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TALKS

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

About these abstracts

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We are unable to make **corrections of any kind** to the abstracts once they are published.

Indexing

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

TALKS

Plenary Lectures

Saturday 9 July
17:00–18:00, Auditorium I

IUBMB Claudina Rodrigues-Pousada Lecture – Opening Plenary Lecture

PL-01–1
Mapping the human body one cell at a time

S. Teichmann
Wellcome Sanger Institute, Cambridge, UK

The 37 trillion cells of the human body have a remarkable array of specialised functions, and must cooperate and collaborate in time and space to construct a functioning human. In this talk I will describe my lab's efforts to understand this cellular diversity through a programme of cell atlasing. Harnessing cutting-edge single cell genomics, imaging and computational technologies, we investigate development, homeostasis and disease states, at scale and in 3D, with a particular focus on immunity. I will illustrate the relevance of cell atlasing for engineering organoids and regenerative medicine, and will share new results providing insights into pacemaker cells from the sinoatrial node of this heart. Overall, I hope to illustrate the power of single cell approaches in unlocking fundamental knowledge about the human body.

Sunday 10 July
11:30–12:30, Auditorium I

FEBS Datta Lecture

PL-02-1
Gut microbiota: Fellow travellers that regulate brain & behaviour across the lifespan

J.F. Cryan
University College Cork, Cork, Ireland

We are living in a microbial world; microbes were here first and there has never been a time when the brain existed without microbes. There is increasing interest in the role of these fellow travellers in all aspects of physiology, including brain health. The microbiota-gut-brain axis is emerging as a research area of increasing interest for those investigating the biochemical and molecular basis of neurodevelopmental, age-related and neurodegenerative disorders. The routes of communication between the gut and brain are being resolved and include the vagus nerve, the immune system, tryptophan metabolism, via the enteric nervous system or via microbial metabolites such as short chain fatty acids. Studies in animal models have been key in delineating that neurodevelopment and the programming of an appropriate stress response is dependent on the microbiota. Developmentally, a variety of factors can impact the microbiota in early life, including mode of birth delivery, antibiotic exposure, mode of nutritional provision, infection, stress as well as host genetics. Stress can significantly impact the microbiota-gut-brain axis at all stages across the lifespan. Recently, the gut microbiota has also been implicated in a variety of conditions, including obesity, autism, schizophrenia, motor neuron disease and Parkinson's disease. Moreover, animal models have been key in linking the regulation of fundamental brain processes, ranging from adult hippocampal neurogenesis to myelination to microglia activation by the microbiome. Finally, studies examining the translation of these effects from animals to humans are currently ongoing. Further studies will focus on understanding the causal mechanisms underlying such brain effects, and developing nutritional and microbial-based intervention strategies.

Sunday 10 July
14:30–15:30, Auditorium I

EMBO Lecture

PL-03-1
New approaches to enhance regeneration of aged skeletal muscle

P. Munoz Canoves
Universitat Pompeu Fabra (UPF), Barcelona, Spain

During ageing, tissue regenerative functions decline. In skeletal muscle, regeneration depends on a normally quiescent population of stem cells (satellite cells). Maintenance of the satellite-cell quiescent state is under tight control, including active proteostasis. Upon injury or stress, satellite cells proliferate, and either self-renew or differentiate to regenerate muscle. When and how these decisions are taken is not known. During the seminar, I will first show that these alternative stem-cell fates are pre-determined in quiescence, before satellite cells encounter stress, and I will discuss the mechanisms underlying these fate choices, how they change with aging and the consequences on the regeneration of aged muscle. Secondly, I will show the important role of senescent cells in regulating skeletal muscle regeneration in young and old age. Finally, based on these findings, I will discuss strategies toward improving muscle regeneration throughout life.

Monday 11 July
11:30–12:30, Auditorium I

FEBS Sir Hans Krebs Lecture

PL-04-1
Metabolic liver disease: leaping forward

C.M.P. Rodrigues
Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal.

Non-alcoholic fatty liver disease (NAFLD) affects 25% of the adult population paralleling obesity and diabetes. Despite the lack of approved therapies, pharmacological inhibition of receptor-interacting protein kinases (RIPK) improves experimental NAFLD in part by increasing mitochondrial respiration (*Majdi et al. J Hepatol. 2020*). In addition, mitofusin 2 is decreased in liver biopsies from NAFLD patients and its deficiency reduces phosphatidylserine transfer and phospholipid synthesis, leading to ER stress and inflammation (*Hernandez-Alvarez et al. Cell. 2019*). RIPK3, in turn, is a key player in

necroptosis and an emerging metabolic regulator, whose contribution to NAFLD is controversial. We have shown that hepatic RIPK3 increases in patients with NAFLD, correlating with hepatic inflammation and fibrosis (*Afonso et al. Gut. 2021*). Accordingly, Ripk3 deficiency ameliorated diet-induced hepatocellular damage, inflammation and fibrosis in a mouse model that recapitulates human NAFLD. Furthermore, Ripk3 deficiency hampered hepatocarcinogenesis. Intriguingly, deletion of Ripk3 increased liver fat accumulation and shifted the hepatic lipidome *in vivo*, although lipid droplets were smaller in fat-loaded Ripk3−/− hepatocytes. Ripk3 deficiency upregulated PLIN1 and PLIN5, which in turn are implicated in mitochondria-lipid droplet interactions. In line, Ripk3 deficiency rescued mitochondrial biogenesis, bioenergetics and function *in vivo* and *in vitro*. Data from preclinical models and patients indicate that the PGC-1α/PPARγ/PLIN1 axis is functionally implicated in improving lipid and mitochondrial metabolic homeostasis by Ripk3 deficiency. Conversely, a pathogenic PLIN1 frameshift variant was associated with NAFLD, fibrosis, and RIPK3 levels in familial partial lipodystrophy. In conclusion, RIPK3 plays a key role in managing liver metabolism, damage and carcinogenesis, whereby RIPK3 inhibition may ameliorate NAFLD. (Funding: H2020 IMI2 and MSC; La Caixa; FCT).

Monday 11 July
14:30–15:30, Auditorium I

FEBS/EMBO Women in Science Award Lecture

PL-05-1
Protein synthesis in remote neuronal spaces

E. Schuman
MPI for Brain Research, Frankfurt am Main, Germany

The complex morphology of neurons, with synapses located hundreds of microns from the cell body, necessitates the localization of important cell biological machines, including ribosomes, within dendrites and axons. Local translation of mRNAs is important for the function and plasticity of synapses. Using advanced sequencing and imaging techniques we have updated our understanding of the local transcriptome and identified the local translatome, identifying over 800 transcripts for which local translation is the dominant source of protein. In addition, we have explored the unique mechanisms neurons use to meet protein demands at synapses, identifying surprising features of neuronal and synaptic protein synthesis.

Tuesday 12 July
11:30–12:30, Auditorium I

IUBMB E.C. Slater Lecture

PL-06-1
Neurovascular pathobiology of vascular cognitive impairment and Alzheimer's disease

C. Iadecola
Weill Cornell Medical College, New York, NY, United States of America

The brain lacks energy reserves and is vitally dependent on continuous and well-regulated delivery of oxygen and glucose through the cerebral blood supply, and alterations of cerebral blood vessels have emerged as key correlates of cognitive impairment. The concept of the neurovascular unit was introduced to highlight the close developmental, structural, and functional interactions between brain cells and microvessels (*Neuron 96:17, 2017*), and was recently expanded to neurovascular complex to include neurovascular interactions at all levels of the cerebrovascular tree, and not just the microcirculation (*Nature Neuroscience 24:1198, 2021*). Comprised of neurons, glia, vascular cells, and perivascular cells (perivascular macrophages, microglia, fibroblasts, etc.), the neurovascular complex is responsible for matching the delivery of blood to the brain with local energy needs dictated by brain activity. The neurovascular complex is also involved in regulating the bidirectional molecular exchange between blood and brain (blood–brain barrier), the clearance of metabolic by-products of brain activity (e.g., beta-amyloid and tau), the trafficking of immune cells, and providing trophic support to brain cells. Neurovascular dysfunction alters the homeostasis of the brain microenvironment and is an early pathogenic event not only in vascular cognitive impairment, but also in Alzheimer's disease and related dementias, attesting to the significant overlap between these conditions. Furthermore, major risk factors for cognitive impairment, such as hypertension, ApoE4 genotype, and high salt intake, are also associated with neurovascular dysfunction. Activation of innate and adaptive immunity, inflammation and vascular oxidative stress are major pathogenic factors. The recent realization that vascular dysfunction and neurodegenerative diseases have common pathogenic drivers has major diagnostic and therapeutic implications for both vascular and neurodegenerative dementias.

Tuesday 12 July
13:30–14:00, Auditorium I

The FEBS Journal Richard Perham Prize Lecture

PL-07-1
Vitamin D effects on human colon normal and tumour organoids

A. Fernández-Barral[1,2], A. Costales-Carrera[1,2], S. P. Buira[3], P. Jung[4,5,6], G. Ferrer-Mayorga[1,2], M.J. Larriba[1,2], P. Bustamante-Madrid[1,2], O. Domínguez[7], F.X. Real[1,7], L. Guerra-Pastrián[8], M. Lafarga[9], D. García-Olmo[3,10], R. Cantero[10], L. Del Peso[2,11], E. Batlle[1,4,12], F. Rojo[1,3], A. Muñoz*[1,2], **A. Barbachano***[1,2]
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Many studies indicate an association between vitamin D deficiency and increased colorectal cancer risk and, specially, mortality.

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Accordingly, the active vitamin D metabolite 1α,25-dihydroxyvitamin D_3 (calcitriol) inhibits the proliferation and promotes the differentiation of colon carcinoma cells and of other tumour cell types, and also has antitumour effects in animal models of colon cancer. These results prompted us to analyse the effects of calcitriol on human colon normal and cancer stem cells. To this end, we established a living biobank of patient-derived colon organoids generated from the tumour mass and from the adjacent healthy tissue obtained from surgical biopsies. Organoids are a three-dimensional culture system of normal or cancer stem cells and their progeny with a self-organized multicellular structure. By immunohistochemistry and RNAscope *in situ* hybridization, we found that vitamin D receptor is expressed in $LGR5^+$ colon stem cells in human tissue and in normal and tumour organoid cultures. RNA-sequencing assays showed that both organoid types respond differentially to calcitriol with profound and contrasting changes in their transcriptomic profiles. This was confirmed in an independent series of patient-derived organoids by RT-qPCR assays. In normal organoids, calcitriol upregulates stemness-related genes and inhibits cell proliferation. In contrast, in tumour organoids calcitriol has little effect on stemness-related genes, while it induces differentiation-associated genes, and variably reduces cell proliferation. Concordantly, electron microscopy analyses showed that calcitriol does not affect the blastic cell phenotype in normal organoids, but it induces a series of differentiated features in tumour organoids. These results indicate that calcitriol maintains the undifferentiated phenotype of human normal colon stem cells (homeostatic action), while it promotes the differentiation of colon cancer stem cells (anticancer action). *The authors marked with an asterisk equally contributed to the work.

Tuesday 12 July
14:00–14:30, Auditorium I

FEBS Letters Award Lecture

PL-08-1
Transcription factor control of pluripotency

I. Chambers
5517 - University of Edinburgh, Edinburgh, United Kingdom

Stem cells all have the capacity to both self-renew and differentiate into more specialised cell types. Self-renewal of mouse embryonic stem cells (ESCs) cultured in serum requires leukaemia inhibitory factor (LIF) signalling. This LIF requirement can be circumvented by overexpression of the homeodomain transcription factor (TF) NANOG. Together with OCT4 and SOX2, NANOG forms the core triumvirate of TFs that regulate ESC self-renewal. The function of these TFs will be discussed, with a focus on NANOG. In addition to the DNA binding domain, chromatin binding by NANOG is mediated by an intrinsically disordered region in which every 5th residue is a tryptophan (the tryptophan repeat; WR). Results demonstrating how the multivalent WR enables NANOG to interact with chromatin and partner proteins to deliver function will be presented.

Tuesday 12 July
14:30–15:30, Auditorium I

FEBS Education Plenary Lecture

PL-09-1
Beating to a different drum: How can education become relevant again?

B. Jokić
Institute for Social Research in Zagreb, Zagreb, Croatia

The COVID-19 pandemic represents the greatest global disruption in recent history, affecting education and the personal and social development of over 2 billion students at various educational levels worldwide. The negative effects of the pandemic on educational processes were manifested in significant learning gaps, the loss of learning habits and motivation, and disruptions in students' aspirations. These negative effects of the pandemic further amplified the already present loss of relevance and importance of formal education in the lives of children and youth. Furthermore, the pandemic intensified existing inequalities between and within education systems. Education systems of low and lower-middle income countries experienced greater negative impact. In all systems, opportunities and learning experiences for gifted students, those at risk or with special education needs, were significantly altered. However, the responses of educational authorities, institutions and practitioners also resulted in many positive elements by opening a space for innovation and creativity. Furthermore, the situation revealed a much-needed flexibility in educational structures, a feature not often associated with such robust and inert systems. Along with a presentation of empirical results on the effects of the pandemic on students, this lecture will present a vision for how education can regain relevance for students, practitioners and society. To achieve this vision, significant changes to educational structures, teaching and learning approaches and assessment practices are of crucial importance. Important in this vision is the strong positioning of science, including biochemistry, from the early years, as well as a systematic effort aimed at making science understandable and interesting to all students. In a post-pandemic world, education can still be the most attractive melody for fostering personal and social growth, but to do so, it needs to beat to new and different rhythms.

Wednesday 13 July
11:30–12:30, Auditorium I

IUBMB Kunio Yagi Lecture

PL-10-1
The KEAP1-NRF2 pathway regulating cellular response against oxidative and electrophilic stresses

M. Yamamoto
Tohoku University, Sendai, Japan

Our body has an ability to sense environmental stress and induce cellular defense enzymes. Transcription factor NRF2 is crucial

for coordinated expression of cellular defense enzymes against oxidative and electrophilic stresses. KEAP1 acts as a sensor for the stresses and as a subunit of ubiquitin-E3 ligase that degrades NRF2 constitutively. Nrf2 gene knockout animals are sensitive to a wide variety of toxic electrophiles and reactive oxygen species, while Keap1 gene knockdown animals show a gain-of-function cytoprotection phenotype. Modifications of KEAP1 cysteine residues abrogate the ubiquitin ligase activity and stabilize NRF2. This mechanism is referred to as the Cysteine Code. Two-Site-Binding and Hinge-and-Latch models between a KEAP1 homodimer and a single NRF2 molecule have been proposed for the regulation of this system. Genetic as well as pharmacological induction of NRF2 protect tissues from oxidative injury. Disruption of KEAP1-NRF2 binding explains how nuclear accumulation of NRF2 is attained in a KEAP1-dependent manner, providing a solid basis for the development of new drugs that induce NRF2. On the other hand, many somatic mutations have been identified in KEAP1 and NRF2 genes of human cancers. These mutations disrupt the KEAP1-NRF2 binding and result in constitutive activation of NRF2, which confers advantages on the malignant growth of cancer cells. Autophagy chaperone p62 is also found to disrupt the KEAP1-NRF2 binding. New topics have been constantly added to this field, including Nrf2 regulation of inflammation, metabolism, ageing and neuroprotection. Historical overview as well as recent progress in the field of KEAP1-NRF2 study will be discussed.

Wednesday 13 July
14:30–15:30, Auditorium I

PABMB Lecture

PL-11–1
Phase separation, phase transition and aggregation of mutant p53 as an emerging target in cancer

J. Silva
Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Biomolecular condensates are membraneless structures that originate from liquid–liquid phase separation. In addition to the physiological roles, condensates can undergo a solid-phase transition, generating amyloid-like structures involved in degenerative diseases and cancer. We will present recent studies on the formation of biomolecular condensates and aggregates in proteins involved in cancer, with a special focus on the tumor suppressor protein p53. More than half of malignant tumors harbor mutations in the TP53 gene and will kill hundreds of million people if new therapies are not developed soon enough. We have explored the findings that mutant p53 not only undergoes misfolding, but also forms biomolecular condensates and aggregates comparable to amyloids formed by other proteins, thereby playing a crucial role in cancer development through loss of function (LoF), negative dominance (ND) and gain of function (GoF) mechanisms. Strikingly, as in the case of toxic amyloids in neurodegenerative diseases, the molecular mechanisms responsible for the GoF of mutant p53 are not yet completely understood. However, we already know that different cofactors, such as nucleic acids and glycosaminoglycans, are at the crossroads between these two classes of diseases. Importantly, molecules that inhibit mutant p53 aggregation reduce tumor proliferation and migration. Thus, phase transitions to solid-like amorphous and amyloid-like states of mutant p53 emerge as formidable targets for the development of novel diagnostic and therapeutic strategies against cancer. (Supported by funds from CNPq, FAPERJ, CAPES and FINEP.)

Thursday 14 July
11:30–12:30, Auditorium I

FEBS Theodor Bücher Lecture – Closing Plenary Lecture

PL-12-1
MINFLUX and MINSTED provide molecule-scale resolution in fluorescence microscopy

S. Hell
Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany

I will show how an in-depth description of the basic principles of diffraction-unlimited fluorescence microscopy (nanoscopy) [1] has spawned a new powerful super-resolution concept, namely MINFLUX nanoscopy [2–5]. MINFLUX utilizes a local excitation intensity minimum (of a doughnut or a standing wave) that is targeted like a probe in order to localize the fluorescent molecule to be registered. In combination with single-molecule switching, MINFLUX and its more recent 'cousin' MINSTED [6] have obtained the ultimate (super)resolution: the size of a molecule. Providing 1–3 nanometer resolution, these novel microscopy concepts are being established for routine fluorescence imaging at the highest, molecular-size resolution levels. Relying on fewer detected photons than popular camera-based localization, MINFLUX and MINSTED nanoscopy are poised to open a new chapter in the imaging of protein complexes and distributions in fixed and living cells. [1] Hell, S.W. Nat. Methods 6, 24–32 (2009). [2] Balzarotti, F., Eilers, Y., Gwosch, K. C., Gynnå, A. H., Westphal, V., Stefani, F. D., Elf, J., Hell, S.W. Science 355, 606–612 (2017). [3] Eilers, Y., Ta, H., Gwosch, K. C., Balzarotti, F., Hell, S. W. PNAS 115, 6117–6122 (2018). [4] Gwosch, K. C., Pape, J. K., Balzarotti, F., Hoess, P., Ellenberg, J., Ries, J., Hell, S. W. Nat. Methods 17, 217–224 (2020) [5] Schmidt R., Weihs T., Wurm C., Janssen I., Rehman J., Sahl S.J., Hell S.W. Nat Commun 12:1478 (2021) [6] Weber, M., Leutenegger M., Stoldt S., Jakobs S., Mihaila T.S., Butkevitch A.N., Hell S.W., Nat. Photonics, https://doi.org/10.1038/s41566-021-00774-2 (2021).

Symposia

Sunday 10 July
9:00–11:00, Auditorium I

Cancer and metastasis

S-01.1–2
Beneficial autoimmunity in cancer control

G. Kroemer
Cordeliers Research Center, Paris, France

Many tumour antigens that do not arise from cancer cell-specific mutations ("neoantigens") are targeted by humoral and cellular immune reactions despite their expression on normal cells. Thus, the immune system does not only detect mutations and stress-associated shifts in the immunoproteome and immunopeptidome (the sum of MHC class I-bound peptides) unique to malignant cells, but also recognizes antigens expressed in normal cells, which can result in autoimmune reactions against normal structures from the tissue of origin. These autoimmune manifestations include, among others, vitiligo, thyroiditis and paraneoplastic syndromes, concurrent with melanoma, thyroid cancer and non-small-cell lung cancer, respectively. Importantly, despite the undesirable effects of these symptoms, such events can have prognostic value and correlate with favourable disease outcomes, suggesting the existence of 'beneficial autoimmunity'. For example, there is a negative epidemiological association between, on one hand, breast cancer and, on the other hand, rheumatoid arthritis or systemic lupus erythematosus. Similarly, the occurrence of dermal and endocrine autoimmune adverse events in patients receiving immune-checkpoint inhibitors can have a positive predictive value for therapeutic outcomes. Neoplasias derived from stem cells deemed 'not essential' for survival (such as melanocytes, thyroid cells and most cells in sex-specific organs) have a particularly good prognosis, perhaps because patients can tolerate autoimmune reactions that destroy tumour cells at some cost to non-malignant tissues. Recently, we obtained evidence that, in mice, experimental induction of autoimmune cholangitis can protect against the development of cholangiocarcinoma, but not that of other malignancies from other cells of origin. Furthermore, we have demonstrated that vaccination against normal mammary or ileal epithelial cells has oncopreventive effects against breast cancer or colorectal cancer, respectively.

S-01.1–1
NRF2 addiction of cancer cells

H. Motohashi
Tohoku University, Sendai, Japan

The KEAP1-NRF2 system is a major inducible defense mechanism against redox disturbance. While increased NRF2 activity is principally beneficial for our health, the outcome of NRF2 activation in cancer cells is detrimental which is observed in almost 15% of non-small cell lung cancer (NSCLC). Multiple lines of evidence suggest that aberrantly activated NRF2 in cancer cells drives their malignant progression and that the cancer cells consequently develop "NRF2 addiction." NRF2 enhances survival of cancers by activating cytoprotective genes. NRF2 also redirects glucose and glutamine into anabolic pathways by activating metabolic genes, which are advantageous for cancer cell proliferation. Under the influence of the microenvironment, NRF2 strongly promotes tumorigenesis by helping cancer cells to evade anti-cancer immunity. To explore new therapeutic targets for NRF2-activated cancers, we conducted an unbiased approach by investigating the NRF2-dependent transcriptome in NSCLC cell lines with NRF2-activated NSCLCs, and in those with NRF2-normal NSCLCs. We identified a battery of genes that are regulated by NRF2 specifically in NRF2-activated NSCLCs and found that these genes are accompanied by unique NRF2-dependent enhancers. CEBPB accumulation in NRF2-activated NSCLCs is found to be one of the prerequisites for the establishment of the unique enhancers, in which NOTCH3 enhancer is critical for the promotion of tumor-initiating activity. In addition, genes involved in drug metabolism and detoxification were found to be co-regulated by NRF2 and CEBPB. These results suggested that enhanced activities of stem-like phenotype, drug metabolism and detoxification are achieved by the cooperative function of NRF2 and CEBPB in NRF2-activated NSCLCs.

ShT-01.1–1
Conversion of arginine to citrulline alters the angiogenic and epigenetic profile of human endothelial cells

O. Ciesielski[1,2], A. Balcerczyk[1]
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Citrullination, a protein posttranslational modification converting peptidyl-arginine to peptidyl citrulline, significantly changes the structure and function of proteins, as it has been shown in several physiological and pathological processes, including cancer metastasis. However, its role in regulating angiogenesis and also modulating the epigenetic profile remain unknown. The purpose of this study was to investigate the effect of pharmacological inhibition of protein citrullination on endothelial cell (EC) viability, histone H3 and H4 citrullination status and the flagship EC function: angiogenesis. Two human EC models were used: primary vein (HUVECs) and microvascular endothelial cells (HMEC-1). Three irreversible commercially available inhibitors of PADs (PADi) were used: BB-Cl-amidine, Cl-amidine and F-amidine. Cellular viability was measured with the resazurin reduction assay. Inhibitor concentrations above IC_{50} were then used to examine the effects on citrullination status by western blotting (WB). Next, the impact of PAD inhibition on angiogenic potential of EC was examined with R&D angiogenesis kit, the expression of selected angiogenic factors was checked via qPCR, and we also performed capillary tube-like formation assay and also migration assay. Also, the profile of other histone PTMs in PADi treated ECs was analysed via WB. Out of the three inhibitors tested, only BB-Cl-amidine exerted a cytotoxic effect on ECs. All the inhibitors decreased the H3cit mark; however, BB-Cl-amidine proved to be the most efficient, followed closely by Cl-amidine. Similar results were observed in the angiogenic profile studies: BB-Cl-amidine exerted the strongest effects out of the other tested compounds. No significant differences were observed between the primary and immortalized EC. The presented results

show that citrullination may play an important part in regulating angiogenesis. The presented study was funded by the Polish Ministry of Higher Education under *Diamond Grant DI2018 018948*.

ShT-01.1–2
Proteasomal degradation of the transcription factor SHARP1 via the SCFßTrCP Ubiquitin Ligase Complex

A. Lauriola[1], J.H. Enriqué Steinberg[2], F.A. Rossi[3], M. Santucci[4], M. Rossi[3], D. Guardavaccaro[4]
[1]*University of Verona, Verona, Italy,* [2]*Department of Biotechnology, University of Verona, Verona, Italy,* [3]*Instituto de Medicina Traslacional (IIMT), CONICET-Universidad Austral, Buenos Aires, Argentina,* [4]*1Department of Biotechnology, University of Verona, Verona, Italy*

A recently identified suppressor of the metastatic behavior of triple negative breast cancer (TNBC) is the bHLH transcription factor SHARP1. SHARP1 blocks the invasive phenotype of TNBC by inhibiting hypoxia-inducible factors. Moreover, loss of SHARP1 expression correlates with poor survival of breast cancer patients and represents a prognostic marker for TNBC. Here we show that SHARP1 is an unstable protein that is targeted for proteasomal degradation by an SCF ubiquitin ligase. To identify the specific F-box protein interacting with SHARP1, we immunopurified SHARP1. Mass spectrometry analysis of immunoprecipitated SHARP1 identified the F-box protein ßTrCP and the SCF subunits SKP1 and CUL1. To confirm the ßTrCP-SHARP1 interaction and test its specificity, we immunoprecipitated various F-box proteins and examined their binding to SHARP1. ßTrCP was the only F-box protein interacting with SHARP1. Most proteins recognized by ßTrCP contain a DSGXX(X)S motif, known as phosphodegron, in which the phosphorylated serine residues recruit ßTrCP. SHARP1 has a similar motif in which the second serine is replaced by glutamic acid. A SHARP1 mutant, in which Ser240 and Glu245 were mutated to Ala, was not able to immunoprecipitate ßTrCP, indicating that Ser240 and Glu245 are required for SHARP1 binding to ßTrCP. Our results raise the possibility that ßTrCP-mediated degradation of SHARP1, a suppressor of TNBC metastasis, may contribute to the metastatic property of TNBC. To test this hypothesis, TNBC cells expressing non-degradable SHARP1 were injected into the mammary fat pad of female mice. Kaplan–Meier curves for tumor-free survival indicated that cells expressing SHARP1(S240A/E245A) generated fewer tumors. Accordingly, a genetic screen employing an shRNA library against ubiquitylation-related genes identified ßTrCP as a potential target of tumor cell invasion. Indeed, silencing of ßTrCP by short hairpin RNAs in TNBC cells reduced their migratory and invasive potential *in vitro*.

ShT-01.1–3
The loss of the DNA polymerase epsilon accessory subunits POLE3 and POLE4 results in PARP inhibitor sensitivity

H. Mamar, A. Mihut, R. Fajka-Boja, G. Timinszky
Laboratory of DNA Damage and Nuclear Dynamics, Institute of Genetics, Biological Research Centre, Eötvös Loránd Research Network (ELKH), Szeged, Hungary

Poly(ADP-ribose) Polymerase inhibitors (PARPi) are selective anti-cancer drugs which target tumors with Breast Cancer Susceptibility protein (BRCA) deficiency, sparing healthy cells. The successful use of PARPi in the clinic is hindered by pre-existing and acquired resistance, such as the reactivation of homologous recombination and the suppression of replication gaps. It is, therefore, crucial to identify mechanisms that re-sensitize such resistant cancer cells to PARPi. In a genome-wide CRISPR knock-out screen, we identified DNA polymerase epsilon 3 (POLE3) and DNA polymerase epsilon 4 (POLE4) as potential inducers of sensitivity towards the FDA-approved PARPi, olaparib. POLE3 and POLE4 are the two accessory subunits of the DNA polymerase epsilon holoenzyme, a major DNA-replicating enzyme with a role in base- and nucleotide excision repair as well. While both accessory subunits are dispensable for replication, they possess H3-H4 histone chaperone activity, facilitating histone deposition on the DNA leading strand. We employed CRISPR/Cas9 to generate knock-out cell lines for the two POLE subunits to validate the results of the screen. Interestingly, deleting one of the subunits severely affects the expression of the other one. More importantly, the knock-out cells are hypersensitive towards olaparib treatment compared to the wild-type. Furthermore, we find that the knock-outs do not exhibit defects in their response to oxidative stress as compared to the wild-type, nor in their poly(-ADP-ribose) levels; however, their susceptibility to replication stress and cell-cycle arrest points to a potential replication-related mechanism of action for their PARPi sensitivity. By using biochemical and microscopy-based assays, we will identify the molecular mechanisms underlying resistance to PARPi in POLE3 and POLE4 proficient cells in detail.

ShT-01.1-4
Multifunctional nanovaccine synergizes with OX40 agonist in triple-negative breast cancer

C. Peres[1,2], A.I. Matos[1,2], L.I.F. Moura[1], B. Carreira[1], M. Verdial[1], J. Conniot[1], M.B. Afonso[1], R. Acúrcio[1], S. Mensurado[2], L. Graça[2], R. Satchi-Fainaro[3], H. Florindo[1]
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Triple negative breast cancer (TNBC) is the most aggressive subtype of breast cancer. Immunotherapy, notably cancer vaccines and immune checkpoint inhibitors, has emerged as a promising alternative therapy. However, limited efficacy has been obtained for cancer vaccines, and severe immune-mediated side effects have been related to immune checkpoint inhibitors under clinical development. This study focused on the development of a multifunctional nanovaccine to re-shape tumor microenvironment (TME), sensitizing TNBC to the immune checkpoint OX40 agonist. Nanoparticles (NP) were designed to target dendritic cells (DC) and the TME by incorporating TNBC-associated antigens, toll-like receptor ligands, and regulators of potent immune suppressor molecules within the TME. NP surface was modified to promote DC activation, but also to potentiate their delivery to the TME. NP size, surface charge and morphology were characterized by Dynamic Light Scattering, Laser Doppler Electrophoresis and Atomic Force Microscopy, respectively. The entrapment efficiency (EE) of each bioactive agent entrapped within NP were fully determined and NP impact on cell viability was assessed by Alamar Blue assay. NP internalization and DC activation profile were evaluated *in vivo* by flow cytometry. The immunotherapeutic potential of the nanovaccines was assessed,

isolated and in combination with αOX40, in 4 T1 and E0771 mammary carcinoma. NP presented a mean diameter close to 200 nm and EE superior to 85%. NP showed no negative effects on DC and tumor viability. Targeted NP were extensively taken up by DC, but also by tumor cells. Nanovaccines induced DC activation and maturation. 4 T1 and E0771 tumor-bearing animals treated with the multifunctional nanovaccine combined with αOX40 showed a noteworthy tumor remission, with a prolonged overall survival. The nanovaccine re-shaped the immune profiling within the TME, which correlated with the overall anti-tumor effect obtained in this combinatorial scheme.

Sunday 10 July
9:00–11:00, Auditorium II

Host–pathogen interactions

S-02.1–1
Exploring the gut microbiota of avian scavengers and its role in pathogen control

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Abstract not available.

ShT-02.1–1
Listerial PC-PLC is a critical virulence factor for phagosomal membrane disintegration

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Broad-range phospholipase C (PC-PLC) is a zinc metalloenzyme and an important virulence factor of the bacterial pathogen *Listeria monocytogenes* (Lm). PC-PLC, together with the cholesterol-dependent pore-forming toxin listeriolysin O (LLO) and other bacterial and host proteins, disintegrates the lipid membrane of the phagosome and releases the pathogen into its replicative niche, the cytosol. To study the structure and function of PC-PLC, we recombinantly produced the active PC-PLC, determined its crystal structure, and constructed mutants exhibiting different degrees of phospholipase activity. The N-terminal tryptophan residue (W1) was found to be critical for enzymatic activity as the backbone is involved in zinc ion binding. Based on the solved three-dimensional structure, we designed two peptide inhibitors, which both notably inhibit PC-PLC activity. Next, we also investigated the interaction between PC-PLC and LLO through the lipid membrane by preincubating 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC)/cholesterol lipid vesicles with PC-PLC. Enzyme activity increased the binding of LLO to liposomes and LLO-induced vesicle leakage, whereas PC-PLC alone did not cause permeabilization. Preincubation with less active PC-PLC mutants resulted in lower LLO binding and vesicle leakage. In addition, preincubation with PC-PLC also increased the rate of LLO-induced hemolysis. We hypothesize that PC-PLC may increase cholesterol availability in the membrane by cleaving polar head groups of lipids, thereby exposing membrane cholesterol. Our results describe important structural features of PC-PLC and a potential specific inhibitor of this virulence factor. In addition, they indicate the importance of PC-PLC in the process of vacuolar escape of Lm and a possible mode of synergy between PC-PLC and LLO.

ShT-02.1–2
Visualizing pyrazinamide action by live single cell imaging of phagosome acidification and *Mycobacterium tuberculosis* pH homeostasis

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Host-Pathogen Interactions in Tuberculosis Laboratory, The Francis Crick Institute, London, United Kingdom

Mycobacterium tuberculosis (Mtb) segregates within multiple subcellular niches with different biochemical and biophysical properties that, upon treatment, may impact antibiotic distribution, accumulation, and efficacy. However, it remains unclear whether fluctuating intracellular microenvironments alter mycobacterial homeostasis and contribute to antibiotic enrichment and efficacy. Here, we describe a live dual-imaging approach to monitor host subcellular acidification and Mtb intrabacterial pH. By combining this approach with pharmacological and genetic perturbations, we show that Mtb can maintain its intracellular pH independently of the surrounding pH in human macrophages. Importantly, unlike bedaquiline (BDQ), isoniazid (INH) or rifampicin (RIF), the drug pyrazinamide (PZA) displays antibacterial efficacy by disrupting intrabacterial pH homeostasis *in cellulo*. By using Mtb mutants with different subcellular localisation, we confirmed that intracellular acidification is a prerequisite for PZA efficacy *in cellulo*. We anticipate this imaging approach will be useful to identify host cellular environments that affect antibiotic efficacy against intracellular pathogens.

ShT-02.1–3
Dual inhibitors of human and fungal sphingosine-1-phosphate lyase as antimicrobial strategy in cystic fibrosis

G. Pampalone, C. Costantini, M. Pariano, E. Camaioni, L. Macchioni, R. Galarini, F. Paoletti, T. Zelante, E. Costanzi, M. Davidescu, C. Stincardini, M. Bellet, J. Saba, S. Giovagnoli, L. Romani, B. Cellini
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Cystic fibrosis (CF) is due to the deficit of the chloride/bicarbonate channel Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), which regulates the electrolyte content of luminal fluid. The progressive decline of respiratory functions in CF patients is due to a vicious circle of inflammation and increased susceptibility to infection. *Aspergillus fumigatus* is one of the most common species isolated from the sputum of CF patients. Thus, targeting a common pathway in the host and the pathogen to balance between inflammation and infection would represent a unique treatment opportunity. Sphingosine-1-phoshate (S1P) lyase (SPL) is a Vitamin B6-dependent enzyme that irreversibly degrades S1P into hexadecenal and phosphoethanolamine at the exit point of sphingolipid metabolism. The inhibition of fungal

SPL would increase S1P, which is highly toxic to fungi. Moreover, S1P levels are reduced in the lungs of a murine model of CF and cause an altered immune response upon inflammatory challenge. Thus, dual inhibitors against host and *Aspergillus* SPL are expected to improve the antifungal response by potentiating the immune response of the host while reducing the fitness of the pathogen. In order to identify dual inhibitors, we setup a purification protocol from both human and fungal SPL and a fluorescence activity assay suitable for a high-throughput screening. Based on the crystal structure of human SPL with a known inhibitor, we performed an *in silico* virtual screening campaign. We identified 50 candidates that were tested and ranked based on their effectiveness in the dual targeting on purified human and fungal SPLs. Kinetic analyses allowed the identification of one hit compound able to inhibit for 40% and 50% the activity of human and fungal SPL, respectively. Overall, our results pave the way for the identification of a potent SPL inhibitor showing a dual-targeting action on human and *Aspergillus* SPL to be tested on *in vivo* CF models.

