, 117-132.

SpT-04-08
Novel fluorescent and photosensitive colchicine derivatives for cancer theranostics and photodynamic therapy

J. Škubník[1], V. Pavlíčková[1], M. Jurášek[2], I. Křížová[3], P. Drašar[4], T. Ruml[1], S. Rimpelová[1]
[1]*Department of Biochemistry and Microbiology, University of Chemistry and Technology Prague, Technická 3, 166 28, Prague 6, Prague, Czech Republic,* [2]*Department of Chemistry of Natural Compounds, University of Chemistry and Technology Prague, Prague, Czech Republic,* [3]*Departement of Biotechnology, University of Chemistry and Technology Prague, Prague, Central African Republic,* [4]*Departement of Chemistry of Natural Compounds, University of Chemistry and Technology Prague, Prague, Czech Republic*

Despite enormous progress in anticancer research, commonly used therapies are still based on classic chemotherapeutics, which often exert severe side effects. Therefore, novel therapeutic options must be sought. In this work, novel conjugates of an antimitotic compound colchicine with fluorescent/photosensitive 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) moieties were synthesized. The design was done with the aim to achieve both imaging and therapeutic properties, so that the conjugates could be suitable for theranostics and photodynamic therapy. The compounds were able to enter cells and localize in the endoplasmic reticulum, which was confirmed by fluorescence microscopy. Their cytotoxicity was assessed in a panel of human cancer cell lines and noncancerous primary lung fibroblasts. All compounds exhibited moderate cytotoxicity in the dark with half maximal inhibitory concentrations in micromolar values after 72-h treatment, which was 3 orders of magnitude higher than for colchicine. Cytotoxicity after light irradiation, however, was potently increased; the half maximal inhibitory concentration was achieved at ten times lower concentrations than in the dark. Besides, the impact of the studied conjugates on the cell cycle was evaluated by flow cytometry. At micromolar concentrations, the compounds caused G2/M phase arrest, however, at nontoxic concentrations, the cell cycle was not affected at all. Importantly, cancer cells were generally more susceptible to the treatment than noncancerous cells. To understand this phenomenon and the mechanism of action of the compounds, the type of regulated cell death and changes in microtubule fibers caused by them were studied. The obtained data confirm that novel fluorescent and photosensitive colchicine derivatives represent a potentially very useful tool for cancer therapy and diagnosis. Owing to their moderate toxicity and selective action against cancer cells, it is very worthy to study them further.

SpT-04-09
Mutually beneficial cooperation of MSC and HUVEC in the early steps of angiogenesis

I. Beloglazova[1], E. Zubkova[1], V. Stepanova[2], K. Dergilev[1], E. Ratner[1], Y. Parfyonova[1,3]
[1]*National Medical Research Center for Cardiology, Moscow, Russia,* [2]*Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, United States of America,* [3]*Faculty of Fundamental Medicine, Lomonosov Moscow State University, Moscow, Russia*

During tissue reparation the rapid restoration of blood supply into the damaged area is very important. The vasculature

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

formation occurs with the joint participation of endothelial (EC) and mesenchymal stromal cells (MSC). Communication between EC and MSC plays an important role in angiogenesis and includes both paracrine exposure and direct cell to cell contacts. The focus of the current study was to examine the mechanism of communication between EC and MSC in the early steps of the process of angiogenesis. We cocultured human umbilical vein EC (HUVEC) with MSCs for 24 h. We found that MSC serves as initiator of angiogenesis through VEGF secretion and HUVEC actively utilized it in the process of capillary like structures formation. HUVEC in turn attract MSC through PDGF secretion. One of the main mechanisms regulating intercellular interactions is the Notch signaling pathway, through which the direction of development of contacting cells is selected, and which also regulates their ability to self-renew, grow, survive, differentiate, and apoptosis. Coculturing of HUVEC with MSCs for 24 h increased mRNA expression levels of Notch 1 receptor and ligands Dll1, Dll4, Jag1 in HUVECs and Jag1, Notch 3 receptor in MSCs. Inhibitory analysis indicates that interaction of Jag1 on HUVEC with Notch3 on MSC leads to upregulation of urokinase in MSC. Excess of urokinase promotes capillary like structures formation through several pathways: advanced extracellular matrix remodeling and upregulation of VEGFR2 by blocking the transcriptional repressor HHEX/PRH after translocation to the nuclei of EC in a kringle-dependent manner. Our data indicate that mutual influence during communication between MSC and EC is necessary for vascular network formation by endothelial cells. This work was supported by the grants from the Russian Foundation for Basic Research 19-015-00511 and Russian Science Foundation 17-15-01368П.

SpT-04-10
Aging of multipotent stem cells (MSC) leads to specific changes in microRNAs secreted within extracellular vesicles potentially promoting insulin resistance

E. Voinova[1], P. Tyurin-Kuzmin[1], K. Kulebyakin[1], O. Grigorieva[2], N. Basalova[2], N. Alexandrushkina[2], M. Arbatsky[2], M. Vigovsky[2], A. Efimenko[2]
[1]*Moscow State University, Faculty of Medicine, Moscow, Russia,* [2]*Moscow State University, Institute of Regenerative Medicine, Moscow, Russia*

Multipotent stromal cells (MSCs) are postnatal stem cells that maintain homeostasis of tissues and regulate their repair and renewal by mesodermal lineage differentiating, e.g. into adipocytes, as well as affecting the functions of differentiated cells. Adipose tissue is the main storage depot and an important endocrine organ. During aging, regulation and renewal of adipose tissue cells may be disrupted, which underlies the development of the metabolic syndrome and obesity. Insulin is a key physiological inducer of MSC differentiation into adipocytes, and disturbances in MSC insulin sensitivity could negatively affect adipose tissue renewal. We explored whether aging could affect the adipogenic potential of human adipose tissue-derived MSCs regulated by insulin. Age-associated senescent MSCs (isolated from donors older than 65 years) and MSCs in replicative senescence (long-term culture) were treated by insulin to induce adipogenic differentiation, and the efficiency of the process was compared to MSCs from young donors. Also, the microRNA profiles of extracellular vesicles (EVs) produced by young and aged MSCs were compared using PCR arrays. Both replicatively and chronologically aged MSCs showed sufficiently decreased adipogenic potential. This was associated with insulin resistance of aged MSCs caused by the increase in the basal level of activation of insulin receptor targets, Erk- and Akt-dependent signaling cascades. To assess the impact of the paracrine cross-talk of MSCs, we analyzed microRNAs profile differences in MSC-secreted EVs and revealed that aged MSCs produced EVs with increased content of miRNAs targeting components of insulin-dependent signaling cascade PTEN, MAPK1, GAREM1. We also confirmed these data by differentiation of young MSCs in the presence of EVs from old cells and vice versa. Thus, aging attenuated the adipogenic potential of MSCs due to paracrine-dependent induction of insulin resistance. The study was supported by RFBR (#19-29-04172).

Tuesday 6 July
18:15–19:15, Marmorna Hall A

Synthetic and systems biology, bioinformatics and biomarkers

SpT-05-05
Method for controlling bacterial cell metabolism

J. Panina[1,2], S. Bruskin[1,2], Y. Nikolsky[3], M. Baev[4]
[1]*Vavilov Institute of General Genetics Russian Academy of Sciences, Laboratory of Functional Genomics, Moscow, Russia,* [2]*Mazef Bioscience Ltd, Moscow, Russia,* [3]*MiLaboratories, LLC, Solana Beach, United States of America,* [4]*MBF, Toronto, Ontario, Canada*

In industrial fermentations and laboratory cultivation, bacterial growth is limited by external factors, such as depleted nutrition, heat generation, lack of oxygen. Fermentations can be more efficient if bacterial strains are redesigned to replace external growth limitations with internal constrains. One then may slow down growth rate, positively affecting consumption of nutrients and reducing accumulation of toxic metabolites in the cell. We developed a new approach for controlling cell growth of a production strain of *E. coli*. Reduction of cell growth rate allows redirecting internal resources to the production of specific proteins of interest. Metabolic regulation of cell growth is achieved at the translation level due to regulated expression of mazF/mazE, toxin/antitoxin module of *E. coli*. MazF is an RNAse which cleaves cellular mRNA at the ACA sites. MazE is its co-expressing antitoxin, which bounds and inactivates the toxin. Varying the ratio of toxin MazF and antitoxin MazE enables tight control of protein translation and thereby, cell growth and metabolism. For controlled expression of mazF in the cell, we positioned mazF and mazE under control of different mutated T7 promotors. We engineered and tested multiple plasmids- and chromosome-integrated inducible expression systems of mazF/mazE. In total, 15 new strains were designed and cultivated in laboratory conditions. The growth patterns of new strains showed, that the growth rate of the culture and the time by that culture reached the stationary phase of growth depended on the amount of toxin MazF in the cell, which was controlled by affinity of T7 RNA polymerase to the promoters. Thus, transcriptional activity was reduced up to 100 folds due to point mutations in nucleotide sequence of the original T7 promotor. We conclude that we can

slow down the culture growth by regulating MazF/MazE expression level by promoter strength. Our approach can be used for optimizing numerous industrial bacterial fermentations.

SpT-05-03
Design of a coiled-coil protein origami with controllable self-assembly

J. Aupič[1], F. Lapenta[1], Ž. Strmšek[1], R. Jerala[1,2]
[1]*National Institute of Chemistry, Ljubljana, Slovenia,* [2]*EN-FIST Centre of Excellence, Ljubljana, Slovenia*

The ability to design proteins whose conformational state can be precisely and reversibly controlled would facilitate the development of smart bio-inspired materials or molecular machines tailored for specific applications. Coiled-coil dimers are present in many natural proteins and have been used to construct synthetic protein nanostructures, such as coiled-coil protein origami (CCPO) cages. Design of coiled-coils with dynamic self-assembly, mediated by a selected environmental signal, would enable the construction of CCPO cages for encapsulation, targeted delivery or controlling the protein activity of packaged cargo. Here, we utilized metal-binding site design to engineer an orthogonal set of Zn(II)-responsive coiled-coil heterodimers. Circular dichroism (CD) spectroscopy and size exclusion chromatography coupled to multi-angle light scattering confirmed the designed peptides assembled into coiled-coil heterodimers only in the presence of Zn(II) ions. Additionally, the designed peptides also acted as pH switches, since low pH prevented coordination of Zn(II) and led to coiled-coil disassembly. The designed set was employed for the construction of several single-chain triangular folds. CD and SAXS analysis demonstrated the designs' dynamic Zn(II)-dependent folding behaviour, indicating the developed coiled-coil set could be used for controlling the assembly and disassembly process of larger CCPO cages and other coiled-coil based nanostructures. Furthermore, we showed that by selecting the building blocks with the appropriate thermodynamic stability, the temperature range where Zn(II) ions exhibit the greatest effect on the equilibrium between the folded and unfolded state could be tuned according to the desired application.