ShT-02.1-4
Molecular determinants of the SARS-CoV-2 fusion peptide activity

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The coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronaviruses 2 (SARS-CoV-2), emerged in late 2019 and quickly spread worldwide. SARS-CoV-2 is an enveloped virus and its entry into host cells is mediated by the spike glycoprotein (S-protein) [1]. The S-protein is composed of two subunits (S1 and S2) that contain essential domains for the viral entry mechanism, such as the fusion peptide (FP) which inserts into and disturbs the host cell membrane promoting the fusion between viral and host membranes. Despite its relevance for viral entry, there is still no consensus among scientists for its location on the S-protein and amino acid sequence, although two major candidate regions have been proposed [2, 3]. To shed light on this matter, we combined computational and experimental methods to characterize and compare the effect of the two putative SARS-CoV-2 FPs. We performed a systematic analysis of the SARS-CoV-2 putative FPs, using Molecular Dynamics simulations, to dissect how these peptides interact with the membrane. In parallel, we evaluated the putative FPs behavior in membrane model systems applying biophysical techniques. Since both FPs revealed modest fusogenic activity, we hypothesized that a longer FP or a cooperation among the individual FPs might be required to achieve fusion between viral and host membranes. Given the pivotal role of the FP to viral entry, our work provides relevant insights on the SARS-CoV-2 entry mechanism. [1] Walls AC et al. (2020) Cell 181, 281-292.e6 [2] Koppisetti RK et al. (2021) J Am Chem Soc 143, 13,205–13,211 [3] Basso LG et al (2021) Biochem Biophys Acta 1863, 183,697.

Sunday 10 July
9:00–11:00, Auditorium VII

Cell division and cell cycle regulation

S-03.1–1
Bacterial cell wall synthesis and its regulation

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To protect themselves against both extracellular stress and intracellular osmotic pressure, most bacteria are surrounded by a net-like elastic polymer called peptidoglycan (PG). PG is a covalently cross-linked single macromolecule made up of multiple overlapping glycan strands interlinked by short peptide chains that forms a mesh-like sacculus around the bacterial cytoplasmic membrane. Because PG completely encases the cytoplasmic membrane, cleavage of the peptide cross-links is a prerequisite to open the mesh for making space to incorporate new PG material for its successful enlargement during growth of a cell. Using a Gram-negative rod-shaped bacterium, *Escherichia coli*, as a model system, we established the fundamental role of PG hydrolysis in growth and enlargement of bacterial cell wall. Here, the significance of PG hydrolytic enzymes and their regulation during expansion of PG will be discussed.

S-03.1–2
Regulation of cell cycle progression in the bacterial pathogen *Staphylococcus aureus*

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The bacterial cell cycle can be described as the sequence of events in a bacterial cell leading to DNA replication, cell division and separation in two daughter cells. Progression of the cell cycle is an interesting and underexplored target for new antimicrobial compounds, as impairing cell cycle progression will necessarily lead to bacterial growth arrest. To identify key players in the regulation of the cell cycle of the bacterial pathogen *Staphylococcus aureus*, we have screened the Nebraska library of ~2000 mutants in all non-essential genes of methicillin-resistant *S. aureus* (MRSA) strain JE2. Mutants were imaged by fluorescence microscopy to look for those with an overrepresentation of cells in a specific stage of the cell cycle, indicating impaired progression from that phase to the next. One of the identified mutants was impaired in the splitting of the division septum, required at the end of division to generate two daughter cells. I will address the characterization of this autolysin mutant and how it led us to identify a new regulation mechanism for cell cycle progression in this important pathogen.

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ShT-03.1–1
Novel perspectives of target-binding by the evolutionarily conserved PP4 phosphatase

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Reversible protein phosphorylation regulates complex cellular processes, including cell division, by modulating the biological function, structure, localization, half-life or activity of regulatory or structural proteins. Although the role of protein kinases that phosphorylate mitotic proteins is relatively well characterized, the function of protein phosphatases that oppose the activity of these kinases, has been less understood. Protein Phosphatase 4 (PP4) is an evolutionarily conserved Ser/Thr phosphatase that is essential for cell cycle progression; however, little is known about its function. The major form of PP4 is the PP4c-R2-R3 heterotrimeric holoenzyme. We and others have shown that the R3 subunit of PP4 is responsible for target-recognition, including substrates and interacting partners. Recently, we have demonstrated in flies that the non-canonical EVH1 domain occupying the N-terminus of the R3 subunit, binds to specific Short Linear Motifs (SLiMs) on the target proteins, in a highly conserved manner. In parallel, we discovered, that a second domain with unknown function (DUF625/SMK1) is also involved in target-recognition; however, the mechanism is completely different from that of EVH1. We found that the SMK1 domain is present exclusively in R3 orthologues, moreover, SMK1 appears in R3 from yeast to humans and its primary sequence is highly conserved in Metazoa. We suppose that the two target-binding domains with different structure in a single regulatory subunit allow the PP4c-R2-R3 holoenzyme to recognize and thus regulate different types of molecules in different biological processes and in various tissues (*Karman et al., 2020*).This work was supported by the Government of Hungary, the Ministry of Human Capacities of Hungary and the Aron Marton College to ZsRN, the Ministry for National Economy of Hungary (GINOP-2.3.2-15-2016-00032) to RS and ZL and the Hungarian Academy of Sciences (Lendület Program Grant (LP2017-7/2017)) to ZL.

ShT-03.1–2
A liaison between functionally similar cytokinesis regulators Rab11 and Rab35 in control of cancer cell division

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Intracellular membrane transport is a key factor for controlling cellular metabolism and survival. Vesicular delivery of membranes and various proteins to the specific cellular sites during each phase of a cell cycle is essential for the maintenance of normal homeostasis. Here, a family of small monomeric Rab GTPases have been shown to play the key roles. According to recent data, Rab11 and Rab35 have been shown to be responsible for the regulation of apoptosis, adhesion, division, survival, and migration. Regulation of these processes occurs via recruitment of Rab effectors and other regulatory proteins that control the interaction of Rab proteins with endocytic membranes, and thus determine their activity in cells. Despite all the work done on the regulation of Rab11 and Rab35, it is not clear whether these proteins can function synergistically. Therefore, it is important to identify interactions and compensatory mechanisms between these proteins in regulating cell-important processes, such as cell division, proliferation, migration, and invasiveness. Thus, the aim of this study was to investigate the functions of Rab11 and Rab35 in regulating the last stage of cell division, cytokinesis, which upon dysregulation, might influence and cause phenotypic alterations in cancer cells. To study the role of Rab11 and Rab35 coherence during the regulation of cell division, different gene silencing methods were applied, which were followed by various functional analysis methods, including multinucleation assay, telophase cells assay, actin fluorescence intensity measurement, and other cell migratory assays. Overall, the presence of either Rab11 or Rab35 during cell division proved to be a vital factor for successful division, having a significant effect on cancer cell phenotype upon dysregulation. Moreover, this work allowed for the existence of synergistic mechanisms between Rab11 and Rab35 to be determined, while applying different study approaches.

ShT-03.1–3
Meloxicam inhibits cell proliferation and disturbs cell cycle in epidermal melanocytes and dermal fibroblasts exposed to UVA radiation

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Meloxicam (Mel) is a non-steroidal, anti-inflammatory drug (NSAIDs) that is extensively and commonly used due to its analgesic, anti-inflammatory, and antipyretic properties. Escalation of musculoskeletal disorders makes meloxicam usage unavoidable. Skin adverse reactions belong to the most frequently occurring adverse effects related to pharmacotherapy with meloxicam. Phototoxicity is one of the reactions and appears as a result of the simultaneous interaction between ultraviolet A radiation (UVAR) and the drug. The study aimed to evaluate the impact of meloxicam on proliferation, viability, and cell cycle of human dermal fibroblasts (HDF) and human epidermal melanocytes, lightly pigmented (HEMn-LP). The obtained results indicated that Mel, proportionally to the concentration, inhibited cell proliferation both in unirradiated cells and cells exposed to UVAR. Melanocytes were found to be more sensitive to the inhibitory effect than fibroblasts. The observation was confirmed by cytometric analysis of the cell number in tested cultures. UVAR itself had no significant effect on melanocytes and fibroblasts; however, it augmented the action of Mel at the highest concentration. The phototoxic effect of meloxicam was revealed especially in a decrease in cell viability, changes in cell morphology, and an increase in ROS level. The greatest differences were noted for melanocytes cultured with 1 mM of meloxicam and exposed to UVA radiation. Co-treatment of melanocytes with Mel and UVAR caused a decrease in cell viability by over 30% and reduced cell number by around 2.5 times. The cytotoxic and phototoxic effects of the tested drug were reflected also in the disturbance of the cell cycle. The irradiation of cells cultured with 1 mM of meloxicam caused cell cycle arrest of fibroblasts in the

S phase and melanocytes in the G1/G0 phase. Phototoxic reactions caused by Mel are associated with the induction of oxidative stress and can lead to severe damage to skin cells.

ShT-03.1-4
Phosphoregulation of the ATP synthase beta subunit contributes to mitochondrial respiration for G2/M progression

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Recently, we showed that the 2A-like protein phosphatase Sit4 promotes the dephosphorylation of ATP synthase catalytic beta subunit (Atp2 in yeast) at T124/T317. Phosphorylation at T124/T317 upregulates Atp2 levels, leading to an increase in ATP synthase activity and ATP production [1]. Using *in silico* analysis, four kinases were predicted as Atp2 regulators, namely Pkc1, Ipl1, Cdc5 and Hrr25. From these kinases, only Cdc5 overexpression increased Atp2 levels, suggesting Atp2 is regulated by Cdc5. As for the phosphatase Sit4, Cdc5 plays a prominent role in cell cycle regulation, suggesting that Atp2 phosphoregulation may be cell cycle related. To investigate this hypothesis, we monitored Atp2 levels during cell cycle progression in synchronous cells. We found Atp2 levels vary during cell cycle phases, with an increase at G1 and at G2/M. Since the Atp2 phosphosites lie within prototypal APC/C recognition motifs, an ubiquitin ligase involved in cell cycle progression, we investigated if phosphorylation prevents APC/C-mediated Atp2 degradation. In the absence of APC/C subunits Atp2 levels increased, but the mutation of the putative APC/C motifs in Atp2 or overexpressing APC/C do not influence Atp2 levels, indicating the stability of phosphorylated Atp2 is not the result of APC/C regulation. Since Atp2 phosphorylation promotes mitochondrial function, we monitored mitochondrial respiration during cell cycle phases. We found that preventing Atp2 phosphorylation, using an Atp2-T124A/T317A mutant, decreased the mitochondrial respiration peak at G2/M. In accordance, cell cycle progressed similarly in the Atp2-T124A/T317A mutant until G2/M, when the transition to G1 was delayed. Our results show that Atp2 phosphorylation is associated with cell cycle regulation, leading to increased mitochondrial respiratory activity that promotes cell cycle progression at G2/M. [1] Pereira C *et al.* (2018) Biochim Biophys Acta Bioenerg 1859, 591–601.

Sunday 10 July
9:00–11:00, Auditorium VIII

Proteins

S-04.1–2
Septin filament assembly: The rules of the game

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Septins represent the fourth component of the cytoskeleton and their importance in a wide range of essential intracellular events, many of which involve membrane remodeling or diffusion barrier formation, have become increasingly apparent over recent years. Thirteen human septins assemble into a wide range of different heteropolimeric filaments which follow specific rules of assembly giving rise to a finite set of combinations. Heteroligomeric core particles first assemble into palindromic hexamers or octamers which subsequently polymerize end-to-end. For over a decade we have been attempting to understand the rules of assembly which guarantee how each individual subunit within a viable combination assumes its rightful position along the filament and how these subsequently unite into higher order structures which associate with membranes. We have used a "divide and conquer" approach by which we have dismantled the core particles into smaller assemblies and reduced individual monomers to isolated domains. By accumulating a large number of crystal and cryo-EM structures, several features are beginning to emerge, including the importance of 1) group-specific residues which guarantee that the correct interfaces are formed along a filament; 2) the metastable properties of the C-terminal domains allowing them to participate in both parallel and antiparallel coiled coils relevant to filament assembly and cross-bridging respectively; 3) the dynamics of a polybasic region important for forming electrostatic interactions with membranes and 4) the presence of a large internal cavity essential for allowing the relative movement of subunits along the filament. These phenomena will be described in an attempt to draw our current knowledge together into a coherent picture of filament assembly and relate it to function and malfunction, such as in the case of the off-target cleavage of SEPT2 by the Zika NS2B3 protease.

S-04.1–1
Chiral proofreading during protein biosynthesis and its evolutionary implications

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A major focus of our laboratory is on translation quality control with a special reference to chirality-based checkpoints in the cell. We earlier elucidated the 'Chiral Proofreading' mechanism,

completely conserved in all bacteria and eukaryotes, by which D-amino acids are prevented from infiltrating the translational machinery and its functional implications. We identified a distinct paralog of DTD1, named as ATD for Animalia-specific tRNA deacylase, that proofreads a unique tRNA selection error in eukaryotes and could be identified from choanoflagellates, the unicellular ancestor of all animals. Our work on elucidating the functional role and its implications for the evolution of multicellular animals will be discussed. DTD2 is another chiral checkpoint of archaeal origin which is completely absent in Opisthokonts (fungi and animals) but present in plants, in addition to the canonical DTD1. We identified and characterized a unique biochemical activity through which DTD2 alleviates anaerobic stress-induced acetaldehyde toxicity, suggesting that the recruitment of archaeal DTD is a key event in the emergence and evolution of land plants. I will also discuss our current efforts in understanding further the evolutionary implications of the 'Chiral checkpoint' in biology. References: Kuncha, S. K. et al., (2020) 9, eLife, 9:e58118, 1–24. Mazeed, M., et al., (2021) Sci. Adv. 7:eabe8890, 1–16. Gogoi, J., et al. (2022) Sci. Adv. 8:eabj7307, 1–9.

ShT-04.1–1
The mysterious active site of Class 3 L-asparaginases

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From among the three classes of L-asparaginases, the *Rhizobium etli*-type (now Class 3) enzyme has been the most recalcitrant to structural characterization. The recently solved crystal structure of the inducible ReAV enzyme from *R. etli* [1] shows a dimeric protein, unlike any of the known asparaginases, similar in fold to some β-lactamases and penicillin binding proteins. Some elements of the active site, such as a tandem of Ser-Lys residues or a triad of water molecules tightly H-bonded to one of the catalytic serines (S48), are preserved from those structural homologs, but some are new and puzzling. The most intriguing is a zinc cation coordinated by a pair of cysteines, a lysine side chain and a water molecule. However, the presence of the zinc cation is not required for catalysis. Another feature is an oxidized Cys residue involved in an intricate network of H-bonds linking all the elements of the active site. *R. etli* does not have the canonical Class 1 and 2 asparaginases but it encodes a constitutive ReAIV enzyme, which despite low sequence similarity has basically the same crystal structure as ReAV. A pan-genomic analysis of all available bacterial genomes detected over 9500 Class 3 asparaginases, and in the vast majority, the active site elements of ReAV are conserved. We also found a number of fungi whose genomes encode Class 3-like proteins. The Nessler test confirms their asparaginase activity, and the first crystal structure of the *Colletotrichum fructicola* protein again shows an ReAV-like active site. Full understanding and possibly engineering of the enzymatic mechanism of Class 3 asparaginases raises hope for their application in antileukemic therapy. On the other hand, finding potent inhibitors of the fungal enzymes might lead to the development of fungicides. Work supported by National Science Centre (NCN, Poland) grant 2020/37/B/NZ1/03250. 1. Loch JI et al. (2021) Nature Commun 12, 6717.

ShT-04.1–2
'Drugging the undruggable' - discovery of unique peptide inhibitors of protein–protein interactions by machine learning and biophysical tools

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Tel Aviv University, Tel Aviv, Israel

A fundamental challenge in structural biology is the discovery of modulators for a selected region on a target protein. This has immense importance for the discovery of modulators of protein–protein interactions (PPIs). Indeed, the large number of PPIs and relevance for a broad range of diseases, marks it as an important therapeutic target. However, physio-chemical characteristics and lack of structural data often prevent the application of traditional drug discovery methodologies. Discovery of new peptides targeting a defined protein interface is an attractive approach for the deciphering of PPI structural aspects. The main challenge stems from the exponential number of peptide sequences of length L which is 20^L. This makes the discovery of such peptides nearly impossible experimentally and a time-consuming challenge computationally. Moreover, computational-based discovery frequently relies on available structural data that is often missing since many protein–protein interfaces are not yet characterized. Herein, we are developing synergistic machine learning algorithms integrated with real-time biophysical data. This unique setup can foster the filtering of initial peptides with novel sequences as the basis for further optimization of high affinity binders in the form of peptides or small molecules. We applied our methodology on the discovery, characterization and optimization of peptides containing the motifs PxIxIT and LxVP known to bind the important protein calcineurin and inhibit its interaction with the transcription factor NFAT. The latter is considered as an imperative T-cell activation switch. We discovered and validated the binding and cellular activity of unique 'out-of-the-box' binding sequences, showing potent activity against calcineurin. These novel peptide sequences enable the development of new immune modulators and further study of this important PPI. Moreover, our approach paves the way for the study of additional unexplored PPI interfaces. *The authors marked with an asterisk equally contributed to the work.

ShT-04.1–3
Protein and peptide type inhibitors of the human dUTPase

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The dUTPase enzyme plays a key role in preventive DNA repair by eliminating dUTP from the DNA biosynthetic pathway; meanwhile it produces the essential dUMP precursor for dTTP biosynthesis. Based on this prominent role of the enzyme it is in the focus of onco-therapeutic drug design projects and targeted to fight pathogenic microorganisms. Proteinaceous inhibitors provide a promising alternative for modulating enzyme function. Such protein inhibitors may also present useful tools for *in cellulo* studies on the effect of enzyme inhibition. For instance, it has been shown that a bacteriophage-related uracil-DNA glycosylase inhibitor, Ugi, can significantly reduce the enzymatic activity of the human enzyme *in vitro* and *in vivo*, and this inhibitory

effect has been exploited by CRISPR base editors as well. We have shown that the human dUTPase (hDUT) also has a potent proteinaceous inhibitor (K_i = 6.7 nM), named Stl (*Nyiri K et al. (2018) Sci Rep. 8, 4326*). Nevertheless, hDUT retained more than one third of its activity upon addition of Stl in large excess, due to the competition of Stl dimerization with Stl-hDUT interaction. We aimed to enhance the inhibitory effect of Stl on human dUTPase to obtain a powerful tool to modulate dUTP levels *in cellulo*. On one hand, we created a HDX-MS and SEC-SAXS based structural model of the dUTPase-Stl complex and designed point mutant Stl proteins based on the structure, which potentially show enhanced affinity to the human dUTPase. On the other hand, we introduced point mutations in Stl, which impair the dimerization of the protein, thus it could be more effective in dUTPase inhibition. Finally, we also tested the effect of Stl orthologs on human dUTPase. These combined efforts led to an Stl variant with enhanced properties. To further aid the design of a more potent Stl variant, we studied the complex of Stl and human dUTPase by X-ray crystallography and Cryo-EM to obtain detailed structural insights of the Stl-dUTPase interaction.

ShT-04.1-4
Structure and 'fuzziness' in the newly discovered WIP-Cortactin Regulatory Complex

C.G. Sokolik, I. Kuzminsky, **J.H. Chill**
BAR ILAN UNIVERSITY, Ramat-Gan, Israel

WASp Interacting Protein (WIP) is a cellular multi-tasker that regulates actin cytoskeleton and remodeling, and participates in a range of protein–protein interactions impacting health and disease [1,2]. One of its central binding partners is cortactin, a key nucleation-promoting factor responsible for actin polymerization and a biomarker for invasive cancers [3]. The WIP-cortactin interaction was previously mapped to the proline-rich region of WIP [4], but to date no structural evidence supports this hypothesis. Employing nuclear magnetic resonance (NMR) techniques suited for studying intrinsically disordered proteins (IDPs) we have uncovered the precise WIP cortactin-binding motif and determined the structure of the WIP/cortactin complex. Strikingly, WIP binds the N-terminal SH3 domain of cortactin in a dual class-I/class-II mode that gives rise to a 'fuzzy' complex of intermediate affinity. The non-canonical class-II pose utilizes an interaction with a second hydrophobic surface ('surface II') adjacent to the canonical polyproline-binding grooves. The existence of multiple binding poses in this 'fuzzy' complex is prototypical of complexes of a regulatory nature. We demonstrate the importance of the various molecular determinants for binding, examine this binding in the context of longer WIP-derived polypeptides, and suggest even tighter ligands that are potential leads for anti-cancer therapies. Solving the structure(s) of this key regulatory complex highlights the power of NMR to provide a fundamental biophysical view of cellular biological events and lays the foundation for further research with potential therapeutic impact. [1] Antón, I. M.; Jones, G. E. Eur. J. Cell Biol. 2006, 85 (3–4), 295–304. [2] Sokolik, C. G.; Qassem, N.; Chill, J. H. Biomolecules 2020, 10 (7), 1–19. [3] Daly, R. J. Cortactin Signalling and Dynamic Actin Networks; 2004, Vol. 382. [4] Kinley, A. W. et al, Parsons, J. T. Curr. Biol. 2003, 13 (5), 384–393.

Sunday 10 July
16:00–18:00, Auditorium I

Food and nutrition in biochemistry

S-05.1–1
Calorie restriction and aging: the mitochondrial connection

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Universidade de São Paulo, São Paulo, Brazil

In humans, obesity is associated with increased incidence of a variety of age-related diseases. Similarly, laboratory rodent lifespans are limited by obesity, including that promoted by *ad libitum* access to standard chow diets. Indeed, a daily limitation of caloric intake (calorie restriction) has been widely shown to enhance lifespans and prevent age-related diseases in rodents. We will discuss the metabolic effects of caloric restriction, and show that mitochondrial form and function are regulated by caloric restriction.

S-05.1–2
Unravelling the molecular mechanisms underlying the healthy effects of common dietary polyphenols

M. Battino*, D. Cianciosi, F. Giampieri*
Department of Clinical Sciences, Università Politecnica delle Marche, Ancona, Italy

Epidemiological studies have already established a close association between a diet enriched in fruits and vegetables and a lower risk for the onset and development of several non-communicable diseases, including types 2 diabetes, cardiovascular diseases and some types of cancer. Bioactive phytochemicals are of increasing interest for their roles both in preventive strategies and as adjuvants in the treatment of different pathologies. During the past decade, our research group has been widely involved in the evaluation of the protective effects of natural compounds present in different food matrices against several types of stressors, by using diverse *in vitro* and *in vivo* experimental models (1–3). In this presentation, the ability of common dietary polyphenols to modulate the expression of several genes and proteins involved in different biological processes, including apoptosis, inflammation, antioxidant defense, lipid metabolism, proliferation, metastatization, cell cycle and mitochondrial biogenesis, will be presented and discussed. 1. Battino M, et al. (2021) Phytomedicine 86, 153,170. 2. Cianciosi D, et al. (2020) Food Chem 325, 126,881. 3. Giampieri F, et al. (2018) J Agric Food Chem 66(3), 581–592. *The authors marked with an asterisk equally contributed to the work.

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ShT-05.1–1
Vitamin D reduces the binding effects of S1 (spike) and N (nucleocapsid) proteins of the SARS-CoV-2 virus to MRC-5 lung fibroblast cells

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In this study, the *in vitro* role of vitamin D on cell proliferation and the toxicity of MRC-5 cells induced by the presence of S1 (spike) and N (nucleocapsid) proteins of SARS-CoV2 virus were investigated. Vitamin D was used in various treatment protocols for COVID-19 before vaccination. The S1 and N protein, produced by molecular biology techniques at the Faculty of Chemistry, University of Belgrade, within the CAPSIDO project, were used to stimulate MRC-5 cells during 24 and 48 h cell culture. S1 protein was used in a concentration from 6.0 $x10^{-2}$ng/µl to 7.5$x10^{-4}$ ng/µl, and N protein from 12.00 $x10^{-2}$ ng/µl to15.00 $x10^{-4}$ng/µl. Cells were analyzed morphologically as well as on the basis of MTT test. The cytokine IL-6, LDH and AST enzymes as well as potassium were determined in the supernatant to analyze the degree of cell membrane damage. The results showed significantly different effects of S_1 protein in relation to concentrations and time of incubation. In addition, S1 showed most prominent effects compared to N protein. The effects of S1 protein showed a statistically significant increase in the release of intracellular enzyme AST and intracellular potassium, depending on the concentration and incubation time (ANOVA, $p < 0.05$), while LDH and IL-6 were not detected under the tested conditions. Vitamin D (Vigantol^{R}) (20,000 IU/mL) significantly reduced the release of AST and potassium after S1 protein-induced treatment, while it was less pronounced on the effects caused by N protein. These preliminary results indicated that S1 protein, most likely due to its specific structure relative to N protein, after binding to appropriate ACE receptors and without the presence of the whole viral particle, can lead to changes in target cells and induce their necrosis depending on concentration. Vitamin D appears to at least partially reduce the direct binding of S1 protein to target cells and reverse its effects.

ShT-05.1–2
Lauric acid decreased brain CD36 levels in mice: preliminary data in diet-induced neuroinflammation

B. Kisioglu Halis, A. Akyol
Hacettepe University, Ankara, Turkey

The role of fatty acid translocase CD36 that is responsive to dietary intake is shown in neuroinflammatory pathways. Neuroinflammation is induced by dietary high fat and fructose intake and prevented by polyphenols such as resveratrol. Although it is high in saturated fat, dietary coconut oil intake might reduce neuroinflammation. Thus, this study aimed to find out the effects of lauric acid, the main fatty acid of coconut oil, on diet-induced neuroinflammation and the role of CD36. C57BL/6 male mice (8 weeks old, n = 32) were divided into 4 dietary groups and fed *ad libitum* for 6 weeks. The control group (C) received a control diet (fat: 10% kcal). The other 3 intervention groups: fructose added high fat diet (FHFD), resveratrol and FHFD (RSV-FHFD), lauric acid and FHFD (LA-FHFD) received a high fat diet (fat: 60% kcal) with fructose added drinking water (5% w/v). RSV-FHFD and LA-FHFD groups received a daily dose of resveratrol (7,5 mg/kg) or lauric acid (750 mg/kg), respectively. The Open Field Test (OFT) was applied at the end of intervention. Mice were then anesthetized; blood and brain tissues were collected. Plasma and brain TNF-α and IL-10 were analyzed by flow cytometry. Brain CD36 levels were analyzed by ELISA/colorimetric methods. FHFD increased plasma and brain levels of TNF-α, but decreased IL-10 compared to C ($p < 0.05$). RSV-FHFD and LA-FHFD decreased plasma and brain levels of TNF-α, but increased IL-10 compared to FHFD ($p < 0.05$). Brain CD36 levels increased in FHFD compared to C ($p < 0.05$). Brain levels of CD36 were lower in RSV-FHFD and LA-FHFD than FHFD ($p < 0.05$). FHFD showed anxiety-like behavior compared to C in the OFT measurements ($p < 0.05$). RSV-FHFD and LA-FHFD decreased anxiety-like behavior compared to FHFD in the OFT measurements ($p < 0.05$). Lauric acid may alleviate neuroinflammation induced by a fructose added high fat diet by decreasing TNF-α and increasing IL-10. CD36 may be a regulator in the context of diet-induced neuroinflammation.

ShT-05.1–3
Bovine miRNA bta-miR-154c withstands *in vitro* human digestion: possible link with colorectal cancer initiation or progression?

M. Pieri[1], H. Dweep[2], G. Papagregoriou[3], C. Papaneophytou[1], L. Papazachariou[1], **T. Panayi**[1], V. Nicolaidou[1], K. Felekkis[1]
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Colorectal cancer (CRC) is the third most frequent human cancer with over 1.3 million new cases globally. CRC is a complex disease caused by the interaction of both genetic and environmental factors with diet and the high consumption of red meat, including beef, being a risk factor for CRC initiation and progression. Recent data demonstrate that exogenous microRNAs (miRNAs) entering the body via ingestion could pose an effect on the consumer. In this study we focused on bovine miRNAs that do not share a seed sequence with humans and mice. We identified bta-miR-154c, a bovine miRNA found in edible parts of beef and other red meats, and predicted via cross-species bioinformatic analysis to affect cancer-related pathways in human cells. When bovine tissue was subjected to cooking and a simulation of human digestion, bta-miR-154c was still detected after all procedures, albeit at reduced concentrations. However, lipofection of bta-miR-154c in three different colorectal human cell lines did not affect their viability as evaluated at various time points and concentrations. These data depict that bta-miR-154c is predicted to affect cancer-related pathways in human cells, it can withstand digestion and be detected after all stages of an *in vitro* digestion protocol, but does not appear to alter epithelial cell viability should it enter the human enterocyte even at supraphysiological

amounts. Further ongoing experiments both in cells and mice will elucidate whether bta-miR-154c could pose a different functional effect either directly on the intestinal epithelial cells, or via an effect on the host gut microbiota affecting in this way CRC progression due to its consumption.

ShT-05.1-4
From proteins to bread: novel tools for the molecular description of protein–protein interactions (and their evolution) in baked products

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DeFENS, Università degli Studi di Milano, Milan, Italy

Cereal-based matrices are deeply studied and characterized from a rheological and technological point of view. Although protein–protein interactions at the base of gluten formation are well known (i.e. disulfide exchange reactions and hydrophobic interactions), the peculiar chemistry of the proteins involved in the formation of the network - gliadins and glutenins - is still a source of discussion. The present work aims at developing new molecular approaches suitable to describe the "thiolome" of the system, in order to provide information on gluten protein structure and protein–protein interactions during processing. The thiolomic approach relies on labeling accessible free cysteine thiols with a specific iodoacetamide derivative fluorescent probe, in the presence of different destructuring agents (SDS and urea). Labeled proteins are separated in mono- and/or bi-dimensional electrophoresis. Image analysis is used to compare the fluorescent emission map and the total protein pattern to detect changes in the distribution of free thiols among the various proteins. The comparison of the information obtained from the thiolomic approach with those obtained from other approaches, such as front-face fluorescence and differential solubility, can provide various insights into the evolution of the protein structure of the matrix. Preliminary results on a soft wheat-based baked product (bread), show that changes in the thiols pattern during kneading—i.e., when disulfide exchange and rearrangement of hydrophobic patches occur—are comparatively modest with respect to those observed after cooking, a step characterized by temperature-induced structural rearrangements and by water transfer from proteins to starch.

Sunday 10 July
16:00–18:00, Auditorium II

Genomics

S-06.1–1
The hunt for dark DNA: identification of long noncoding RNAs in vertebrate genomes

B. Uszczynska-Ratajczak[1], M. Kwiatkowska[1], S. Carbonell-Sala[2], V. Gadekar[1], R. Johnson[3], R. Guigo[2]
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Vertebrate genomes produce tens of thousands of long noncoding RNAs (lncRNAs) – long transcripts with limited protein coding potential. Although an increasing number of lncRNAs are linked to fundamental physiological processes in the cell, the vast majority of them (>97%), even for the human genome, still remain functionally uncharacterized. Understanding biological roles of lncRNAs requires accurate genome annotations describing their precise location, gene boundaries and transcript structures. However, current lncRNA catalogues show evident signs of incompleteness with many gene models being fragmented or uncatalogued. To overcome this issue, the present work aims to advance towards a complete and accurate annotation of lncRNAs in human and mouse genomes. By developing and applying targeted long-read RNA sequencing methodology, this study provides accurate lncRNA annotations at high-throughput rates. Presented methodology eliminates the need for the noisy transcriptome assembly and requires minimal manual curation. Produced transcript models uncover thousands and hundreds of novel, full-length lncRNAs for human and mouse genomes, respectively, also substantially increasing the annotated transcript complexity within targeted loci. Resulting lncRNA catalogues are of quality comparable to present-day manually curated annotations. Moreover, we detected human and mouse lncRNA orthologues in the zebrafish genome using a newly designed synteny-based approach. Improved annotation of mammalian lncRNA orthologues in the zebrafish genome is expected to largely facilitate their functional characterization.

S-06.1–2
How to maintain genomic stability through cell division

Claudio Sunkel
Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Porto, Portugal.

The fidelity of chromosome segregation relies on the attachment of sister kinetochores to microtubules of opposite spindle poles and on the Spindle Assembly Checkpoint (SAC), a biochemical pathway that prevents anaphase onset until the former state is achieved. Mitotic kinases are key regulators of both processes, which are frequently deregulated in malignant cells. Our team combines Drosophila genetics, human cultured cells, high-resolution imaging and biochemical approaches to provide a detailed understanding of the mechanisms orchestrating kinetochore-microtubules interactions and SAC signaling. We aim to unveil the molecular and mechanical switches that fine-tune the action of key mitotic kinases at kinetochores and how these are relayed to microtubule attachment regulation and translated into biochemical signals that control SAC activity. Our findings generate critical knowledge to understand how dividing cells prevent aneuploidy.

ShT-06.1–1
Genome sequence of *Paenarthrobacter nicotinovorans* strain ATCC 49919, a nicotine-degrading soil microorganism

A. El-Sabeh[1], I. Honceriu[1], F. Kallabi[1,2], R. Boiangiu[1], M. Mihăşan[1]
[1]*Faculty of Biology, Alexandru Ioan Cuza University of Iasi, Iasi, Romania,* [2]*Laboratory of Human Molecular Genetics, Faculty of Medicine of Sfax, University of Sfax, Sfax, Tunisia*

Paenarthrobacter nicotinovorans ATCC 49919 is a nicotine-degrading microorganism isolated from soils on which tobacco is cultivated. The bacterium's pyridine pathway for nicotine degradation could be used for the conversion of nicotine-containing waste into valuable green chemicals such as 6-hydroxy-nicotine or 3-succinoyl-pyridine. Here, the strain's genomic DNA was sequenced using two technologies: Illumina NovaSeq 6000 produces short reads and the Oxford Nanopore Technology MinION yields long-read sequences. A hybrid assembly was performed using Unicycler and the complete genome was obtained. The genome is organized in two circular replicons. The largest replicon represents the chromosome of 4 316 184 bp, with an overall GC content of 63.2%. The second replicon is the pAO1 megaplasmid of 165 141 bp, with an overall GC content of 59.7%. A total of 3953 coding sequences, 54 tRNAs, 2 ncRNAs, 1 tmRNA and 6 identical ribosomal operons were identified in the chromosome. The plasmid shows 99.99% identity with the previously described pAO1 megaplasmid sequence (GenBank entry AJ507836.131). Comparisons between the complete genome of *P. nicotinovorans* ATCC 49919 and genomes of nicotine-degrading microorganisms that also use the pyridine pathway for nicotine degradation indicated low identity at the nucleotide level. Therefore, the complete genome of *P. nicotinovorans* ATCC 49919 will provide a much-needed reference genome for microorganisms using the pyridine pathway for nicotine degradation.

ShT-06.1–2
In silico evaluation of antibiotic resistance and CRISPR systems of *Paenarthrobater nicotinovorans*

I.T. Munteanu*[1], A.M. Mleşniţă *[1], F. Kallabi[2], M. Mihăşan[1]
[1]*BioActive Research Group, Faculty of Biology, Alexandru Ioan Cuza University of Ia?i., Iasi, Romania,* [2]*University of Sfax, Faculty of Medicine of SFAX, Sfax, Tunisia*

The ability to metabolize nicotine brought the Gram-positive bacterium *Paenarthrobacter nicotinovorans* into the researchers' limelight since the 60's. Many advancements have been made since then and the potential applications of *P. nicotinovorans* nicotine catabolic pathway for the conversion of nicotine-containing waste into useful chemicals have been proved. Yet, its applications in biotechnology are hampered by the lack of knowledge on the antibiotic resistance and by the lack of reliable gene editing systems that would permit rational engineering of the nicotine degradation pathway. CRISPR systems have been extensively used for genomic editing of eukaryotic cells and proved to be reliable and accurate. The applicability of the CRISPR system for genomic editing of *Paenarthrobacter* strains remains elusive. Our long-term goal is to develop a gene editing system that would work in this strain. The current work aims to provide insights into the antibiotic resistance of *P. nicotinovorans* and to evaluate the applicability and functionality of the CRISPR-Cpf1 system in this strain using an *in silico* approach. The draft genome of *P. nicotinovorans* was sequenced by NGS using the Illumina NovaSeq 6000 and the reads assembled with SPAdes 3.14.1. Contigs files were uploaded to CARD and CRISPRs web servers. Four CRISPR candidates were found on different contigs, but none were related to CRISPR-Cpf1. CARD gave a total of 255 loose hits (individual antibiotics and major antibiotic classes). All hits were filtered to 14 major hits based on an amino acid identity level higher than 50%. *P. nicotinovorans* was found to be resistant to 7 antibiotics (Kanamycin, Ceftriaxone, Erythromycin, Gentamicin, Neomycin, Spectinomycin, and Vancomycin) corresponding to 6 CARD major hits. Hence, we concluded that the CRISPR-Cpf1 system found on the pJYS3_ΔcrtYf plasmid might work and we have a solid selection of antibiotics to develop custom plasmid vectors that would work in *P. nicotinovorans*. *The authors marked with an asterisk equally contributed to the work.

ShT-06.1–3
Omics strategies on natural plant biomass-degrading systems for development of biocatalytic routes for renewable feedstocks conversion into value-added products

F. Squina, J.P. Franco Cairo, R. Tramontina
Universidade de Sorocaba, Sorocaba, Brazil

Plant feedstocks are at the leading front of the biomass-to-bioproducts industries. These activities can promote economic, social, and environmental development worldwide through sustainable energy production scenarios and replace petroleum-based materials. In this sense, we have revealed novel enzymes and pathways based on genomics, transcriptomics, proteomics analyses from natural plant biomass-degrading systems, such as fungi (https://doi.org/10.1186/s13059-017-1151-0), termites (https://doi.org/10.3389/fmicb.2016.01518), hyperthermophilic bacteria (https://doi.org/10.1007/s00792-017-0942-2), soil-derived microbial communities, and soil metagenome (https://doi.org/10.1186/s13068-016-0525-y). These studies have assigned novel biocatalysts to biotechnological applications and proposed new routes to produce high-value chemicals. In this sense, the aldo-keto reductase (AKR) from the lower termite *Coptotermes gestroi* has been applied to develop synthetic routes based on the reduction of organic aldehydes, along with the carboxylic acid reductases (CAR). Based on the CAR/AKR system using *E. coli* as a host, along with the expression of auxiliary activities, we have promoted the production of coniferol, which is a high-value chemical, from lignocellulosic biomass such as wheat straw. Furthermore, we demonstrated that an ancient redox-active enzyme encoded by *C. gestroi*, a Cu/Zn superoxide dismutase (CgSOD-1), plays a previously unknown role in plant biomass degradation. We discovered that this CgSOD-1 degrades polysaccharides through an oxidative mode of action, thereby boosting the action of canonical GH enzymes and adding a new member to the group of 'Auxiliary Activity' enzymes used by nature in biomass utilization, opening opportunities for scouting other redox enzymes related for applications on biomass into bioproducts conversion.

ShT-06.1-4
Genome-wide CRISPR screen identifies BUB1 as a cancer-dependency gene in malignant pleural mesothelioma

E. Cakiroglu, S. Senturk, G. Karakulah
Dokuz Eylul University, Izmir Biomedicine and Genome Institute, Izmir, Turkey

Malignant pleural mesothelioma (MPM) is a rare cancer associated with an increasing incidence. Existing treatment options are limited to chemotherapy and/or multimodal therapy. Despite such an aggressive therapy regimen, MPM has poor prognosis and low survival rates. Several genes and pathways have been associated with MPM so far, yet the absence of a defined dependency or vulnerability hinders targeted treatment alternatives. In this study, we sought to elucidate the genes that were essential for MPM cell survival using a genome-wide CRISPR/Cas9 screening strategy. To this aim, clonal cells with stable Cas9 expression were engineered in non-tumorigenic mesothelial cell line (MeT-5A) and 3 MPM cell lines (H2052, H2452, and H28). After validating Cas9 protein expression and genome editing efficiencies, cell lines were transduced with Brunello genome-wide gRNA library at MOI ~ 0.3, selected with puromycin, and cultured for 14 doublings. T0 and T14 samples were sequenced, and the results were analyzed using MaGeCK Flute tool. As expected, the analysis revealed hits in common essential genes that have roles in prominent cellular processes such as replication, translation, and transcription. In addition, as an indicator of a successful screen, depleted gRNAs included those that target genes with oncogenic functions such as CCT6A, KPNB1, HSPA5, while enriched gRNAs were targeting tumor suppressor genes such as PTEN, TP53, and CDKN1A. Our screening results demonstrated that, when compared to the mesothelial cell line, MPM cells show selective sensitivity to depletion of BUB1 gene, a kinase promoting proliferation of diverse cancer cells. These findings were further validated by BUB1 knockout or small molecule inhibitor treatments. Importantly, increased BUB1 expression in MPM patients correlates with poor overall survival. Based on our preliminary findings, we anticipate that further molecular studies may highlight BUB1 as a promising druggable target in MPM.

Sunday 10 July
16:00–18:00, Auditorium VII

Atomic and molecular imaging

S-07.1–1
AFM as a structural biology tool: from membrane protein architecture and ligand binding to mechano-ciliary sensing

N. Barrera
Pontificia Universidad Católica de Chile, Santiago, Chile

Atomic Force Microscopy (AFM) has proved to be a versatile tool to characterize structural and biophysical properties of molecules and cells. This symposium will enlighten recent progress in AFM imaging for the molecular architecture of heteromeric connexin channels and P2X receptor mobility after activation. In addition, via force spectroscopy configuration, AFM will reveal single molecule pharmacology of P2X receptors and mechano-ciliary sensing in cultured ciliated cells. AFM has become an established approach for biologists where new avenues are constantly emerging. Funded by Millennium Science Initiative P10-035F grant, Fondecyt 1211060 and 1120169 grants, Newton-Picarte DPI-20140080 grant and Anillo ACT210057 grant.

S-07.1–2
Small, but powerful and attractive: 19F in biomolecular NMR

A. Gronenborn
University of Pittsburgh School of Medicine, Pittsburgh, United States of America

Nuclear magnetic resonance (NMR) spectroscopy is a versatile tool for probing structure, dynamics, folding, and interactions at atomic resolution. While naturally occurring magnetically active isotopes, such as ^{1}H, ^{13}C, or ^{15}N, are most commonly used in biomolecular NMR, with ^{15}N and ^{13}C isotopic labeling routinely employed at the present time, ^{19}F is a very attractive and sensitive alternative nucleus, which offers rich information on biomolecules in solution and in the solid state. This presentation will summarize the unique benefits of solution and solid-state ^{19}F NMR spectroscopy for the study of biological systems. Particular focus will be placed on the most recent studies and on future unique and important potential applications of fluorine NMR methodology.

ShT-07.1–1
Assembly of recombinant tau into filaments identical to those of Alzheimer's disease and chronic traumatic encephalopathy using high-throughput cryo-EM

S. Lovestam, M. Goedert, S. Scheres
MRC-LMB, Cambridge, United Kingdom

Abundant filamentous inclusions of tau are characteristic of more than 20 neurodegenerative diseases collectively termed tauopathies. Electron cryo-microscopy (cryo-EM) structures of tau amyloid filaments from human brain revealed that distinct tau folds characterise many different diseases. A lack of laboratory-based model systems to generate these structures has hampered efforts to uncover the molecular mechanisms that underlie tauopathies. Here, using high-throughput cryo-EM, we report *in vitro* assembly conditions with recombinant tau that replicate the structures of filaments from both Alzheimer's disease (AD) and chronic traumatic encephalopathy (CTE), as determined by cryo-EM. Our results suggest that post-translational modifications of tau modulate filament assembly, and that previously observed additional densities in AD and CTE filaments may arise from the presence of inorganic salts, like phosphates and sodium chloride. *In vitro* assembly of tau into disease-relevant filaments will facilitate studies to determine their roles in different diseases, as well as the development of compounds that specifically bind to these structures or prevent their formation.

ShT-07.1–2
Structural basis for viral fold recognition by bacterial inflammasomes

M. Wilkinson, L. Gao, J. Strecker, F. Zhang
Broad Institute, Cambridge, United States of America

Bacteria are in a constant arms race with their viral foes and have developed remarkable mechanisms to resist infection. Here, we uncover a new mode of innate immunity in bacteria, where a diverse family of inflammasome-like ATPases detect phage proteins to trigger cell death. Cryo-EM shows that the ATPases generally recognise phage protein folds rather than specific residues, explaining how a single ATPase protein can confer defence against a wide variety of phage: viral protein structure is more conserved than sequence. Additionally, the ATPases can avoid escape mutants by directly recognising active site residues and ligands of phage enzymes. Pattern recognition then triggers ATPase tetramerisation, leading to activation of an N-terminal effector domain, DNA degradation, and cell death. This work reveals remarkable similarity between the defence strategies of prokaryotes and eukaryotes and extends the paradigm of pattern recognition of pathogen-specific proteins across all domains of life.

ShT-07.1–3
Ultrastructural changes of red blood cells derived from women with preeclampsia

I. Giosheva[1,2], V. Strijkova-Kenderova *[1,3], A. Langari[1], A. Danailova[1], R. Komsa-Penkova[4], S. Krumova[1], **S. Todinova***[1]
[1]*Institute of Biophysics and Biomedical Engineering, BAS, Sofia, Bulgaria,* [2]*University Obstetrics and Gynecology Hospital "Maichin Dom", Sofia, Bulgaria,* [3]*Institute of Optical Materials and Technologies "Acad. Yordan Malinovski", BAS, Sofia, Bulgaria,* [4]*Department of Biochemistry, Medical University, Pleven, Pleven, Bulgaria*

Preeclampsia (PE) is one of the leading causes of maternal and perinatal morbidity and mortality with unknown etiology. Early-onset preeclampsia is presented by hypertension and significant proteinuria. The disease is associated with severe changes in the red blood cells (RBCs) morphology with early destruction of its membrane. This study aims to apply atomic force microscopy (AFM) imaging in order to examine the ultrastructural changes of RBCs derived from women with PE relative to normotensive pregnant subjects (PC). Freshly isolated RBCs from PC do not differ from those of nonpregnant women (NPC) and have a typical biconcave shape with a gently folded membrane surface. The membrane surface of fresh PE cells differs from "healthy" ones and is characterized by invaginations and enhanced roughness (Rrms) value (4.7 $\pm$ 0.8 nm for PE vs 3.8 $\pm$ 0.4 nm and 2.7 $\pm$ 0.4 nm for PC and NPC). These alterations become more drastic with cell aging, resulting in more pronounced and larger protrusions and concavity, with exponentially increasing membrane roughness, unlike controls in which Rrms decreases over time. The Rrms for senescent PE cells (13 $\pm$ 2.0 nm) is significantly higher ($p < 0.01$) than that of PC (1.5 $\pm$ 0.1 nm) and NPC (1.9 $\pm$ 0.2 nm). After 20–30 days of storage, cells with unusual nanomorphology are observed for PE cases. Moreover, the RBCs from PE patients appear very fragile and often only ghosts are observed instead of intact cells at around 30 days of aging. The results demonstrate strong morphological alterations of RBCs from preeclamptic women associated with structural rearrangement of the cell's cytoskeleton. Acknowledgments: This work is supported by grant KP-06-H21/4, Competition for financial support for basic research projects – 2018, National Science Fund. Research equipment of Distributed Research Infrastructure INFRAMAT, part of Bulgarian National Roadmap for Research Infrastructures, supported by Bulgarian Ministry of Education and Science was used in this investigation. *The authors marked with an asterisk equally contributed to the work.

Monday 11 July
9:00–11:00, Auditorium I

Neurodegeneration and regeneration

S-01.2-2
Boosting protein quality control: A strategy against neurodegenerative diseases

A. Bertolotti
MRC-Laboratory of Molecular Biology, Cambridge, UK

The deposition of misfolded proteins is a defining feature of many age-dependent human diseases, including the increasingly prevalent neurodegenerative diseases. Cells normally strive to ensure that proteins get correctly folded and have powerful and sophisticated protein quality control mechanisms to maintain protein homeostasis (proteostasis). However, with age, the cellular defense systems against misfolded proteins get overwhelmed, leading to the accumulation of misfolded proteins with devastating consequences for cells and organisms. Improving the cells' ability to deal with misfolded proteins should represent a generic approach to reduce pathology in diverse protein misfolding diseases. My lab has identified powerful strategies to help cells survive when protein quality control fails and implemented some of these strategies in mice. Through unbiased approaches, we have identified small drug-like molecules that safely boost a natural defense system against misfolded proteins. The small molecules we have identified inhibit serine/threonine phosphatases controlling the termination of a proteostatic pathway, an interesting finding because phosphatases were previously thought to be undruggable. The selective inhibitors discovered in the lab have demonstrated therapeutic effects in various models of neurodegenerative diseases. This work demonstrates that generic approaches aimed at helping cells to survive protein quality control failures can be useful to prevent protein misfolding diseases, including the devastating neurodegenerative diseases. One of these inhibitors, Sephin1, has passed through favorable Phase 1 clinical trials in 2019 and is being developed for Charcot-Marie-Tooth disease. Last year, Guanabenz was found to be beneficial in a phase 2 clinical trial in ALS, 10 years after we reported its activity in helping cells to survive protein misfolding insults.

S-01.2-1
Macro problems of microvessels in ischemic stroke

M. Yemisci Ozkan
Hacettepe University, Ankara, Turkey

Ischemic stroke is a leading cause of mortality and disability worldwide, and is a major public health problem. Currently approved treatment methods for acute ischemic stroke patients are primarily targeted to opening the occluded cerebral vessels by tPA or endovascular thrombectomy. Thus, improving the functionality of the ischemic cerebrovasculature will foster better outcome after ischemic stroke. However, recent clinical trials consistently show that despite achieving this goal and providing recanalization in the main cerebral vessels, blood flow might not improve at the microcirculatory level, hence reperfusion of the ischemic tissue is often incomplete. The success of recanalization therapies can be improved by diminishing incomplete reperfusion caused by loss of patency of some microvessels during ischemia that persists after recanalization. Recent experimental studies demonstrated the importance of pericyte cells, which are responsible for the regulation of cerebral and retinal microcirculation by wrapping the vessels at the capillary level, in this so called 'no-reflow phenomenon'. It is shown that alpha smooth muscle actin is the critical protein which is closely related to the contractile properties of pericytes, and thereby plays an important role in cerebral and retinal ischemia/reperfusion pathophysiology. Reducing 'no-reflow' by pharmacological treatments or by *in vivo* alpha smooth muscle actin targeted small interfering RNA (siRNA) seems to be an important and viable target for recanalization therapies. As the importance of microcirculation is not only limited to stroke, all these findings in the cerebro-retinal microcirculation under physiological and pathological conditions would probably have implications in the field of other brain pathologies such as neurodegenerative diseases, and would provide opportunities for new therapeutic approaches in all these diseases.

ShT-01.2-1
Anti-apoptotic and neuroprotective effects of thiamine high dose in the cornea of rats exposed to chronic ethanol consumption

O. Pavlova*, V. Bilous, A. Tykhomyrov*
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Chronic ethanol (EtOH) abuse is associated with gross thiamine (vitamin B1) deficiency that can play a major role in some pathological eye conditions, including dry eye, epithelial defects of ocular surface, and optic neuropathies, leading to deterioration of vision quality. The purpose of this study was to investigate the effects of vitamin B1 high dose administration on specific epithelial adhesive, apoptotic, angiogenic, and neurological markers in cornea of rats chronically affected to a long-term EtOH consumption. Adolescent male white albino rats (n = 20) were given EtOH water solution (15%, v/v) for 9 months or distilled water (control group). One week before the experiment termination, half of EtOH-exposed rats were administered with the single dose of thiamine (25 mg/kg b.w.). Western blot analysis of corneal tissue lysates revealed that vitamin B1 treatment significantly reduced hypoxia marker HIF-1alpha, angiogenic inhibitor angiostatin and MMP-9 levels, while up-regulated levels of pro-angiogenic regulator VEGF in cornea of EtOH-exposed rats. Thiamine enhanced pro-survival signaling through elevating Bcl-xl/Bax ratio and reducing pro-apoptotic marker NF-kb and caspase-3 levels. Thiamine reversed epithelial-mesenchymal transition and improved adhesive capacities of corneal epithelial cells through increasing E-cadherin and ZO-1 expression in cornea of chronically EtOH-affected rats. We found dramatically increased levels of reactive glia marker GFAP in cornea of EtOH rats, while microglial marker Iba-1 appeared to be down-regulated. Vitamin B1 exerted beneficial neuroprotective effects through alleviating excessive glial reactivity and improving microglial function in EtOH-related neuropathological condition in cornea. Thus, thiamine high dose can relieve manifestation of some eye symptoms after long-term alcohol impact in cornea by improving aerobic metabolism, inhibiting apoptosis, modulating epithelization, and diminishing EtOH neurotoxicity. *The authors marked with an asterisk equally contributed to the work.

ShT-01.2-2
Secretome from anti-miR-124-treated ALS motor neurons shows therapeutic potential after intrathecal injection in the early symptomatic ALS mouse model

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Dysregulation of inflammatory (inflamma)-miRNAs in cells and their dissemination via secretome contribute to Amyotrophic Lateral Sclerosis (ALS) pathophysiology, and their regulation may constitute a therapeutic approach. We showed that transfection of mutant SOD1G93A (mSOD1) motor neurons (MNs) with anti-miR-124 prevented neurodegeneration and its secretome counteracted spinal pathogenicity in organotypic cultures from early symptomatic (12-week-old) mSOD1 mice (*Vaz AR et al. (2021) Int J Mol Sci 22:6128*). Therefore, we aimed to test the potential beneficial properties of the modulated concentrated secretome after intrathecal injection in mSOD1 mice at 12-weeks old. We evaluated motor performance, inflamma-miRNA profile and glial/synaptic markers. WT and mSOD1 mice were injected with the MN basal media as control. Two weeks later, we performed the limb clasping/grasping tests to evaluate the corticospinal function and footprint test to assess gait quality. The lumbar spinal cord (SC) was isolated from 15-week-old mice and molecular evaluation was performed by RT-qPCR. The mSOD1 mice developed hind-limb clasping and grasping and performed shorter strides. Homogenates of their lumbar SC showed overexpressed GFAP and miR-146a/−155, together with iNOS, arginase 1, IL-10, TREM2, post-synaptic PSD-95 and PLP downregulation, evidencing neuro-immune dysregulation and myelination deficits. The injection of the treated secretome not only recovered the motor performance and corticospinal function in the mSOD1 mouse model, but also normalized the expression of inflamma-miRNAs and genes related with glial activation, synaptic function and myelination. Overall, this study validates the potential of anti-miR-124 MN secretome in recovering early

motor disabilities associated with MN/glia pathogenicity, opening new promises for the treatment of patients with mutations in the SOD1 gene. FCT (PTDC/MED-NEU/31395/2017, LISBOA-01-0145-FEDER-031395, UIDB/UIDP/04138/2019–20, SFRH/BD/129586).

ShT-01.2-3
Deciphering frataxin implication in endoplasmic reticulum-mitochondria associated membranes (MAMs)

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Intracellular Ca^{2+} homeostasis is a key event regulated by specific structures termed ER-mitochondria associated membranes (MAMs). Traditionally, FXN, the protein responsible for the neurodegenerative disease Friedreich's Ataxia (FRDA), has been characterized as a mitochondrial matrix protein. We have recently described that in FXN-deficient cells, MAMs' communication is disrupted both structurally and functionally. Remarkably, we found that FXN is located in MAMs. Besides canonic FXN (FXN I), a cytosolic isoform of FXN (FXN II) has been described in the last years, but little is known about its role. Thus, our main objective is to elucidate the function of FXN associated with its spatial compartmentalization to establish new pathways involved in the regulation of its function and the pathophysiology of FRDA. We have optimized the Split-TurboID technique to confirm the subcellular location of FXN isoforms and map their interactomes in the ER-cytosol. TurboID biotin-ligase activity reconstitution was evaluated by imaging and western blotting in the ER with both isoforms. Biotinylated proteomes were identified by LC–MS/MS. After confirming the expression of both isoforms in cell lines and immortalized lymphoblasts, we evaluated the reconstitution of TurboID activity in the ER. We observed that both FXN I and II are present in the MAMs' domain, showing a different pattern of biotinylated proteins in the ER-cytosolic domain. We identified 59 significant proteins for FXN I and 113 for FXN II, 30 of them being shared by the two isoforms. The analysis of the proteomic datasets confirms over-represented pathways in both isoforms are related to fatty acid metabolism, necroptosis, translation, and autophagy. Interestingly, we can confirm that FXN II is the isoform implicated in intracellular Ca^{2+} homeostasis. These results shed light on the differential functionality of both isoforms, describing new pathways that could be relevant for FRDA therapy design.

ShT-01.2-4
Non-cell autonomous toxicity of ALS/FTD astrocytes to motoneurons is mediated by an excessive release of inorganic polyphosphate

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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are devastating, incurable diseases. While ALS/FTD is characterized by the degeneration of motoneurons in the spinal cord and frontal lobes, independent investigations have shown that non-neuronal cells – specifically astrocytes – release one or more toxic factors that contribute to motoneuron death by increasing neuronal activity. The nature of this toxic factor(s) that mediates this so-called non-cell autonomous toxicity has been elusive. Here we provide evidence that the offending toxic factor is inorganic polyphosphate (polyP), which is enriched in mouse and human astrocytes with diverse ALS/FTD-linked mutant genes (SOD1, TARDBP, and C9ORF72), and derived astrocyte-conditioned media (ACM). PolyP is a ubiquitous, negatively charged inorganic biopolymer of hundreds of PO4 residues and found in every tested cell type in nature and conserved across more than a billion years of evolution. While studies in bacteria and yeast have revealed numerous vital physiological functions for polyP, its role in mammalian cells is poorly understood. We found that exposure of spinal cord neurons to polyP reproduced the toxic effects of ALS/FTD-ACM, causing hyperexcitability and enhanced motoneuron death. Conversely, motoneurons can be rescued from the toxic ALS/FTD-ACM by degrading (i.e., with yeast polyphosphatases) or sequestering (i.e., with nano-sized polycationic compounds) polyP. These findings establish excessive astrocyte-derived polyP as a critical factor in non-cell autonomous motoneuron degeneration and a potential therapeutic target for ALS/FTD. Additional studies further reveal that cerebrospinal fluid (CSF) from ALS patients exhibits increased polyP concentrations, indicating that polyP might serve as a new biomarker for ALS/FTD.

Monday 11 July
9:00–11:00, Auditorium II

Looking for new antibiotics

S-02.2-1
Activity of viral-derived peptides against *Staphylococcus aureus* biofilms and insights into their mechanism of action

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Bacterial infections are a major human health threat given both the increasing incidence of drug-resistant bacteria and the ability of bacteria to form biofilms. Biofilm-related infections are particularly difficult to treat due to their reduced susceptibility to conventional antibiotics. Antimicrobial peptides (AMPs) are a group of natural molecules that have been considered as alternatives to conventional antibiotics and a promising option against bacterial biofilms. In the quest for new antimicrobial agents, we have recently shown the potential of structural viral proteins, particularly viral capsid proteins, as a source for peptides with antibacterial and anti-biofilm properties. Here, we show that the cationic peptide from the capsid protein of Torque teno douroucouli virus, vCPP2319, is active against preformed *Staphylococcus aureus* biofilms of both an ATCC strain and a strain isolated from a diabetic foot ulcer, mainly by the killing of biofilm-embedded bacteria. The direct effect of the peptide on biofilm-associated cells was imaged using atomic force (AFM) and confocal laser scanning (CLSM) microscopies, showing that the peptide induces morphological changes on bacterial cells and membrane disruption. Importantly, vCPP2319 exhibits low toxicity towards human cells and high stability in human serum. Overall, our studies contribute to the development of new anti-biofilm agents and shed light on their anti-biofilm mechanism of action.

S-02.2-2
Abstract withdrawn

ShT-02.2-1
Functional and biophysical studies of Microcin V mechanism of action using suicide probes

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The study of alternative antimicrobials is a primary concern due to the increasing resistance to common antibiotics. Bacteriocins seems to be a promising solution and the understanding of their mode of action is key for their potential use as new antibiotics. Microcin V (MccV) was the first bacteriocin reported in the academic literature (in 1925). It is a membrane-active peptide produced by *E. coli* and is active against other Gram-negative strains through the recognition of a specific membrane receptor and pore formation, causing membrane permeabilization and cell death. In addition, MccV is encoded by the genome of some probiotic strains of *E. coli*. Despite its great potential, interest in this bacteriocin has faded away over the years and its research has been neglected. Here we present a strategy to study MccV mechanism of action, using hybrid proteins called "suicide probes". This system fuses the bitopic membrane protein EtpM with the MccV sequence. When the fusion EtpM-MccV is heterologeously expressed in receptor-free *E. coli* strains, the host cell dies and that is why it is called a suicide probe. This suggests that the receptor is more likely to act as a docking molecule and it would be dispensable for the pore-mediated membrane disruption after the bacteriocin is anchored. The aim of this work was the design

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and development of suicide probes containing different MccV-derived domains (truncated peptides) to: 1) determine the function of each domain of MccV in the mechanism of action of this bacteriocin and 2) study the effect of MccV and each domain in membrane fluidity and transmembrane potential using fluorescence spectroscopy. Our results shows that MccV is able to increase phospholipid order and depolarize the membranes of receptor-free bacterial cells. Moreover, suicide probes prove to be useful to study and compare the effect of different domains of the peptide, in order to gain more information about the toxicity process in *E. coli* membrane.

ShT-02.2-2
Reaction hijacking ATP-activated enzymes as a strategy to develop new anti-infectives

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Diseases caused by infectious organisms pose an enormous threat to global health, food security and sustainable development. Malaria is one such debilitating disease, caused by protist parasites of the genus *Plasmodium*. New treatments with novel modes of action are urgently needed to overcome existing resistance, expand possible treatment options and enable more effective combination therapies. Aminoacyl tRNA synthetases (aaRSs) are adenylate-forming enzymes (AFEs) that represent attractive drug targets. Here, we show that class I and II aaRSs are previously unrecognized targets for AMP-mimicking nucleoside sulfamates. The target enzyme catalyzes the formation of an inhibitory amino acid-sulfamate conjugate, via a reaction-hijacking mechanism. We identified adenosine 5′-sulfamate (AMS) as a broad specificity compound that hijacks a range of aaRSs; and ML901 as a specific reagent that hijacks a single aaRSs in the malaria parasite, *Plasmodium falciparum*, namely, tyrosine RS (PfYRS). ML901 exerts whole-of-life-cycle killing activity with low nanomolar potency and single dose efficacy in a mouse model of malaria. X-ray crystallographic studies of plasmodium and human YRSs reveal differential flexibility of a loop over the catalytic site that underpins differential susceptibility to reaction-hijacking by ML901. The work points to the possibility of designing bespoke small molecular weight, membrane-permeable AFE inhibitors with adjustable specificity. In addition to charging tRNA and activating ubiquitin, AFEs are involved in activating fatty acids for degradation, biosynthesis of natural products, and other diverse pathways. Thus, nucleoside sulfamates may find applications in a broad range of infectious, metabolic and neurodegenerative diseases. *The authors marked with an asterisk equally contributed to the work.

ShT-02.2-3
A novel derivative of quinoxaline-carboxylic acid 1,4-dioxide as an anti-tubercular drug-candidate

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Tuberculosis (TB), a disease caused by *Mycobacterium tuberculosis*, remains the leading killer among bacterial infections, accounting for over 1.4 million deaths annually. The spread of multidrug and extensive drug resistant TB (MDR- and XDR-TB) drives the need to develop novel anti-TB drugs. We have previously described 25 novel 2-acyl-3-trifluoromethylquinoxaline 1,4-dioxides with prominent inhibitory properties against *M. smegmatis* (minimal inhibitory concentration, MIC = 2–8 μg/mL); however their activity was limited on *M. tuberculosis* (MIC = 5–10 μg/mL) [Buravchenko GI *et al.* (2022) Pharmaceuticals 15, 155]. We have designed a novel series of quinoxaline-2-carboxilic acid 1,4-dioxides with alteration of substituents in the positions 3, 6 and 7. The compounds were tested on *M. smegmatis* and autoluminiscent *M. tuberculosis* H37Ra and H37Rv (AlRa and AlRv) strains. The 2-carboethoxy- and 2-carbonitrile-derivatives with chlorine atoms at the 6 and/or 7 positions of quinoxaline ring demonstrated the highest anti-tubercular potency, showing MICs on *M. tuberculosis* strains <1 μg/mL, and 2-carboethoxyquinoxaline 1,4-dioxide LCTA-3368 was selected as the lead compound (*M. smegmatis* MIC = 8 μg/mL, *M. tuberculosis* AlRv MIC = 0.08 μg/mL). The comparative genomic analysis of spontaneous *M. smegmatis* mutants resistant to LCTA-3368 revealed mutations in genes encoding redox enzymes of pyruvate metabolism, which can act as pro-drug activators. Resistance to LCTA-3368 can be also provided by MmpS5-MmpL5 mediated efflux. The *in vivo* studies on a rapid model of murine infection showed no differences with negative control, however no toxic effects were observed. Thus, LCTA-3368 is a promising lead for further development of anti-TB drug candidates with a focus on enhancing its *in vivo* activity. This work is supported by the Russian Science Foundation (grant 21–45-00018) and the National Natural Science Foundation of China (grant 82061138019).

ShT-02.2-4
Development, characterization and engineering of anti-LexA nanobodies as suppressors of the bacterial SOS response

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The pro-mutagenic SOS response, regulated by the genotoxic stress sensor RecA and the transcriptional repressor LexA, is a bacterial pathway driving the evolution of antimicrobial resistance. Its suppression has been validated as a feasible strategy to inhibit the onset of resistance, even though currently known SOS antagonists are very limited in number and activity. In particular, the inhibition of LexA autoproteolytic activity might be highly beneficial for SOS response suppression, but the LexA active site proved to be very hard to target with exogenous molecules. Llama immunization with *Escherichia coli* LexA led to the isolation of a nanobody library that was enriched in LexA-specific binders by phage display technology and ELISA screening. A fluorescence polarization-based screening procedure was applied to select nanobodies able to inhibit RecA-induced LexA autoproteolysis. The same technique was employed to quantify the inhibitory potency of three best hits (NbSOS1-3; IC_{50} = 1–2 μM), while their affinity for the antigen was determined by surface plasmon resonance (K_D = 0.1–0.2 μM). X-ray structures of LexA-NbSOS complexes revealed an unprecedented inhibition mechanism on LexA: instead of targeting the active site, NbSOSs trap the LexA cleavage loop in an inactive conformation unable to reach the catalytic dyad. The ability of NbSOSs to suppress the SOS response in bacterial cultures treated with DNA damaging antibiotics was validated by RT-PCR expression profiling of key SOS genes. Further NbSOSs amelioration is being addressed by means of rational protein engineering, in particular by linking two NbSOSs targeting non-overlapping LexA epitopes to obtain biparatopic constructs, which showed improved binding and inhibitory efficiency. Presented nanobody-based LexA inhibitors can pave the way to develop biotherapeutics with an adjuvant effect to traditional antibiotics by delaying the onset of resistance and so extending our current arsenal of antimicrobial drugs. *The authors marked with an asterisk equally contributed to the work.

ShT-02.2-5
Structure-based design of NAD^+ analogues targeting bacterial NAD kinases, promising targets for new antibiotics

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Multi-drug resistance is a major public health problem that requires the urgent development of new antibiotics and therefore the identification of novel bacterial targets. The activity of nicotinamide adenine dinucleotide kinase, NADK, is essential in all bacteria tested so far, including many human pathogens that display antibiotic resistance leading to failure of current treatments. Inhibiting NADK is therefore a promising and innovative antibacterial strategy since there is currently no drug on the market targeting this enzyme. Through a drug design approach based on substrate-derived fragments, we have recently developed NAD^+-competitive inhibitors of NADKs, which displayed *in vivo* activity against *Staphylococcus aureus* or *Pseudomonas aeruginosa* in animal models of infection [1–3]. Funding and supports: Agence Nationale de la Recherche (ANR-17-CE18-0011-02), Institut Pasteur, Centre National de la Recherche Scientifique (CNRS), Institut National de la Santé et de la Recherche Médicale (INSERM), University of Montpellier. References: [1] Clément DA, Leseigneur C, Gelin M, Coelho D, Huteau V, Lionne C, Labesse G, Dussurget O, Pochet S (2020) New chemical probe targeting bacterial NAD kinase. Molecules. doi: 10.3390/molecules25214893. [2] Gelin M, Paoletti J, Nahori MA, Huteau V, Leseigneur C, Jouvion G, Dugué L, Clément D, Pons JL, Assairi L, Pochet S, Labesse G, Dussurget O (2020) From substrate to fragments to inhibitor active *in vivo* against *Staphylococcus aureus*. ACS Infect Dis. doi: 10.1021/acsinfecdis.9b00368. [3] Rahimova R, Nogaret P, HuteauV, Gelin M, Clément D, Labesse G, Pochet S, Blanc-Potard A, Lionne C (2022) Structure-based design, synthesis and biological evaluation of a NAD^+ analogue targeting *Pseudomonas aeruginosa* NAD kinase. In Preparation.

Monday 11 July
9:00–11:00, Auditorium VII

Apoptosis

S-03.2-2
Autophagy as a key cellular mechanism in health and disease

D. Gozuacik

Koç University School of Medicine, Istanbul, Turkey

Autophagy is a key biological event that occurs at low basal levels in all cell types from yeast to mammals under non-deprived

conditions, performing homeostatic functions such as protein degradation and organelle (e.g., mitochondria) turnover. It is rapidly upregulated during cellular stress, providing cells with recycled intracellular building blocks and substrates for energy generation and survival. Autophagy dysregulation or abnormalities play a critical role in the pathogenesis and progress of several human health problems, including neurodegenerative disorders, inflammation, and cancer. In our laboratory, we focus on the discovery of novel autophagy regulators and study implications of our findings in disease pathogenesis and diagnosis. Moreover, we investigate means to modulate autophagy for treatment purposes. In this talk, selected results of our research on autophagy and cancer will be presented.

S-03.2-1
The mitochondrial permeability transition: An old player back in the game

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Major progress has been made in defining the basis of the mitochondrial permeability transition, a Ca^{2+}-dependent permeability increase of the inner membrane that has puzzled mitochondrial research for almost 70 years. Initially considered an artifact of limited biological interest by most, over the years the permeability transition has been raised to the status of regulator of mitochondrial ion homeostasis and of druggable effector mechanism of cell death. The permeability transition is mediated by opening of channel(s) modulated by matrix cyclophilin D, the permeability transition pore(s) (PTP). The field has received new impulse (i) from the hypothesis that the PTP may originate from a Ca^{2+}-dependent conformational change of F-ATP synthase; and (ii) from the re-evaluation of the long-standing hypothesis that it originates from the adenine nucleotide translocator (ANT). I will provide an account of how ANT and F-ATP synthase may form high-conductance channels, and discuss how unravelling the molecular components of the PTP will allow a reassessment of the role of the permeability transition in cell death.

ShT-03.2-1
Structural insight into regulation of apoptosis signal-regulating kinase 1

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Apoptosis signal-regulating kinase 1 (ASK1) is a member of the mitogen-activated protein kinase family. Its activity is triggered by reactive oxygen species, cytokines, endoplasmic reticulum stress or osmotic stress, and leads to activation of p38 and c-Jun N-terminal kinase pathways and subsequently to inflammation or cell death. Dysregulation of ASK1 is linked to several serious pathologies (cardiovascular, neurodegenerative, tumour diseases), which makes this kinase an important therapeutic target. ASK1 activity is known to be regulated through oligomerization and protein–protein interactions, but the precise molecular mechanism is unclear. Also, despite the structures of individual domains, a more complex view of the full ASK1 itself, whether in complex with its important regulatory binding partners or in context of the assumed dimer, is still missing. To better understand the principle of ASK1 activation, we employed several techniques of integrative structural biology to characterize ASK1 alone and in complex with its regulator thioredoxin (TRX). Our results from analytical ultracentrifugation measurements revealed that the N-terminal part of ASK1 forms dimers in solution and that this dimerization is affected by redox conditions. Moreover, using hydrogen-deuterium exchange coupled to mass spectrometry we also identified the regions that form the dimerization interface. In addition, our data suggest that the interaction between ASK1 and its inhibitor TRX is considerably weaker in strong reducing conditions and that TRX does not prevent ASK1 dimerization. Altogether, our findings provide an important insight into the mechanism of ASK1 inhibition by TRX. This work was supported by the Czech Science Foundation (project 19-00121S).