SpT-05-02
Programmable External Network based Compartmentalized Self-Replication (PEN CSR): a new method for *in vitro* directed evolution of enzymes

A. Dramé-Maigné[1,2], R. Espada[2], G. MacCallum[3], R. Sieskind[2], Y. Rondelez[2]
[1]*Institut de la Vision, Paris, France,* [2]*ESPCI, Paris, France,* [3]*Center for Research and Interdisciplinarity (CRI), Paris, France*

Directed evolution is a well-established method for enzyme engineering. Mimicking the process of Darwinian evolution with iterative cycles of genetic diversification and selection, researchers can find new enzyme variants with enhanced or completely new properties. Despite engineered enzymes are often intended for use in unnatural environments, existing methods allowing high-throughput *in vitro* selection conditions can only be applied to polymerases replicating their own genetic sequence in microdroplets. Here, we used an external DNA-based artificial network to create a feedback loop linking the activity of a nicking enzyme to the replication of its own gene. Taking the enzymatic activity at the input, the molecular program is producing a correlated amount of specific primers that are necessary for the PCR amplification of the gene. Bacteria carrying and expressing the mutants are co-encapsulated and lysed with the molecular program in individual droplets using microfluidics. The isothermal primers amplification by the network is initiated by raising the temperature to 45°C. The yield of the PCR then launched in each droplet depends on the amount of primers, therefore on the enzyme activity. After emulsion breakage, we retrieve a gene pool enriched in the best mutant genes. We applied the method to select for faster or more thermostable enzymes. After one selection cycle, next generation sequencing using MinION allowed us to detect key mutations involved in the improvement of these two traits. We generated mutants of the nickase by introducing some of these mutations in the wild-type sequence and could confirm that indeed these mutants had improved properties with sometimes additive effects. This work is the first demonstration of the Programmable External Network based CSR (PEN CSR) method. Programs detecting other types of activity can be envisioned and would allow not only to greatly expand the scope of the CSR but also to implement smart selection functions.

SpT-05-07
De novo designed minimal blue fluorescent protein

V. Prakash[1,*], R. Ranbhor[2], V. Ramakrishnan[1,*]
[1]*Indian Institute of Technology, Guwahati, Assam, India, Guwahati, India,* [2]*Indian Institute of Technology Bombay, Mumbai, India*

Peptides have been used as an integrant in the biological analysis and fabrication of novel biosensors. Many sensitive fluorophores respond rapidly to environmental changes as a change in spectral characteristics. We have designed the hetero-chiral artificial protein employing automated design tools such as automated repetitive simulated annealing molecular dynamics and IDeAS. The designed peptide is further examined to explore the possibility of converting it to a fully functional molecule. An unnatural amino acid β-(1-azulenyl)-L alanine is impregnated in a heterotactic protein scaffold which allows a spectral separation from the native tryptophan signal. Unnatural amino acid β-(1-azulenyl)-L alanine can be selectively excited at 342 nm, which emits in the visible region. Designed proteins are synthesized by solid-phase peptide synthesis method. Structure and stability are examined by spectroscopic and calorimetric methods. Previously published in: Prakash V et al. (2020) ACS omega, 5(41), 26382-26388. *The authors marked with an asterisk equally contributed to the work.

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

SpT-05-09
A platform for creating relevant models of hereditary diseases using human induced pluripotent stem cells and the CRISPR-Cpf1 system

T. Malankhanova[1,2,3], **A. Paulissen**[4], K. Valetdinova[1,3], S. Medvedev[1,2,3]
[1]*Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia,* [2]*Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, Russia,* [3]*E.N. Meshalkin National Medical Research Center Ministry of Health of the Russian Federation, Novosibirsk, Russia,* [4]*Thomas More University of Applied Sciences, Mechelen, Belgium*

Gene editing systems based on the CRISPR/Cas system made it possible to create transgenic and/or isogenic cell models more efficiently. Also, the technology of cell reprogramming allows obtaining various types of differentiated cells for modeling specific pathologies. In our work, we have combined both these technologies by creating a universal cell platform based on induced pluripotent stem cells (iPSCs) containing the Cas12a (AsCpf1) programmable nuclease transgene in the genome. We obtained cell clones with two AsCpf1 variants, which have different PAM consensus - 5'-TTTV-3 ' and 5'- TYCV-3 '. Transgenes were introduced into the genome using CRISPR/Cas9-mediated homologous recombination into the AAVS1 locus. AsCpf1 was inserted into one locus, and a doxycycline-dependent transactivator into the other locus. Western blot analysis showed that the addition of doxycycline to the culture medium activates the expression of AsCpf1 in cells. Quantitative PCR and immunofluorescence analysis showed a high level of pluripotency marker expression. Transgenic clones differentiated into cell types of three germ layers after spontaneous differentiation *in vitro*. These results confirmed that transgenic iPSCs corresponded to the main characteristics of human iPSCs. Next, we carried out experiments to edit 11 target genes by delivering the plasmids expressing guide RNAs into the cell clones. Using T7EI assay and Sanger sequencing, we have shown that some genes have been edited. But some genes remained intact, presumably due to the epigenetic inaccessibility of the locus. So, we used the agents reducing DNA methylation/ histone methylation in cells that made it possible to edit the silent genes. Thus, AsCpf1 iPSC model can be used to create isogenic models for a variety of hereditary diseases. Moreover, we can use these cells to study the protein functions by knocking them out. The study was funded by RFBR and Novosibirsk region according to the research project № 19-44-540002.

SpT-05-08
Establishment of CRISPR/Cas9-mediated microRNA knock-out in potato

T. Lukan[1], F. Veillet[2], L. Chauvin[2], A. Coll[1], T. Mahkovec Povalej[1], K. Pogačar[1], K. Stare[1], M. Križnik[1], J. Chauvin[2], K. Gruden[1]
[1]*National Institute of Biology, Ljubljana, Slovenia,* [2]*INRA, Agrocampus Ouest, Université Rennes 1, UMR 1349 IGEPP, Domaine de Kéraiber, 29260 Ploudaniel, France*

To date, CRISPR/Cas9-mediated microRNA editing has not been established in potato. However, there is a growing evidence that small noncoding RNAs can be targeted by CRISPR/Cas9 system in plants. The novel gene-editing strategy is a challenge yet worth accepting, due to the compelling robustness, specificity, and stability for the modification of microRNA expression. In potato, CRISPR/Cas9 technology was mostly used in combination with Agrobacterium-mediated stable transformation. This could pose a problem, especially in case of time-consuming stable transformation with sgRNAs not previously confirmed as functional. On the other hand, protoplasts transfection is a faster method, but protoplasts isolation and plant regeneration remain bottlenecks. Therefore, we established a protocol, which consists of the design of CRISPR/Cas9 constructs, transient transfection of protoplast to select functional sgRNAs, followed by stable transformation of potato explants. This was achieved through the optimisation of protoplasts isolation from potato, protoplasts transfection and high-resolution melting analysis (HRM) to confirm functionality of tested constructs already one week after transfection. In the last step, functional constructs were used for stable transformation. Transgenic lines with desired mutations were selected by Sanger sequencing. Thus, we established a fast and efficient protocol for CRISPR/Cas9-mediated microRNA knock-out in potato. In addition, we confirmed our hypothesis that protoplasts transfection followed by HRM is an optimal strategy to test functionality of designed sgRNA constructs in general targeting mRNAs or miRNAs prior to stable transformation.

SpT-05-06
Ancient brothers of the human p53 tumour suppressor

V. Brazda[1,2], M. Bartas[3], J. Červeň[3], P. Pecinka[3]
[1]*Faculty of Chemistry, Brno University of Technology, Brno, Czech Republic,* [2]*Institute of Biophysics Czech Academy of Sciences, Brno, Czech Republic,* [3]*Department of Biology and Ecology, University of Ostrava, Ostrava, 710 00, Czech Republic, Ostrava, Czech Republic*

The p53 family plays key roles in development, senescence, and tumour development. Moreover, the p53 protein is the most often mutated tumour suppressor in human cancers. Even though is this protein investigated for many years, its original evolutionary history is not entirely clarified. Using the BLAST algorithm and structure prediction methods, we examined newly sequenced Holozoan genomes as well as non-annotated sets of protein sequences. Interestingly, we discovered p53 homologs outside the Metazoa group, with six new characterized homologs in the unicellular clades Filasterea, Ichthyosporea, and Corallochytrea (the most distant homolog identified). Validation and structure prediction of these new p53 homologs revealed that they contain domains that form structures with high similarity to the human p53-family DNA-binding domain, and some of them also show similarities to the oligomerization and SAM domains. The results of this research describe in detail the presence of p53 family homologs in unicellular evolutionary remote branches of Holozoa and give valuable insights for further research on this essential family of transcription factors.

SpT-05-04
Exploring rare events of water transport in and out of buried active sites of hydrolytic enzymes

A.S. Thirunavukarasu[1,2], C.J. Dongmo Foumthuim[1,2], J. Brezovsky[1,2]
[1]*Laboratory of Biomolecular Interactions and Transport, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, Poznan, Poland,* [2]*International Institute of Molecular and Cell Biology in Warsaw, Warsaw, Poland*

Hydrolytic enzymes require the presence of water molecules in their active sites to perform their action. However, many enzymes have their active sites buried, which requires molecules of their cognate ligands and water to travel through transport pathways, called tunnels, to reach these sites. Since the tunnels relevant for water transport are often gated and only transiently opened molecular dynamics simulations are considered a method of choice for their study. Nonetheless, such investigation is hindered by the rare nature of tunnel opening and water transit. Here, we have intensively explored transient tunnels' conformations in three distinct hydrolytic enzymes with adaptive sampling simulations. These simulations revealed rare gating events comprising domain and loop movements, as well as several novel tunnels employed by water molecules that were not identified by traditional simulations so far. By mapping the flow of waters to the identified tunnel networks, we could link tunnels' capacity to transport water with their properties. Additionally, we have evaluated our findings with various available water models to control for the dependency of our findings on the model used. The presented research will provide novel insights into mechanisms by which enzymes facilitate the utilization of water molecules during their catalytic action. Since many hydrolytic enzymes are biotechnologically and medically relevant, such knowledge can be beneficial for the engineering of enhanced biocatalysts or the development of new drugs. This research was supported by POWR.03.02.00-00-I006/17 project, National Science Centre, Poland (grant no. 2017/25/B/NZ1/01307), and grant of the Dean of faculty of biology, UAM (grant no. GDWB-05/2020). The computations were performed at the Poznan Supercomputing and Networking Center.

SpT-05-01
The impact of repetitive scuba dives on the cardiovascular, muscular and immune system function and integrity biomarkers

M. Žarak[1], A. Perović[2], M. Njire Bratičević[2], S. Šupraha Goreta[3], J. Dumić[4]
[1]*Dubrava University Hospital, University Department for Laboratory Diagnostics, Zagreb, Croatia,* [2]*Dubrovnik General Hospital, Department of Laboratory Diagnostics, Dubrovnik, Croatia,* [3]*University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia,* [4]*University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Cuba*

Recreational SCUBA (self-contained underwater breathing apparatus) diving is a special form of physical activity, which due to specific environmental conditions, triggers a stress response of the organism. To explore whether repetitive recreational SCUBA (rSCUBA) diving triggers an adaptive response of the cardiovascular (CV), muscular, and immune system, we measured the cardiac damage (NT-proBNP, hs-TnI, and CK-MB), muscle damage (myoglobin (Mb), galectin-3, CK, and LDH), vascular endothelial activation (ET-1 and VEGF), and inflammatory (leukocyte count (Lkc), CRP, and IL-6) biomarkers. A longitudinal intervention study included divers (N=14) who conducted one dive per week over 5 weeks at the depth of 20-30 m for 30 min after a non-dive period of 5 months. The blood samples were collected before and after the 1st, 3rd, and 5th dives and specific biomarkers were measured in plasma or serum by the standard laboratory methods. The concentrations of the majority of measured biomarkers increased after every single dive; the exception was ET-1 concentration that decreased. The cumulative effect of five dives has been reflected in diminishing changes in hs-TnI, Mb, galectin-3, ET-1, VEGF, and IL-6 levels, and more pronounced increases in NT-proBNP and hs-CRP levels. The median values of all measured biomarkers in all time points, except Mb, remained within the corresponding reference range. This study showed that rSCUBA diving causes changes in specific biomarkers that reflect (patho)physiological changes in CV, muscular, and immune system after the dive. However, it is shown for the first time that continuously preformed SCUBA diving caused decrease of specific biomarkers highlighting possibly positive effect of diving on CVS.

SpT-05-10
Decreased expression of N-cadherin in the plasma membrane of bladder cancer urothelial cells does not prevent their adhesion to the urothelium

U.D. Jerman[1], T. Višnjar[1], N. Resnik[1], I. Hafner Bratkovič[2], M. Pavlin[3], P. Veranič[1], **M.E. Kreft**[1]
[1]*University of Ljubljana, Faculty of Medicine, Institute of Cell Biology, Ljubljana, Slovenia,* [2]*National Institute of Chemistry, Ljubljana, Slovenia,* [3]*Institute of Biophysics, Faculty of Medicine, University of Ljubljana, Group for nano and biotechnological applications, Faculty of Electrical Engineering, University of Ljubljana, Ljubljana, Slovenia*

Urinary bladder cancer is one of the most common cancers worldwide. It is highly recurrent and often arises in a form of multifocal tumors. According to the luminal spreading theory, recurrent and multifocal tumors can result from the release of cancer urothelial cells (UCs) from the primary tumor into the bladder lumen and their adhesion to the urothelium at a distant site. The proteins involved in the adhesion of cancer UCs to the urothelium have not been identified yet. The present study evaluates the role of N-cadherin in the adhesion of cancer UCs T24 to the urothelium. We established a T24 Ncadlow cell line with decreased expression of N-cadherin at the plasma membrane, which was confirmed by cell surface biotinylation and immunolabelling. The T24 Ncadlow cells formed smaller and less firmly connected aggregates in suspension and secreted less pro-matrix metalloproteinase-2 compared to the T24 cells. The T24 and T24 Ncadlow cells were labeled with fluorescent lipophilic dye or with PAA cobalt ferrite nanoparticles and were seeded on the *in vitro* model of the urothelium. After 24 hours in co-culture, the non-adherent cancer UCs were rinsed and urothelia with attached cancer UCs were processed for fluorescence and electron microscopy. Both, the T24 and T24 Ncadlow cells, attached to the superficial UCs. Immunolabeling showed that the attachment of T24 cells to the urothelium is not mediated via heterotypic E-N

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

cadherin junctions. Additionally, no adherens junctions between cancer and superficial UCs were found at the ultrastructural level. The present study shows that decreased expression of N-cadherin in the plasma membrane of T24 cells does not interfere with their adhesion to the urothelium. This suggests that N-cadherin, although generally believed to be involved in the dissemination of cancer cells, most probably does not mediate the adhesion of cancer UCs to the urothelium.

Tuesday 6 July
18:15–19:15, Marmorna Hall B

Molecular medicine

SpT-06-07
Modular approach to CAR-T regulation based on the barnase-barstar complex for therapy of oncological diseases

R. Kalinin[1], V. Shipunova[1], I. Chernikov[2], D. Volkov[1], A. Shulga[1], S. Deyev[1], A. Stepanov[1], A. Gabibov[1]
[1]*Institute of Bioorganic Chemistry, Moscow, Russia,* [2]*Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences (ICBFM SB RAS), Novosibirsk, Russia*

Cellular immunotherapy using T cells modified with chimeric antigen receptors (CART) is one of the most promising directions in the therapy of oncological diseases. CART therapy yielded promising results in studies focused on hematological malignancies. However, against the background of the complications associated with uncontrolled hyperactivation and non-specific cytotoxicity, there is a high risk of developing life-threatening side effects, primarily cytokine storm and tumor lysis syndrome. Therefore, the opportunity to monitor the therapeutic cells during therapy in real-time is problem number one at present. One possible approach to the regulation of CART activity can be controlled over the intensity CART recognition of tumor antigens. Adoptive cell therapy involves direct contact of the tumor cell with CART cell. Our approach is to use a third component, which is an intermediate link between cancer and therapeutic cells. The mediator molecule includes a darpine specific to the tumor antigen HER2, and a fused barnase (bacterial RNase), which specifically binds to its natural inhibitor-the barstar exposed on the CAR. We demonstrated the works of our approach on a reporter cell system based on the Jurkat NFAT Luc, which had dose-dependent activation. In addition, we have demonstrated the dose-dependent cytotoxicity of natural human CAR-T in the lactate dehydrogenase assays and cytokine expression assays. Moreover, we made BT-474 luciferase cells and evaluated their tumorigenicity in immunodeficient mice for further experiments *in vivo*. The developed adjustable system allows controlling the intensity of the T-cell response by dosing the mediating molecules. Furthermore, it will help to estimate perspectives of universal CAR-T cells. This study was supported by Russian Scientific Foundation project №. 17-74-30019.

SpT-06-03
miRNA assisted discriminative diagnosis between benign prostatic hyperplasia and localized prostate cancer via human blood examination

H.M. Said[1], G. Al Kafaji[2], M.A. Alam[2], Z.T. AL Naieb[3]
[1]*Dokuz Eylül University - Higher Institute of Health Sciences - Department of Molecular Medicine, Izmir, Turkey,* [2]*Department of Molecular Medicine and Al Jawhara Centre for Molecular Medicine, Genetics and Inherited Disorders, College of Medicine and Medical Sciences, Arabian Gulf University, Manama 329, Kingdom of Bahrain, Manama, Bahrain,* [3]*Department of Surgery, College of Medicine and Medical Sciences, Arabian Gulf University, Manama 329, Bahrain*

Prostate cancer (PCa) is the second most diagnosed malignancy and the leading cause of cancer-associated mortality among males. Prostate-specific antigen (PSA) has long been used for the detection of PCa. However, PSA levels increase in PCa and benign prostatic hyperplasia (BPH), and are associated with a poor disease outcome. Circulating microRNAs (miRNAs) have been determined to be highly stable in the circulation and could be utilized as biomarkers to improve disease diagnosis and management. Here, the effectiveness of four PCa-associated miRNAs in the discrimination of PCa from BPH and the risk-stratification of PCa was assessed. 100 individuals, where: 35 patients with localized PCa, 35 patients with BPH and 30 healthy subjects were analyzed. RT-PCR was employed to assess the miRNA expression in peripheral blood samples. Significantly reduced expression of miR-15a, miR-126, miR-192, and miR-377 was observed in patients with PCa compared with patients with BPH and healthy subjects. In addition, the expression of the four miRNAs was lower in high-risk PCa patients than in low-risk PCa patients, with miR-126 being the most downregulated. The expression of the four miRNAs was also significantly and independently associated with PCa. Receiver operating characteristic curve analysis revealed a significant ability of the miRNAs to distinguish patients with PCa from those with BPH, patients with PCa from controls and low-risk PCa from high-risk PCa. Our findings suggested that these miRNAs expression in the blood circulation represent potential non-invasive biomarkers for the early detection of localized PCa, and for PCa risk stratification.