ShT-03.2-2
Cigarette smoke extract induced extrinsic eryptosis initiated via p38 MAPK

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Mature red blood cells (RBCs) undergo a controlled suicidal death program, also known as eryptosis, which is in many aspects comparable to apoptosis of nucleated cells. Excessive eryptosis presumably participates in the pathophysiology of several diseases involving vascular dysfunction because it is associated with vaso-occlusive complications (Restivo I, 2021). Cigarette smoke (CS) is an established risk factor in the etiology of vascular diseases and recently we reported that cigarette smokers have a higher level of circulating eryptotic erythrocytes than non-smokers (Attanzio *et al.* 2019). In this *in vitro* study we investigate whether CS extract (CSE) may directly affect RBC function, and determine the mechanism by which this occurs. Treatment for 24 h of human isolated RBCs with CSE (10%–20%) caused PS externalization and cell shrinkage, hallmarks of eryptotic cells. CSE treatment did not affect the Ca^{2+}, ROS or glutathione levels. During 0–6 h of treatment, CSE (10%–20%) caused a time- and concentration-dependent increase of ceramide. Concomitantly, assembly of the DISC in membrane, phosphorylation of p38 MAPK, as well as caspase 8 and caspase 3 cleavage were evident. Inhibition of caspase 8 with Z-IETD-FMK significantly blunted the formation of ceramide, caspase 3 cleavage and PS externalization. These results demonstrated that CSE-induced DISC recruitment initiated eryptosis by a direct involvement of the initiator caspase 8 in the activation of the executioner caspase 3. Also, following the inhibition of p38 MAPK with SB203580, we found a significant reduction of the externalization of PS and a decreased amount of procaspase 8 in the immunoprecipitates with anti-FAS, as well as its cleaved active p18 subunit in the cell cytosol. These data demonstrated that CSE-induced extrinsic eryptosis is initiated via p38 MAPK. Restivo I *et al.* (2021) Antioxidants 10(2), 154. Attanzio A *et al.* (2019) Toxicology 411, 43–48.

ShT-03.2-3
Apoptosis and mitochondrial domestication: Co-evolution?

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Apoptosis, a common type of programmed cell death initiated by mitochondrial permeability transition, is a tool that allows cells to self-destruct when stimulated by the appropriate trigger. It is present both in higher and unicellular eukaryotes as well as in prokaryotes. In our previous study, we identified a core apoptotic machinery containing: protease metacaspase Mca1, nuclease Nuc1, and apoptosis-inducing factor Ndi1 and HTRA/Omi protease Nma111. These genes were used for this study as their deletion decreases apoptotic activity while overexpression induces apoptosis. We aimed to experimentally verify that the apoptotic machinery is a primordial adaptation acquired during mitochondrial domestication. We also tested the retention of ancient functions of these gene orthologs across the various kingdoms i.e., bacteria, protists, plants, animals etc. in yeast by their ability to replace their yeast orthologs. The codon-optimized orthologs were commercially synthesized and were cloned in front of the *CPS1* terminator of the self-constructed common vector. Yeast mutants containing ortholog replacements were constructed by homologous recombination. Furthermore, GFP-tagged versions of these orthologs were used to verify the expression and localisation of foreign proteins in the yeast. Fluorescence microscopy analysis showed that the GFP-fusion proteins localized to the predicted compartments. The expression and the size of GFP-tagged proteins was verified by western blot. The apoptotic activity of ortholog-bearing strains was tested by the phenotypic assay as well as Annexin-V/PI co-staining assay after apoptotic induction with 200 mM acetic acid. The majority of analyzed orthologs were able to significantly revert the sensitivity to apoptotic induction as compared to the respective deletion strains.

ShT-03.2-4
Interactions of FGF2 with novel binding partners play an essential role in its anti-apoptotic response

K. Służalska[1], J. Szymczyk[1], M. Biaduń[1], M. Kostaś[2], A. Lampart[1], J. Otlewski[1], D. Krowarsch[3], M. Zakrzewska[1]
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Fibroblast growth factor 2 (FGF2) increases cell survival not only through activation of specific receptors on the cell surface and signal transduction, but also through translocation across the cell membrane. However, the mechanism of the anti-apoptotic action of FGF2 inside the cell has still not been elucidated. The aim of our work was to identify novel intracellular FGF2 binding partners with which interactions would be important for the unique pro-survival activity of FGF2. Using several techniques, such as yeast two-hybrid and mass spectrometry analysis of complexes obtained by co-precipitation from cell lysates with recombinant FGF2, we identified eight FGF2 partner proteins, including p53, Sirt1, PCAF, CDK4, MDM2, NPM, API5, and HSP90. Their direct interaction was confirmed by pull-down assays and SPR measurements. In order to identify regions involved in interactions with binding partners nineteen surface mutational variants of FGF2 were generated. The biological activity of all FGF2 variants was confirmed by assessing their ability to bind and activate the FGF receptors (FGFRs) and the downstream ERK signaling pathway, and by verifying their proliferative potential. Their anti-apoptotic responses were then assessed using measurements of caspase 3/7 activity after starvation and staurosporine treatment. To ensure that the data obtained were solely from the translocated protein, the specific FGFR inhibitor PD173074 was used. Our data revealed that several variants showed a reduced ability to inhibit caspase 3/7 activity upon apoptosis induction, suggesting that the mutated residues are essential for the pro-survival activity of FGF2. Furthermore, some of these mutants bound more weakly to identified partner proteins, including p53 and its DNA binding domain. These data suggest that interaction with p53 is critical for the anti-apoptotic properties of FGF2. Acknowledgments: The work was supported by the NCN, Poland (CEUS-UNISONO nr 2020/02/Y/NZ3/00028).

Monday 11 July
9:00–11:00, Auditorium VIII

Lipids

S-04.2-2
Kinase-independent synthesis of 3-phosphorylated phosphoinositides by a phosphotransferase

G. Walpole
Dalhousie University, Halifax, Canada

Despite their low abundance, phosphoinositides play a central role in membrane traffic and signaling. PtdIns(3,4,5)P3 and PtdIns(3,4)P2 are uniquely important, as they promote cell growth, survival, and migration. Pathogenic organisms have developed means to subvert phosphoinositide metabolism to promote successful infection and their survival within host organisms. We demonstrate that PtdIns(3,4)P2 is a major product generated in host cells by effectors of the enteropathogenic bacteria Salmonella and Shigella. Pharmacological, gene silencing and heterologous expression experiments revealed that, remarkably, the biosynthesis of PtdIns(3,4)P2 occurs independently of phosphoinositide 3-kinases. Instead, we found that the Salmonella effector SopB, heretofore believed to be a phosphatase, generates PtdIns(3,4)P2 *de novo* via a phosphotransferase/phosphoisomerase mechanism. Recombinant SopB is capable of generating PtdIns(3,4,5)P3 and PtdIns(3,4)P2 from PtdIns(4,5)P2 in a cell-free system. Through a remarkable instance of convergent evolution, bacterial effectors acquired the ability to synthesize 3-phosphorylated phosphoinositides by an ATP- and kinase-independent mechanism, thereby subverting host signaling to gain entry and even provoke oncogenic transformation.

S-04.2-1
The complexity of the sphingolipid biosynthetic pathway

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Modern cell membrane contains a bewildering complexity of lipids, among them sphingolipids (SLs). Advances in mass spectrometry have led to the realization that the number and combinatorial complexity of lipids, including SLs, are much larger than previously appreciated. Irrespective of their complexity, SLs are generated by three enzymes in the anabolic pathway, namely serine palmitoyltransferase (SPT), 3-ketodihydrosphingosine reductase (KDSR) and ceramide synthase (CerS). All three enzymes depend on the availability of substrates and specific cofactors, which are themselves supplied by other complex metabolic pathways. The evolutionary pathway of these three enzymes is poorly understood, and likely depends on the co-evolution of the metabolic pathways that supply the other reaction components. We now introduce the concept of the 'anteome', from the Latin ante, to describe the network of metabolic ('omic') pathways that must converge in order to allow these pathways to co-evolve to permit SL synthesis, and suggest that current origin of life and evolutionary models are insufficient to explain the appearance of such complexity.

ShT-04.2-1
Decreased hydration causes nanoscale structural rearrangement within biomimetic cell membranes

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Many biological processes, such as endo- and exocytosis, neurotransmission, viral entry, fertilization, or cell fusion during embryogenesis depend on the merging of two lipid membranes. Each of these membrane fusion events involves mutual interactions between lipids, proteins, and water molecules surrounding two merging membranes. Although lipid membranes in natural conditions exist in excess of water, many crucial biological processes require partial dehydration at the boundary of two lipid bilayers. In this study, we used the combination of fluorescence microscopy and atomic force microscopy (AFM) to analyze the structural changes in biomimetic cell membranes under a wide range of hydration. Model lipid membranes were measured without applying any chemical or physical modifications, that so far were reported in the literature as crucial for maintaining the membrane structure under dehydration. Thus, the obtained results reveal the native properties of lipid membranes. We show that the removal of bulk water leads to the mixing of phases, extensive migration of liquid ordered (Ld) into liquid disordered (Lo) phase, and changing of the boundary between them. Finally, we observed that the process of dehydration leads to the decrease of hydrophobic mismatch between Ld and Ld phases, and as a consequence, lowers the line tension at their boundary. Importantly, this process is fully reversible and upon subsequent rehydration, both phases regain their initial height. Under low hydration conditions, which is present during events such as cell–cell fusion, the membrane becomes more flexible in terms of its structural organization. The presented here pioneering method of AFM measurements under controlled humidity can be applied for studying other model cell systems under varying hydration conditions. Part of the results were previously published in M. Chattopadhyay, E. Krok et al. 2021 JACS 143, 36, 14,551–14,562.

ShT-04.2-2
Effects of "minor" phytocannabinoids on the endocannabinoid system of human keratinocytes

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Cannabis contains more than 400 bioactive components, especially phytocannabinoids (pCBs). The recreational and medicinal activity of cannabis extracts has been known for centuries, and today medical cannabis has been legalized in many countries under regulated terms; though, controversial issues remain about its therapeutic efficacy to treat human diseases (previously published in: Pattnaik et al. (2022)). Growing interest has been recently focused on "minor" pCBs, that are non-psychotropic compounds like Cannabigerol (CBG), Cannabichromene (CBC), Tetrahydrocannabivarin (THCV) and Cannabigerolic acid (CBGA). Of note, pCBs can act via an endocannabinoid system (ECS), that is an ensemble of bioactive lipids, their receptors and metabolic enzymes involved in the regulation of key physiological and pathological processes, also in the skin. In this study, we used human keratinocytes (HaCaT cells) as an *in vitro* model that expresses all major ECS elements (CB_1, CB_2, GPR55, TRPV1 and PPAR$\alpha/\gamma/\delta$ receptors; NAPE-PLD, FAAH, DAGLα/β and MAGL enzymes), to systematically interrogate the effects of CBG, CBC, THCV, and CBGA. To this end, we analyzed gene and protein expression of these ECS elements through quantitative real-time reverse transcriptase-polymerase chain reaction (qRT-PCR) and western blotting, as well as their functionality through radioligand binding and enzymatic activity assays. Our results demonstrate that "minor" pCBs differently modulate gene and protein expression of distinct ECS elements. Of note, all pCBs significantly increased CB_2 binding and FAAH and MAGL activity. In addition, they all significantly increased TRPV1 channel activity. These unprecedented observations should be considered when exploring therapeutic potential of cannabis extracts for human diseases, at least in the skin. Pattnaik F., Nanda S., Mohanty S., Dalai A.K., Kumar V., Ponnusamy S.K., Naik S. Cannabis: Chemistry, extraction and therapeutic applications. Chemosphere. 2022, 289:133012. *The authors marked with an asterisk equally contributed to the work.

ShT-04.2-3
Plasma membrane and metabolic alterations as novel actors in the onset of GCase-related pathologies

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GCase-related pathologies are caused by mutations affecting the GBA gene that codes for β-glucocerebrosidase (GCase), a lysosomal glycohydrolase responsible for the physiological catabolism of the sphingolipid glucosylceramide (GlcCer). Among GCase-related pathologies, biallelic mutations of GBA are causative of Gaucher Disease (GD), while heterozygous mutations represent the major genetic risk factor for the development of GBA-dependent Parkinson's Disease (PD). Nowadays, the relation between GCase loss of function and neurodegeneration is still debated. We developed an *in vitro* model of the neuronal form of GD represented by iPSC-derived dopaminergic neurons obtained from healthy subjects' fibroblasts and treated for 30 days with 500 μM conduritol B epoxide (CBE), a specific inhibitor of GCase. CBE-treated neurons accumulate GlcCer with the onset of neuronal damage, whereby the volume of intracellular acidic organelles appears increased. In addition, by the isolation and characterization of plasma membrane (PM) detergent-resistant portions, we discovered that the accumulated GlcCer is not only confined to the lysosome but affects also the PM, where it is associated with an enrichment of the active form of the non-receptor tyrosine-kinase c-Src. We also investigated changes in the energetic metabolism, through LC–MS/MS targeted metabolomics analysis, which suggested the slow-down of both the glycolytic pathway and Krebs cycle upon CBE treatment. In addition, impaired neurons show an increased uptake and use of aminoacids as energetic substrates. The obtained data let to speculate that in Gcase-related pathologies, the enzymatic deficit and GlcCer accumulation impair the lysosomal compartment, leading to: i) the establishment of an aberrant lysosome-plasma membrane axis which alters the plasma membrane architecture with consequences on intracellular signalling pathways and ii) severe alterations in the neurons' energetic metabolism.

ShT-04.2-4
An expanded palette of photoswitchable sphingolipids for optical control of lipid microdomains

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Sphingolipids are a complex class of lipids largely found at the plasma membrane of cells and commonly linked to lipid rafts. From a biophysical standpoint, sphingolipids laterally segregate with other lipids and cholesterol into phase-separated liquid-ordered (L_o) domains; a model system widely used to gauge basic principles of lipid rafts *in vitro*. Hence, owing to the importance of sphingolipids for lipid phase-separation, controlling their lateral localization on membranes is of utmost significance. In this work (https://doi.org/10.1101/2021.10.11.463883), we took advantage of the light-triggerable *trans-cis* isomerization of azobenzene-modified acyl chains and developed novel photoswitchable sphingolipids with varying headgroup (hydroxyl, galactosyl, phosphocholine) and sphingoid backbone (sphingosine, phytosphingosine, tetrahydropyran (THP)-blocked sphingosine) functionalities. By combining atomic force and fluorescence microscopies, we then assessed how these photolipids can reversibly remodel membranes upon irradiation with UV and blue lights, altering structural properties such as phase-separation area, height mismatch, line tension, and membrane piercing forces. Overall, we show that all sphingosine- and phytosphingosine-based photolipids promote a reduction in L_o domain area when in the UV-adapted *cis*-isoform; while photolipids with a protective THP group (blocking H-bonding) at the sphingosine backbone induce an increase in the L_o domain area instead, accompanied by a major rise in domain height mismatch and line tension. Such changes were fully reversible upon blue light-triggered isomerization of the various azobenzene-modified photolipids back to *trans*, thus pinpointing the importance of sphingoid base functionality for a stable L_o domain formation and differential optical control of various membrane properties.

Monday 11 July
16:00–18:00, Auditorium I

Sensors and nanotechnology

S-05.2-1
Lab-on-chip platforms for biological analysis – applications in agrofood

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Microfluidic lab-on-chip sensing platforms are currently being intensively studied for detection of bioanalytes (such as DNA, proteins, cells, and metabolic products) in applications such as food safety, health monitoring and environmental control. These systems have compelling potential advantages, such as portability, speed, sensitivity, multiplexing, no need for highly skilled operators or laboratory infrastructure, and low cost. I will present two applications as case studies of integrated lab-on-a-chip analytical systems: (1) detection of mycotoxins produced by fungi that contaminate several sources of food and drink; and (2) detection of metabolites (hormones) in grapes and vines as biomarkers of plant infection and hydric stress. To take full advantage of the miniaturization of the biosensor, it is crucial to also address the following issues: (i) fluidic handling directly from sample; (ii) consideration of the interfering effects of the often chemically and physically complex biological sample matrix and on-chip sample preparation; (iii) on-chip transducer integration – in our case typically thin-film silicon photosensors; (iv) methods of signal enhancement; and (v) strategies for simultaneous (multiplex) detection of various target molecules.

S-05.2-2
Signaling and sensing NO

J. Moura
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Nitric oxide (NO) is a signaling molecule with a critical role in several physiological and pathological pathways. In mammals, NO's role includes vasodilatation, neurotransmission, apoptosis, gene expression regulation, platelet aggregation, and immune response. In humans, NO synthase produces NO from arginine and dioxygen. However, under anoxia or hypoxia conditions, NO formation can be impaired. Under such conditions, "non-respiratory" nitrite reduction to NO is carried out by "non-dedicated" nitrite reductases (NIR). On the other hand, under the N-biocycle, bacteria reduce nitrate and nitrite into ammonia or NO, since the nitrite reduction step is still diverse being carried out by different enzymes. When formed, NO is further reduced to N_2O (nitric oxide reductase - NOR) that is converted to N_2 (nitrous oxide reductase - N_2OR), under the denitrification pathway (DEN). Considering the variety of NO generating/consuming enzymes, the role of NO in biological signaling is discussed and methodologies for sensing in the following cases:
-NO formation from nitrite, by a new class of nitric oxide-forming nitrite reductases, xanthine oxidase and aldehyde oxidoreductase (molybdenum enzymes) using EPR and specific electrodes;
-NO formation from nitrite in DEN by cytochrome cd1 (cdNIR) by electrochemical sensing;
-NO sensing by NOR (and O_2 reduction) by modified electrochemical electrodes;
-and a brief account of nitrite reduction to ammonia by a multiheme nitrite reductase (ccNIR) using electrochemical sensing. References: Maia and Moura, Redox Biology, 2018, 19, 274–289; Maia *et al*. Biochemistry, 2015, 54, 685–710; Lopes H *et al*. J Biol Inorg Chem. 2001, 55–62; Gomes *et al*. Bioelectrochemistry. 2019, 125, 8–14; Gomes *et al*. Bioelectrochemistry. 2019, 127, 76–86; Duarte *et al*. Biochim Biophys Acta. 2014, 375–84; Almeida *et al*. Sensors (Basel) 2010, 10, 11,530–55.

ShT-05.2-1
A nanobody-based immunosensor for the detection of C-reactive protein

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C-reactive protein (CRP) is a homopentameric oligoprotein inflammatory biomarker that plays an essential role as a predictor of infections, cardiovascular diseases, and stroke. The advent of nanobody (VHH) technology has thus far revolutionized medical research, therapeutics, and diagnostics fields owing to VHH's small size, good tissue penetration, and stability. In this study, nanobodies specific for CRP were selected from phage display naïve library, and the best candidates were identified based on their binding properties. Among the eight (8) selected clones, Clone NbE12 showed the highest yield (11 mg/L) with an excellent binding affinity (KD = 13 nM) for its antigen. NbE12 was expressed fused to SpyTag and further characterized as an immunocapture reagent for detecting and quantifying CRP using an impedance electrochemical biosensor activated via SpyTag/SpyCatcher conjugation system. A linear CRP concentration range was achieved between 0.2–1 μg and the biosensor sensitivity was of 0.21 μg/mL, with a measurement time of about 6 min. This study indicates that electrochemical biosensor based on nanobody-SpyTag/SpyCatcher fusion could be a useful and sensitive diagnostic tool for quantifying CRP in clinical samples. Previously published in: Oloketuyi S et al. (2021) Biosensors. 11 (12):496, 1–14.

ShT-05.2-2
Selection of histamine binding peptides as potential biorecognition elements

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Hacettepe University, Ankara, Turkey

Histamine is one of the most common biogenic amines in foods and has threatened public health due to its physiological and toxicological effects after consumption. In this study, we aimed to identify peptides that bind specifically to histamine by phage display method. The screening was performed with a 12mer M13 phage display library. The last panning cycle was modified with the elution of histidine binding phages, which have a similar structure to histamine for enhancing the selectivity of the histamine binding phages. Then the affinities of the selected phages were tested via an enzyme-linked immunosorbent assay and four different peptide sequences were obtained after this step; HBF5, WETCVHLWDCQR; HBF10, SGFRDGIEDFLW; HBF14, TTQDMWNFWWHS and HBF26, IPLENQHKIYST. In order to understand the thermodynamic properties of binding between peptides (HBF10, HBF26) and histamine, we used isothermal titration calorimetry. While the peptides showed high affinities to histamine with a K_a value of $2.56x10^4$ (M^{-1}) and $8.94x10^4$ (M^{-1}), respectively, there were no affinities observed to structurally similar histidine. The alteration of the secondary structure of HBF26 peptide with relation to the binding of HBF26 to free histamine was analyzed by Circular Dichroism Spectrometry. At the end of the study, the binding of HBF26 peptides to histamine molecules was investigated using an SPR sensor system, and the calibration curve was obtained using the sensorgram data of the HBF26 peptide. Considered as a whole, the structural and thermodynamic studies of peptides have the potential to be used to develop novel selective diagnostic agents or effective antihistaminics.

ShT-05.2-3
Nanoparticles for brain drug delivery – overcoming the blood–brain barrier

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Poly(lactic-co-glycolic) acid (PLGA) nanoparticles enhance drug pharmacodynamics and bioavailability, and when loaded with

superparamagnetic iron oxide nanoparticles (SPIONs) they can act as contrast agents for magnetic resonance imaging (MRI) [1]. These characteristics make them attractive for brain imaging and therapy. However, the application of nanoparticles (NPs) for brain drug delivery is hindered by the presence of the blood–brain barrier (BBB). The BBB is a natural defense against circulating toxic and infection agents that also prevent most therapeutic compounds from reaching the brain. BBB peptide shuttles (BBBpS) are small peptides that engage adsorptive mediated transport (AMT) across the BBB and allow brain uptake [2]. In this work, we propose SPIONs-loaded PLGA nanoparticles functionalized with a BBBpS, as a platform for brain drug delivery. We produced SPIONs-loaded PLGA NPs through simple-emulsion solvent evaporation technique with a size range of 110–145 nm and iron content of SPIONs in NPs of 80%. In the functionalization step, 15% of fluorescently labeled BBBpS was conjugated to the NPs surface, resulting in alteration of size and charge. The size increased in 60 nm and zeta potential from -21.2 ± 0.6 before functionalization, to -4.2 ± 1.2 after functionalization. The increase in charge was expected due to the presence of cationic BBBpS at NPs surface. To test the activity of NP we first investigated the interaction with human brain endothelial cells (BEC) that make up the BBB. NPs internalization in BEC was evaluated through flow cytometry and fluorescence microscopy. The results reveal that BBBpS promotes internalization, with an increase of 6-fold in BBBpS modified NPs, in comparison with naked NPs, at 24 h. We will further test these NPs in *in vitro* models of the BBB and *in vivo* brain uptake. [1] Chaparro C. I. P et al. (2019) 6th IEEE Port. Meet. Bioeng. ENBENG 2019. [2] Neves V et al. (2017) ACS Chem. Biol. 12, 1257–1268.

Monday 11 July
16:00–18:00, Auditorium II

Proteomics

S-06.2-2
Mass spectrometry-based clinical proteomics indicates prognostic signature in oral cancer

A. Busso-Lopes*, C. Carnielli*, D. Granato, J. Sá*, E. Santos, A.G. Normando, L. Trino*, **A. Paes Leme**
Brazilian Center for research for Energy and Materials, Campinas, Brazil

Head and neck cancer (HNC) is ranked the eighth leading cause of cancer worldwide and exhibits high prevalence and morbidity. Despite the efforts in improving diagnosis, prognosis, and therapeutic modalities, lymph node (LN) metastasis, local relapses, and poor survival rates represent a clinical challenge for the disease. In this talk, I will present the application of discovery and targeted mass spectrometry-based clinical proteomics to search for biomarkers in multisite samples from primary and matched LN-negative or -positive tissues, biofluids and circulating extracellular vesicles. These approaches have enabled us to gain a deeper insight into the HNC biology and indicate robust signatures of metastasis, which may enhance prognostic decisions in HNC. Finally, I will discuss potential strategies to translate our findings into clinical practice. *The authors marked with an asterisk equally contributed to the work.

S-06.2-1
How mechanical force regulates talin function

V. Hytönen
Tampere University, Tampere, Finland

Cells continuously interact with their environment and receive numerous chemical and physical signals. Among those, mechanical cues have been found to be essential for cellular differentiation and maintenance of central cellular functions. Talin is a cytoplasmic integrin adapter protein which transmits mechanical signals between extracellular matrix and cell cytoskeleton. Talin head interacts with cytoplasmic tails of integrins and large talin rod binds actin and several other proteins linked with the cytoskeleton. It has been observed that talin not only transmits mechanical load, but its conformation is regulated by mechanical force. There are numerous binding sites within talin which become activated or inactivated as a response to the amount of mechanical load applied. To study how talin behaves under applied mechanical load, single-molecule biophysical experiments have been performed. Atomistic dynamic models have been generated with the help of molecular dynamics simulations. These studies have revealed how cryptic binding sites within talin may become activated to enable binding of ligands such as vinculin. Under mechanical load, intermediate states of talin have been observed, where unique conformation of the protein is only reached under applied load. Talin-mediated mechanosensing is essential for cell polarization and matrix remodelling. Therefore, it is not surprising that talin is also associated with diseases such as cancer. While we understand the basics of mechanoregulation of talin conformation, there is still a long way to go to understand the complex process of cellular mechanoregulation.

ShT-06.2-1
Comparison of SPEED, S-Trap, and In-Solution based sample preparation methods for tandem mass spectrometry

E.M. Templeton[1], A.P. Pilbrow[1], T. Kleffmann[2], J.W. Pickering[1], M.T. Rademaker[1], N.J.A. Scott[1], L.J. Ellmers[1], C.J. Charles[1], Z. Endre[3], A.M. Richards[1,4], V.A. Cameron[1], M. Lassé[1]
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Bottom-up proteomic analyses rely on efficient protein extraction from tissue and proteolysis into peptides for mass spectrometry. Commonly used detergent-based strategies aid cell lysis and protein solubilization but are poorly compatible with downstream protein digestion and liquid chromatography-coupled mass spectrometry. Consequently, additional sample purification and buffer exchange steps are required, which are time-consuming and introduce additional technical variability. This study provides the first direct quantitative comparison of two well-established detergent-based methods for protein extraction and solubilization (In-solution and suspension trapping, S-Trap) with the recently developed Sample Preparation by Easy Extraction and Digestion (SPEED) method, which uses a strong acid for denaturation. Identification rates and quantitative performance of each method were compared with data-dependent acquisition (DDA) and

label-free SWATH-MS (sequential window acquisition of all theoretical mass spectra) in both sheep kidney cortical tissue and plasma. In kidney tissue, SPEED outperformed In-solution and S-Trap by quantifying the highest number of unique proteins (SPEED 1250; S-Trap 1202; In-solution 1197) and displayed the most efficient proteolysis (SPEED 93.8% of peptides fully tryptic in DDA; S-Trap 88.5%; In-solution 87.3%). In plasma, S-Trap produced the most unique protein quantifications (S-Trap 151; In-solution 148; SPEED 138), indicating it may be the optimal method for this biofluid. Protein quantifications were reproducible across biological replicates in both tissue (R^2 from 0.85 to 0.90), and in plasma (SPEED $R^2 = 0.84$; In-solution $R^2 = 0.76$, S-Trap $R^2 = 0.65$). Our data suggest SPEED as the optimal method for proteomic preparation in kidney tissue and S-Trap or SPEED as the optimal method for plasma, depending on whether a higher number of protein quantifications or greater reproducibility is desired.

ShT-06.2-2
Analysis of CD 19-CAR NK cells secretome unfolds probable molecular effectors in the management of hematological malignancies

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Hematological malignancies (leukemia and lymphoma), accounted for about 571,000 deaths globally in 2020. A notable therapeutic option is the Chimeric Antigen Receptor Natural killer (CAR NK) cell therapy which has emerged as a novel and effective therapeutic practice for the treatment of hematological malignancies. Understanding the tumor microenvironment is crucial to identifying key players in cellular communication and signaling mediated by secreted proteins. These proteins are involved in a variety of physiological processes, including cell signaling and matrix remodeling. These proteins could help uncover molecular effectors of CD-19 CAR NK cell therapy. Sample preparation procedure included: protein quantification, immunodepletion, reduction, alkylation, tryptic digestion, isobaric tag for relative and absolute quantification (iTRAQ) labeling, visualized with SDS gel electrophoresis, and were analyzed with LC–MS/MS (Liquid Chromatography-Mass Spectrometry). Gels of protein were analyzed and from the MS Spectrum, gene ontology and molecular network of interactors were analyzed. Data from this study shows some secretome profiles that can be explored, validated, and translated to enhance the understanding of the molecular mechanisms of action of CAR NK cell therapy. Some notable players identified include proteins involved in IF-gamma, TCR, Caspase cascade of apoptosis, regulation of telomerase, and p53 pathways among others. Our study showed secreted proteins could help uncover probable molecular effectors of CD19-CAR NK cells which can enable more understanding of the mechanism and signaling of the CAR NK therapy. With the continuous advancement of technology, the secretome profiles will become more evident and fundamental to provide answers to many relevant biological questions and hypotheses relating to the CAR NK-cell therapy in hematological malignancies.

ShT-06.2-3
Characterization of cystatin B interactome in saliva of Alzheimer's disease patients

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Cystatin B is a small protein with cathepsin inhibitory activity, widely expressed in different biofluids and tissues, including the brain. Cystatin B has been associated with a neuroprotective role against oxidative stress, which is a typical condition linked to Alzheimer's disease (AD). The ability of cystatin B to form polymeric structures has been studied *in vitro* and in cellular systems including its ability to interact with other proteins to form a multi-protein complex in rats' cerebellum. In this study, we characterized a cystatin B multi-protein complex in human saliva, in which cystatin B is usually detectable as several Cys-oxidized proteoforms. Whole saliva pools from 24 AD patients and 24 elderly healthy controls (HC) were submitted to immunoprecipitation in triplicate with cystatin B antibody followed by SDS-PAGE under reducing and not-reducing conditions, tryptic digestion, and nano-HPLC-high-resolution-MS/MS analysis. Protein identification was performed by Proteome Discoverer software, while statistical analysis between AD and HC groups was performed with Perseus tool. We identified 81 cystatin B protein partners, present in both AD and HC groups, mainly involved in the innate immune system and neutrophil degranulation (*e.g.* S100A6, A8 and A9, annexins, calmodulin and others), and antimicrobial activity (*e.g.* bactericidal permeability-increasing protein (BPI), elastase, cathepsin G and others). In the cystatin B interactome of AD patients, we observed an increase of BPI, matrix Gla protein, and mucin-7 with respect to HC, and a decrease of grancalcin and triosephosphate isomerase (TPI). This last result is in agreement with the low efficiency of glycolytic enzymes, which is due to nitrotyrosination of TPI and others under stress oxidative conditions. This study highlights for the first time the ability of cystatin B to form a multi-protein complex in human saliva.

ShT-06.2-4
Highly efficient exploration of conformational spaces using robot mechanics - A helical bundle case study

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We show here that robot mechanics may be used to efficiently explore transitions between various functional states in biomolecules by taking advantage of patterns such as secondary structures that can be used as constraints. Here we describe results obtained with a recently developed molecular simulation program Robosample, described in: Spiridon L et al. Biochim Biophys Acta Gen Subj. 2020, 1864(8):129616, on a set of Coiled-coil systems present in NLR proteins that we have recently phenotyped and published in Wróblewski et al. PLoS Biol. 2018, 16(12): e2005821. Robosample is based on a previously developed method GCHMC published in: Spiridon L et al. (2017) J Chem Theory Comput. 13, 4649–4659, shown to efficiently cover conformational spaces by mixing constrained and fully flexible Generalized Coordinates Hamiltonian Monte Carlo moves via Gibbs sampling. Also, through the use of robot mechanics and parallel tempering, Robosample enables large rigid body moves and allows a broad range of joint types, such as Ball or Slider, which eases the usage of custom constraints. Helical bundles, in their closed form, have energy surfaces marked by high energy barriers, mainly due to the 'knobs into holes' packing of the side-chains, presenting a challenge for sampling methods. Here we developed a simple method that generates large helical bundle start conformation ensembles in a pre-folded 'molten-globule' type of state which led to their highly efficient GMHMC sampling. GCHMC simulations analysis based on molten globule conformations yielded free energy profiles that allowed the identification of predominant states and their relative population within different members of the NLR-CC family. This, in turn, sheds light on the link between their biological roles and the corresponding conformational space; Acknowledgement: This work was supported by a grant of the Romanian Ministry of Education and Research, CNCS - UEFISCDI, project number PN-III-P4-ID-PCE-2020-2444, within PNCDI III. *The authors marked with an asterisk equally contributed to the work.

Monday 11 July
16:00–18:00, Auditorium VII

Cellular imaging

S-07.2-1
Quantitative immune surveyance in cancer

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Immune checkpoints, such as PD-1/PD-L1 and CTLA-4/CD80, are regulatory mechanisms in the immune system designed to promote self-tolerance and avoid autoimmune diseases. In cancers these immune checkpoints modulate, by upregulating their cognate ligands, to prevent immune detection. Immune checkpoint blockade therapies have enhanced cancer therapy. However, only a small subset of patients experience a sustainable effect, and most acquire primary or secondary resistance. Most patients are stratified for anti-PD-1 or anti-CTLA-4 treatments based on their PD-L1 ligand expression. Ligand expression is currently assessed by traditional immunohistochemistry (IHC) approaches, which lack quantitation and a dynamic range. We have developed a quantitative imaging platform, underpinned by time-resolved Förster resonance energy transfer (FRET) determined by frequency-domain fluorescence lifetime imaging microscopy (FLIM) to spatially quantitate these immune checkpoint interactions at a nanoscopic (< 10 nm) resolution. This assay is termed immune-FRET (iFRET). We applied iFRET to determine PD-1/PD-L1 interactions in a retrospective study with malignant melanoma and malignant non-small cell lung carcinoma (NSCLC). In both melanoma and NSCLC, it was observed that increased PD-1/PD-L1 interaction state, determined by iFRET, correlated with a worsened overall survival. PD-L1 expression, the current stratification criterion of immunotherapy, failed to correlate with patient outcome. In tandem with imaging mass spectrometry, iFRET can be applied to a range of immune checkpoints. The new notion of quantitative immune surveyance raises from the combination of both these methods, which can monitor the functional proteomics of patients. The implementation of iFRET to carry out quantitative immune surveyance may change the way patients are selected for immunotherapies and may provide a precise mechanism to monitor their response to treatment.

S-07.2-2
Seeing is believing

T. Kirchhausen
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Frontier optical-imaging modalities exemplified by the lattice light-sheet microscope sets new visualization standards to see, analyze and understand three dimensional processes at diffraction limited resolution and high-temporal precision with unprecedented duration of minutes or hours in the complex and dynamic environment of living cells in isolation and within tissues of an organism. We are also witnessing an artificial intelligence (AI) inspired transforming revolution that is helping set up robust training and inference robust tools aimed to reveal biological insights from these massive data sets. We believe this ability for large-scale imaging with minimal perturbations combined with use of AI methods is ideally suited to support hypothesis-generating research geared towards new discoveries. This talk will illustrate how we uncovered a templating process mediating the formation of nuclear pores during mitosis and unexpected entry pathways leading to infection by SARS-CoV-2 by combined use of these approaches.