SpT-06-04
Endothelial apoptosis after ionizing radiation mainly occurs in non-perfused vessels leaving vascular function intact

B. Markelc[1,2,3,*], J. Kaeppler[2,*], M. Buono[4], J. Chen[2], W. Cheng[2], J. Thompson[2], M. Hill[2], F. Buffa[2], R. Muschel[2]
[1]*Institute of Oncology Ljubljana, Ljubljana, Slovenia,* [2]*CRUK/MRC Oxford Institute for Radiation Oncology, Department of Oncology, University of Oxford, Oxford, United Kingdom,* [3]*University of Ljubljana, Faculty of Health Sciences, Ljubljana, Slovenia,* [4]*MRC Molecular Hematology Unit, University of Oxford, Oxford, United Kingdom*

The aberrant tumour vasculature is a crucial part of the tumour microenvironment, contributing to tumour growth and response to treatment e.g. radiotherapy. Radiotherapy affects not only cancer cells, but also the tumour microenvironment, including endothelial cells. However, their response to radiotherapy

including is incompletely understood. Our aim was to investigate the biological underpinnings of tumour blood vessel response to radiotherapy. We used intravital microscopy combined with transgenic mice with a VECadherin-Cre-ERT2 construct that in combination with a loxP-flanked STOP tdTomato cassette results in the expression of fluorescent proteins in endothelial cells as well as subcutaneously grown tumours. For intravital microscopy, an abdominal window chamber was surgically implanted and GFP labelled tumours were induced. Upon imaging, a blood pool labelling agent was injected. This setting enabled us to follow tumour growth, blood vessel development and perfusion within the same tumour over time. We followed the response of tumour vasculature to single high dose (15 Gy) and fractionated radiotherapy (5x 3Gy). Unexpectedly, even these high doses led to little structural change of the perfused vasculature. However, non-perfused vessels and blind ends were substantially impaired after radiation accompanied by apoptosis of their endothelium. Additionally, proliferation of endothelium was blocked. These changes did not alter perfusion of the less vascularized B16F10 tumor, but led to increased fraction of perfused vessels in the more vascular MC38 tumor. Further, RNAseq confirmed that irradiation upregulates the expression of genes in apoptotic and cell cycle regulation pathways. Therefore, we show that apoptosis of tumor endothelial cells after radiation is not detrimental to function. *The authors marked with an asterisk equally contributed to the work.

SpT-06-09
The role of the transcription factor Ets-2 in the pathogenesis of primary autoimmune thrombocytopenic purpura

I. Aggeletopoulou[1], I. Panagoulias[1], A. Varvarigou[2], A. Mouzaki[1]
[1]*Laboratory of Immunohematology, Division of Hematology, Department of Internal Medicine, Medical School, University of Patras, Patras, Greece,* [2]*Department of Pediatrics, Medical School, University of Patras, Patras, Greece*

In primary idiopathic/autoimmune thrombocytopenic purpura (pITP), autoreactive effector B and T cells initiate and sustain platelet destruction in a milieu of T helper (Th) 1 and Th17 effector cell polarization. In parallel, regulatory Th cells (Tregs) malfunction and fail to maintain tolerance. We have previously shown that in healthy individuals the IL-2 gene is repressed in naive Th cells by the transcription factor Ets-2, highlighting Ets-2 as a critical factor influencing early events of Th cell differentiation. In this work, we examined Ets-2 and cytokine gene expression profiles in naive and memory effector Th cells (Teffs) and Tregs isolated from pITP patients and healthy controls to investigate the role of Ets-2 in pITP pathogenesis. Blood samples were collected from 6 pITP patients and 6 age/sex-matched controls. Phenotypic analysis revealed increased levels of naive Teffs and decreased levels of memory Teffs and Tregs in pITP patients compared to controls. Naive (CD4+CD45RA+CD25-) and memory (CD4+CD45RO+CD25-) Teffs and Tregs (CD4+CD25+) were isolated for analysis. Bioinformatic analysis revealed multiple Ets-2 binding sites at the promoter sequences of Th cell signature cytokines. In pITP naive Teffs, Ets-2 mRNA and protein synthesis was significantly lower than in control naive Teffs. pITP naive Teffs constitutively expressed IL-2 and IFN-γ and memory Teffs IL-17, whereas control Teffs did not constitutively express any of these cytokines. pITP Tregs constitutively expressed IL-2 and IFN-γ, whereas control Tregs did not constitutively express these cytokines. Compared to control Tregs, pITP Tregs constitutively expressed lower IL-10 mRNA levels. In summary, our results strongly suggest that low expression and synthesis of Ets-2 in naive Teffs of pITP patients leads to impaired downstream events in Th cell plasticity. This manifests as high constitutive gene expression of Th1/Th17 cytokines in Teffs and abnormal cytokine gene expression in Tregs.

SpT-06-10
Evaluation of new progestogen and selective estrogen receptor modulator effect on matrix metalloproteinases activity in experimental endometrioid heterotopias

I. Voronkina[1,*], P. Evstigneeva[1,*], L. Smagina[1], E. Baziian[2], L. Polyanskikh[2], M. Petrosyan[2,3]
[1]*Institute of Experimental Medicine, Sankt-Petersburg, Russia,* [2]*D.O. Ott Institute of Obstetrics, Gynecology and Reproductology, Sankt-Petersburg, Russia,* [3]*Almazov National Medical Research Centre, The Laboratory of Myocardial Metabolism, Sankt-Petersburg, Russia*

The search for new approaches to treatment of patients with endometriosis treatment is an extremely urgent task for modern gynecology. We studied the effect of new progesterone analog (PA) and a selective estrogen receptor modulator (SERM) on activity of matrix metalloproteinases (MMPs) types 1, 2 and 9 in endometrioid heterotopias on model of surgically induced endometriosis in rats. MMP involved in processes of pathogenesis of endometriosis, destroy connective tissue matrix proteins and provide invasion of heterotopias in the underlying tissues, causing the prevalence and clinical manifestations. Due to this mechanism, the possibility of growth and autonomy of endometrioid heterotopias is ensured. Both drugs showed high efficacy in the treatment of endometriosis comparable to dienogest in experimental model. Modeling of endometriosis was made on female Wistar rats by auto transplantation of uterine horn fragments to the mesentery and abdominal wall of the same animal. After successful pathology development, new PA and SERM were administered to the model animals for 21 days. Control group received an adequate volume of solvent. After removing of all animals from experiment, visual evaluation and measurements of implants the tissue fragments was sent to histology and biochemical analysis. The activity of MMP-1, MMP-2, and MMP-9 in tissues was determined by zymography and the amount of collagen type I by Western blotting. The zymograms and blots images were processed by QuantiScan program and statistical processing was made by Statistics 8.0 program. Comparisons of MMP activity between groups were performed using the Wilcoxon-Mann-Whitney test with The Benjamin-Hochberg correction for multiple comparisons. The tested compounds show different effects on the activity of MMPs. In the case of new PA, the mechanism of suppressing the growth of endometriosis foci is manifested among other things due to anti-angiogenic properties of PA through the suppression of MMP activity while SERM does not affect the activity of the studied enzymes. The results obtained indicate that SERM as well as progestins can be used in the treatment of endometriosis. *The authors marked with an asterisk equally contributed to the work.

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

SpT-06-06
Cell-to-cell transmission of SARS-CoV-2 is resistant to neutralizing antibodies

N. Kruglova[1], A. Siniavin[2], D. Mazurov[1]
[1]*Institute of Gene Biology Russian Academy of Sciences, Moscow, Russia,* [2]*Gamaleya Center for Epidemiology and Microbiology, Moscow, Russia*

The COVID-19 pandemic caused by SARS-CoV-2 has had a tremendous impact on our lives. The development of vaccines has raised hopes that the thread may be controlled but looking for a treatment option has not become less important. An invaluable tool in these studies including identification of entry inhibitors and assessing humoral immunity to SARS-CoV-2 is a pseudovirus system. It represents a safe and more widely accessible alternative to the tests utilizing replication-competent virus. In our study, we assembled a SARS-CoV-2 pseudotyped system using HIV-1 packaging and intron-regulated reporter vectors. These reporter constructs are silent in producer cells and become active when one cycle of target cell infection is completed. This feature allows measuring not only cell-free infection, but also cell-to-cell viral replication in mixed producer-target cell co-cultures. In order to study SARS-CoV-2 transmission, we made several modifications in the C-terminus of spike and selected ones that mediated the highest level of infection. Concurrently, we generated the target 293T cell line stably expressing the ACE2 receptor. The system was highly sensitive to detect infectious events using three different reporter genes, luciferase, GFP and mCherry. We evaluated our system with convalescent sera and found that samples which demonstrated high inhibitory activity against cell-free infection and syncytia formation were drastically less potent against fusion-independent cell-to-cell infection. The experiments with the wild-type virus SARS-CoV-2 on Vero cells confirmed the differences observed for neutralization of cell-free vs cell-to-cell infection. The mechanisms of SARS-CoV-2 cell-to-cell transmission are unknown, and our findings underline the importance of studying this unusual route of coronavirus transmission which has crucial implications for the treatment and prevention of COVID-19. This work was supported by RSF grant 21-14-00237.

SpT-06-02
Implication of graded reductions in CLN6's anti-aggregate activity as a pathomechanism of the neuronal ceroid lipofuscinoses

Y. Shiro, T. Yamazaki
Graduate School of Pharmaceutical Sciences, Tokushima University, Tokushima, Japan

Ceroid Lipofuscinosis Neuronal protein 6 (CLN6), spanning the endoplasmic reticulum membrane, is a protein of unknown function. Mutations in the CLN6 gene are associated with an autosomal recessively inherited disorder termed CLN6 disease, a form of the neuronal ceroid lipofuscinoses (NCL). Coupled with a lack of knowledge of the physiological role CLN6 plays, the pathomechanism of CLN6 disease, however, has remained unresolved. We previously demonstrated that CLN6 can prevent aggregate formation mediated by the R120G αB-crystallin (αBC) mutant, highly prone to aggregate. The finding prompted us to hypothesize that an impairment of CLN6's anti-aggregate activity is the chief cause of CLN6 disease. The aim of this study is to explore the hypothesis. Using a series of truncated and missense mutants of CLN6, we showed that the amino acids 148-150 are essential for CLN6's anti-aggregate activity. We subsequently investigated if pathogenic mutations in the amino acid stretch, described in patients with CLN6 disease, indeed attenuate CLN6's anti-aggregate activity toward four aggregation-prone αBC mutants (D109H, R120G, G154S, and R157H). The R149C and the R149H CLN6 mutants, both associated with adult-onset CLN6 disease, blocked aggregation of two out of and all the four αBC mutants, respectively. In contrast, the R106PfsX26 CLN6 mutant, linked to late infantile-onset CLN6 disease, inhibited none of the four αBC mutants from aggregating. Together, CLN6's anti-aggregate activity is differentially modulated according to the substitution pattern at the same amino acid position. Based on the findings, we here propose that graded reductions in the CLN6's anti-aggregate activity governs the clinical course of late infantile- and adult-onset NCL.

SpT-06-01
Human aquaporin-5 selectivity and peroxiporin activity modulate cell survival and cancer cell migration

C. Pimpão[1], C. Rodrigues[1], A.F. Mósca[2], P.A. Pedersen[3], F. Antunes[4], G. Soveral[1]
[1]*Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal,* [2]*Instituto de Medicina Molecular João Lobo Antunes (iMM), Faculdade de Medicina da Universidade de Lisboa, Lisbon, Portugal,* [3]*Department of Biology, University of Copenhagen, Universitetsparken 13, Copenhagen OE, Denmark,* [4]*Centro de Química e Bioquímica, Centro de Química Estrutural e Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal*

H_2O_2 is the main reactive oxygen species (ROS) involved in oxidative sensing and signaling. This molecule has a dual effect: at low concentrations can act as a second messenger in redox signaling, while at high concentrations contributes to oxidative stress. A few mammalian aquaporins (AQPs) called peroxiporins, are known to facilitate H_2O_2 permeation through membranes, controlling its intracellular concentration and participating in tumorigenesis. Both AQP3 and AQP5 are overexpressed in cancer tissues and AQP3 peroxiporin activity has been related with cancer cell migration. Here, we report that human AQP5 also facilitates H_2O_2 diffusion through membranes and has a role in modulating cell growth and resistance to oxidative stress. By mutagenesis studies we found that His173 located in AQP5 selectivity filter is crucial for AQP5 permeability, and its proximity to phosphorylated Ser183 may impair permeability through pore constriction. Moreover, AQP5-silenced pancreatic cancer cells showed impaired cell migration. Our data disclose the important residues for AQP5 water conductance and reveal a major role of this channel in the fine-tuning of intracellular H_2O_2 concentration with impact in cell survival under oxidative stress, suggesting that human AQP5 is a promising target for cancer therapeutics.

SpT-06-08
Deciphering redox-centered regulation of DJ-1 activity and its importance for DJ-1 neuroprotective mechanisms

S. Anjo[1,2], M. Melo[1], L. Loureiro[1,3], L. Sabala[1,3], N. Jordão[1,3], M. Neves[1,3], V.M. Mendes[1], T. Faria[1], P. Castanheira[4], R. Brito[1], M. Grãos[1,4,5], B. Manadas[1,5]
[1]Center for Neuroscience and Cell Biology - University of Coimbra, Coimbra, Portugal, [2]Faculty of Sciences and Technology - University of Coimbra, Coimbra, Portugal, [3]University of Aveiro, AVEIRO, Portugal, [4]Biocant - Biotechnology Transfer Association, Cantanhede, Portugal, [5]Institute for Interdisciplinary Research, University of Coimbra (IIIUC), Coimbra, Portugal

DJ-1 mutations cause a genetic form of Parkinson's Disease (PD) but little is known about the physiological function of DJ-1 and the mechanisms by which its mutations lead to PD. Evidence suggest that these familiar forms are likely to cause loss of DJ-1 stability. Under oxidative stress conditions, the proposed cause of death of dopaminergic neurons in PD, DJ-1 shifts to oxidized species which are detected in the brain of patients with sporadic PD. Moreover, DJ-1 may act as a "sensor" of cellular ROS levels, and the oxidized DJ-1 may subsequently acquire a new function of protecting against cellular damages partly by modulating its network of interactions. To clarify this redox-centered regulation of DJ-1 activity and its importance for DJ-1 neuroprotective mechanisms, several approaches were performed, including a dynamic interactome characterization of endogenous DJ-1 under resting and oxidative stress conditions. To study the role of extracellular DJ-1 and to evaluate the impact of the modulation of some specific DJ-1 residues, recombinant DJ-1 proteins were produced and characterized, including an evaluation of the effect of cysteine-oxidation in protein structure and stability. Moreover, the impact of the PD-related mutation on DJ-1 oxidation was also addressed by using our recent method OxSWATH[1]. Pull-down assays were used to establish the importance of DJ-1 stress regulation and PD mutations on the newly interactions/mechanisms identified, and rescue assays were performed to evaluate the neuroprotective capacity of these different DJ-1 forms. The data obtained from the combination of all these approaches largely contributes to elucidate DJ-1 neuroprotective mechanisms and to identify several novel DJ-1 binding partners, pointing to new processes for DJ-1-mediated neuroprotection and contributing with new insights for PD-related mechanisms. [1]Previously published in: Anjo SI. (2019) Redox Biology 22, 101130.

SpT-06-05
miRNA-directed dendritic cell reprogramming

N. Arh[1,2], F. Rosa[1,2], F. Pereira[1,2,3]
[1]Molecular Medicine and Gene Therapy, Lund Stem Cell Center, Lund University, Lund, Sweden, [2]Wallenberg Center for Molecular Medicine, Lund University, Lund, Sweden, [3]Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal

Direct cell reprogramming is an emergent way of understanding and controlling somatic cell fate. Our group has previously described direct reprogramming of fibroblasts to induced dendritic cells (iDCs) through overexpression of the transcription factors (TFs) PU.1, IRF8 and BATF3 (PIB), providing evidence that immunity can be induced with direct reprogramming. While the role of TFs is well established during DC specification the contribution of other RNA species remains unclear. We hypothesized that miRNAs would have a critical role in DC specification and may be employed to improve the efficiency of the direct reprogramming process. First, we combined miRNA overexpression with TF-mediated reprogramming of mouse embryonic fibroblasts to dendritic cells. We identified 15 individual candidate miRNAs or clusters by combining literature mining with published gene expression data. Reprogramming efficiency was quantified using flow cytometry with Clec9a-tdTomato reporter expression and activation of CD45 and MHC-II, as well as the DC1-specific marker XCR1. We identified two miRNAs that improved iDC reprogramming by distinct mechanisms: one upregulated the population co-expressing CD45 and MHC-II while the other led to an increase in the DC1-specific XCR1. These phenotypic changes were corroborated by morphologic differences as quantified by fluorescence microscopy and their combination demonstrated an additive impact on iDC reprogramming. To address species-conservation and discover novel candidates in human dermal fibroblasts, we then utilized a pooled lentiviral miRNA library and quantified enrichment within reprogramming marker subpopulations using amplicon sequencing. The pooled screen identified new miRNA candidates not previously associated with DC1 specification. Our work sheds light on miRNA regulatory networks underlying the establishment of dendritic cell fate and may serve as an improved platform for the development of human patient-specific DCs for immunotherapy.

Early Bird Sessions

Sunday 4 July
8:00–8:45, Gallery Hall

NGS is everywhere

EB-01-1
Changes in DNA methylation and gene expression of zinc finger 714: a possible blood biomarker of suicidal behaviour

K. Kouter[1], T. Zupanc[2], A. Videtič Paska[1]
[1]University of Ljubljana, Faculty of medicine, Institute of Biochemistry, Ljubljana, Slovenia, [2]University of Ljubljana, Faculty of Medicine, Institute of Forensic Medicine, Ljubljana, Slovenia

DNA methylation is a well-studied epigenetic modification in mammals. Changes in the methylation pattern can be associated with various disease states, including psychopathologies. Suicide is a global public health problem, with Slovenia being ranked as one of leading European countries regarding suicide rate. Currently the field of psychiatry is one of the few fields of medicine where biochemically supported diagnostics is scarce despite the emerging evidence of biological involvement in mental disorders. Therefore, we strive to identify biomarkers that would be suitable for clinical use in psychiatry; one possibility could be the use of DNA methylation as a biomarker. Zinc finger 714 (ZNF714) is a protein belonging to a large group of DNA binding proteins, zinc fingers. We have identified ZNF714 as a new candidate gene in a pilot study of a global methylation pattern (previously published

in: Kouter K, Zupanc T & Videtic Paska A (2019) J Affect Disord 253, 419-425.). Our sample included blood and tissue of four different brain areas (hippocampus, insula, amygdala and Brodmann area 46. Using next-generation targeted bisulfite sequencing we obtained methylation information of a CpG island, residing in the promoter region of ZNF714. We observed significantly decreased levels of methylation in suicide victims in all four brain regions and blood, with highly comparable methylation pattern between all brain regions and blood. When analysing gene expression, in hippocampus there was a significantly higher expression of ZNF714 in suicide victims. With suicidal behaviour and other mental disorders being highly prevalent, the need for additional diagnostics and treatment is grave. ZNF714 could therefore serve as a possible blood biomarker of suicidal behaviour.

EB-01-2
NGS in diagnostics of neurological disorders

K. Gotovac Jerčić[1,*], F. Borovečki[1,2,*]
[1]*Clinical Hospital Center Zagreb, Zagreb, Croatia,* [2]*Center for Translational and Clinical Research, University of Zagreb, School of Medicine, Clinical Hospital Center Zagreb, Department of Neurology, Zagreb, Croatia*

Rapid progress of genetic diagnostic methods and application of genomic technologies has revealed the genetic basis of many neurological diseases that were traditionally characterized as idiopathic. Neurological disorders present a significant public health problem and include a number of clinical symptoms from delay in cognitive and linguistic abilities, presence of anatomic abnormalities to epileptic events and muscle weakness. Despite extensive clinical and laboratory tests, specific genetic cause remains unexplained in more than 50% of cases of these diseases. Previous diagnostic methods enabled analysis of single genetic mutations, making determination of the causal mutation time consuming, expensive, and very often not detectable. The precise differential diagnosis of neurological disorders is challenging due to their genetic heterogeneity, phenotypic similarities and overlapping symptoms. Most commonly used molecular diagnostic technique applied in the diagnosis of complex diseases is next generation sequencing (NGS). We have applied our custom designed epilepsy panel that consists of 142 genes and exome sequencing in patients with epilepsy and neuromuscular disorders which enabled the identification of causative variants in patients in whom standard diagnostic procedures failed to identify a clear genetic cause of disease. These results offer further proof that NGS approaches represent powerful tools for establishing a definitive diagnosis and can improve treatment efficacy. Genomic approach in neurological diseases opens new horizons in understanding disease mechanisms, leading to the development of new diagnostic tools and their increasing application in medicine. *The authors marked with an asterisk equally contributed to the work.