ShT-07.2-1
A novel PET-reporter gene system for the specific and sensitive imaging of therapeutic and/or virally transduced cells *in vivo*

K. Fritschle*[1], V. Morath*[1], L. Krumwiede[1], M. Zivanic[1], M. Anneser[2], S. Robu[1], S. Dötsch[3], L. Warmuth[3], T. Bozoglu[4,5], S. Kossatz[1], C. Kupatt[4,5], K. Steiger[6,7], D. Busch[3], A. Skerra[2], W. Weber[1]

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Advanced medical treatments, such as cell and gene therapies, necessitate a reliable diagnostic method for imaging transgenes and monitoring the localization in a quantitative manner over time. Generally, PET imaging offers the most sensitive detection and is depth independent. Therefore, we developed a novel reporter gene system encoding a radiochelate-binding protein for the preclinical and clinical quantification of the biodistribution and proliferation of chimeric antigen receptor T-cells as well as to monitor the *in vivo* transduction of cells by AAV vectors. The reporter protein, called DTPA-R, comprises an extra-cellular binding protein, the Anticalin CL31d, which binds rare earth Me•DTPA complexes with subnanomolar affinity [previously published in: Kim HJ et al. (2009) J Am Chem Soc 131 (10): 3565–76], and a membrane anchor domain. The modular design of this reporter protein allows addition of an intracellular fluorescent protein, thus enabling multi-modal imaging. T-cells were transduced with different versions of the reporter gene, and high expression levels were observed which were significantly higher compared to a similar reporter gene based on a single chain antibody fragment. The reporter protein did not alter the proliferation, viability, or cytotoxic effector function of T-cells, as confirmed by flow cytometry assays and a real time killing assay. The specificity of DTPA-R to the corresponding radioligand was confirmed in comparative binding studies in cell culture. PET-studies with CD1-nude mice revealed high accumulation of the radioligand in DTPA-R^+ PC3 xenograft tumors with exceptional contrast. In AAV9 treatment studies, we could clearly detect viral transduction of even tiny anatomic structures such as adrenal glands, which correlated with immunohistochemistry. In summary, this novel reporter gene provides a promising tool to elevate the understanding of cell and gene therapies to a new level and support the development of precision medicine. *The authors marked with an asterisk equally contributed to the work.

ShT-07.2-2
Exocrine dysfunction and beta cell stress in zebrafish larvae: A tale of two pancreatic cells

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Introduction: Type 1 diabetes (T1D) is caused by the loss of insulin-producing beta cells in the endocrine pancreas due to an autoimmune attack. These cells, located in Islets of Langerhans, are embedded in the exocrine pancreas that produces enzymes for the digestive tract. Surprisingly, T1D patients exhibit 'intermediate cells' that have the characteristics of both hormone-producing cells as well as exocrine cells. Moreover, the exocrine pancreas volume is significantly lower in (pre)diabetic patients. *Aim*: The pancreatic exocrine-endocrine miscommunication may have a pivotal role in the onset of T1D. However, a cause-consequence study cannot be performed in human. Therefore, we developed a zebrafish model that allows to address if exocrine damage will induce beta cell stress. *Methods*: Generation of a transgenic zebrafish line that allows modulation of exocrine cells and intravital monitoring of beta cell function. Nitroreductase-based ablation method is used to selectively ablate exocrine cells. Combing light sheet microscopy with fluorescent probes allows monitoring of beta cell function such as calcium dynamics, ER/ mitochondrial stress, neoantigen production and glucose response. *Results*: Nitroreductase positive exocrine cells are destroyed in our zebrafish model after nifropirinol administration, as is obvious from a caspase-dependent fluorescent reporter and large-scale electron microscopy analysis to analyze exocrine damage and beta cell stress (endoplasmic reticulum dilation, mitochondrial stress), which will be presented here. *Conclusion and future outlook*: Our zebrafish model will provide a dynamic tool to modulate the exocrine pancreas. We set up beta cell readouts such as calcium dynamics with GCaMP and light sheet microscopy that will reveal beta cell functionality and stress at single cell resolution in living larvae. These new transgenic fish will help to address a cause-consequence relationship in exocrine malfunction and beta cell stress. *The authors marked with an asterisk equally contributed to the work.

ShT-07.2-3
Defining inflammation through image-based behavioral landscapes

M. Palomino Segura[1], J. Sicilia[1], G. Crainiciuc[1], M. Molina-Moreno[2], I. González-Díaz[2], F. Díaz-de-María[2], F. Sánchez-Cabo[1], A. Hidalgo[1]

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Transcriptional or proteomic profiling of individual cells have revolutionized interpretation of biological phenomena by providing cellular landscapes of healthy and diseased tissues. These approaches, however, fail to describe dynamic scenarios in which cells can change their biochemical properties and downstream "behavioral" outputs every few minutes. To overcome this problem, we have performed 4D intravital microscopy to record hundreds of morpho-kinetic parameters describing individual

leukocytes at sites of active inflammation. By analyzing over 100,000 cells over time, we were able to recognize leukocyte identities and define behavioral changes of the same cell in the inflamed skin and trachea. By examining inflammation inside blood vessels, we also uncovered a continuum of neutrophil states, including a large, sessile state that is associated with pathogenic inflammation (referred to as "B3 behavior"). Our studies suggest that immune behaviors are deterministic, thereby raising the possibility of defining for the first time the molecular build-up of inflammatory leukocytes *in vivo*. To this end, we have embarked on correlative behavioral, proteomic and transcriptomic analysis at the single cell level that should help us to unveil the signals that trigger transitions between immune states and reprogram complex immune behaviors in multiple pathogenic scenarios, from inflammation, infection and tumors.

ShT-07.2-4
Activity of a designed cyclic analogue of gomesin against *Staphylococcus aureus* biofilms

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Biofilms have great impact for public health because biofilm-associated microorganisms exhibit reduced susceptibility to conventional antibiotics. The use of antibiotics even at high dosages often fails, making the treatment of these infections very challenging. Thus, novel antimicrobial agents with distinct mechanisms of action are needed. Here, we explored the use of [G1K, K8R]cGm, a cyclic antimicrobial peptide stabilized by two disulfide bonds, as an alternative approach to treat biofilm infections. The activity of [G1K,K8R]cGm was studied against biofilms of *Staphylococcus aureus*, an opportunistic pathogen associated with several biofilm-related infections. The results show that the peptide has potent activity against 24 h-preformed biofilms through a concentration-dependent capacity to kill biofilm-embedded cells. Mechanistic studies using atomic force and real time confocal laser scanning microscopies showed that [G1K,K8R]cGm causes morphological changes on bacterial cells and permeabilizes their membranes across the biofilm. We also tested an analogue of [G1K,K8R]cGm without disulfide bonds, and a linear unfolded analogue, but both are inactive against 24 h-preformed *S. aureus* biofilms. This finding suggests that the three-dimensional structure of [G1K,K8R]cGm and its stabilization by disulfide bonds are essential for its antibacterial and antibiofilm activities. Our findings support the potential application of this stable cyclic antimicrobial peptide to fight bacterial biofilms. *The authors marked with an asterisk equally contributed to the work.

Tuesday 12 July
9:00–11:00, Auditorium I

Diabetes and obesity

S-01.3-1
Novel players in the regulation of muscle mass, strength, and regeneration

J. Ruas

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Chronic unresolved inflammation is an important player in the etiology of metabolic diseases such as obesity and diabetes. Constantly elevated pro-inflammatory cytokines and immune cell infiltration in skeletal muscle, liver, and adipose tissue can be observed in the early stages of metabolic disease and greatly contribute to the development of insulin resistance and muscle atrophy, among other effects. On the other hand, select cytokine secretion and immune cell recruitment is fundamental for tissue adaptation, remodeling, and regeneration. Thus, identifying tissue regulators of adaptive immune cell recruitment could help develop tools to improve metabolism, tissue function, and whole-body insulin sensitivity. We have identified in skeletal muscle a previously uncharacterized protein as a novel Tissue Remodeler and Activator of INflammation, which we have called TRAIN. TRAIN expression is increased in skeletal muscle of mouse models of genetic- and diet-induced obesity and highly correlated with the mRNA levels of pro-inflammatory cytokines and immune cell markers. AAV-mediated TRAIN delivery to muscle improves exercise performance, whereas skeletal muscle-specific TRAIN knockout mice show compromised recovery in a model of disuse-induced muscle atrophy and compensatory hypertrophy. TRAIN's biological activity seems to depend, at least in part, on its ability to bind specific RNAs (such as miRNAs). This work identifies TRAIN as a novel RNA-binding protein involved in the regulation of tissue remodeling, immune cell recruitment, and regeneration.

S-01.3-2
Polycystic ovary syndrome and gut microbiota

B. YILDIZ

Hacettepe University, Ankara, Turkey

Polycystic ovary syndrome (PCOS) is the most common female endocrine disorder, affecting up to 1 in 5 women. The syndrome is characterized by clinical or biochemical androgen excess, ovulatory dysfunction and polycystic ovarian morphology. At least two of these three features are required to establish a diagnosis after excluding mimicking disorders. PCOS is associated with an adverse cardiometabolic risk profile, including insulin resistance, obesity, dyslipidemia, and increased prevalence of cardiovascular risk factors. Pathogenesis of the syndrome is not fully elucidated. Alterations in composition, diversity and metabolites derived from the

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gut microbiota have been reported in preliminary animal and human studies of PCOS, suggesting that microbiota might potentially be involved in the development of the syndrome and its long-term metabolic health consequences. Larger studies are needed to investigate whether dysbiosis has an implication in pathogenesis of PCOS and whether it could give rise to novel treatment opportunities.

ShT-01.3-1

Abstract withdrawn.

ShT-01.3-2

Understanding the crosstalk between muscle-brain: p38MAPK as a novel therapeutic approach for obesity comorbidities

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Obesity is currently a serious epidemic health problem associated with other metabolic disorders, including type 2 diabetes. Therefore, understanding the mechanisms that lead to the development of obesity comorbidities is paramount to decrease their incidence and mortality. The p38MAPK pathway has been shown to be an important modulator of homeostasis, and excessive activation of this cascade could be responsible for diseases as important as cancer and diabetes. Although p38 function in obesity and its associated pathologies is starting to be addressed, its role in inter-organ communications in the context of obesity and its associated diseases remain unsolved. Using a mouse model lacking p38α in striated muscle, we found that p38α deletion protects mice against high-fat diet (HFD)-induced obesity by increasing energy expenditure and skeletal muscle metabolic remodelling. This phenotype is accompanied by an hyperactivation of p38γ, which improves glucose and energy homeostasis through an increase in the locomotor activity. According to these results, we found a significant decrease in the risk of liver steatosis. Our data highlight local and systemic manifestations of p38 α signalling as a novel muscle-to-brain communication and identified this pathway as a new therapeutic approach to the current obesity and metabolic disorders.

ShT-01.3-3

Impaired TFEB activation and mitophagy as a cause of calcineurin inhibitor-induced pancreatic β-cell dysfunction

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Autophagy or mitophagy play crucial roles in the maintenance of pancreatic b-cell function. Calcineurin can modulate activity of TFEB, a master regulator of lysosomal biogenesis and autophagy gene expression, through dephosphorylation. We studied whether calcineurin inhibitors can affect mitophagy of pancreatic β-cells and pancreatic β-cell function employing FK506, an immunosuppressive drug against graft rejection. FK506 suppressed rotenone- or oligomycin/antimycin A-induced mitophagy measured by mito-Keima localization in acidic lysosome or RFP-LC3 puncta colocalized with TOM20 in INS-1 insulinoma cells. FK506 diminished nuclear translocation of TFEB after treatment with rotenone or oligomycin/antimycin A. Forced TFEB nuclear translocation by a constitutively active TFEB mutant transfection restored impaired mitophagy by FK506, suggesting the role of decreased TFEB nuclear translocation in FK506-mediated

mitophagy impairment. Probably due to reduced mitophagy, recovery of mitochondrial potential or quenching of mitochondrial ROS after removal of rotenone or oligomycin/antimycin A was delayed by FK506. Mitochondrial oxygen consumption was reduced by FK506, indicating reduced mitochondrial function by FK506. Probably due to mitochondrial dysfunction, insulin release from INS-1 cells was reduced by FK506 *in vitro*. FK506 treatment also reduced insulin release and impaired glucose tolerance *in vivo*, which was associated with decreased mitophagy and mitochondrial COX activity in pancreatic islets. FK506-induced mitochondrial dysfunction and glucose intolerance were ameliorated by an autophagy enhancer activating TFEB. These results suggest that diminished mitophagy and consequent mitochondrial dysfunction of pancreatic β-cells contribute to FK506-induced β-cell dysfunction or glucose intolerance, and autophagy enhancer could be a therapeutic modality against post-transplantation diabetes mellitus caused by calcineurin inhibitors.

ShT-01.3–4
The effect of taurine supplementation on the development of beige adipose tissue in high-fat diet fed mice

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In recent years, a new type of adipose tissue (beige adipose tissue) has been mentioned, unlike white adipose tissue (WAT) and brown adipose tissue (BAT). Beige cells are capable of thermogenesis like BAT. In response to various agents, beige cells can develop within WAT through a process called "browning". Therefore, the prevention of obesity and related diseases by providing WAT browning with new potential agents has been extensively studied in recent years. Taurine has many physiological functions in the body and has beneficial effects on obesity and related metabolic disorders. For this reason, we aimed to investigate whether taurine supplementation has effects on browning of WAT and attenuating obesity. Thirty-two male C57BL/6 mice were used for the study. Mice were divided into 4 groups as control, control + taurine, high fat diet (HFD) and HFD + taurine, and fed for 20 weeks. Taurine was given in drinking water (5%). Epididymal WAT samples were obtained from mice and RNA was extracted from these tissues. Expression levels of FLCN, mTOR, TFE3, PGC-1α, PGC1-1β, AMPK, S6K and UCP1 genes were measured by real-time PCR. Taurine supplementation reduced HFD-induced obesity. No UCP1 expression was detected in any of the groups studied. Gene expression was not significantly different between HFD and HFD + taurine groups. Reduced PGC-1α and PGC-1β expression were observed in both HFD and HFD + taurine groups. In conclusion, taurine reduced the obesity in HFD fed mice, but had no effect on browning of epididymal WAT in this study.

Tuesday 12 July
9:00–11:00, Auditorium II

Molecular evolution

S-02.3-1
The role of virulence genes in the diversification of *Pseudomonas aeruginosa* clades

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National University of Mexico, Mexico City, Mexico

Pseudomonas aeruginosa is an environmental bacterium, but it is also an opportunistic pathogen which represents an important health hazard due to its production of virulence factors and to its high antibiotc resistance. Recently, the existence of 4 phylogroups of this bacterial species has been reported by the analysis of hundreds of whole genome sequences of diverse isolates. All clades contain both clinical and environmental isolates which have diverse geographical origin and time of isolation. The most abundant phylogroup is clade 1, comprising about two thirds of all isolates. Clade 2 is the second largest, with one third of the isolates, and clade 3 and 5 are only represented by a reduced number of strains. Clade 3 shows a much higher genetic diversity than the rest of the phylogroups. It has been reported that some virulence factors vary among the 4 clades. For example, clades 1 and 2 present different effectors secreted by the type three secretion system (T3SS) which is a major virulence factor; strains belonging to clade 1 produce ExoS, while those belonging to clade 2 produce ExoU. Clades 3 and 5, even being distantly related, have in common various virulence related traits; they produce a pore forming exolysin (ExlA), and have deletions of the genes encoding the T3SS, RhlC, the enzyme involved in the synthesis of the virulence related dirhamnolipid surfactant and of the PhzH which produces phenazine-1-carboxamide (PCN) using phenazine-1-carboxilic acid (PCA) as substrate, which is also the substrate for the synthesis of the toxin pyocyanin by a diverging route. In this work we analyzed the Pseudomonas Data Base (www.pseudomonas.com) which has 4955 *P. aeruginosa* genomes to classify the strains with a deposited genome sequence in these 4 phylogroups. This analysis enabled us to present some hypotheses on the origins of the *P. aeruginosa* phylogroups and the role that some virulence factors have in the lifestyle of this fascinating bacterial species.

S-02.3-2
Determinants of HIV-2 receptor use and cell tropism lie in the V3 region of the envelope glycoprotein

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Instituto Universitário Egas Moniz and iMed.ULisboa, Fac. Pharmacy of Lisbon, Lisbon, Portugal.

HIV-2 affects about 1–2 million individuals worldwide. Like HIV-1, untreated infection with HIV-2 leads to AIDS and death. The efficacy of most available drugs is limited against HIV-2 and the genetic barrier to drug resistance is lower in HIV-2 relative to HIV-1. A better knowledge of the interaction of HIV-2 with the cell is important for drug and vaccine development. The

HIV-2 envelope glycoproteins mediate binding to the receptor CD4 and co-receptors at the surface of the target cell, enabling fusion with the cell membrane and viral entry. In HIV-1, the V3 region in the envelope surface glycoprotein is a key functional domain as it contains determinants of co-receptor use, cellular tropism and antibody neutralization. We investigated the role of the V3 region in HIV-2 binding to cell receptor and co-receptors, replication capacity in CD4+ T cells and macrophages, and susceptibility to antibody neutralization. Six V3 mutations (H18L, H23Δ + Y24Δ, K29T, H18L+ H23Δ + Y24Δ, H18L + K29T, and H18L + H23Δ + Y24Δ + K29T) were introduced in HIV-2ROD which uses the CXCR4 co-receptor and replicates only in CD4+ T lymphocytes. The mutations decreased surface exposure and increased the width of the V3 loop. The H18L mutation was sufficient for X4-to-R5 tropism switch in the context of the short version of the V3 loop (H23Δ + Y24Δ). K29T mutation was sufficient to confer HIV-2ROD replication capacity in macrophages. Double deletion H23Δ + Y24Δ also enabled macrophage replication capacity albeit at the cost of some capacity to replicate in CD4+ T cells. Macrophage tropism was associated with improved binding to the CD4 receptor. No clear association was found between mutations in the V3 region and susceptibility to antibody neutralization. Thus, selected mutations in the V3 region have a major role in the interaction of the HIV-2 envelope complex with the cell surface receptors and in cell tropism. These results may help guide the development of new entry inhibitors for this virus.

ShT-02.3-1
Widespread positive epistasis shapes molecular recognition in a human kinase pair

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Human kinase cascade networks play a central role in regulating organism development, the stress response and inflammation. This outcome is achieved though specific yet flexible molecular interactions between kinases in the signalling network: which kinases are activated depends on the stimulus and subsequent molecular recognition of possible downstream kinases. We recently developed a new microdroplet-based compartmentalised assay for screening kinase-kinase phosphorylation and activation *in vitro*. This enabled us to screen a large combinatorial library of MKK1 variants with 500,000 members, in which six residues were simultaneously randomised in the D-domain which governs governing molecular recognition in kinases. Contrary to an expectation of high specificity, we identified >29,000 sequence permutations that efficiently phosphorylate and activate the MKK1 downstream target kinase ERK2. A closer investigation of the sequence-function relationship in the randomised library shows that the molecular pattern in active variants is not governed by a handful of conserved residues. Instead, a flexibly placed hydrophobic sequence motif emerges which is defined by higher order epistatic interactions between six residues, suggesting synergy that enables high connectivity in the sequence landscape. We confirm the presence of positive epistasis through statistical tests and investigation of inactive variant sequence patterns, and furthermore examine the implications in a sequence similarity network of active MKK1 variants. Our work shows that positive epistatic interactions enable MKK1 to maintain function during mutagenesis, which suggests a mechanism for the molecular evolution of specificity in mammalian protein kinase-substrate interactions. Background references: 1. Podgornaia, A. I. & Laub, M. T. Science (80-.). 347, 673–677 (2015). 2. Burotto, M., Chiou, V. L., Lee, J.-M. & Kohn, E. C. Cancer 120, 3446–3456 (2014). *The authors marked with an asterisk equally contributed to the work.

ShT-02.3-2
Limited impact of genetic interaction network on the evolutionary trajectories in yeast *S. cerevisiae*

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The impact of genetic interaction networks on evolution is a key issue. Previous studies have demonstrated that functionally related genes frequently interact with one another, and they establish functional modules, i.e., groups of genes involved in the same biological process. We experimentally tested the hypothesis that compensatory evolutionary modifications, such as mutations and transcriptional changes, occur frequently in genes from perturbed modules of interacting genes using *S. cerevisiae* as a model. We investigated modules lacking *COG7* or *NUP133*. Strains lacking each of these genes were subjected to experimental evolution in continuous cultures and the evolved populations were examined at the genomic and transcriptomic levels. It was found that for both functional modules: genetic interactions, the modular structure of genetic networks, and the adaptive landscape described by the genetic interaction network did not have a significant impact on the process of evolution of yeast populations after gene deletion [1]. In fact, a majority of the gene inactivations were predicted to be neutral. Similarly, transcriptome changes mostly signified adaptation to growth conditions rather than compensation for the absence of the tested genes. Our findings show that modular structure of the network and the genetic interactions described by others have very limited effects on the evolutionary trajectory following gene deletion of module elements in tested experimental conditions and have no significant impact on short-term compensatory evolution. Interestingly, we identified a few genes that were mutated more than once across all yeast populations. Mutations in these genes may be beneficial in our experimental conditions, regardless of genetic background. The issue of potential driver mutations is subjected to further research. The work was supported by the Polish National Science Center: 2014/13/B/NZ8/04719, 2018/29/N/NZ2/00902. [1] Klim J et al. (2021) BMC Ecol Evo 21, 99.

ShT-02.3-3
Structure, function and evolution of Developmentally regulated GTP-binding protein 1 (DRG1): Lessons learned from studies in sponge and humans

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Cancer is a disease of multicellular organisms caused by errors within the multicellular system. Although cancer research has advanced substantially, new approaches focusing on fundamental aspects of cancer origin and mechanisms of spreading are necessary. Comparative genomic studies have shown that most genes/proteins linked to human cancer emerged during the early evolution of Metazoa. One of these proteins is Developmentally regulated GTP-binding protein 1 (DRG1), a GTPase stabilized by interaction with DRG family regulatory protein 1 (DFRP1). In this study, analysis of DRG1 gene/protein evolutionary history shows its high conservation in metazoans, from sponges (Porifera), basal multicellular animals without true tissues and organs, to humans. Our biochemical analysis and structural predictions imply that both recombinant sponge and human DRG1 are predominantly monomers that form complexes with DFRP1 and bind non-specifically to RNA and DNA. We reveal the evolutionary conservation of sponge and human DRG1 biological features, including intracellular localization and DRG1:DFRP1 binding, function of DRG1 in α-tubulin dynamics, and its role in cancer biology demonstrated by increased proliferation, migration and colonization in human cancer cells. This study indicates that the ancestor of all Metazoa already possessed DRG1 that is structurally and functionally similar to the human DRG1, even before the development of real tissues or tumorigenesis, suggesting an important function of DRG1 in one of the fundamental cellular pathways.

ShT-02.3–4
R-RAS2 oncogene shows structural and functional conservation between sponges and humans

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Human Ras-related protein R-RAS2/TC21, a member of the Ras family of proteins, is a ubiquitously expressed small GTPase with an important role in signal transduction that controls multiple cellular processes. Functional dysregulation of R-RAS2 has been shown to contribute to oncogenesis, as it triggers critical biological processes for cancer cells, including proliferation, survival and migration. Although the evolution of cancer is still not fully understood, there is increasing interest in studying the cancer-related genes in non-bilaterian basal animals, considering that many oncogenes and tumor suppressors appeared early in the evolution of Metazoa. Therefore, we used sponges, the basal organisms that changed only slightly during the evolution, to better understand the biological functions and evolution of R-RAS2. We identified a homolog of human R-RAS2 in the freshwater cave sponge *Eunapius subterraneus* using bioinformatic tools and our evolutionary analysis showed high conservation of this protein among metazoans. Biochemical characterization revealed that sponge R-RAS2 possesses intrinsic GTPase activity, the same as human R-RAS2. Intracellular localization of both sponge and human R-RAS2 was in endosomal and plasma membranes indicating their similar biological characteristics. The roles of human and sponge R-RRAS2 in cell proliferation, migration and invasion confirmed their similar cancer-related functions. All in all, this study implies that biological functions of R-RAS2 and their biochemical background were established early in metazoan evolution and possibly much earlier in the evolution of life.

Tuesday 12 July
9:00–11:00, Auditorium VII

Molecular Immunology

S-03.3-1
Chemokines controlling adaptive immune cell migration

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The immune system is dependent on the coordinated migration and positioning of leukocytes. The orchestrated recruitment of immune cells is guided by chemotactic cytokines, the so-called chemokines, and their cognate chemokine receptors expressed by the target cells. Chemokine receptors belong to the superfamily of G-protein coupled receptors (GPCRs). The chemokine receptor CCR7 is key in coordinating adaptive immune responses by guiding antigen-bearing dendritic cells and lymphocytes to lymphoid organs. Thereby, CCR7-expressing cells migrate along guidance cues established by defined local chemokine gradients. How local chemokine gradients are established, and how chemokine receptor signaling guides adaptive immune cells to fight invading pathogens will be discussed.

S-03.3-2
Macrophage polarization to M1-like profile during infection with arthritogenic alphaviruses as an antiviral response mechanism

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Arthritogenic alphaviruses are mosquito-borne viruses that cause incapacitating and long-lasting articular disease. Outbreaks of viral arthritis, as those recently caused by Chikungunya virus, point to the emergence of these pathogens as an important public health problem. The development of the chronic phase of the disease is related to the extension of viral replication and the maintenance of the articular inflammation, in which the cellular infiltrate is predominantly composed by macrophages. Mayaro virus (MAYV) is an arthritogenic alphavirus firstly restricted to South America, but

with great potential for emergence. MAYV-induced arthritis in humans is well documented, but the molecular mechanisms that contribute to its pathogenesis remain poorly understood. We demonstrated that macrophages, key players in arthritis development, are target cells for MAYV infection. MAYV replication in these cells induces TNF-α production and switches cell metabolism to a glycolytic profile, with an inhibition of cellular respiration and increased lactate secretion. The production of reactive oxygen species, superoxide and peroxide, is significantly increased at the early times of infection, which coincides with the peak of virus replication and precedes TNF-α secretion. Also, iNOS expression is induced, as well as NO production of nitric oxide. Altogether, these findings show that MAYV infection leads the macrophages to have an activation profile similar to the classically activated M1 phenotype. On the other hand, when the macrophages are polarized to the M1 profile previously to infection, MAYV replication is completely restricted, suggesting the cellular activation to the M1-like profile is an antiviral mechanism that controls viremia. Since we detect high inflammatory cytokine expression (TNF-α and IL-6) and NO production in this profile, identifying which of these factors have a role in restricting viral replication would be of interest to the development of antiviral strategies.

ShT-03.3-1
The role of S100A8, S100A9 and Calprotectin in skin inflammation

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Inflammatory skin diseases (ISDs) such as atopic dermatitis (AD) are common conditions affecting 5–10% people worldwide. Microbiota dysbiosis and deregulation of antimicrobial peptides (AMPs), that limit growth of pathogens in the host, including S100A9 (A9) and A9/A8 heterodimer Calprotectin (CP), have been documented in ISDs. However, their function in skin inflammation is still not understood. Therefore, to decipher the role of AMPs in skin inflammation, genetically engineered mouse models (GEMMs) were used. AD-like mouse model with constitutive Keratin 5-dependent epidermal deletion of *JunB* ($JunB^{\Delta ep}$) present skin inflammation with macroscopic lesions and *Staphylococcus aureus* colonization[1], as well as systemic inflammation with multi-organ involvement[2,3]. In the lesional skin of $JunB^{\Delta ep}$ mice, increased expression of A8 and A9 was observed as well as significant increase of A8, A9 and CP in serum and stool. Fecal CP has been broadly used as a clinical biomarker for inflammatory bowel disease. Hence, we elucidate the contribution of skin inflammation-derived CP in ISD and inter-organ communication. Global inactivation of A9 in $JunB^{\Delta ep}$ mice substantially improved skin macroscopic lesions. Inflammatory mediators, including G-CSF, IL-17 and IL-6, as well as *S. aureus* colonization were locally decreased. However, global inactivation of A9 in $JunB^{\Delta ep}$ mice exacerbated systemic inflammation and induced prominent swelling of digits. These results reveal a complex involvement of A8, A9 and CP in the development of local and systemic inflammation originating in the skin, supporting a role of AMPs in inter-organ crosstalk. Ongoing experiments aim to identify the specific A8- and A9-expressing cells involved in ISD and to determine the critical targets connected to A8, A9 and/or CP to prevent or ameliorate ISD. [1]*Uluckan O et al. (2019). Cell Rep 29, 844–859.* [2]*Meixner A et al. (2008). Nat Cell Biol 10, 1003–1011.* [3]*Pflegerl P et al. (2009). Proc Natl Acad Sci U S A 106, 20,423–20,428*

ShT-03.3-2
Cytokine gene expression regulation by leptin in childhood immune thrombocytopenia (cITP)

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High plasma leptin levels have been observed in patients with certain autoimmune diseases, suggesting the involvement of leptin in their pathogenesis. In this work, we investigated the effect of leptin in cITP, a typical type-1 autoimmune disease. We measured plasma leptin levels in 39 children with ITP before and after treatment with intravenous immunoglobulin and/or methylprednisolone and in 33 healthy sex/age/body mass index-matched controls. We also cultured isolated peripheral blood CD3+ T-cells and CD14+ monocytes with recombinant leptin to investigate its effect on pro-inflammatory (type-1) and anti-inflammatory (type-2) cytokine gene expression. Our results showed that plasma leptin levels were significantly elevated in patients with active disease compared with controls, and correlated negatively with platelet count. Intravenous immunoglobulin treatment had no significant effect on leptin levels, whereas steroid treatment decreased leptin levels below control levels. In remission, leptin levels were in the control range [Previously published in: Thomas I et al. (2021) Int J Mol Sci 16, 7636]. Culturing T-cells and monocytes with leptin resulted in a significant decrease in IL-6 constitutive expression in patient T-cells and monocytes, a significant decrease in TNF-α expression in patient monocytes and a significant increase in IL-10 expression in both patient and control monocytes. Notably, the increase in IL-10 expression in patient monocytes was significantly higher than the increase in IL-10 expression in control monocytes. In conclusion, in cITP, leptin levels correlate with disease activity. Leptin acts as an active anti-inflammatory agent through its direct effect on pro- and anti-inflammatory cytokine gene expression in patient T cells and monocytes, resulting in an overall type-2 polarization.

ShT-03.3-3
Survival of vaccine-induced human milk SARS-CoV-2 IgG and IgA immunoglobulins across simulated human infant gastrointestinal digestion

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Four vaccines have been approved to date by the European Medicines Agency for the management of the COVID-19

pandemic in Europe, with all four licenced for administration from 5 years of age. One way to protect the vulnerable younger population such as newborns and infants is through passive immunity via breastfeeding. Many studies have revealed that human milk contains immunoglobulins (Ig) against the SARS-CoV-2 virus, both after natural infection or vaccination. It is not known however, whether these antibodies can resist enzymatic degradation during digestion in the infant gastrointestinal (GI) tract or indeed protect the consumers. Here, we describe the validation of commercially available ELISA kits to detect IgA, secretory IgA (sIgA) and IgG antibodies in human milk and subsequently evaluated the vaccine-induced immunoglobulin profile of breastmilk from a cohort of lactating mothers vaccinated with either the Pfizer/BioNTech, Moderna or the Astra Zeneca vaccine. We also investigated the effect of a static *in vitro* digestion protocol representing the gastric and intestinal phases of infant digestion on the IgA and IgG concentrations. Our data show that there is an increase in Ig levels in human milk following vaccination and provide important information regarding the extent to which these antibodies can resist digestion in the infant GI tract.

ShT-03.3–4
C-terminal portion of Amblyomin-X interacts with Tom70 and acts as an immunomodulatory molecule in macrophages

A. Maia Lobba, D. Souza Oliveira, M. Medina de Souza, R. Nascimento Gomes, A.M. Chudzinsk-Tavassi
Butantan Institute, São Paulo, Brazil

Amblyomin-X is a Kunitz-type recombinant protein identified from the transcriptome of the salivary glands of *Amblyomma sculptum* tick that displays antitumor properties. Amblyomin-X drives tumor cell death by proteasome inhibition and also appears to play a role in the tumor microenvironment activating the immune system against tumor progression. Amblyomin-X seems to be restricted to tumor cells, not affecting non-tumorigenic cells, making it an attractive molecule for anticancer therapy. Previous studies demonstrated that while the N-terminal Kunitz-type domain of Amblyomin-X leads to tumor cell death, the C-terminal portion, CAmbly, acts as a carrier of the molecule and appears to present an immunomodulatory function. Aiming to understand the role of CAmbly as an immunomodulatory molecule, we further explored its molecular mechanism of action in macrophage. To this end, yeast two-hybrid screening was performed in an effort to identify the molecular ligands of CAmbly. Among the macrophage proteins that were able to interact with CAmbly, the mitochondrial receptor Tom70 – a critical adaptor linking MAVS to TBK1/IRF3 in MDA5 signaling upon RNA virus infection – was identified and further confirmed through FRET microscopy as a direct ligand of CAbmly. In order to investigate if CAmbly is able to change the pattern of cytokines and chemokines released by macrophage, a multiplex assay was performed. CAmbly was able to induce an increase of VEGF, CCL2 and CCL22 and a decrease of IL-10 and CXCL10 in the media of macrophages. CAmbly also induced an increase of Il-6 and TNF-a in the media of inflammatory macrophages and IL-4 in the media of anti-inflammatory macrophages. These results demonstrated that CAmbly seems to be an immunomodulatory molecule, modulating an innate immune pathway by interaction with Tom70 and changing the cytokine profile and chemokine released by macrophages.

Tuesday 12 July
9:00–11:00, Auditorium VIII

Saccharides

S-04.3-1
Action and mechanism of herbal glycans

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University of Macau, Macau, China

Extensive evidence shows that natural glycans from medicinal plants, fungi and marine organisms have a variety of biological activities with beneficial effects to the human body. Discovering target molecules, such as glycan receptors, growth or angiogenesis factors and enzymes, is a key step to track the signaling pathway involved in the succession of glycan effects and understand the pathological mechanisms. Actually, glycans are still the "sleeping giant" of research in herbal medicine, and how they contribute to phytotherapeutic effects are still not clear. Generally, the therapeutic efficacy of a drug mainly depends on its concentration at the site of action. Therefore, how polysaccharides are absorbed and distributed to the site of action is always questionable, as well as their mechanisms of action. Technological advances in glycobiology and glycochemistry are paving the way for a new era in developing sweet solutions to sticky situations. The action modes and mechanisms of herbal polysaccharides which may be sweet medicine of tomorrow will be summarized and discussed, which is also beneficial to comprehensively understanding the holistic effects of herbal medicine. Acknowledgements The research was partially funded by grants from the Science and Technology Development Fund, Macau SAR (File no. 0017/2019/AKP), the Key-Area Research and Development Program of Guangdong Province (File no. 2020B1111110006) and the University of Macau (File no. MYRG2018-00083-ICMS /MYRG2019-00128-ICMS / CPG2021-00009-ICMS). *The authors marked with an asterisk equally contributed to the work.

S-04.3-2
Lactate-driven metabolic and epigenetic prostate tumor-stroma crosstalk

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Glucose utilization within the tumor microenvironment leads to anaerobic glycolysis coupled with lactate exploitation by neighboring cells. The consequence for this metabolic partnership among stromal and cancer cells is that lactate is an abundant oncometabolite in the tumor environment. In prostate cancer (PCa), cancer-associated fibroblasts are major donors of secreted lactate, which can be uploaded by cancer cells to sustain their mitochondrial metabolism. However, how lactate impacts transcriptional regulation in tumors has yet to be fully elucidated. We have recently reported a mechanism by which cancer-associated fibroblast-secreted lactate is able to increase the expression of genes involved in lipid metabolism in prostate cancer cells. This epigenetic regulation enhances intracellular lipid accumulation into lipid droplets and provides acetyl moieties for histone acetylation, establishing a regulatory loop between metabolites and epigenetic modification. Inhibition of this loop by targeting

the BET bromodomain and extra-terminal protein family of histone acetylation readers suppresses the expression of Perilipin-2, a crucial component of lipid droplets, disrupting the lactate-dependent lipid metabolic rewiring. *In vivo*, the inhibition of the metabolic-epigenetic regulatory loop, induced by cancer-associated fibroblasts, reduces growth and metastatic dissemination of prostate cancer cells, thus confirming its translational relevance as a therapeutic target in prostate cancer progression. Clinically, expression of Perilipin-2 is elevated in tumors with a higher Gleason grade and in castration-resistant prostate cancers, compared to primary cancer lesions. As a take-home message, I underline that lactate delivered in the tumor microenvironment due to Warburg metabolism plays both a metabolic and an epigenetic role in promoting prostate cancer malignancy.