EB-01-3
Non-coding RNA of extracellular vesicles in type 1 diabetes

T. Tesovnik
University Medical Centre Ljubljana, Ljubljana, Slovenia

Type 1 diabetes is an autoimmune disease associated with pancreatic Langerhans beta-cells destruction, resulting in a lack of endogenous insulin, impaired glucose homeostasis, and a decrease in the quality of patient's life. The etiology of the disease is still not well established due to the inaccessibility of the affected endocrine pancreatic beta-cells. The insight into the physiological processes in the affected beta-cells can be achieved with the study of extracellular vesicles, which can be biomarkers and mediators of the disease development. In our study, a fraction of blood plasma extracellular vesicles positive for membrane proteins potentially associated with insulin-producing beta-cells was observed, which indicates beta-cell communication with other tissues and the immune system. Next-generation sequencing was used to analyze blood plasma extracellular vesicle RNA-fractions of type 1 diabetes individuals and intensive beta-cell destruction in Langerhans islets transplantation. The immunomodulatory role of the selected differentially expressed extracellular vesicle miRNAs was evaluated with the whole human blood samples *in vitro* stimulation, which resulted in vesicle-miRNAs accumulation in phagocytes endolysosomal pathway and the activation of the immune system. The immune system activation presented with the increased degranulation and proliferation of NK and T-cells, as well as increased cytokine/chemokine release, while the miRNA transfection together with the chloroquine inhibitor resulted in the decreased inhibition of the immune system response. The cloroquine inhibition indicated TLR7/8 responsible for vesicle delivered miRNA recognition. Vesicle delivered miRNAs in type 1 diabetes show the complexly of extracellular vesicles RNA in the regulation of the immune system and a potential involvement in the development of autoimmunity. The TLR7/8 signaling also emphasizes the implications for developing strategies for disease prevention.

EB-01-4
cfDNA methylation as a stable diagnostic biomarker

J. Krasić[1], I. Abramovic[1], A. Vrtaric[2], N. Gabaj[3], S. Kralik-Oguic[4], A. Katusic-Bojanac[1], D. Jezek[1], N. Sincic[1]
[1]*Centre of Excellence for Reproductive and Regenerative Medicine, School of Medicine, University of Zagreb, Zagreb, Croatia,* [2]*Department of Clinical Chemistry, Sestre Milosrdnice University Hospital Center, Zagreb, Croatia, Zagreb, Croatia,* [3]*Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia, Zagreb, Croatia,* [4]*Clinical Institute of Laboratory Diagnostics, University Hospital Centre Zagreb, Zagreb, Croatia, Zagreb, Croatia*

Tissue biopsy has for the longest time been the gold-standard in cancer research and patient management. However, in the age of precision and personalized medicine, a shift is being made towards the employment of liquid biopsies and further analysis by NGS technology. While both the genetic and epigenetic markers of cell-free DNA (cfDNA) reflect the molecular status of its tissue-of-origin it is known that preanalytical procedures impact the downstream results. Calls for greater standardization are continuously being made, yet studies that comprehensively assess the impact of different methods on downstream diagnostic parameters, including NGS, are lacking. An analysis of blood plasma is presented with the aim to assess the variability of their respective cfDNA diagnostic parameters. The most popular methods of cfDNA isolation were assessed. Quantification of cfDNA was performed by qPCR as well as cfDNA fragment analysis. cfDNA methylation was analysed by pyrosequencing, the first next-generation sequencing instrument which is still a gold-standard in DNA methylation research. Different isolation methods gave a

wide range of cfDNA yields. cfDNA fragmentation was also impacted with different methods producing different fragmentation indexes. Still, cfDNA methylation data have remained consistent across different methods used. While both cfDNA yield and cfDNA fragmentation are highly impacted by preanalytical methods employed, cfDNA methylation analysed by pyrosequencing has remained unchanged. cfDNA methylation has been shown as a stable biomarker that could reduce pressure of method and protocol inter-lab standardization and help cfDNA find clinical use sooner rather than later.

Monday 5 July
8:00–8:45, Gallery Hall

How to improve immunotherapy of cancer?

EB-02-1
An enhanced CRISPR tool for treating chronic myelogenous leukemia

D. Lainšček[1], V. Forstnerič[1], Š. Malenšek[1], M. Sever[2], R. Jerala[1,3]
[1]*Department of Synthetic Biology and Immunology, National Institute of Chemistry, Ljubljana, Slovenia,* [2]*University Medical Centre Ljubljana, Department of Haematology, 1000 Ljubljana, Ljubljana, Slovenia,* [3]*EN-FIST Centre of Excellence, Ljubljana, Slovenia*

The CRISPR/Cas system is a highly potent tool which has revolutionized genome engineering and regulation of gene transcription in various cells and organisms. This gene-editing tool consists of a guide RNA (gRNA) and Cas9 endonuclease. Cas9 catalyzes the formation of double-strand DNA breaks, which are then repaired by different cell mechanisms. Error-prone Non-homologous end joining occurs, resulting in random indel (insertion-deletion) mutations, which can lead to functional gene inactivation by either frameshift or deletions. To achieve greater indel mutations, CRISPR system can be coexpressed in cells with DNA exonucleases, which cause increased recessions of DNA following DNA breaks. We show that joint action of the CRISPR system with different exonucleases significantly increases the percentage of indel mutations at various targeted genes. Of the different exonucleases tested, the *E. coli*-derived exonuclease III (EXOIII) exhibited the best performance in terms of indel formation. To further improve the rate of indel mutations, Cas9 and EXOIII were brought into the proximity via coiled-coil forming heterodimeric peptides (CCExo). This resulted in increased indel formation compared to the classical CRISPR/Cas system as well as more efficient than cointroduction of non-interacting and genetically fused Cas9-EXOIII. We performed a case study for the use of the CRISPR-EXO system as a potential anti-cancer therapeutic tool. The Philadelphia chromosome, which occurs in leukemic cancer cells, is the result of characteristic the reciprocal genome translocation t(9:22) and is responsible for higher proliferation of tumorous cells. Using the CCExo system, we achieved a higher degree of indel mutations at the translocation site, which resulted in greater killing of cancer cells, thus providing a useful potential anti-cancer therapeutic tool.

EB-02-2
Increasing natural killer cell cytotoxicity by targeting cystatin F

M. Perišić Nanut[1], E. Senjor[1], J. Sabotič[1], A. Jewett[2], J. Kos[3]
[1]*Institute Jozef Stefan, Ljubljana, Slovenia,* [2]*Division of Oral Biology and Medicine, The Jane and Jerry Weintraub Center for Reconstructive Biotechnology, UCLA, Los Angeles, California, United States of America,* [3]*University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia*

Natural killer (NK) cells show strong cytolytic function against tumor cells and virus-infected cells. Due to their effective lysis of tumor cells and tumor stems cells NK cell-based immunotherapies were tested in several clinical trials delivering promising results. Increasing evidence suggests that tumor microenvironment regulate the phenotype and inactivates the cytolytic function of NK cells. Understanding the mechanisms of NK cell inactivation is crucial for development of more effective antitumor therapy. Cystatin F is endogenous inhibitor of cysteine peptidases cathepsins. It is produced and secreted as a disulphide-linked dimer inactive as an inhibitor of C1 cathepsins. Due to this N-linked glycosylation, it is targeted to endo/lysosomes where it is proteolyticaly cleaved by cysteine peptidase cathepsin V and activated to become a strong inhibitor of major granzyme convertases, cathepsins C and H. The secreted dimeric protein can be taken up from the microenvironment via mannose-6-phosphate receptors and directed to the endo/lysosomes of recipient cell. Using recombinant cystatin F proteins, we showed that dimeric and activated N-terminally truncated cystatin F were taken up by both NK-92 cells and primary human NK cells and translocated to endo/lysosomes. There they inhibited the activity of cathepsins C and H, and decreased the activity of effector granzymes A and B leading to a decrease in cytotoxic efficiency of NK-92 cells and primary human NK cells. Therefore, altered extra- and intra-cellular availability of active cystatin F could significantly affect NK cells' cytolytic function. We confirmed that N-glycosylation pattern affects the secretion, uptake and subcellular sorting of cystatin F in different cell lines. By targeting its expression and activation through inhibition of cystatin F activating peptidase or by modulating its glycosylation, we will be able to enhance/maintain the cytotoxicity of NK cells and increase their antitumor activity.

EB-02-3
Treatment induced upregulation of DNA sensing pathways as a mechanism for inducing anti-tumor immune response

T. Jesenko[1,2], M. Bosnjak[1], B. Markelc[1,3], G. Sersa[1,3], K. Znidar[1], L. Heller[4], M. Cemazar[1,5]
[1]*Institute of Oncology Ljubljana, Ljubljana, Slovenia,* [2]*University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia,* [3]*University of Ljubljana, Faculty of Health Sciences, Ljubljana, Slovenia,* [4]*University of South Florida, Tampa, Florida, United States of America,* [5]*University of Primorska, Faculty of Health Sciences, Ljubljana, Slovenia*

Radiation therapy is a mainstay of cancer treatment with approximately one-half of all cancer patients receiving it as a part of their standard of care. In addition, new treatment modalities are entering clinical practice, among which, gene therapy, more specifically gene electrotransfer (GET) of therapeutic plasmid DNA to tissues, shows potential. The effects of these

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

therapies are not only specific on their targeted mechanism, but, it is becoming more and more evident that they can also modulate the immune response in tumors, which in conjunction with immune checkpoint inhibitors could mediate the tumor's response to therapy or even incite systemic rejection of cancer. The mechanisms of these effects remain unclear; however, DNA sensing pathways were demonstrated to be involved. The DNA, which accumulates in the cytosol of cells following irradiation due to the damage induced by X-ray radiation is sensed by specific pattern recognition receptors, i.e. DNA sensors, which through several signaling pathways induce the production of various cytokines - type I interferons, tumor necrosis factor alpha, interleukin 1 beta and others, depending on the cell type. A similar effect can be seen when plasmid DNA enters the cell after GET, which is also sensed by DNA sensors in the cytoplasm. The released cytokines, which are expressed following the sensing of DNA in the cytoplasm can then mediate the anti-tumor immune response in tumors. Understanding the timing of the activation of the DNA sensing pathways and subsequent cytokine expression induced by different treatment modalities could improve the schedules for combining immune checkpoint inhibitors with standard of care treatment modalities in order to improve the immunotherapy of cancer.