ShT-04.3-1
Determining the structure of the enterococcal polysaccharide antigen

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Enterococcus faecalis is an opportunistic pathogen, commonly found in the healthy human gut, which can also cause life-threatening diseases such as meningitis and septicaemia. The enterococcal polysaccharide antigen (EPA) is a surface exposed polymer produced by all *E. faecalis* strains, and is a major virulence factor, being essential for host immune evasion during infection. It is comprised of a repeating rhamnose backbone which is decorated with strain specific teichoic acid decoration subunits. Work by our group (Smith R et al. (2019) PLoS Pathog 15, e1007730) has shown that these strain-specific decoration subunits are responsible for the biological activity of EPA, rather than the highly conserved polyrhamnose backbone. Structural characterisation of EPA decoration subunits could therefore provide key insights into strain–strain phenotype variation and pathogenicity. Recently, the structure of EPA produced by a clinical isolate (V583; Guérardel Y et al. (2020) mBio 11, e00277-20) has been solved, which has paved the way for more detailed analysis of EPA structural diversity and molecular activity. Here, for the first time, we have solved the structure of the EPA decoration subunits in the model clinical isolate, OG1RF. EPA was purified enzymatically from the cell wall of OG1RF, then broken into three uniform fragments, corresponding to the polyrhamnose backbone and two decoration subunits, using HF acid treatment. A specialised pipeline of 2D NMR experiments (HSQC, COSY, TOCSY, ROESY and HMBC) was then employed to allow the assignment of each sugar residue, and subsequently solve the structures of the purified decoration subunits. OG1RF decoration subunits were found to differ in size, composition, and level of branching, when compared to V583. This variant structure may help to explain the increased pathogenicity of OG1RF, as compared to V583, and is hopefully one of the first steps towards understanding how EPA impacts *E. faecalis* pathogenesis.

ShT-04.3-2
β-glucans derived from mushroom *Coriolus versicolor* for applications on skin wound healing

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The beneficial effects of natural compounds in cosmeceutical and biopharmaceutic fields have been extensively studied over the years, and gained popularity because of their distinct advantages, including fewer side effects, better tolerance, and relatively low expenses. Currently, with the growing demand for the use of nature-derived molecules, the research aiming for new biomolecules has increased. Beta-glucans have proved their pluripotent bioactivity (antioxidant, anti-inflammatory, antimicrobial, anti-cancer, regenerative effects, immunomodulation, healing properties) in skin cells. These properties are dependent on several aspects, such as the source, molecular weight, solubility, degree of branching, charge of polymers, and structure in aqueous media. The versatility of these molecules makes them a challenge for the studies of structure–activity relationships, once each different compound (with a unique structure) will show different biological activity. Regarding the high levels of environmental and endogenous stresses that the skin is exposed leading to premature aging and chronic inflammation, this ongoing work aims to explore the ability of β-glucans extracted from *C. versicolor* to act as antioxidant and anti-inflammatory molecules in the skin and to eventually promote wound healing and tissue cicatrization. Therefore, assays exploring cytotoxic, antioxidant, and anti-inflammatory activities of different β-glucans in keratinocytes (HaCaT) and human fibroblast (HFF) cell lines were performed. The effects of β-glucans on angiogenesis were assessed by the migration (wound healing activity) and the tube formation assay (differentiation and vascular formation) using cell models of human umbilical vein endothelial cells (HUVEC) and mouse macrophage cells (RAW 264.7). Lastly, two well-known ECM components, hyaluronic acid, and collagen were evaluated to understand the effects of β-glucans in the production of these components in a human fibroblast cell line (HFF).

ShT-04.3-3
Unravelling the molecular recognition of the cancer and pathogenic glycobiomarker LacdiNAc by human immune-related lectins

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Cancer and infection diseases have a negative impact on society and it is well established that lectin/glycan interactions modulate how the human immune system faces these major diseases. In this perspective, discovering exactly how lectins enrolled in immune response interact with the glycan epitopes is crucial for the discovery of new and alternative therapies towards these diseases (E. Rodríguez, et al. (2018) Nat. Rev. Immunol. 18, 204–211; D. A. Wesener, et al. (2017) Curr. Opin. Struct. Biol. 44,

168–178). Herein, we report a multidisciplinary approach, that includes NMR binding techniques ($^{1}H/^{15}N$-HSQC NMR, saturation transfer difference NMR (STD-NMR) and TrROESY experiments), X-Ray Crystallography, isothermal titration calorimetry (ITC) and molecular dynamics (MD) to disentangle the molecular recognition mechanism of the cancer and pathogenic glycobiomarker LacdiNAc (GalNAcβ1-4GlcNAc, LDN), as well as its comparison with the ubiquitous and exogenous epitope, LacNAc glycan epitope (Galβ1-4GlcNAc, LN), by two immune-related lectins, the human Galectin-3 (hGal-3) and the human Macrophage Galactose-type Lectin (hMGL) (C. D. L. Lima, et al. (2021) Chem. Eur. J. 27, 7951–7958). Our integrative methodology indicates that the mechanism of recognition of LDN and LN by hGal-3 and hMGL is substantially different, which explains the difference in the binding specificity of LDN and LN by these two lectins. Acknowledgements: FM, EC, JD, HC, AD and CL acknowledge FCT-Portugal for funding projects: PTDC/QUI-OUT/2586/2020, UCIBIO project (UIDP/04378/2020 and UIDB/04378/2020), Associate Laboratory Institute for Health and Bioeconomy i4HB project (LA/P/0140/2020). CL and AD thank the PhD grants 2021.06789.BD and PD/BD/142847/2018, respectively. FM and HC also thank the contracts 2020.00233.CEECIND and 2020.03261.CEECIND, respectively. The authors thank NMR spectrometers Infrastructure Project No 22161. ALC and FT thank the ALBA synchrotron (Barcelona, Spain).

Wednesday 13 July 9:00–11:00, Auditorium I

Cardiovascular diseases

S-01.4-1 Collagen VI and the failing heart

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Mutation of collagen VI is linked to muscular dystrophy in humans. However, little is known about its function in the heart. We have previously demonstrated increased nanoscale deposition of collagen VI within the transverse(t)-tubules, leading us to hypothesize a role in their structural remodelling. Knockout of alpha 1 chain of type VI collagen ($Col6A1^{-/-}$) in mice has conveyed substantial protection against myocardial infarction (MI) induced heart failure. To investigate the role of collagen VI in the heart we have created a novel $Col6A1^{-/-}$ rat using CRISPR/Cas9 genetic engineering. Heart failure was then induced by MI in both $Col6A1^{-/-}$ and wild type rats and compared to sham-operated rats. Echocardiography demonstrated a reduced ejection fraction in sham Col6A1−/− rats compared to wild type shams ($44 \pm 2\%$ vs $56 \pm 2\%$, mean ± SE respectively). Moreover, the knockout conveyed no protection against heart failure showing an ejection fraction of $34 \pm 4\%$ after MI in $Col6A1^{-/-}$ rats compared to an ejection fraction of $44 \pm 4\%$ in wild type rats with MI. Echocardiography also showed sham $Col6A1^{-/-}$ rats had diastolic dysfunction with an increased E/A doppler signal compared to sham wild type (2.4 ± 0.3 vs 1.5 ± 0.1 respectively). To investigate the role of Ca2+ signalling in the observed cardiovascular defects, cardiac myocytes were isolated from $Col6A1^{-/-}$ and wild type rats. The cardiac myocytes were loaded with the calcium indicator Fura-2 and stimulated Ca^{2+} transient recorded. This analysis demonstrated a profound change in Ca^{2+} regulation with a large increase in peak systolic Ca^{2+} in $Col6A1^{-/-}$ myocytes compared to wild type (1.8 ± 0.2 vs $1.2 \pm 0.03340/380$ ratio). Caffeine induced calcium transients revealed $Col6A1^{-/-}$ myocytes had increased sarcoplasmic reticulum Ca^{2+} store (0.8 ± 0.06 vs $0.6 \pm 0.03340/340$ ratio). Our results indicate that collagen VI has a role in Ca^{2+} signalling in the cardiac myocyte that may be mediated through its presence in the t-tubules.

S-01.4-2 Transcription factor EB controls epicardial EMT

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During heart development, epithelial-mesenchymal transition (EMT) sustains differentiation of epicardial cells into vascular smooth muscle cells and cardiac interstitial fibroblasts. We have shown that the oncogenic Transcription Factor EB (TFEB), a master gene of autophagy, regulates epicardial EMT. By exploiting epicardial specific mouse genetic models, transcriptomic and massive chromatin immunoprecipitation sequencing and cell biology approaches, we have brought evidence that TFEB regulates the EMT activity of transforming-growth factor (TGF)β and this effect results from the activation of the transcription of Thymine-Guanine-interacting factor 1, a TGFβ/Smad pathway repressor. Interestingly, the EMT regulatory activity of TFEB is not restricted to epicardial cells, but it is extended to different endodermal and mesodermal cell types.

ShT-01.4-1 Frequency of congenital heart disease in oculoauriculovertebral spectrum and assessment of two biomarkers

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Oculoauriculovertebral spectrum (OAVS) is the second most frequent malformative disorder of head and neck with clinically heterogeneous phenotype. The etiology and pathogenesis of OAVS is highly heterogeneous. Genetic causes have been brought up; furthermore several non-genetic factors have been described as possible environmental causes. The retinoic acid (RA) signaling pathway has been implicated in various developmental processes and is essential for neural differentiation and craniofacial development. In addition to the classic features of the syndrome comprising hemifacial microsomia, ear anomalies, eye malformations and vertebral defects, multisystem affection is common. Our study included 36 patients with suspected OAVS in an attempt to study the characteristic clinical features and the frequency of associated systemic anomalies in this syndrome. Congenital heart disease (CHD) was found in 10/36 patients (27.7%). Ventricular septal defect (VSD) was the most common anomaly (60%) followed by atrial septal defect (ASD). Mitral valve

prolapse, patent foramen ovale, and hypertrophic cardiomyopathy with subaortic stenosis was also found in our cohort. Cardiac affection which was frequent in the studied cases was consistent with previous studies. Moreover, the occurrence of CHD appears to increase the risk of additional malformations, which should then be screened for. Hemodynamic abnormalities in patients with CHD can cause activation of some biochemicals. High CRP was found in 40% of the cases while increased lactate level was found in 30%. With follow up, lactate level normalized in most of the cases with mild condition. These biomarkers which in turn assist in the prediction of pathological changes could provide information about the prognosis of the cases. Moreover, assessment of some biomarkers at an earlier stage can reduce unwanted complications.

ShT-01.4-2
Fibroblast growth factor receptor-2 modulates the MMP2 and MMP14/TIMP1 ratio in human cardiac myofibroblasts

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Cardiac fibrosis is characterized by differentiation of fibroblasts (Fb) into myofibroblasts (MyFb), the main source of excessive accumulation of extracellular matrix (ECM) proteins. The increased ECM deposition undergoes remodeling controlled by matrix metalloproteinases (MMPs) and tissue inhibitor of matrix metalloproteinases (TIMP). The ratio of MMP/TIMP guides the rate of ECM turnover in normal and pathological conditions. A strong correlation between fibroblast growth factors and their receptors (FGF/FGFR) and MMP/TIMP balance occurs in various diseases. We hypothesize that in cardiac MyFb, inhibition of FGFR could alter the MMP/TIMP balance. *Experimental design*: Cardiac MyFb generated by exposure of Fb to TGFβ (5 nM, 24 h) were exposed (2 h) to FGFR2 inhibitor (LY2874455) followed by 24 h stimulation with 1 μM Basigin (a MMP stimulator). In controls, cells were not exposed to inhibitor, nor to Basigin. Harvested cells and culture media were subjected to RT-PCR, ELISA and proteolytic activity quantification (zymography), respectively. *Results:* Compared to controls, in MyFb exposed to FGFR inhibitor, a significant decrease of MMP2 (−44%) and MMP14 (−71%) and no modification for TIMP1 genes occurred. In the secretome, the presence of LY2874455 decreased the proteolytic activity of MMP2 by ~105%, whereas TIMP1 activity was not modified. The inhibitor of FGFR decreased the concentration of MMP14 by ~47%. The gene expression ratios for MMP2/TIMP1 and MMP14/TIMP1 were lower in FGFR2-inhibited MyFb. *Conclusion:* Pharmacological inhibition of FGFR2 acts as an important down regulator of gene expression for MMP2 and MMP14, but does not affect either the gene or the activity of TIMP1. Therefore, the MMP/TIMP1 ratio is modified in MyFb, suggesting a decreased synthesis of MMPs. Hence, the inhibition of FGFR on MyFb could represent a novel target in the treatment of late stages of cardiac fibrosis. Supported by UEFISCDI PN-III-P1-1.1-PD-2019-1234 (PD160/2020) and Romanian Academy.

ShT-01.4-3
Long non-coding RNA KLRK1-AS1 is involved in neonatal endothelial function and correlates with maternal weight gain in pregnancy

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The endothelium has unique functions, including barrier formation, angiogenesis, vascular tone regulation, blood coagulation and inflammation. Metabolic derangements affect endothelial function, which underlies cardiovascular disease (CVD). Maternal metabolic disturbances in pregnancy also affect neonatal endothelial cells and increase the offspring's risk to develop CVD later in life. Amongst various other functions, some long non-coding RNAs (lncRNA) have recently emerged as epigenetic regulators of endothelial function. We hypothesized that maternal metabolism during pregnancy affects neonatal endothelial function via lncRNAs. Therefore, we employed cord blood derived endothelial colony forming cells (ECFC), i.e., circulating endothelial progenitors, as a model for neonatal endothelial cells and determined expression and function of lncRNAs in respect to maternal metabolic characteristics. Neonatal ECFC were isolated after healthy pregnancies of women with a pre-gestational BMI ranging from lean to obese (n = 40; BMI: 19–36). Expression of lncRNAs was analyzed by RNA-Seq and revealed that gestational weight gain inversely correlated with the expression of lncRNA *KLRK1-AS1* (*Killer Cell Lectin Like Receptor K1 Antisense RNA* 1; $r = -0.362$, $p = 0.033$). To determine the role of *KLRK1-AS1* in neonatal ECFC (n = 6), we used two different siRNAs for silencing and performed functional analyses: Barrier function was measured by Electric Cell-substrate Impedance Sensing (ECIS) at 4000 Hz and angiogenesis by a spheroid sprouting assay. *KLRK1-AS1* downregulation with both siRNAs (by 84% and 73% respectively) impaired barrier function (FC = 0.68, $p = 0.005$; FC = 0.56, $p < 0.001$), but enhanced the number of sprouts (FC = 1.32, $p = 0.099$; FC = 1.62, $p = 0.039$). In conclusion, lncRNA *KLRK1-AS1* regulates the function of neonatal endothelial progenitor cells and it is affected by maternal metabolism i.e. weight gain in pregnancy.

ShT-01.4-4
The development of dual C-domain ACE/neprilysin inhibitors for hypertension and heart failure

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Combined inhibition of NEP (neprilysin) and ACE (angiotensin-converting enzyme), without adverse effects, remains an attractive therapeutic strategy in cardiovascular medicine. Here, we aimed

to determine whether lisinopril-tryptophan (lis-Trp), a C-domain specific ACE inhibitor that preserves the N-domain catalytic activity, together with sacubitril (NEP inhibitor), differentially influences cardiovascular function and vascular permeability in hypertension compared with omapatrilat and lisinopril+sacubitril, which inhibits both the ACE C- and N-domains. Ang II–dependent hypertensive mice (LinA3) received vehicle, sacubitril, lis-Trp, lisinopril, lisinopril+sacubitril, or 1 lis-Trp + sacubitril for 4 weeks. Systolic blood pressure was increased in LinA3 mice, along with cardiac hypertrophy/dysfunction, impaired endothelium-dependent vasorelaxation, hypercontractile responses, and vascular remodeling. Lis-Trp + sacubitril, lisinopril+sacubitril, and omapatrilat reduced systolic blood pressure and normalized cardiovascular remodeling and vascular hypercontractile responses in LinA3 mice. Although lisinopril+sacubitril and omapatrilat improved Ach-induced vasorelaxation, lisW-S + sacubitril had no effect. Endothelial permeability was increased in omapatrilat but not in lis-Trp + sacubitril–treated mice. In conclusion, lisW-S combined with sacubitril reduced systolic blood pressure and improved cardiac dysfunction in LinA3 mice, similar to omapatrilat but without effects on endothelium-dependent vasorelaxation. Moreover, increased vascular permeability induced by omapatrilat was not evident in mice treated with lis-Trp + sacubitril. In addition, we have developed novel C-domain/NEP dual inhibitors and solved the X-ray crystal structures of these compounds in complex with N- and C-domains. Targeting ACE C-domain and NEP as a combination therapy may be as effective as omapatrilat in lowering systolic blood pressure, but without inducing vascular permeability and endothelial injury. *The authors marked with an asterisk equally contributed to the work.

Wednesday 13 July
9:00–11:00, Auditorium II

Microbial metabolism

S-02.4-2
A molecular view of energy metabolism in anaerobes

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Anaerobic microorganisms play key ecological roles in many environments, such as the human gut, in freshwater and in marine sediments. In the latter, sulfate-reducing bacteria are particularly important due to the high sulfate concentration in sea water, being responsible for up to 50% of carbon remineralization. We in the last years we have studied how these bacteria obtain energy from the process of sulfate respiration and have identified several membrane complexes and soluble proteins that are essential for this process. These include the QmoABC [1] and DsrMKJOP [2] complexes, involved in electron transfer pathways to AprAB and Dsr proteins, respectively, as well as the QrcABCD complex responsible for menaquinone reduction in Deltaproteobacterial sulfate reducers [3]. In this talk, I will discuss the role of these complexes in sulfate reduction, their evolution and how they contribute to energy conservation, as well as the mechanism of sulfite reduction by the Dsr system [4]. References: 1. Chernyh NA*, Neukirchen S, Frolov EN, Sousa FL*, Miroshnichenko ML, Merkel AY, Pimenov NV, Sorokin DY, Ciordia S, Mena MC, Ferrer M, Golyshin PN, Lebedinsky AV, Pereira IAC* & Bonch-Osmolovskaya EA (2020) Dissimilatory sulfate reduction in the archaeon 'Candidatus Vulcanisaeta moutnovskia' sheds light on the evolution of sulfur metabolism. *Nature Microbiology*, 5, 1428–1438. 2. Santos AA, Venceslau SS, Grein F, Leavitt WD, Dahl C, Johnston DT, Pereira IAC (2015) *Science*, 350, 1541. 3. Duarte AG, Catarino T, White GF, Lousa L, Neukirchen S, Soares CM, Sousa FL, Clarke TA & Pereira IAC (2018) An electrogenic redox loop in sulfate reduction reveals a likely widespread mechanism of energy conservation. *Nature Comm.* 9, 5448. 4. Ferreira D, Barbosa ACC, Oliveira GP, Catarino T, Venceslau SS, Pereira IAC (2022) The DsrD functional marker protein is an allosteric activator of the DsrAB dissimilatory sulfite reductase. *Proc. Nat. Acad. Sci.*, 119 (4) e2118880119.

S-02.4-1
The control of RpoS stability and its role in the expression of genes for the synthesis of polyhydroxybutyrate in the soil bacterium *Azotobacter vinelandii*

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Azotobacter vinelandii is a soil nitrogen-fixing bacterium belonging to the *Pseudomonadaceae* family that produces polyhydroxybutyrate (PHB), a polymer of industrial importance utilized as biodegradable plastic. The regulation of PHB synthesis in *A. vinelandii* is complex and involves the transcription of PHB biosynthetic and regulatory genes from RpoS recognized promoters. PHB synthesis is also under the control of the bacterial global regulatory PTS^{Ntr} system, since the presence of an unphosphorylated $EIIA^{Ntr}$ protein induces degradation of the sigma factor RpoS by the ClpAP chaperone-protease complex during stationary phase, resulting in a negative effect on PHB synthesis. As in most bacteria studied, in exponentially growing cells, RpoS is also degraded by the ClpXP complex in *A. vinelandii*. However, unlike many other bacteria, the molecular mechanisms involved in degradation of RpoS by ClpP in bacteria of the *Pseudomonadaceae* family remain largely unknown. In this work, we report the identification and characterization of the *avin32720* gene encoding a protein annotated as a response regulator that possesses in its N-terminal a receptor domain (REC) and a domain with phosphatase activity (PP2C) in the C-terminal. Immediately downstream of *Avin32720* and forming a bicistronic operon, *Avin32710* a gene encoding a 483 amino acid protein possessing a STAS domain (sulphate transport and anti-sigma factor antagonist) is present, and is annotated as an anti-anti-sigma factor. We constructed strains carrying mutations in *Avin32720*, *Avin32710* or both genes, and found that their inactivation has a positive effect on PHB synthesis and in the levels and stability of RpoS. These and other results indicate that the proteins encoded by these genes are involved in the degradation of RpoS by the ClpP protease.

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ShT-02.4-1
How a methanogen assimilates sulfate

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By growing on sulfate (SO_4^{2-}) as sole sulfur source, *Methanothermococcus thermolithotrophicus* breaks a dogma: methanogenesis and sulfate-reduction should not co-occur in one organism due to toxic intermediates and energetic barriers [Daniels L et al. (1986) Appl Environ Microbiol 51, 703–709]. Using a complementary approach of physiological, biochemical and structural studies, we offer an unprecedented view of the complete sulfate-reduction pathway of this methanogenic archaea. While the first two reactions catalyzed by the ATP-Sulfurylase and APS-Kinase are common to other microorganisms, a new class of PAPS Reductase converts the product 3'-Phosphoadenosine-5′-phosphosulfate (PAPS) into sulfite (SO_3^{2-}) and 3'-Phosphoadenosine-5′-phosphate (PAP). This reductase shares similarity with enzymes involved in dissimilatory process, but different residues involved in substrate recognition shift the substrate specificity and allow the reduction of PAPS. Next, PAP is specifically hydrolyzed by a novel type of PAP Phosphatase, probably derived from an RNA-exonuclease. Finally, SO_3^{2-}, a poison for methanogens, is quickly converted to Sulfide (HS^-) by the coenzyme F_{420}-dependent Sulfite Reductase (Fsr); a single enzyme composed of a $F_{420}H_2$-oxidase and a new class of Sulfite Reductase [Johnson EF, Mukhopadhyay B (2005) J Biol Chem 280, 38,776–38,786]. Our crystal structures revealed an architecture similar to the dissimilatory Sulfite Reductases but an active site and enzymatic properties identical to assimilatory ones. Because of its primitive organization, Fsr would provide a plausible picture of a Sulfite Reductase prototype. These results show the first insights into how a methanogen can turn SO_4^{2-} into an elementary block of life.

ShT-02.4-2
Regulation of catalase and PerR expression by the RNA chaperone Hfq unveils a novel pathway of the bacterial oxidative stress response

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The RNA chaperone Hfq is an important bacterial post-transcriptional regulator. In Gram-negative bacteria, its main role is in riboregulation, promoting the sRNA/mRNA interaction. However, in Gram-positive bacteria like *Listeria monocytogenes* the main role of Hfq remains elusive as this protein seems to be expendable for riboregulation. Here, we show that Hfq is essential in the oxidative stress response of *Listeria*. Disruption of the *hfq* gene results in a hypersensitive phenotype to hydrogen peroxide (H_2O_2). In the presence of sub-inhibitory concentrations of H_2O_2, the wild-type (WT) strain was barely affected and recovered immediately upon stress. However, the *Δhfq* mutant could not resume growth and presented a loss of viability. Interestingly, this phenotype is specific to H_2O_2 stress given that superoxide stress imposed by plumbagin had no effect. H_2O_2 is one of the reactive oxygen species (ROS) within cells and its decomposition is mediated mainly by catalase. Enzymatic activity assays show that catalase (encoded by *kat* gene) is less active in the *Δhfq* mutant when comparing to the WT strain. This correlates with lower levels of the *kat* mRNA and Kat protein levels found inside cells not expressing *hfq*. This was shown to be result of higher levels of the PerR transcription factor that acts as a repressor of the *kat* expression. Accordingly, *Listeria* infection of macrophages, which use ROS to eliminate pathogens, revealed that the *Δhfq* mutant is less virulent than the WT strain in this cell line. These results shed light on a novel regulatory pathway of the oxidative stress response found in the human pathogen *Listeria monocytogenes*. We demonstrate that Hfq regulates indirectly the expression of the H_2O_2 scavenging enzyme catalase through regulation of the oxidative stress transcriptional regulator PerR. These findings may help to uncover new strategies used by intracellular bacteria to fight ROS in the infection process.

ShT-02.4-3
From the inner membrane to the cell's exterior: biochemical characterization of *Geobacter sulfurreducens*' cytochrome CbcL, an entry gate for electrons in extracellular electron transfer

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Geobacter bacteria can couple their oxidative metabolism to the reduction of electron acceptors located in the cells' exterior, including electrode surfaces. This respiratory mechanism called extracellular electron transfer (EET) has potential application in several biotechnological fields, namely in bioremediation, bioenergy production and microbial electrosynthesis. The EET pathways use different multiheme cytochromes located in the inner membrane, periplasm, and outer membrane. CbcL is an inner-membrane associated cytochrome that plays an essential role in EET to final electron acceptors with a low redox potential, as iron oxides and electrodes poised at −100 mV. CbcL has a transmembrane domain with six helices and two *b*-type hemes, and a periplasmic domain containing nine *c*-type hemes. The complementary usage of different spectroscopic techniques, such as ultraviolet–visible, circular dichroism, and nuclear magnetic resonance, allowed us to structurally and functionally characterize the entry gate for electrons into the periplasm of *G. sulfurreducens*. The reduction potential of CbcL was determined, and both the interaction and the electron transfer reaction between CbcL and the periplasmic triheme cytochrome PpcA were studied. The results revealed that CbcL and PpcA form a redox complex in the periplasm and that the electron transfer reaction is thermodynamically favourable. Taken together, this study showed for the first time how electrons are injected into the periplasm of *G. sulfurreducens* and initiate their journey to the cell's exterior. This work was supported by Fundação para a Ciência e Tecnologia (FCT) through the grants PTDC/BIABQM31981/2017 (CAS) and PTDC/BIABQM/4967/2020 (CAS). This work was also supported by national funds from FCT within projects UIDP/04378/2020 and UIDB/04378/2020 (UCIBIO) and project LA/P/0140/2020 (i4HB). The NMR spectrometers are part of the National NMR Network and are supported by FCT (ROTEIRO/0031/2013 and PINFRA/22161/2016).

ShT-02.4-4
Protein-carbohydrate recognition in the biodegradation of plant cell wall: functional studies using carbohydrate microarrays

D. O. Ribeiro[1], B. A. Pinheiro[1], J. L.A. Brás[2], L. M. Silva[3], Y. Zhang[3], Y. Liu[3], T. Feizi[3], C. M.G.A. Fontes[2], A. L. Carvalho[1], W. Chai[3], A. S. Palma[1]
[1]UCIBIO, NOVA School of Science and Technology, NOVA University Lisbon, Caparica, Portugal, [2]NZYTech, Lda - Genes & Enzymes, Lisboa, Portugal, [3]Glycosciences Laboratory, Department of Medicine, Faculty of Medicine, Imperial College London, London, United Kingdom

Plant cell wall is mostly constituted of complex and structurally diverse polysaccharides that are valuable resources for industrial and biotechnological applications. Anaerobic microbial organisms are highly efficient for plant cell wall polysaccharide biodegradation and their enzymes are usually modular with appended non-catalytic Carbohydrate Binding Modules (CBMs) that highly potentiate the enzymes' catalytic efficiency. Elucidation of function and carbohydrate-binding by these CBMs is essential to understand plant cell wall carbohydrate recognition and deconstruction by different cellulolytic bacteria. Carbohydrate microarrays allow for the systematic array of carbohydrate probe libraries and to identify the specificity and biological role of carbohydrate-binding proteins in a high throughput manner[1]. The detailed characterization at epitope level requires the availability of focused microarrays with purified and well characterized oligosaccharide sequences. Here we will present an approach to develop microarrays of naturally derived linear and branched plant-related oligosaccharides featuring diverse structures and degrees of polymerization. The developed microarrays were applied to screen for carbohydrate binding all assigned CBMs from two major cellulolytic bacteria, *Ruminococcus flavefaciens* FD-1 and *Clostridium thermocellum*[2], revealing novel CBM carbohydrate interactions and ligand specificities. The information derived from these results is crucial to assess the structural analysis of CBMs and their oligosaccharide ligands to elucidate cellulolytic capabilities of these bacteria at the molecular level, while the identification of novel CBM-targets for modulation of carbohydrate recognition will promote potential new applications. 1. Ribeiro DO et al. (2017) Carbohydrate Chemistry: Chemical and biological approaches, RSC 159–176. 2. Ribeiro DO et al. (2020) FEBS J. 287, 2723–2743. Funding: PTDC/BIA-MIB/31730/2017, UIDP/04378/2020 and UIDB/04378/2020.

Wednesday 13 July
9:00–11:00, Auditorium VII

Cell signalling

S-03.4-2
Disease-association of cell-ECM adhesion adaptor Talin-1

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Talin is a core component of the focal adhesion complex that mediates the communication between cell interior and extracellular space. It is a large multidomain protein that is essential for cell migration, survival, and at large scale, for the development of multicellular organisms. Talin connects and activates integrin receptors and forms a link to actin filaments and other cytoskeletal components via its long rod-domain. In addition to these connections that introduce mechanical force into the molecule, long rod domain contains several other binding sites that are regulated by the amplitude of applied mechanical load. Talin-disease association is an interesting emerging topic. For example, there is an overexpression of talin observed in several cancers and this correlates with cancer invasiveness. When looking at talin's role in cell migration and cell-ECM communication, these findings are not unexpected. Now with current understanding of the structure–function relationship, we are ready for the next steps towards the identification of disease-causing mutations and defining talin's potential as a therapeutic target.

S-03.4-1
Adding a piece to the leaf epidermis puzzle

S. Robert
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In plants, the epidermis plays an essential role in shaping the entire organism, as it is thought to be limiting for growth and contains stiff and cohesive cell walls, which hinder cell–cell movements. Puzzle-shaped leaf pavement cells displaying inter digitated lobes and indents in the two-dimensional plane of the leaf epidermis provide a powerful model system to investigate the cellular and subcellular processes underlying cell polarity and shape determination in plant tissues. The formation of these multipolar cells is regulated by auxin at the plasma membrane via the subcellular compartmentation of Rho GTPase of plants (ROP) signaling pathways, but it remains unclear how such local molecular heterogeneities are translated into local cell wall properties to generate local shape changes. I will present our data on cell shape acquisition using jigsaw puzzle-shaped leaf epidermal pavement cells of *Arabidopsis* as a remarkably synchronous model of cell shape development.

ShT-03.4-1
The FLT3-ITD receptor could be regulating the process of protein folding in acute myeloid leukemia

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Acute myeloid leukemia (AML) is the most common myeloid tumour in adults. The FLT3-ITD receptor, present in 30% of the cases of AML, accumulates in the endoplasmic reticulum (ER) due to a defect in its post-translational processing. We decided to examine whether this affects the functioning of the organelle. The ER depends on GSH to neutralize reactive oxygen species (ROS) produced in the process of protein folding. We found FLT3-ITD cell lines to have lower levels of protein aggregates, ROS and GSH,

additionally to greater levels of ERO1, an ER protein folding oxidorreductase. We verified that FLT3-ITD cell lines were less sensitive to protein folding blocking agents, DTT and 2-mercaptoethanol, reflected by a slight decrease in cell proliferation compared to wild-type cell lines, which showed high levels of cell death after the treatment. Under ER stress conditions, such as an accumulation of misfolded proteins in the ER, the unfolded protein response (UPR) is activated. We found FLT3-ITD cell lines to have lower protein expression of the active forms of PERK and IRE, together with the protein BiP, the main chaperone found in the ER. Altogether, our data suggest that FLT3-ITD cell lines have less ER stress, possibly because they rely on more efficient protein folding mechanisms. To confirm the importance of protein folding process in acute myeloid leukemia, we tested the effect of ERO1 inhibitor and found it to have a greater impact in FLT3-ITD cell lines. In order to determine a possible relation between the mutated FLT3 receptor and ERO1, we used stably transfected Ba/F3 cell line carrying the normal or mutated FLT3 receptor. In agreement with results in human cell lines, we found ERO1 to be overexpressed on FLT3-ITD Ba/F3 cells. FLT3-ITD cell lines, with greater resistance to treatments, have more efficient protein folding mechanisms. This process should be studied in more detail to determine its potential as a therapeutic target for AML treatment.

ShT-03.4-2
BDNF-induced synaptic potentiation requires Slitrk5-Shank3 complex in the corticostriatal circuits

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Obsessive compulsive disorder (OCD) is highly heritable, yet there are few molecular genetic risk markers. Slit- and NTRK-like family 5 (Slitrk5) has been identified as the most relevant single gene for OCD in human and genetic animal studies. Slitrk5 is a synaptic adhesion molecule that mediates neurite outgrowth, synaptogenesis, and neuronal survival in the corticostriatal circuits. The molecular mechanisms that underpin these functions, however, are elusive. Here, we show that Slitrk5 controls brain-derived neurotrophic factor (BDNF)-dependent biological responses by interacting with Shank3, a postsynaptic scaffolding protein. In endogenous conditions, Slitrk5 forms a physical complex with Shank3, but not with other Shank isotypes. TrkB and downstream PI3K activity are both necessary for BDNF-induced recovery of Shank3 fluorescence. Slitrk5 was required to induce fluorescence recovery of Shank3 in striatal neurons in both BDNF-treated and resting circumstances. Furthermore, the interaction between Slitrk5 and Shank3 was required for BDNF-induced synaptic potentiation in the corticostriatal circuits. These findings show that proper structure and function of the corticostriatal circuit require the cooperative function of the Slitrk5-Shank3 complex in response to bdnf stimulation from the presynapses.

ShT-03.4-3
Interplay between C terminal mitophagy receptor NIX phosphorylation and dimerization as a new mechanism of receptor-mediated mitophagy regulation

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A form of autophagy specialized for selective removal of mitochondria, mitophagy, is essential for elimination of dysfunctional mitochondria whose accumulation can lead to the development of neurodegenerative diseases and tumors. Programmed mitophagy of healthy mitochondria is crucial for differentiation of particular cell types, such as erythrocytes. Autophagy receptor BNIP3L/NIX is shown to be important for the removal of healthy mitochondria during terminal erythropoiesis but molecular mechanisms of selectivity are still unclear. Here, we have investigated BNIP3L/NIX dimerization and phosphorylation as a novel molecular mechanism underlying receptor-mediated mitophagy. Stable BNIP3L/NIX homodimers provide the formation of new and strong interactions between the receptor and autophagosomal protein, more robust recruitment of autophagosomes and efficient removal of mitochondria. This dimerization is achieved by specific Ser212 dephosphorylation located in the intermembrane mitochondrial space and has the same effect on mitophagy initiation and progression as LIR phosphorylation, previously published in: Marinković et al. (2021) Autophagy 17(5), 1232–1243 and Rogov V et al. (2017) Sci Rep 7, 1131. Thus, the interplay between BNIP3L/NIX phosphorylation and dimerization indicates that the combined mechanism of LIR phosphorylation and receptor dimerization is needed for proper BNIP3L/NIX-dependent mitophagy initiation and progression. Currently, the focus of our research is on detailed analysis of interactions between BNIP3L/NIX and identified kinases and phosphatases to unveil upstream signaling pathways that trigger and regulate mitophagy especially in erythroid cell lines. Lastly, this knowledge of the molecular basis of BNIP3L/NIX-dependent mitophagy regulation is crucial for better understanding the mechanisms of an individual cell's differentiation and the development of pathological conditions that underlie the disturbed mitophagy process.

ShT-03.4-4
KinCon biosensors: Tracking mutation and drug-driven alterations of kinase activity conformations

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Kinases act as central nodes of cellular signaling networks. As such, many of these enzymes function as molecular switches for coordinating spatiotemporal signal transmission. Genomic alterations affect kinase abundance and/or their activities which contribute to the malignant transformation, progression, and metastasis of human cancers. Thus, major drug discovery efforts have been made to identify lead molecules targeting clinically relevant onco-kinases. The concept of personalized medicine aims to apply the therapeutic agent with the highest efficacy towards a patient-specific mutation. We demonstrated that cell-based reporters assist in the decision-making process to identify the most

promising lead-molecules. We have generated a modular kinase conformation (KinCon) biosensor platform for live-cell analyses of kinase activity states (Enzler et al., IUBMB Life 2020). First, we have unveiled allosteric effects of anti-cancer drug-driven intramolecular communication between the mutated oncokinase BRAF and RAS, which may further promote paradoxical kinase activation and drug resistance mechanisms (Röck et al., Science Advances 2019). Second, we showed that FDA-approved melanoma inhibitors may have the potential to reduce mutated BRAF functions in non–small-cell lung cancers (Mayrhofer et al., PNAS 2020). Third, we recently extended the KinCon platform for efficacy analyses of lead molecules and key kinases involved in inflammation responses and the etiology of Parkinson's disease. We were surprised to identify that targeting kinases such as p38, RIPK1 and PINK1 with bioactive small molecules alters their conformational state, thus impacting decisive molecular interactions and phosphorylation-independent signal propagation. These observations underline that the KinCon technology may open new avenues for systematic and patient-tailored drug discovery efforts for selecting and identifying the most efficient lead molecule for the respective mutated kinase.

Wednesday 13 July 9:00–11:00, Auditorium VIII

DNA and RNA

S04.4 2 Stochastic vs. deterministic models of chromosome capture during mitosis

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Accurate chromosome segregation during mitosis relies on the formation of a thick bundle of microtubules (MTs) that attach at the kinetochore (KT) region of each chromosome to form KT fibers (k-fibers). While the molecular basis of end-on KT-MT attachments has been elucidated in recent years, the mechanism by which mammalian KTs attach up to dozens of MTs within a matter of minutes remains poorly understood. For years, this process, known as k-fiber maturation, was thought to rely on stochastic rounds of "search-and-capture" by centrosomal MTs. However, this proved to be highly inefficient. Moreover, "search-and-capture" by centrosomal MTs cannot explain k-fiber formation and maturation in cells that are naturally devoid of centrosomes, or after experimental centrosome inactivation in animal somatic cells. The Indian muntjac (IM) is a placental mammal whose females have the lowest known diploid chromosome number of their class (2 N = 6). IM cells have long and morphologically distinct chromosomes with unusually large KTs (up to 2 μm linear length) that bind up to 60 MTs. These unique cytological features, combined with recent large-scale ruminant genome sequencing efforts, create the ideal conditions to directly dissect the molecular mechanism underlying k-fiber maturation in mammals. Here we used RNAi and high-resolution live-cell microscopy to investigate the role of more than 60 conserved mitotic proteins in mitotic spindle assembly and chromosome segregation in IM fibroblasts. Assisted by sub-second live-cell super-resolution CH-STED nanoscopy analysis of MT growth within individual k-fibers and direct perturbation of k-fiber structure by laser microsurgery, we identified Augmin as the main driver of k-fiber self-organization and maturation, regardless of pioneer centrosomal microtubules. Thus, unlike stochastic models that dominated the field for decades, chromosome capture is rather deterministic and driven by microtubule self-organization at kinetochores.

S-04.4-1 Post-transcriptional regulation of LINE-1 retrotransposition by enzymes modifying RNA ends

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LINE-1 retrotransposons are one of the major constituents of human genomic repetitive sequences. These retrotransposons might still create new genomic insertions in genomic DNA in modern humans by a copy-paste mechanism involving RNA intermediates. LINE-1 is tightly regulated by multi-layered regulatory processes to prevent the detrimental effects of LINE-1 insertions on the integrity of the genome. Although transcriptional regulation of LINE-1 expression has been widely studied, the specific influences of general post-transcriptional regulatory processes on LINE-1 remain less well understood. In this work, we investigated the role of XRN1 and other factors including DCP2, TUTases, DIS3L2 and deadenylases involved in post-transcriptional regulation of RNA 5′ and 3′ ends on LINE-1 biology. By using multiple experimental approaches, we demonstrate that LINE-1, unlike many other cellular mRNAs, is very susceptible to 3′ end modifications, but less so to degradation. In result of the interconnected processes of deadenylation, uridylation, decapping and reduction of translation of retrotransposonal proteins, LINE-1 retrotransposition is grossly diminished. This mostly results from incapability of deadenylated, uridylated LINE-1 mRNA to initiate reverse transcription in the genomic DNA context, and likely reduction in retrotransposonal proteins' levels, but not from a decrease in LINE-1 mRNA abundance, which is not observed. We conclude that the general post-transcriptional processes shaping LINE-1 mRNA 5′ and 3′ ends play the major role in LINE-1 biology. This work was supported by National Science Centre of Poland, grant numbers: UMO-2017/26/D/NZ1/00887 (SONATA-13), UMO-2019/33/B/NZ1/02260 (OPUS-17).

ShT-04.4-1 Target binding triggers hierarchical phosphorylation of human Argonaute-2 to promote target release

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Argonaute (Ago) proteins play a central role in post-transcriptional gene regulation through RNA interference (RNAi). In this

process, Ago binds to a small RNA, such as a small interfering RNA (siRNA) or a microRNA (miRNA), and uses it as a guide to target messenger RNAs containing regions of complementarity and down-regulates production of their corresponding proteins. It was previously shown that the kinase CK1α phosphorylates a cluster of residues in the eukaryotic insertion (EI) of Ago, leading to the alleviation of miRNA-mediated repression through an undetermined mechanism. We show that binding of miRNA-loaded human Argonaute-2 (hAgo2) to target RNA with complementarity to the seed and 3′ supplemental regions of the miRNA primes the EI for hierarchical phosphorylation by CK1α. The added negative charges electrostatically promote target release, freeing hAgo2 to seek out additional targets once it is dephosphorylated. The high conservation of potential phosphosites in the EI suggests that such a regulatory strategy may be a shared mechanism for regulating miRNA-mediated repression.

ShT-04.4-2
An ATPase filament bridge: how a transposon and CRISPR stick together

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The primary role of CRISPR-Cas systems in prokaryotes is adaptive immunity against mobile genetic elements. Conversely, several Tn7-like elements co-opted CRISPR-Cas RNA-guided machineries to direct transposon DNA insertion into specific target sites. If CRISPR-associated transposons could be repurposed for mammalian genome editing, they would provide the first truly programmable, site-specific gene insertion technology. In type V CRISPR-transposons systems, RNA-directed DNA insertion relies on the cross-talk between the pseudonuclease Cas12k, the transposase TnsB, the zinc-finger protein TniQ and the ATPase TnsC. Yet, the molecular mechanisms underpinning this interplay have remained elusive. Here, we present a cryo-electron microscopy structure of DNA-associated TnsC in its ATP-bound state. The structure reveals that the AAA+ ATPase forms an ATP-dependent helical filament that encloses and remodels the underlying target DNA. One strand only of the duplex is tracked by consecutive TnsC protomers with an unexpected two-nucleotide periodicity, resulting in a DNA helix with 12 base pairs per turn. Biochemical studies show that TnsC polymerization is a critical aspect of the system that enables the coupling of RNA-guided target recognition by Cas12k with the downstream recruitment of TnsB by direct protein interactions. In turn, the TnsB transposase triggers filament disassembly upon ATP hydrolysis, establishing target immunity. By determining structures of TniQ and TniQ-TnsC complexes, we further dissected the role of TniQ in TnsC regulation. Together, our data point to a mechanistic model whereby TnsC oligomers bridge between the RNA-guided target selector Cas12k and the transposition machinery, promoting target DNA remodeling and ultimately transposon integration. This work discloses the first mechanistic insights into targeting and regulation of type V CRISPR-associated elements and will guide the rational design of these systems as genome editing tools. *The authors marked with an asterisk equally contributed to the work.

ShT-04.4-3
Possible effect of miR-505-5p which targets CYP3A4 in the colchicine resistance of Familial Mediterranean Fever patients

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Familial Mediterranean Fever (FMF) is the most common autosomal recessive autoinflammatory disease. Daily colchicine uptake is a well-known treatment of the disease. However, nearly 2–5% of patients are non-responsive despite using the highest tolerable colchicine. These patients receive biological treatments like anakinra and canakinumab to suppress inflammation. This study aims to analyze the potential impact of miRNAs on colchicine resistance seen in FMF patients. Microarray data were evaluated for both colchicine-resistant and colchicine-responsive patients. Bioinformatics analyses determined candidate miRNAs involved in drug metabolism. Validation of miRNA expression and miRNA-target gene studies were performed by qRT-PCR. 3'UTR luciferase activity experiments were carried out to determine the target gene. A colchicine-resistant HEPG2 cell line was generated. Then, functional analyses related to the miRNA-target gene were performed. As a result of miRNA array analysis, miR-186-3p, miR-548a-3p, miR-7-5p were decreased, and miR-505-5p and miR-4482-3p were increased in colchicine-resistant FMF patients. miR-505-5p was found to be a regulator of drug metabolism and drug resistance-related genes by bioinformatical tools and validated by qRT-PCR. Target gene studies performed in HEPG2 cells showed that miR-505-5p regulates CYP3A4 expression. miR-505-5p – CYP3A4 interaction was demonstrated by 3'UTR luciferase assay. Similar to colchicine-resistant FMF patients, increased miR-505-5p and decreased CYP3A4 expression levels were shown in colchicine-resistant HEPG2 cells. The results can contribute to understanding the drug resistance seen in colchicine-resistant FMF patients. In addition, it will enable the development of new drug targets and biomarkers in addition to existing colchicine and similar treatments in the future. This project has been funded by The Technical and Scientific Research Council of Turkey, 218S522.

ShT-04.4-4
Inhibition of respiratory syncytial virus infection by tryptophan-like side chain holding aptamers

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The respiratory syncytial virus (RSV) is the most common cause of acute lower respiratory tract infections during infancy and adulthood that often leads to hospitalization. Currently, there is no licensed vaccine available to prevent RSV infection. A multitude of human antibodies were raised against the fusion glycoprotein (F protein) of RSV, including the prophylaxis providing approved molecule, palivizumab. A more potent virus neutralizing capacity was achieved by isolating antibodies that stabilize

the F protein in its prefusion conformation. We hypothesise that a similar effect can be achieved using aptamers, the single stranded oligonucleotides of antibody-like specificity and affinity. To date, a fraction of aptamers made their way into therapeutics due to various limiting factors, e.g., their short half-life in biological matrices. This shortcoming can be salvaged by application of non-natural nucleotides to increase the nuclease resistance of aptamers. These modifications may also extend the range of potential target-aptamer interactions by the introduction of hydrophobic side chains. In this study, our goal was to produce aptamers of therapeutic potential for RSV infection. A stabilized version of the prefusion F protein was targeted by aptamer selection using an oligonucleotide library holding a tryptophan-like side chain. The selection process resulted in aptamers that bind the F protein with nanomolar dissociation constant and differentiate the pre- and post-fusion conformation. Furthermore, the best aptamer candidates inhibit the viral infection of lung epithelial cell culture with similar capability to that of palivizumab. Introduction of the modified nucleotide also resulted in the extended half-life of aptamers in the studied cell culture. We believe that our results testify that virus surface-targeting modified aptamers could yield efficient and cost-effective drug candidates, which could keep up with the pace of continuously-evolving pathogens.

Wednesday 13 July
16:00–18:00, Auditorium I

Synthetic biology

S-05.3-1
Implementing biological computation with re-programmable distributed multicellular consortia

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Engineering approaches to synthetic biology have shown that there are a number of strategies allowing us to build complex functional constructs with computational abilities. There are a number of efforts towards building artificial computational devices that could be used for a wide range of applications, including bioremediation, food production or biomedicine. Using yeast as a model organism, we have been able to implement complex circuits by distributing computation within cellular consortia. This approach to biological computation has opened the possibility to develop a novel method of properly designed general purpose which can be combined in multiple ways to create complex computational circuits. The potential use of this approach is demonstrated by implementation of complex logical functions responding to up to six inputs. The generation of re-programmable circuits can increase circuit flexibility and the scalability of complex cell-based computing devices. We have reprogrammable biological circuits that allow the development of a variety of different functions with minimal cell engineering. We demonstrate the feasibility of creating several circuits using only a small set of engineered cells, which can be externally reprogrammed to implement simple logics in response to specific inputs. The reprogrammability of biological circuits is an intrinsic capacity that is not provided in electronics and one that should be exploited to encourage the use of biocomputing as a tool to solve complex biological problems.

S-05.3-2
How to build biological complexity from the bottom-up

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In order to arrive at a self-sustaining minimal system of molecular interactions with the ability to evolve – a minimal living system – we likely need to build it from scratch. Our lab has focused on the emergence of cell division as a genuinely physicochemical process. With compartmentation being a key facilitator of biological identity, the challenge is to reveal the mechanistic origin of self-replication of membrane compartments. We employ Giant Unilamellar Vesicles (GUVs) as bases for protocells, because of their facile deformability and comfortable sizes, allowing us to study membrane transformations by light microscopy. A huge breakthrough was made when we accomplished the reconstitution of a minimal self-organizing biological machinery at the basis of bacterial cell division. These proteins, MinD and MinE from *E.coli*, are supposed to orchestrate the positioning of the divisome to mid-cell by establishing oscillating concentration gradients on the membrane between the two poles and the center through spatiotemporal self-organization. We showed that the Min proteins, a membrane, and a metabolic energy source yielded emergent behavior of pattern and gradient formation. Intriguingly, the ability of these spatial patterns to act as a spatial cue for other proteins of the bacterial divisome could be confirmed in cell-shaped compartments, and lately also in deformable vesicles. This demonstrates that apparently complex phenotypic features of living organisms do have a functional core, the elucidation of which may not only allow us to better understand biology as a whole, but may also inform us about the most efficient ways of designing new biological functionality.

ShT-05.3-1
Investigation on the ability of hydrophilic carbon nanomaterials to mimic antioxidant defense system enzymes

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Antioxidant nanozymes have been studied for many biological applications. Compared with antioxidant nanozymes prepared with other materials, carbon-based nanozymes have several advantages: low-cost, easy mass production, robustness and stability in biological environments, and low toxicity. This work aims to evaluate the ability of a water-soluble carbon-based nanomaterial to mimic the native superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). This nanomaterial, named EHC@malic, was produced from graphite by an electrochemical approach, using malate buffer as electrolyte. EHC@malic exhibits a string-assembly organization dominated by

amorphous carbon nanoparticles as revealed by TEM and AFM studies. Cyclic voltammetry showed that EHC@malic nanomaterial has electron-donating properties with an oxidation peak at 0.173 V, suggesting the ability to operate in the range of redox potentials compatible with the substrates used by several natural antioxidant enzymes. The SOD-like activity was evaluated using a hypoxanthine-xanthine oxidase system for $O_2^{\bullet-}$ generation and NBT as the detector. Results indicate that EHC@malic nanomaterial (0–50 μg/mL) exhibits a SOD-like activity comparable to native SOD. CAT-like activity, evaluated using a Clark-like electrode, revealed EHC@malic nanomaterial has a semi-CAT-like activity since it shows the ability to react with H_2O_2 without O_2 generation. Lastly, the POD-like activity was appraised spectrophotometrically by measuring the ability of the nanomaterial to accelerate the TMB oxidation in the presence of H_2O_2. The results indicate that it does not exhibit POD-like activity. Summing up, the specific SOD-like activity exhibited by EHC@malic nanomaterial turns it into a promising nanotool to fight the oxidative stress associated with pathophysiological conditions resulting from SOD deficiency. Acknowledgments to Fundação para a Ciência e Tecnologia (SFRH/BD/138425/2018, UIDB/00616/2020, UIDP/00616/2020, UIDB/50006/2020).

ShT-05.3-2
Synthetic biology to the rescue: On-demand biopharmaceuticals in extreme environments

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Astropharmacy, pioneered at the University of Nottingham, focusses on solving challenges related to human health in extreme environments, in particular space. Biopharmaceuticals expire within 6–12 months and require cold-storage and transportation; degradation is further accelerated in extreme environments (radiation, lack of cold-storage, microgravity etc.). These contribute to difficulty in expanding human presence in extreme environments and is a highly unsustainable approach (due to high carbon footprint). One solution for these challenges is enabling on-site, on-demand manufacturing of biopharmaceuticals enabled by cell-free protein synthesis (CFPS). CFPS surpasses the limitations of cell-based expression and is evolving to be the system of choice for many therapeutic, technological, and environmental applications. We founded our in-house CFPS system on bacterial strain BL21 Star (DE3), and further optimised this for high-level chromogenic reporter expression driven by T7 polymerase. We then constructed a platform by freeze-drying the CFPS components on cellulose stacks, which were layered and rehydrated with water to kickstart protein synthesis. Our results show that paper-encompassed reactions are capable of robust expression of various therapeutics following drying and rehydration, by simply changing the DNA element. Lastly, we aimed to obtain pure protein of pharmaceutical standard by using two approaches: on-cellulose purification and nanobody-driven functional purification – this critical aspect of our work will provide pioneering contributions toward on-site and on-demand purification of biopharmaceuticals. Such a platform could form a part of a futuristic Astropharmacy capable of producing life-saving therapeutics in a few hours (4–6 h) at the point-of-care. We envision other Earth-based applications for our system, such as promoting biopharmaceutical access to low-resource environments and extreme human habitats in the absence of cold storage.

ShT-05.3-3
Enhanced poly(ethylene terephthalate) degradation by modified *Yarrowia lipolytica* strains

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Polyethylene terephthalate (PET) is classified as a non-biodegradable polymer whose large accumulation in the environment is a serious threat to the ecosystem. The ability to hydrolyze the ester bonds present in this plastic has been proven for enzymes from the class of hydrolases such as cutinases, lipases and PETase. In this work, we have tested two *Y. lipolytica* strains expressing cutinase from *Fusarium solani* and PETase from *Ideonella sakaiensis*. These unconventional yeasts are known for its capacity to assimilate atypical carbon sources and large amounts of extracellularly produced lipases. In our study, we have verified the PET degradation capabilities of the modified *Y. lipolytica* strains AJD 2 pAD CUT_FS and AJD 2 pAD PET_IS directly in shake culture. In addition, we have checked the influence of culture supplementation with various salts and olive oil on the degradation efficiency. Level of PET hydrolysis was verified with the use of ultraperformance liquid chromatography (UPLC) based on the amount of degradation products released to the medium during cultivation. Changes in the structure of the PET film after culture were also investigated using scanning electron microscopy (SEM). The results indicate that PET degradation is possible directly in the culture of the modified *Y. lipolytica* yeast. The structure of PET film shows significant damage in the form of numerous cracks and pits. This work was financially supported by the National Science Centre, Poland, project UMO-2017/27/B/NZ9/02218. *The authors marked with an asterisk equally contributed to the work.

Wednesday 13 July
16:00–18:00, Auditorium II

Systems biology

S-06.3-2
How far does hydrogen peroxide travel in the microcirculation and in tissues?

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H_2O_2 is a key auto/paracrine signalling agent in vascular adaptation and neural development. However, its extracellular concentrations and action ranges are uncertain. We have examined them for the microvasculature and brain tissue *in silico* and

in vivo. Reaction–diffusion modelling of the spatial distribution of superoxide ($O_2^{\bullet-}$) and H_2O_2 in the lumen of arterioles and capillaries considered: (i) $O_2^{\bullet-}/H_2O_2$ release by a 20 μm-long ring of endothelial cells (EC) and uptake by erythrocytes and EC throughout the vessels; (ii) $O_2^{\bullet-}$ dismutation; (iii) $O_2^{\bullet-}/H_2O_2$ diffusion and transport by the blood flow. *Results*: For plausible EC $O_2^{\bullet-}$ production rates, H_2O_2 concentrations in blood plasma reach just ~1 nM and ~ 10 nM in arterioles and capillaries, respectively, making signaling unviable in the former case. In capillaries, the H_2O_2 concentration is maximal within the $O_2^{\bullet-}$ production zone and 50% lower just 20 μm downstream, allowing mostly autocrine signalling. We used an implanted H_2O_2 microelectrode to follow the concentration in the brain of anesthetized rats upon supply of a 1 mM H_2O_2 bolus through a nearby-implanted micropipette. Fitting a model of H_2O_2 diffusion and clearance to this data yielded a 2500 $\mu m^2 s^{-1}$ effective diffusion constant and 2.2 s half-life. Thus, H_2O_2 can diffuse 112 μm in this tissue within its half-life, allowing it to transmit signals across cells that are not physically connected. A simple model accounting for the brain tissue's morphometry suggests that this long action range is due to the virtual impermeability of myelinated neurons to H_2O_2. Extracellular H_2O_2's action range thus strongly depends on niche features. Work financed by the European Regional Development Fund, through COMPETE2020-Operational Program for Competitiveness and Internationalization, and Portuguese funds via FCT-Fundação para a Ciência e a Tecnologia, under projects UIDB/04539/2020, UIDP/04539/2020, UIDB/00313/2020, UIDP/00313/2020 and POCI-01-0145-FEDER-028261 (Portugal).

S-06.3-1
Putting biochemistry into models of cellular physiology

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For unicellular organisms and highly proliferative cells, growth rate is an important determinant for success. An arguably naive view of cellular growth is the synthesis of all the biomass components in the right proportions. When biomass composition is known, the accumulated biochemical knowledge on enzymes and metabolic pathways can be collected and stored in computable knowledge bases known as genome-scale metabolic models. They link genes to mRNAs to proteins to protein complexes to metabolic activity. They are an indispensable tool in advanced multi-omics data integration to understand (patho)physiology of cells, and to map phenotype from genotype. A key to the successful use of these models in predicting phenotypes, however, is knowing their assumptions and limitations. I will show that it is essential to understand the constraints that act on the metabolic network, and that these models can help in identifying which of these constraints actively limits growth – or some other function that can be described by metabolic fluxes. I will illustrate the use of these models in understanding the Crabtree effect in yeast (known as the Warburg effect in cancer), catabolite repression of amino acid metabolism in lactic acid bacteria, and liver cancer metabolism.

ShT-06.3-1
Understanding yeast colony growth through a modelling – experiment loop

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Colonies built by natural isolate strains of the budding yeast, *Saccharomyces cerevisiae,* show various morphologies. To understand the differences in the growth behaviour of these strains we study the quantitative features of yeast colonies in isolation or in mixed cultures. We investigate the limiting factors which slow down colony growth, and study how various strains interact with each other. We quantify the number of the cells, the density, the size, the shape and internal structure of the colonies, initiated from various inoculation scenarios to reveal the key factors affecting the proliferative capacity of yeast strains. This quantitative data is used to train an agent-based mathematical model that is capable of capturing growth differences of various yeast strains. We use the fitted models to design experiments which can distinguish between alternative scenarios explaining why colony growth slows down in time. Imaging of the internal structure of mixed colonies of two strains also help us to test how our models can capture local interactions inside colonies.

ShT-06.3-2
Role of coding region mutations on gene expression noise in yeast

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Genetically identical cells show variation in expression of genes even under identical environmental condition – a phenomenon referred to as gene expression noise. Noise is generated by fluctuations in the synthesis and degradation processes of mRNA and protein molecules. Promoter sequence of a gene, specifically presence of TATA box, and promoter nucleosome occupancy, have been shown to be key regulators of noise. However, how the coding sequence of a gene can impact noise has still not been empirically quantified. Mutations in a gene can affect mRNA secondary structure and its stability which can lead to variations in mRNA level from one cell to another. In addition, codons encoding for the same amino acid can impact translation efficiency due to changes in availability of specific tRNA molecules. Prior work on codon usage has indeed shown that synonymous substitutions can change the overall expression level of a gene. In this work, we empirically quantify the impact of coding region mutations on gene expression noise. To do so, we integrated a promoter-fluorescent gene construct in the genome of yeast *Saccharomyces cerevisiae*. We introduced coding region mutations in the fluorescent gene and quantified their impact on noise through flow cytometry. Our results show that mutations in the 5′ end of the gene are more likely to impact noise than the 3′ end mutations. Thus, our work provides a first glimpse of how the gene sequence itself can control noise in an organism.

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ShT-06.3-3
Light quality modulates plant cold response

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The last decades of plant research revealed that an efficient plant cold-acclimation process is critical for surviving the following cold period. Plant cold-acclimation is regulated by external factors like ambient temperature, day length, light intensity, and internal factors like hormones. Here, the role of light quality in the cold response was studied in one-month-old *Arabidopsis thaliana* (Col-0) plants exposed for one week to 4 °C at short-day conditions under white (100 and 20 μmol m^{-2} s^{-1}), blue or red (20 μmol m^{-2} s^{-1}) light conditions. An upregulated expression of *CBF1*, inhibition of photosynthesis, and an increase in membrane damage showed that cold treatment under blue light enhanced the effect of low temperature. Interestingly, blue and red light cold-treated plants showed only limited freezing tolerance compared to white light treated plants. Next, the specificity of the light quality signal in cold response was evaluated in *Arabidopsis* accessions from different latitudes. In all but one accession, blue light increased the effect of cold on photosynthetic parameters and electrolyte leakage. This effect was not found for WS-0, which lacks functional CRY2 protein, indicating its role in the cold response. Proteomics data confirmed significant differences between red and blue light-dependent cold responses and showed that the cold response was accession specific. In general, blue light increased mainly the cold-stress-related proteins, and red light induced higher expression of chloroplast-related proteins, which correlated with higher photosynthetic parameters in red light treated plants. Altogether, our data suggest that light modulates two distinct mechanisms during the cold period: red light-driven cell function maintaining program and blue light-activated specific cold response. The importance of mutual complementarity of these mechanisms was demonstrated by the significantly higher freezing tolerance of plants treated under white light. *The authors marked with an asterisk equally contributed to the work

ShT-06.3–4
Microfluidics in systems biology and biodiversity exploration

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The world of living systems is filled with intricate molecular interactions and is incredibly difficult to analyze in detail. Classical methods of molecular and cellular biology study averaged effects, which gives a highly distorted picture, full of artifacts. Alternatively, methods based on the analysis of individual biological objects do not have these limitations. Droplet microfluidics provides a universal platform for single-cell compartmentalization and high-throughput screening, allowing deep phenotypic and genotypic profiling of biodiversity. General principles of compartmentalization were applied to various biological objects to preserve biodiversity and isolate rare functional subpopulations. The versatility of this concept enables deep functional profiling of natural and synthetic biodiversity. By encapsulating individual cells in emulsion droplets, we performed directed enzyme evolution, the isolation of antibiotics and probiotics, personalized screening of resistomes, and functional populations of lymphocytes. The obtained results indicate that microfluidic technological platforms based on the principles of compartmentalization and ultrahigh-throughput screening allow attaining a new level of understanding the functioning of living systems and biocommunities. The research was supported by the Ministry of Science and Higher Education of the Russian Federation grant No. 075–15–2020-0773.

The next two Talks have been moved to the following Symposium: Thursday 14 July 09:00–11:00, Auditorium VII

Cell–cell recognition

S-07.3-1
Advanced fluorescence microscopy to study bacterial response to environmental challenges at the single-cell level

C. Flors
IMDEA Nanociencia, Madrid, Spain

In this talk, I will discuss advanced microscopy tools to study the physiological response of *E. coli* to a range of environmental challenges. Simultaneous fluorescence imaging and nanoindentation with an atomic force microscope can be used to monitor bacterial response to controlled force, which is relevant for a mechanistic understanding of mechanobactericidal nanomaterials.[1] We find that about 20 nN are necessary to produce critical damage to the cell wall, and that the repeated application of much lower forces leads to fatigue effects. The latter is revealed by monitoring the oscillation period of the Min system, a protein complex that is involved in cell division. The performance of this live-cell reporter is also tested in assessing the effect of reactive oxygen species on bacterial physiology in real time.[2] Overall, the combination of advanced instruments and complementary fluorescence labelling strategies is powerful for a full mechanistic understanding of bacterial processes at the single-cell level.

1. A. del Valle, J. Torra, P. Bondia, C.M. Tone, P. Pedraz, V. Vadillo-Rodríguez, C. Flors, Mechanically Induced Bacterial Death Imaged in Real Time: A Simultaneous Nanoindentation and Fluorescence Microscopy Study, *ACS Appl. Mater. Interfaces* **2020**, 12, 31235.
2. I.V. Ortega, J. Torra, C. Flors, Min Oscillations as Real-time Reporter of Sublethal Effects in Photodynamic Treatment of Bacteria, *ACS Infect. Dis.* **2022**, 8, 86

S-07.3-2
Whole-brain biodistribution analysis of adeno-associated virus (AAV) by tissue clearing and light-sheet microscopy

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Gene therapy has emerged as a promising approach for treating a spectrum of neurodegenerative disorders by delivering a healthy cargo to the central nervous system (CNS). Of all gene therapy vectors, recombinant adeno-associated viruses (rAAVs) became a powerful system, transducing a wide range of cell types, through different routes of administration and with an impressive safety profile. However, a key challenge is the lack of tools to efficiently evaluate the biodistribution of AAVs, after intravenous administration, at whole-brain level. Therefore, we established a new pipeline, based on tissue clearing and light-sheet microscopy, that allows to study the delivery and biodistribution of rAAVs in the brain, and that can be applied as a bioimaging tool in tropism and gene therapies studies. *The authors marked with an asterisk equally contributed to the work.

Thursday 14 July
9:00–11:00, Auditorium I

Ageing

S-01.5-1
Molecular basis of successful therapies to delay progression to Alzheimer's disease

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Alzheimer's disease has, as it is well known, devastating effects on the individual health, that of the caregivers and finally, on the whole of society, not only in personal terms, but also in huge economic terms. Any attempt to delay the onset of Alzheimer's dementia deserves full attention. It is well known now that brain damage, including plaque deposition and even changes in brain volume, start well before the onset of clinical symptoms. The lag period may be as long as one or even two decades. On the other hand, Alzheimer's pathology is of such seriousness that it involves not only one mechanism, but a series of molecular mechanisms leading to amyloid-β accumulation and tau hyperphosphorylation. Attempts to treat Alzheimer's by delaying the onset of dementia, i.e., changing the course of the disease, must be multimodal, because the pathogenetic mechanisms leading to the disease are also multimodal. Of critical importance is that the fact that oxidative stress, inflammation and probably senescence, are all involved in the pathogenetic mechanisms of Alzheimer's pathology. Another important condition for the treatment of a disease that has to be performed over the course of decades is that the interventions be practically devoid of side effects. For instance, intravenous effects of substances that are meant to last for twenty years may not be indicated as they are inconvenient for the patients. In the past twenty years, we have analysed the characteristics of oxidative stress associated with Alzheimer's disease and found that these changes in redox signalling contribute to link amyloid-β pathology with tau hyperphosphorylation. Some time ago we realised that genistein, a soya isoflavone that binds to PPAR-γ, activates production of ApoE, which in turn clears amyloid-β from brain. We tested this hypothesis in an animal model, i.e. the APP-PS1, and observed that genistein very significantly lowers the amount of amyloid-β in brain, decreases brain inflammation associated with Alzheimer's and improves cognition in animal tests. Now, we wish to report the results of a pilot clinical trial that show that genistein is effective in delaying the transition of minimal cognitive impairment patients to dementia. The mechanisms for these results will be discussed.

S-01.5-2
Metabolism and redox signaling in brain aging and neurodegeneration

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Deficits in glucose availability, mitochondrial function, and inflammatory responses are well-known hallmarks of the aging brain and are particularly accentuated in neurodegenerative disorders, such as Alzheimer's disease. The decrease in energy metabolism associated with brain aging may be assessed in terms of a coordinated metabolic triad encompassed of mitochondria, insulin signaling (IKIS), and JNK (c-Jun N-terminal kinase) signaling. Impairment of these coordinated responses leads to the cognitive decline that occurs with aging and neurodegenerative disorders. The complexity of the antagonism and cross-talk between IIS- and JNK signaling and how they converge on mitochondrial function underscores the significance of an insulin resistance state. Moreover, diabetes and obesity are considered risk factors to the brain hypometabolic state, thus placing insulin resistance as a major driver that coordinates the development of these insufficiencies. The hypometabolic state inherent in brain aging and a mouse model of Alzheimer's disease is accompanied by decreased brain glucose uptake, an imbalance between IIS- and JNK signaling, decreased rate of glycolysis and flux of metabolites to the TCA cycle, and diminished synaptic plasticity. These effects are not cell specific because astrocytes developed an age-dependent energy phenotype (increased mitochondrial oxidative metabolism and biogenesis) and augmented responses to inflammatory cytokines. Multiple mechanisms account for the bioenergetics deficits and microglia activation as the driving forces that contribute to cognitive decline during aging. Brain mitochondrial H_2O_2 – through thiol/disulfide exchange mechanisms – serves as a link between bioenergetics and neuroinflammation, the latter entailing NFkB signaling and inflammasome assembly and activation.

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ShT-01.5-4
Protein misfolding in primary chondrocytes is modulated by nutraceuticals and autophagy regulators

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The deposition of misfolded proteins is linked to a group of age-related diseases, known as amyloidosis, leading to functional degeneration of interested organs. Age is the main risk factor of osteoarthritis (OA), the most common form of arthritis and first cause of disability in the elderly. Although the mechanisms involved in the onset and progression of OA have only been partially elucidated, amyloid deposits have been found in both human advanced OA and aged non-OA cartilage. Autophagy (ATG), a stress response system that plays a pivotal role in anti-aging cell homeostasis in chondrocytes, is deregulated in OA, possibly concurring to an increase in protein misfolding. Our study aims to investigate the functional connection between amyloid deposition and ATG in OA and the potential activity of dietary nutraceuticals in reducing aggregate formation. Primary chondrocytes derived from OA patients were treated with ATG and endoplasmic reticulum (ER) stress modulators and nutraceuticals. Pre-fibrillar oligomer and fibril formation was then detected through western blot and amyloid-specific thioflavin T assay. Our results showed an increase in aggregate deposition in cells treated with ATG inhibitors (BAF - bafilomycin and CQ - chloroquine) and ER stress inducer (DTT), that, conversely, was rescued by epigallocatechin gallate (EGCG - content in green tea) pre-treatment. Then rapamycin (RAPA – ATG inducer) or CQ showed a reduced or increased presence of pre-fibrillar Aß1-42 oligomers, respectively. Furthermore, an increased aggregation of vimentin, an intermediate filament protein of chondrocytes, was observed following stimulation with lipopolysaccharide. In conclusion, these results open an interesting outlook to better understand the role of ATG and the beneficial role of EGCG in clearance mechanisms of misfolded proteins and/or their formation in OA pathogenesis. This study is supported by Fondazione Cassa di Risparmio in Bologna (Carisbo): project number #19348.

ShT-01.5-3
HSF1 regulates mitochondrial proteotoxic stress response in mammals

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Protein homeostasis or proteostasis is highly related with organismal health and fitness. Environmental toxins and metabolic perturbations alter the proteostasis network and challenge the subcellular compartments including the mitochondria. Mitochondria is a central hub of cellular metabolism, and its dysfunction is associated with age-related disorders and protein misfolding diseases. However the cellular mechanisms by which cells sense mitochondrial proteotoxic stress is not well elucidated. Our studies suggest heat shock transcription factor 1 (HSF1) is required for activation of mitochondrial chaperone genes, which is primarily activated during the mitochondrial unfolded protein response (UPRmt). We show HSF1-dependent induction of mitochondrial chaperones HSP60, HSP10, and mtHSP70 at the transcriptional level in MEF cells in the presence of mitochondrial stress inhibitors which target different functional sites of mitochondria: GTPP (mtHSP90 inhibitor), CDDO (Lon protease inhibitor), and Rotenone (ETC inhibitor). We also uncover the HSF1 activation process during the mitochondria stress response, Our experimental findings suggest that HSF1 significantly shuttles into the nucleus, exists in DNA binding dimer-trimer formation, and the active mark of transcriptional activation HSF1Ser326 phosphorylation was significantly increased. Our chip data suggest that HSF1 was remarkably enhanced at different levels during the UPRmt. Functional assays have also supported the role of HSF1 in maintenance of mitochondrial oxygen consumption and membrane potential during stress response. Thus, considering therapeutics of HSF1, we have extended our studies on understanding the genetic cause of classical mitochondrial ISCA gene in neurodegeneration by incorporating patient-specific homology directed mediated mutation in mammalian cells using the CRISPR/Cas system. We speculate that HSF1 could be a critical molecule in maintaining the mitochondrial proteostasis network and combating the neurodegeneration phenotype.