EB-02-4
Amniotic membrane as a multimodal therapy of bladder cancer: targeting the growth and invasive potential of urothelial cancer cells

T. Železnik Ramuta[1], A. Janev[1], L. Tratnjek[1], U. Dragin Jerman[1], M. Magatti[2], E. Vertua[2], P. Bonassi Signoroni[2], A. Silini[2], O. Parolini[2,3], M. Erdani Kreft[1]
[1]*Institute of Cell Biology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia,* [2]*Centro di Ricerca E. Menni, Fondazione Poliambulanza Istituto Ospedaliero, Brescia, Italy,* [3]*Istituto di Anatomia Umana e Biologia Cellulare, Università Cattolica del Sacro Cuore, Facoltà di Medicina e Chirurgia, Rome, Italy*

The human amniotic membrane (hAM) is a multi-layered membrane that comprises the innermost part of the placenta. It possesses many properties that make it suitable for use in regenerative medicine, namely promotion of epithelization and decrease of scarring, low immunogenicity, immunomodulatory properties and also antimicrobial and anticancer properties. The aim of this study was to investigate the effect of hAM-derived preparations, namely hAM scaffolds, hAM homogenate and hAM-derived cells, on the urothelial cancer cells. We demonstrated that all hAM-derived preparations diminished the proliferation of urothelial cancer cells. Moreover, hAM scaffolds also altered the dynamics of urothelial cancer cells' growth, decreased expression of N-cadherin, Snail and Slug and hindered their muscle-invasive potential. Individual urothelial cancer cells even began expressing epithelial markers E-cadherin and occludin. We demonstrated that the hAM homogenate induced detachment of urothelial cancer cells and limited their attachment to the surface. Furthermore, the hAM homogenate also disrupted the architecture of 2D and 3D urothelial cancer models. In conclusion, our results demonstrate the detrimental effect of hAM-derived preparations on urothelial cancer cells and provide important fundamentals for further research of hAM potential as a novel anticancer therapeutic.

Tuesday 6 July
8:00–8:45, Gallery Hall

New approaches in medicinal chemistry

EB-03-1
Inhibiting cysteine cathepsins by targeting sites outside of the active site

M. Novinec
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia

Human cysteine cathepsins are appealing drug targets in numerous diseases, including autoimmune, inflammatory and metabolic diseases. Unfortunately, no cathepsin inhibitor has been successfully launched on the market so far. Traditional cathepsin inhibitors target the active site and are often plagued by low selectivity and off-target effects. Therefore, alternative strategies of these peptidases are emerging that target sites outside of the active site. We are investigating mechanisms of allosteric inhibition of selected cysteine cathepsins, focusing on synthetic and natural small molecules (effectors). Using a combination of *in silico* screening and experimental methods, we discovered and characterized the first allosteric effectors of cathepsin K, a target in osteoporosis and osteoarthritis. Two compounds from the US NCI/DTP Open Chemical Repository (NSC13345 and NSC94914) were shown to bind to a novel allosteric site on cathepsin K and acted as partial inhibitors of its activity. Based on the structure of compound NSC13345, we synthesized novel effectors with improved activity and affinity in the low micromolar range. Recently, a succinimide-amino acid scaffold initially designed to target cathepsin K was also diversified to target cathepsin S, which is associated with cardiovascular and pulmonary diseases, resulting in the first partial inhibitors of this peptidase. The identified effectors showed good selectivity over other closely related peptidases and our current efforts are aimed at improving their affinity. Moreover, we recently discovered that selected natural derivatives of cinnamic acid are reversible, full inhibitors of all cysteine cathepsins that act via kinetic mechanisms other than specific (competitive) inhibition, indicating that they bind outside of the active site. We are currently investigating their structure-activity relationships and the potential of this scaffold for development of specific inhibitors of individual cathepsins.

EB-03-2
Cathepsin X inhibition – new approach for improvement of cathepsin B directed antipeptidase therapy

A. Mitrović[1], J. Završnik[2], G. Mikhaylov[2], U. Pečar Fonović[3], D. Knez[3], M. Butinar[2], S. Gobec[3], B. Turk[2,4,5], J. Kos[1,3]
[1]*Department of Biotechnology, Jožef Stefan Institute, Ljubljana, Slovenia,* [2]*Department of Biochemistry and Molecular Biology, Jožef Stefan Institute, Ljubljana, Slovenia,* [3]*University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia,* [4]*Center of Excellence for Integrated Approaches in Chemistry and Biology of Proteins, Ljubljana, Slovenia,* [5]*University of Ljubljana, Faculty of Chemistry and Chemical Technology, Ljubljana, Slovenia*

Cathepsin B is lysosomal cysteine peptidase that is one of the crucial peptidases in cancer. It is unique among cysteine

cathepsins due to its dual endo- and exopeptidase activity that is a consequence of the presence of extra structural element the occluding loop. Cathepsin B has been validated as a promising target for antitumor therapy. However, decrease in the effectiveness of cathepsin B inhibition has been observed over time. This can be attributed to the compensation of its activity by increased expression and activity of cathepsin X, another lysosomal cysteine carboxypepstidase that is also involved in progression of cancer. Here we showed that triazole-based selective reversible inhibitor of cathepsin X (1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-((4-isopropyl-4H-1,2,4-triazol-3-yl)thio)ethan-1-one) (Compound 22) significantly reduces tumor growth and lung metastasis formation in vivo in transgenic FVB/PyMT breast cancer mouse model and in orthotopic MMTV-PyMT mouse breast cancer model. Therefore, the use of cathepsin X inhibitors presents novel strategy for improvement of cathepsin B directed therapy. Addition of cathepsin X inhibitors to the cathepsin B directed antipeptidase therapy, additionally decreased tumor invasion and migration in vitro on multiple cell-based models of tumor invasion and migration both in two- and three-dimensional settings and in vivo on mouse model. Taken together, our data show that potent selective reversible inhibitors of cathepsin X impair tumor progression both in vitro and in vivo and can be used in combination with other antipeptidase inhibitors as an innovative approach for overcoming resistance to the antipeptidase therapy.

EB-03-3
Potentiation of the antitumor effect of electrochemotherapy with bleomycin, cisplatin or oxaliplatin by immunostimulation with IL-12 gene electrotransfer depends on the tumor immune status

K. Ursic[1], S. Kos[1], U. Kamensek[1], M. Cemazar[1,2], S. Miceska[3], B. Markelc[1,4], S. Bucek[3], B. Staresinic[1], V. Kloboves Prevodnik[3,5], R. Heller[6], G. Sersa[1,4]
[1]Institute of Oncology Ljubljana, Department of Experimental Oncology, Ljubljana, Slovenia, [2]University of Primorska, Faculty of Health Sciences, Izola, Slovenia, [3]Institute of Oncology Ljubljana, Ljubljana, Slovenia, [4]University of Ljubljana, Faculty of Health Sciences, Ljubljana, Slovenia, [5]University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia, [6]University of South Florida, Tampa, Florida, United States of America

The therapeutic effectiveness of electrochemotherapy (ECT) in the clinics is up to 90% of local tumor control; however, a systemic antitumor effect (abscopal effect) has not yet been observed. The aim of the study was to test a new combined therapy including ECT with cisplatin, bleomycin or oxaliplatin and gene electrotransfer (GET) of plasmid encoding interleukin-12 (IL-12) in three immunologically different tumors. We hypothesized that in the combination, IL-12 boosts the in situ vaccination effect of ECT by recruiting effector immune cells. A malignant melanoma (B16F10), mammary carcinoma (4T1) and colon carcinoma (CT26) were treated (U34401-1/2015/7). Growth of primary treated tumors and of distant untreated tumors in a dual-flank model mimicking systemic disease was followed. After the therapy, cytometric and immunohistochemical analysis were performed to detect immunologically important biomarkers. In poorly immunogenic B16F10 melanoma, IL-12 potentiated the antitumor effect of ECT with biologically equivalent low doses of cisplatin, oxaliplatin or bleomycin. However, we observed the most pronounced potentiation after ECT with cisplatin, resulting in 38% of complete responses as well as an abscopal effect. The antitumor effectiveness of this treatment combination could be ascribed to the induction of the local and systemic immune responses. Namely, infiltration of granzyme B positive effector immune cells was observed in both, primary and distant tumors. Furthermore, we observed better responsiveness to ECT in more immunogenic 4T1 and CT26 tumors, where the addition of GET led to the lowest potentiation. To conclude, we showed that IL-12 boosts the effect of ECT by recruiting effector immune cells in poorly immunogenic melanoma. Effectiveness of the tested treatment combinations depends on the immunological status of the tumor; ECT was more effective in more immunogenic tumors but the contribution of GET was higher in less immunogenic tumors.

EB-03-4
PROTACS as tools for the modulation of pharmacological targets

I. Sosič[1], C. Steinebach[2], A. Bricelj[1], Y.L.D. Ng[3], M. Gobec[1], J. Krönke[3], R.J. Pieters[4], M. Anderluh[1], M. Gütschow[2]
[1]University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia, [2]University of Bonn, Pharmaceutical Institute, Bonn, Germany, [3]University Hospital Ulm, Department of Internal Medicine III, Albert-Einstein-Allee 23, D-89081 Ulm, Germany, Ulm, Germany, [4]Department of Chemical Biology & Drug Discovery, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, Netherlands

The proteolysis targeting chimera (PROTAC) concept is currently receiving major attention in drug discovery field as it holds tremendous potential in the therapy of different human diseases (Scudelari, M (2019) Nature 567, 298-300). PROTAC strategy uses a heterobifunctional molecule comprised of a small-molecule ligand (binding to a protein of interest), another small molecule ligand (binding to an endogenous E3 ligase), and a linker moiety connecting both compounds. If designed successfully, PROTAC molecules hijack the endogenous ligase and the ubiquitin proteasome system to degrade, rather than just inhibit, protein(s) of interest (Burslem, GM et al. (2020) Cell 181, 1-13). Our work is focused on several important pharmacological targets, namely the O-GlcNAc transferase (OGT), B-cell lymphoma 2 (BCL-2), and cyclin-dependent kinase 6 (CDK6). The cellular levels of all three proteins are dysregulated in many diseases; therefore, development of their degraders is a viable strategy in the development of both possible therapeutics and useful chemical probes to study involvement of these proteins in various molecular pathways inside cells. During the development of PROTACs for these targets, we utilized their known inhibitors as starting points, i.e. OSMI-4 for OGT, venetoclax for BCL-2, and palbociclib for CDK6. Moreover, for the construction of chimeras we used ligands for at least three different E3 ligases, such as cereblon, von Hippel-Lindau, and Inhibitor of Apoptosis Protein. Such modularly assembled PROTACs did yield potent and selective degraders of CDK6 (Steinebach C et al. (2020) Chemical Science, https://doi.org/10.1039/D0SC00167H), which represent extremely valuable tools for future development of anti-cancer therapeutics.

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206

Wednesday 7 July
8:00–8:45, Gallery Hall

Molecular toxicology and environment

EB-04-1
Crosstalk of proteases in DNA–protein crosslink repair

M. Popovic, C. Supina, N. Ivić, I. Anticevic, C. Otten
Ruder Boškovic Institute, HR-10000 Zagreb, Croatia

DNA-protein crosslinks (DPCs) are severe DNA lesions which occur when a protein becomes irreversibly covalently linked to DNA. On a cellular level, aberrant DPC repair leads to the formation of DSBs, genomic instability and/or cell death, while on the organismal level impaired DPCR causes premature aging phenotypes and cancer. Despite the fact that DPCs are frequently occurring in the nucleus and cause severe damage, not much is known about its repair mechanisms at a molecular and organismal level. Our discovery of proteolysis-coupled DPC repair centred on SPRTN protease led to recognition of DNA-protein crosslink repair as a separate DNA damage repair pathway. We and others have shown that SPRTN removes proteinaceous part of DPC, thus initiating subsequent removal of peptide remnant crosslinked to DNA. Considering that SPRTN is a replication-specific protease, we searched for another protease which would act in lowly proliferative cells where DPCs pose a threat to transcription progression. Indeed, we have identified a SPRT-domain containing protein, ACRC (acidic repeat containing) using phylogenetic analysis. In line with the phylogenetic proximity, the 3D structure of the protease core of ACRC is very similar to that of SPRTN. The putative protease core of ACRC includes two α-helices bearing three Zn-binding histidines and a catalytic glutamate residue which together form a HEXXH motif, a characteristic of all Zn-dependent metalloproteases. Our goal is to determine if ACRC is proteolytically active, if it bears a role in DPC repair and what is its relation to SPRTN. To address functionality of ACRC, protein purification and biochemical analysis are under way. Using CRISPR gene manipulation, we created an enzymatic impaired version of ACRC through mutation knock-in in mammalian cells and in zebrafish (Danio rerio) model organism. Our study reveals the contribution of ACRC to the DPC removal on the cellular and organismal level.

EB-04-2
Mechanisms of rapid adaptation to changed environment

H. Bilandžija
Ruder Boskovic Institute, Zagreb, Croatia

A widely accepted model for the evolution of cave-dwelling animals posits cave colonization by a surface ancestor followed by the acquisition of adaptations over many generations until a fully cave-adapted form is achieved. However, the speed of adaptive evolution in some species is difficult to reconcile with this conventional viewpoint, suggesting the importance of alternative mechanisms that operate over a shorter timescale. To obtain insight into these mechanisms, we used *Astyanax mexicanus*, a single teleost species consisting of two morphs, an ancestral surface-dwelling morph (surface fish) and a derived cave-dwelling morph (cavefish). We exposed Astyanax surface fish (SF) to completely dark (D/D) conditions as embryos and compared them to siblings placed on a normal photoperiod (L/D SF). We identified a number of traits that change in D/D SF raised for several months to couple of years in complete darkness compared to L/D SF, including endocrine, stress-related, weight-related, and metabolic changes, as well as a change in the expression of approximately 350 genes. Remarkably, most of these changes are associated with known adaptations to the cave environment and alter in the direction of the cavefish phenotype. Our results indicate that appearance of many cave-associated traits can occur within a single generation by phenotypic plasticity. By comparing gene expression in D/D SF, which were raised in darkness beginning a few hours after fertilization, with L/D SF, we determined that all of the molecular mechanisms known to facilitate phenotypic plasticity, including upregulation of HSP90, endocrine modifications, and epigenetic changes, may be involved in this process in Astyanax. The results suggest that phenotypic plasticity followed by genetic assimilation may be an important mechanism for the rapid evolution of cave-related traits during the colonization of *A. mexicanus* to dark cave environments.

EB-04-3
New antimicrobial candidates – quaternary ammonium compounds based on natural scaffold quinuclidine

L. Mastelić[1], A. Maravić[2], L. Krce[3], B. Soldo[1], R. Odžak[1], V. Bučević Popović[1], I. Aviani[3], I. Primožič[4], P. Bošković[1], **M. Šprung**[1]
[1]University of Split, Faculty of Science, Department of Chemistry, Split, Croatia, [2]University of Split, Faculty of Science, Department of Biology, Split, Croatia, [3]University of Split, Faculty of Science, Department of Physics, Split, Croatia, [4]University of Zagreb, Faculty of Science, Department of Chemistry, Zagreb, Croatia

Quaternary Ammonium Compounds (QACs) are amphiphilic molecules of antimicrobial properties with applications in numerous industries. Recent studies show that environmental bacteria acquire resistance to QACs at an alarming pace, and that this resistance is mostly but not exclusively related to Qac efflux pump expression. Motivated by these findings, our research group has been mainly focused in developing new potent QACs derived from natural precursor(s). One such precursor that caught our attention is quinuclidine, a bicyclic part of alkaloids isolated from the bark of the Cinchona tree. In our two recent studies we have shown that quaternization of quinuclidine improves its bioactivity by several hundred folds (Previously published in: Odžak R et al. (2017), Open Chem. 15, 320-331 and Bazina L et al. (2019) Eur. J. Med. Chem. 163, 626-635). The most active identified candidate was QOH-C14. This compound, with the longest alkyl chain, exhibited the lowest MIC values across a selected panel of the bacteria, had good activity toward *Staphylococcus aureus* biofilms and had the lowest toxicity toward healthy human cell lines. Moreover, atomic force microscopy and flow cytometry indicated that QOH-C14 acts by disrupting the cell membrane provoking membrane disintegration and cell death. In addition, physicochemical characterization showed that derivatives with longer alkyl chains spontaneously form micelle and that their stability is also related to the number of carbon atoms in the chain (Previously published in: Bošković P et al. (2019) J. Surfact. Deterg. 23, 207-214). From these studies two main conclusions could be drown. First, quaternization

of natural precursors could be a promising strategy in new QACs development. Second, alkyl chains are an important part of the structure whereby antimicrobial activity directly correlates with the number of carbon atoms in the chain.

EB-04-4
Roles of Rrp6/EXOSC10-targeted lncRNAs in anti-cancer drug toxicity and cell wall architecture

I. Stuparevic[1], A. Novacic[1], M. Oskomic[1], L. Strbac[1], V. Beauvais[2], M. Primig[3], R. Rahmouni[2]
[1]*University of Zagreb, Faculty of Food Technology and Biotechnology, Zagreb, Croatia,* [2]*Center for Molecular Biophysics (CBM), CNRS, ORLEANS, France,* [3]*Univ Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail)- UMR_S 1085, Rennes, France*

Saccharomyces cerevisiae is a versatile model organism that has been used extensively to study mitotic cell growth and division in the presence of numerous toxic compounds, including chemotherapeutical agents and toxic molecules that affect cell wall formation and maintenance. The widely used anti-cancer drug 5-fluorouracil (5-FU) was initially identified as a DNA replication inhibitor but was later found to also inhibit the conserved 3′-5′ exoribonuclease Rrp6/EXOSC10, a catalytic subunit of the nuclear RNA exosome. The protein plays a role in degradation and processing of protein-coding and non-coding transcripts and its absence renders cells hypersensitive to 5-FU. Transcriptome analysis of 5-FU treated yeast cells revealed a negative effect on the expression of the transcriptional activators Swi5 and Ace2 that induce cell cycle regulated genes involved in mitotic cell division. Moreover, we observed that different types of non-coding RNAs (ncRNA) accumulated in 5-FU treated cells, which are typically present at high levels in a strain lacking Rrp6/EXOSC10. Interestingly, comparison of transcriptome data from wild type and rrp6 mutant strains showed altered expression levels both of genes that encode proteins crucial for cell wall integrity and lncRNAs that either overlap their promoters or that are in a sense/antisense configuration. Consistently, a yeast strain lacking Rrp6 is more sensitive than the wild-type to cell wall stressing compounds. Our data offer interesting leads for the discovery of novel protein-coding and non-coding RNAs that may be involved in 5-FU toxicity and cell wall defects caused by cell wall stressing compounds. These results are potentially important for improving antifungal therapies and 5-FU based chemotherapies. *The authors marked with an asterisk equally contributed to the work.

FEBS Open Bio **11** (Suppl. 1) (2021) 2–102 DOI: 10.1002/2211-5463.13206