ShT-01.5-2
Grape seed extract improves intestinal barrier integrity and increases lifespan in various model systems

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A compromise of the intestinal barrier is strongly associated with ageing. Among others, oxidative stress contributes to rupture of intestinal integrity. For this study, we tested the effects of a grape seed extract (GSE) rich in polyphenols for its potential to strengthen the intestinal barrier *in vitro* and *in vivo* and to prolong lifespan of the model organisms. Therefore, an *in vitro* trans-well insert-based assay with intestinal cells was applied. Furthermore, lifespan and Smurf assays using the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* were performed. Treatment of IPEC-J2 intestinal enterocytes with GSE led to significant increase of transepithelial electrical resistance values (TEER) in unstressed cells and to a significantly faster recovery of intestinal barrier in cells upon the induced oxidative stress in a dose-dependent manner. In the temperature sensitive *C. elegans* mutant SS104, lower concentrations of GSE led to a significant improvement of lifespan, while treatment with higher concentration of GSE did not provide positive effects on the maximal lifespan. GSE supplementation also significantly decreased the fraction of Smurfs (organisms with compromised intestinal barrier) for both SS104 mutant and wild-type worms. In *D. melanogaster*, the GSE-enriched diet significantly increased maximal lifespan in a dose-dependent manner. Similar to the results obtained from *C. elegans* experiments, a significant decrease of the number of Smurfs

was observed in ageing flies that were treated with GSE during the entire lifetime. Our results suggest that GSE has a protective effect on intestinal barrier *in vitro* and *in vivo* during ageing or upon oxidative stress, respectively. We conclude that life-long supplementation with GSE prolongs lifespan of model organisms, such as *C. elegans* and *D. melanogaster*, which could also provide health benefits in higher organisms.

ShT-01.5-1
Inhibition of glycogen phosphorylase counteracts age-related defects in memory formation

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Inhibition of glycogen degradation in the brain of young animals has been shown to block memory formation and disrupt long-term potentiation (LTP). Unexpectedly, inhibition of glycogen phosphorylase (Pyg) activity, an enzyme indispensable for glycogen breakdown, significantly improved LTP induction in hippocampal brain slices of old animals [PMID:26101857]. Based on this we hypothesized that inhibition of Pyg activity using BAY U6751 (Pyg inhibitor) may be used for the improvement of age-associated deficits of memory. To verify this hypothesis, young (1 mth) and old (18–21 mth) mice were treated for two weeks with BAY. To evaluate the long-term general memory formation, we performed a Novel Object Recognition test with a 6 h inter-trail interval (ITI). Our results revealed significantly improved memory formation in old mice treated with BAY (OLD+BAY, $p = 0.004$). Analysing the dendrite spines, we found that BAY influenced their morphology in the hippocampus only. Thus, to measure hippocampus-dependent spatial memory formation we performed the Object Location Test (30' ITI). BAY-treated old mice spent significantly more time exploring the familiar displaced object than the familiar non-displaced one ($p = 0.0001$), compared to the control group ($p = 0.42$). Moreover, we also observed a shortening of time to the first exploration in the OLD+BAY group ($p = 0.0001$). Pyg is an enzyme of basal energy metabolism, and thus it might have been expected that its inhibition would disturb physiological parameters. However, two-week treatment of animals with BAY had no effect on body mass index and blood glucose level, and furthermore, we didn't observe differences in mice behaviour in the Rotarod and Open-field tests. Our proteomic data identified 2670 hippocampal proteins, that significantly change with aging. Interestingly, in OLD+BAY samples, the titers of 765 of them were modified by the inhibitor toward the concentrations which were measured in young control mice.

Thursday 14 July
9:00–11:00, Auditorium II

Human microbiome

S-02.5-1
Microbiota gut-brain axis regulation of hippocampal neurogenesis and behaviour: Implications for stress-related psychiatric disorders

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University College Cork, Cork, Ireland

There is a growing appreciation that bidirectional signalling between the gut and the brain plays a role in the regulation of stress-related responses and thus may contribute to stress-related disorders such as depression. Moreover, there is accumulating evidence that the microbes resident in the gut are key players in the gut-brain axis and may thus play a role in stress-related disorders such as depression and anxiety disorders. This presentation will focus on our preclinical data showing that gut microbes can alter neurogenesis, the production of new brain cells, in the hippocampus area of the brain and the implications that this may have for brain health, particularly stress-related psychiatric disorders.

S-02.5-2
From disease to therapeutic response: microbiota-host interaction

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An unbalanced microbiome (dysbiosis) is known to give rise to intestinal barrier dysfunction leading to translocation of bacterial and/or bacterial derived pro-inflammatory metabolites that, ultimately, result in systemic inflammation with metabolic dysfunction. Dysbiosis has been observed in many diseases, particularly metabolic, neurological and auto-immune disorders. Microbiota and its metabolites, particularly theones produced from dietary components, have been implicated in these disorders. The aryl hydrocarbon receptor (AhR) ligands derived from dietary tryptophan such as indole derivatives, the kynurenine pathway intermediates, and short chain-fatty acids seem to have a role in the microbiota-derived metabolic consequences. The taxonomic changes of microbiota, including diversity and richness are important features of a eubiotic vs dysbiotic microbiota, but microbiota metabolic products can play key roles in the host-microbiota interactions. Interventions with specific nutrients, probiotics, prebiotics, synbiotic or, fecal microbiota transplant that re-establishes the balance in gut microbiota and in its metabolites have been described. These interventions could represent an efficient strategy for disease management, therapeutic response, and subsequently an improvement of clinical outcomes. Thus, the gut microbiota as a new therapeutic target for disease prevention or treatment has been considered as a key medical tool.

ShT-02.5-1
Dietary nitrate prevents the loss of intestinal claudin-5 induced by broad-spectrum antibiotics and may modulate gut microbiota at lower taxonomic levels

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Dietary nitrate is bioactivated in the gastrointestinal tract via stepwise reduction to nitrite and nitric oxide along the gut. Although a weight of evidence support the effect of nitrate on oral microbiota, encompassing its reduction to nitrite, the impact of nitrate on colon bacteria remains elusive. Nitrate is likely to interact with the local colon microbiota, modulating not only the structure and function of local bacterial communities, but also epithelial barrier function. This study investigates the impact of nitrate on intestinal microbiota and the expression of local tight junction proteins. Wistar rats were randomly distributed into 4 groups and the drinking water was supplemented for 7 days as follows: 1) antibiotics, 2) antibiotics+nitrate, 3) nitrate and 4) tap water. Occludin and claudin-5 were analysed by immunoblotting in the colon. Nitrate and nitrite were measured in colon mucosa by HPLC and fecal bacterial DNA was studied by DGGE before and after treatment. Nitrate increases claudin-5 expression in rats exposed to a therapeutic dose of broad-spectrum antibiotic in comparison to animals exposed to antibiotics alone ($p = 0.016$), but decreases the expression of occludin ($p = 0.003$). Increases of tissue nitrate were noticed by c.a. six fold in comparison to both controls and rats exposed to antibiotics without supplementation ($p < 0.0001$). Overall, antibiotics eradicate most of the gut flora ($p = 0.0016$), reducing microbiota richness by 56% and nitrate showed a tendency to attenuate such microbial loss (48%, $p = 0.068$). In conclusion, nitrate might preserve critical tight junction proteins and bacterial communities during antibiotherapy. However, functional studies are mandatory to ascertain the impact of this anion on intestinal barrier function and bacterial metabolic pathways with impact on gut metabolome and overall host homeostasis.

ShT-02.5-2
Engineering anticancer microbes: Recombinant *Lactococcus lactis* can simultaneously target tumor antigens and bind proinflammatory cytokines

A. Zahirović*, T.V. Plavec*, **A. Berlec**
Jozef Stefan Institute, Ljubljana, Slovenia

Lactococcus lactis is used in the food industry and is the most prevalent lactic acid bacterium in the gut microbiota. It has been engineered as a vector for delivery of therapeutic proteins with some examples already in clinical trials. Co-display of cytokine-binding proteins with ligands for tumor antigens could facilitate the specific interactions of the bacteria with cancer cells and targeted delivery of cytokine-binding proteins. We introduced BglBrick cloning to *L. lactis* to achieve straightforward assembly of up to three expression cassettes. IL-8-binding evasin and IL-6-binding affibody were displayed on *L. lactis* for cytokine removal, while EpCAM-binding affitin and HER2-binding affibody were displayed for targeting tumor antigens. Infrared fluorescent protein was concomitantly expressed to enable bacterial detection and imaging. Engineered *L. lactis* removed IL-8 and IL-6 in human colon adenocarcinoma cells Caco-2 and HT-29 and in monocyte-like cells THP-1 and U-937. The engineered *L. lactis* removed >65% of IL-8 from the supernatant of Caco-2 and HT-29 cells and > 90% of IL-6 from the supernatant of THP-1 and U-937 cells. Specific adhesion of the engineered bacteria was observed in HEK293 cells transfected to overexpress EpCAM or HER2 receptors using fluorescence microscopy. App. 40 and app. 10 *L. lactis* cells bound per single EpCAM- and HER-2-expressing HEK293 cell, respectively. Apart from static conditions, targeting ability of engineered *L. lactis* was also demonstrated in conditions of constant flow in microfluidic system. In summary, *L. lactis* was engineered to simultaneously express multiple proteins with different functionality, including tumor antigen binders, cytokine binders, and infrared fluorescent protein. Activity of the recombinant bacteria was confirmed in several cell models, and the microbiota-based approach resulted in a novel therapeutic strategy against colorectal cancer. *The authors marked with an asterisk equally contributed to the work.

ShT-02.5-3
In vitro evaluation of the effects of different foods on the gut microbiota

A. Lerma-Aguilera[1], S. Pérez-Burillo[2], B. Navajas-Porras[2], E.D. León[1], S. Ruíz[1], S. Pastoriza[2,3], N. Jiménez-Hernández[1,4], B. Cämmerer[5], J.Á. Rufián-Henares[2,3], M.J. Gosalbes[1,4], M.P. Francino[1,4]
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The gut microbiota has a key role as a mediator in the impact of diet on health. The potential of diet to modify microbial composition in the short and long term has also been demonstrated. Furthermore, recent findings have pointed to the fact that cooking methods, as an important way of altering the chemical composition of food, also directly influence the gut microbiota. The aim of this study was to further study the gut microbiota growing on foods representative of the Mediterranean and Western diets during *in vitro* fermentations with fecal inocula. To this end, we performed *in vitro* digestions and fermentations of 56 foods using up to 6 cooking methods, making a total of 159 combinations, employing fecal material from healthy adults as inoculum. The resulting microbial composition was determined by sequencing the 16 s rRNA gene. The great heterogeneity among individuals led us to analyze them individually. We detected significant changes in the composition produced by foods using PERMANOVA analysis. The food categories of starchy tubers, fish and animal and vegetable fats stood out as the ones with the greatest differences in composition compared to the rest. In addition, we found changes in the alpha-diversity of the microbiota growing on some foods, although few of them were detected in all individuals. Furthermore, we compared the

differential abundance of taxa among foods using ANCOM methodology. Animal and vegetable fats, fish and dairy products led to the greatest shifts in microbial composition when compared to fruits, grain-based products, legumes and vegetables. Specifically, an increase in *Blautia*, *Lachnoclostridium*, *Faecalibacteirum*, *Lachnospira* and *Roseburia* abundance was identified in animal and vegetable fats. The characterization of the fermentative microbiota would allow us to refine dietary interventions with the aim of modulating the composition of the gut microbiota and move towards the goal of personalized nutrition as therapeutic treatment.

ShT-02.5-4
Gut microbiota changes are associated with gender in paediatric oncology patients in conditioning regime before bone marrow transplantation

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Bone marrow transplantation (BMT) in cancer is still challenging and associated with graft-versus-host-disease (GvHD) characterized by organ inflammation and febrile neutropenia (FN) associated with bloodstream infections. Gender-based changes in gut microbiota can play an important role in both. This study aimed to determine the association of gender and related gut microbiota composition alterations in patients undergoing BMT (T;n = 21) compared to healthy controls (CTRL;n = 14). Samples CTRL and patients (T) collected at Transplantation Unit at University hospital (2018–2020) included both females (F) and males (M) (CTRL-8F/6M; T-8F/13M) aged 8,5 ± 4,7 years (CTRL) and 7 ± 4,8 years(T). Stool samples were collected before BMT, *16S rRNA* gene shotgun sequencing (Illumina MiSeq, 2x300bp) was performed. Data were analysed by QIIME2 and SPSS. Remarkable abundance changes of 43 bacterial genera in males (including *Roseburia, Barnesiella, Akkermansia, Colinsella, Coprobacter*) compared to 14 in females (incl. *Enterococcus, Streptococcus, Faecalibacterium, Lachnospiraceae*-FCS020, *Eubacterium_hallii*) between CTRL and T groups were detected. Changes in the abundance of *Bacteroides, Catenibacterium, and Agathobacter* were found in females with FN/GvHD, but not in males. In conclusion, changes in gut microbiota caused by conditioning therapy affects more male gut microbiota than female. FN/GvHD development-related changes are in female oncology patients associated with additional butyrate-producing taxa disbalance and diminishment of *Bacteroides*.

Thursday 14 July
9:00–11:00, Auditorium VII

Cell–cell recognition

S-03.5-1
Understanding of damage signal propagation, required to build resilient cell walls in plant roots

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Cyst nematodes are biotrophic endoparasites that have developed a very complex infection strategy that involves manipulating plant developmental systems in order to generate neoplastic feeding cells within the host root. The second stage juveniles (J2s) of beet cyst nematode, *Heterodera schachtii*, invade generally near the elongation zone, move across numerous tissue layers to reach the nutrient-rich vascular cylinder to develop permanent feeding sites. During the migratory phase, nematodes release cell wall degrading enzymes, which compromise the cellular integrity of distinct cell files. As a result, when plants detect the collapse of their own cell wall components and the release of cytoplasmic fluid, a number of defensive reactions are triggered. Here, we identified that restricted, single-cell wounding in different root tissues caused by cyst nematodes or laser ablation, causes plant roots to rapidly localize lignin accumulation leading to delay in nematode development. Surprisingly, nematode and laser ablation does not induce a robust lignin accumulation response in the root, but regionally activates genes involved in the phenylpropanoid pathway, which is implicated in the biosynthesis of lignin. This lignin activation depends on ethylene biosynthesis activities, as well as of NADPH oxidases. Overall, the regional signals caused by single-cell wounding stimulation of lignin deposition is not a default effect for general healing, but a specific mechanism to strengthen defense responses around vascular cylinder, and thus appear to constitute a relevant root immune response against small invaders.

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S-03.5-2
Neuron-astrocyte communication: Astroyctic β3 integrin controls life and death decisions in neurons

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The abundance of astrocyte glial cells in the brain and their response to inflammation make them crucial cells to study outcomes related to injury and neurodegeneration. We study "gliosis", a process where astrocytes exposed to inflammation undergo hypertrophy, cell motility, and upregulate reactivity markers, such as GFAP. Naïve astrocytes express low levels of aVβ3 integrin and are unresponsive to its ligand in neurons, the protein Thy-1, whereas under inflammation, reactive astrocytes overexpress this integrin and respond to Thy-1. Importantly, ectopic expression of β3 integrin is sufficient to permit Thy-1-induced responses regardless of the inflammatory setting, implying a key role for the integrin in astrogliosis. However, the mechanisms involved in β3 integrin upregulation during inflammation remain unclear. We re-analyzed public gene expression data from non-reactive/reactive astrocytes to find molecular candidates involved in such mechanisms. We used astrocytes treated with the pro-inflammatory cytokine TNF to evaluate astrocyte reactivity markers, β3 Integrin, mRNA and protein levels of dysregulated molecules, astrocyte migration and the effects of TNF-treated astrocytes on neurons. Proteins of the endosome pathway were found downregulated according to *in silico* and *in vitro* analysis. Reduced expression of endocytic proteins in TNF-treated astrocytes was necessary to upregulate β3 Integrin, promote neuron-induced astrocyte migration, and to induce neurite retraction/death, since all these outcomes were prevented by overexpression of such proteins. In summary, inflammation-induced downregulation of proteins in the endocytic pathway increases β3 Integrin levels and promotes astrocyte reactivity. Hence, molecular mechanisms that control the expression of endocytic pathway components are important regulators of astrogliosis and neuronal fate. ACKNOWLEDGMENTS, work funded by ANID grants FONDECYT 1200836 (LL), 1210644 (AFGQ), 3170169 (JD), FONDAP 15130011 (AFGQ). For further talks in this session - see pp.53-54

Thursday 14 July
9:00–11:00, Auditorium VIII

Metabolism and metabolic regulation

S-04.5-1
Mechanisms of endocrine disruptors impact on metabolic outcomes

R. Barouki
Inserm unit 1124, Université de Paris, Paris, France

The incidence of metabolic diseases (obesity, diabetes, metabolic syndrome, chronic liver diseases...) has been increasing for several years. The role of environmental stressors in these diseases has been supported by recent epidemiological and toxicological studies. I will review the most important metabolic disruptors by discussing their mechanisms of action and will propose an integrated pathophysiological vision of their effects on several organs. The relevance of the Adverse Outcome Pathways in this context will be developed. The impact of these mechanistic approaches in regulatory toxicology and in decision making will be discussed and put into perspective.

S-04.5-2
Riboregulation of human serine hydroxymethyltransferase enzymatic activity reveals a novel strategy to shape cell metabolism

F. Cutruzzolà
Sapienza University of Rome, Rome, Italy

RNA-binding proteins are known to regulate RNA metabolism and function. A novel mechanism, named riboregulation, suggests that also RNAs can regulate protein's function (1). By studying human Serine hydroxymethyltransferase (SHMT), a key enzyme in the metabolic reprogramming of cancer cells, we demonstrated that an enzyme's catalytic activity can be controlled by RNA (2). We have also shown *in silico* and in cell lines that the levels of cellular metabolites (serine/glycine) can be modulated by these RNAs (3). The newly determined cryo-EM structure of the SHMT-RNA complex reveals how protein and RNA interact and unveils the molecular basis of RNA-mediated allosteric regulation. Moving a step forward, we demonstrate that RNA molecules can act as metabolic switches, controlling the survival of lung cancer cells. Given that many enzymes in intermediary metabolism are RNA-binding proteins, these results suggest that novel regulatory networks connecting intermediary metabolism and cellular RNAs can be discovered and exploited to control cell fate. 1) Horos R, *et al.* The Small Non-coding Vault RNA1-1 Acts as a Riboregulator of Autophagy. Cell. 2019; 176:1054–1067. 2) Guiducci, *et al.*, The moonlighting RNA-binding activity of cytosolic serine hydroxymethyltransferase contributes to control compartmentalization of serine metabolism, Nucleic Acids Research, 2019, 47:4240–4254. 3) Monti M, *et al.* Modelling of SHMT1 riboregulation predicts dynamic changes of serine and glycine levels across cellular compartments. Comput Struct Biotec 2021; 19: 3034–3041.

ShT-04.5-1
Cholesterol synthesis unleashed: hypoxia induces the proteasomal truncation and constitutive activation of squalene monooxygenase

H. Coates, I. Capell-Hattam, A. Brown
University of New South Wales, Sydney, Australia

Squalene monooxygenase (SM, also known as squalene epoxidase), a rate-limiting and oxygen-dependent enzyme of the committed cholesterol synthesis pathway, is rapidly emerging as an oncogene in numerous malignancies. The SM protein is subject to feedback regulation *via* cholesterol-induced proteasomal degradation, which depends on its lipid-sensing N-terminal degron. However, many details of the SM degradation mechanism remain unknown, and this knowledge will be essential for designing

therapeutic strategies to target the enzyme. Here, we characterize a truncated form of SM that is endogenously expressed by a variety of human cell types. We find that it differs from full-length SM in two major ways: (1) complete resistance to cholesterol-induced degradation, and (2) an altered endoplasmic reticulum membrane topology. However, thin layer chromatography assays reveal that truncated SM retains full catalytic activity and is therefore constitutively active. SM is truncated via partial proteasomal degradation, a rare phenomenon observed for no other eukaryotic enzymes to date, and only two other human proteins. Through targeted mutagenesis, we show that truncation eliminates cholesterol-sensing elements within the N-terminal degron of SM, and is dependent on both an intrinsically disordered region near the truncation site and the stability of the adjacent catalytic domain, which is spared from degradation. Importantly, ongoing work has uncovered that hypoxic conditions dramatically induce SM truncation. This truncation is correlated with the accumulation of the SM substrate, squalene, implicating the metabolic intermediate in partial proteasomal degradation of SM. Our findings shed further light on the complex mechanisms governing post-translational regulation of cholesterol synthesis. They also suggest a role for truncated SM in maintaining metabolic homeostasis under environmental stresses, as well as in the oncogenic effects of SM overactivity.

ShT-04.5-2
Smoothelin-like protein 1: new quarterback in the field of insulin resistance

A. Ungvari, I. Tamas, I. Keller, **B. Lontay**
Department of Medical Chemistry, University of Debrecen, Debrecen, Hungary

Insulin resistance occurs as a variety of disorders including type 2 diabetes, hyperinsulinemia and obesity. Pregnancy can also diminish sensitivity to insulin by the action of progesterone. We hypothesize that the smoothelin-like 1 protein (SMTNL1) as a highly selective progesterone receptor-B (PR-B) corepressor can regulate the gene expression of metabolic enzymes and the elements of insulin signaling. The phenotype of the skeletal muscle (SKM) by its mass determines the metabolic state of the body and its transformation is regulated by progesterone specifically through PR-B. We demonstrated that pregnancy promotes fiber-type changes from an oxidative to glycolytic isoform in SKM. Smtnl1−/− mice were metabolically less efficient and showed impaired glucose tolerance. Pregnancy antagonized these effects by inducing metabolic activity and increasing glucose tolerance. We also studied the role of SMTNL1 in insulin signaling in differentiated C2C12 cells in an insulin resistance model. The Ser-phosphorylation of insulin receptor substrate 1 (the marker of insulin resistance) was decreased upon SMTNL1-overexpression and the PKC, Akt and mTOR-related signaling pathways were involved in the regulatory mechanism of SMTNL1 by Proteome Profiler analysis. The metabolic properties of WT and SMTNL1 overexpressing-differentiated C2C12 cells were investigated by measuring the bioenergetic parameters of cell metabolism, such as oxidative phosphorylation and glycolysis. The overexpression of SMTNL1 in the presence of progestin seemed to increase the metabolic properties of C2C12 cells. Finally, we developed PR-B selective membrane permeable inhibitory peptides which can mimick the coregulatory action of SMTNL1, resulting in a change of gene expression and promoting tissue-specific insulin sensitivity in the SKM. We suggest potential therapeutic application of the SMTNL1-mimick peptides that might improve the metabolic condition of patients with insulin resistance.

ShT-04.5-3
The effect of the Akt-AMP kinase signaling pathway on glycolysis metabolism in the differentiation process of mesenchymal stem cells to neural progenitor cells

I.N. Gokbayrak Atay[1], R.U. Bora[2], T. San[1], P. Akan[1]
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In our study, we aimed to evaluate the relationship between possible changes in glycolysis metabolism and the energy-dependent AMP kinase and Akt signaling pathway during neuronal differentiation of human wharton jelly-derived mesenchymal stem cells (WJ-MSC). During the differentiation process, time-dependent changes in cellular lactate, pyruvate, AMPK enzyme and Acetyl CoA levels were measured by colorimetric photometric and fluorometric method in the presence and absence of Akt, which inactivates the AMPK enzyme. On the 7th day of neuronal differentiation induction, AMPK enzyme levels were significantly decreased ($p < 0.05$), and the AMPK levels increased after Akt inhibitor application. In WJ-MSCs, it was shown that cellular lactate levels decreased depending on the induction time ($p < 0.05$). On the 7th day of neuronal differentiation, it was determined that pyruvate levels decreased significantly compared to cells without induction group, and acetyl CoA levels were increased. While this decrease observed in pyruvate levels was prevented by Akt inhibition, it was observed that acetyl CoA levels increased even more. The significant decrease in AMPK levels on the 7th day when neural progenitor cells are formed in the differentiation process supports the hypothesis that this enzyme, which suppresses lipid synthesis, may play a key role in the formation of mature neurons. However, the change in acetyl CoA and pyruvate levels observed with inhibition of Akt suggested that the Akt-AMPK signaling pathway may play a dominant role in acetyl CoA metabolism. On the other hand, in the presence of Akt, the capacity of WJ-MSCs to transform into mature neurons may be promoted, along with a decrease in the AMPK enzyme level and an increase in the utilization of pyruvate. Further studies will guide the use of lactate-acetyl CoA/pyruvate levels as a biomarker in neuronal differentiation steps in WJ-MSCs.

ShT-04.5-4
Interaction of cell metabolism and oxidative stress in photodynamic therapy

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Photodynamic therapy is a promising therapeutic tool for the treatment of solid tumors, but also for the eradication of microbial biofilms. Interestingly, both tumor and biofilm

microenvironment resemble common metabolic features, including lower supplementation with nutrients, hypoxia, accumulation of metabolic products, and the vital need for metabolic symbiosis between cells. We have developed a class of photosensitizers based on octahedral molybdenum cluster complex, where the coordination of carboxylate ligands to the {Mo6I8}4+ core allows a fine-tuning of physical and biological properties and has the potential for additional functionalization. We have also demonstrated the photodynamic effect of Mo clusters against bacterial biofilms and cancer cells. Here we demonstrate a strong interaction between the metabolic status of target cells and their sensitivity to photodynamic therapy. Specifically, low levels of nutrients and high levels of metabolic products dramatically increased the efficiency of photodynamic therapy due to the effect on NADPH levels that further determine the antioxidant capacity of cancer and bacterial cells. Therefore, metabolic manipulation including the application of approved inhibitors is a promising tool enhancing the therapeutic efficiency of photodynamic therapy. This work was supported by grant no. 21–16084 J given by the Czech Science Foundation.

Special Sessions

Sunday 10 July
16:00–18:00, Auditorium VIII

FEBS Special Session on Research and Career Skills

SS-RCS-1.
How to write a good review article

P. Dhillon
The FEBS Journal, Cambridge, United Kingdom

The writing of a review article is a useful skill to develop early on in your career as a scientist, whether pursuing an academic career path or not. In any field of science, it's important to read widely to keep up to date with the latest developments, and the process of writing a review encourages you to critically evaluate the strengths and weaknesses of the literature to extract the most pertinent information. Writing a review can provide inspiration for your own work as well as enhancing your publication record and highlighting your in-depth knowledge of a research area. A good review can be a valuable resource that is widely accessed by the scientific community and cited even years after publication, but it takes time and plenty of practice to develop the art of writing such an article. Here, I provide tips on planning and writing a review article, with examples of good and bad practice.

SS-RCS-2.
How to get the most out of an academic conference

F. Michelangeli
University of Chester, Chester, United Kingdom

My short presentation will highlight the different types of academic conferences that are available and the benefits, both direct and indirect, that can be gained from attending these events. I will also give a personal perspective of how attending conferences greatly aided my career, especially when I was an early career scientist.

SS-RCS-3.
How to prepare a proper lab book

J. Perret
Faculty of Medicine - UNIVERSITE LIBRE DE BRUXELLES (ULB), Brussels, Belgium

Keeping a lab book is one of the most overlooked skills during university studies, be it undergraduate, graduate or postgraduate studies, be you a lab scientist, architect, engineer, medical doctor, nurse, etc. However, astonishingly, all academics and professionals will agree that this is an essential skill at all levels. Failure to write, keep and "upgrade" a lab notebook may lead to damageable (scientifically and even legally) and sad situations (crucial data loss). Furthermore, in many settings, namely industry, it is mandatory to provide a "lab notebook" responding to defined criteria. Not to be overlooked is that the lab notebook is also an essential tool of communication with your PI, colleagues and management. The talk and workshops will try to make you apprehend the importance of your lab notebook. We will also address the emergence of the digital Lab Notebooks and discuss the issues surrounding this evolution. The lab book is your "LOGBOOK" : it should reflect what you plan to do, what you did (and all changes to the plan), what happened while you were doing it (this is of critical importance), the observations made all along, the outcome(s), remarks and finally the interpretation – "what does it mean?", leading to "what next?". When all is going smoothly then there is no problem. However, when the going gets rough and things do not look so good, then those details in your lab notebook will allow you to evaluate where the problems could be and thereby propose solutions, and most likely save you precious time and effort. These are just some of the requisites behind the art of consigning the "the science" being done at the bench.

Monday 11 July
13:30–14:30, Auditorium II

Biochemical Society International Award Lecture

SS-BSIA-1
The kiss of death: understanding how the zombie protein, MLKL, is triggered to kill cells by necroptosis

J. Murphy
Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

In 2012, Mixed lineage kinase domain-like (MLKL), a catalytically-dead ("zombie") cousin of conventional protein kinases, termed a pseudokinase, was implicated as the key effector in the programmed necrosis (or necroptosis) cell death pathway. This pathway has been implicated in innate immunity, the pathogenesis of inflammatory diseases, and tissue injury arising from

ischemia–reperfusion. As a result, an improved fundamental knowledge of MLKL's activation mechanism is of enormous interest as we and others look to target the pathway therapeutically. Here, I will describe our recent work dissecting the chronology of events in this pathway using novel tools, structural biology, biochemistry, microscopy and proteomics.

Monday 11 July
16:00–18:00, Auditorium VIII

FEBS Special Session on Gender Issues in Science

SS-GIS-2
Woman and leader: to be or not to be

M. Pizza
GSK Vaccines, Siena, Italy

During my career I have contributed to the discovery and licensure of two innovative bacterial vaccines. Although moving from pure to applied research and from academia to industry scared me, I was aware that it was an opportunity not to be missed owing to the invaluable experience I could gain. This experience opened new horizons for me, and over the years I developed the perception of being able to contribute concretely to the resolution of public health problems. The transition has not been easy, but the success of this exciting new adventure has been driven by the way of working in that environment and being part of the team with whom I have shared motivation, dedication, and attitude of facing challenges together and which have contributed to who and where I am today. I have postponed the joy of motherhood for years, thinking I was too busy and that it was never the right time. I was afraid of not being able to dedicate time at work at the same pace as a man. The feeling of inadequacy as a scientist and as a mother is still hard to overcome. I still have the perception of the struggles that we are facing as women. Although we are increasingly successful in many fields, in many tables and occasions we are still under-represented. We should gain awareness that we can be equally brilliant and successful also at higher levels not necessarily by adopting a male attitude. We can be more powerful building on our incredible innate strengths: perseverance, ability to multi-task, ability to listen, ability to create strong support networks, and to bring values. We can be equally brilliant, but we need the courage to go out of our comfort zone and take leadership roles, believe in our potential and not be afraid to be evaluated. Science and society are moving exceptionally fast and as women we must feel not the right but rather the duty to play a key role as scientists and as key leaders that sit at the same table with men to design a better and sustainable future.

SS-GIS-3
Being a woman in science in the 20th century and beyond

M. Pais
Academy of Sciences of Lisbon, Lisboa, Portugal

In my presentation I intend to share with you my experience as a woman scientist and University Professor since 1963 until 2003 and thereafter; followed by my active participation as General Secretary of the Academy of Sciences of Lisbon. Being a scientist, to me is a passion and although being a woman, gender never compromised the accomplishment of my objectives and my career. As a scientist the most grateful achievement was the contribution to plant science improvement in particular woody plants, through development of research in molecular and cell biology. As a Professor, I am thankful to my students and coworkers that followed me and will continue in Portugal or abroad the passion as scientists and in particular as plant scientists contributing to the understanding of the role of plants in the biosphere and its role to cope with the global changes and to the sustainability of man in our endangered planet.

SS-GIS-1
Women in science: what is the problem? Is the situation changing?

P. Cossart[1,2]
[1]*Institut Pasteur, Paris, France,* [2]*EMBL, Heidelberg, Germany*

For many years now, women in science have been struggling to be considered as well as men in science but we are obliged to realize that while some situations are really improving, other are not. One of the most striking situations is the number of women as speakers at small and highly searched meetings, another is the number of women on some prestigious evaluation committees. Even more striking is the number of women as advisors in industry. During the talk, I will discuss my own view on these issues.

SS-GIS-4
Gender and science in Brazil

L. Quercia Vieira
Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Although graduate programs in Biological Sciences have about 50% of women as students, as in the rest of the world higher positions in academia and financial support are not distributed equally. The data will be presented and the new strategies to correct these distortions will be discussed. Financial agencies and scientific societies are more aware of gender biases, however, Brazil is still largely gender prejudicial.

Tuesday 12 July
16:00–18:00, Auditorium VII

FEBS Special Session on Science & Society – RNA solutions to genetic and infectious diseases

SS-S&S-2
Applications, challenges and opportunities of RNA drugs

L. Desviat
Centro de Biología Molecular Severo Ochoa-Universidad Autónoma de Madrid, Madrid, Spain

RNA-based therapies were considered a curiosity until recently, used mainly in research in rare diseases and personalised therapies. However, this research field has gained new momentum, as it has effectively increased the "druggable" space; RNA drugs are easy to design and are cost effective, with greatly improved pharmacokinetic properties thanks to progress in oligonucleotide chemistry over the last few years. Moreover, the massive clinical scientific success of COVID-19 RNA vaccines has boosted the interest of both scientists and lay public for this type of molecules. Currently, there are more than a dozen RNA-based drugs approved for clinical use and many others in different stages of development. RNA-based therapeutics include antisense oligonucleotides, aptamers, small interfering RNAs, microRNAs and messenger RNAs. To date, the future of RNA therapeutics relies on overcoming the major challenges in delivery, requiring further research and collaborations in the fields of chemistry, biology and medicine.

SS-S&S-1
The Dutch Center of RNA Therapeutics: developing mutation-specific antisense oligonucleotide therapies for patients with eye and brain diseases carrying unique mutations

A. Aartsma-Rus[1,2], M. Lauffer[1], R. Collin[3], Y. Elgersma[4], W. van Roon-Mom[1]
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Antisense oligonucleotides (AONs) offer the potential to treat patients with genetic diseases. Notably, for tissues allowing local injection, such as the brain and eye where high local exposure can be achieved with 3–4 infusions of low amounts of AONs annually. Proof-of-concept has been shown for example in spinal muscular atrophy and Leber congenital amaurosis. This approach can also benefit patients with private mutations, as was recently evidenced by the development of the custom-made AON milasen for a patient with Batten's disease. This underlines the potential of AONs as personalized medicines, specifically for patients with private mutations that are associated with brain or eye phenotypes. However, pharmaceutical companies are usually not interested in the development of such approaches, due to the extreme rarity of these variants. The Dutch Center of RNA Therapeutics (DCRT) is a collaboration of Dutch academic centers with a track record in AON development that aims to develop therapies for patients with nano-rare variants and to offer these therapies in a not-for-profit manner. The DCRT works in alignment with the N-of-1 collaborative (global) and the 1 mutation 1 medicine (1M1M, European) initiatives. In the first two years, we have identified several patients with mutations that are suitable for splice modulation by AONs. Here, we outline the pre-clinical development of AON-based splice correction for a cryptic splicing mutation underlying Stargardt disease affecting the eye, and Beta-propeller Protein-Associated Neurodegeneration (BPAN) affecting the brain. We describe the Dutch roadmap towards clinical implementation, highlighting also the efforts to align developments internationally.

SS-S&S-3
Nucleic acid vaccines: A new era for RNA and DNA vaccines and immunotherapies

G.N. Pavlakis
National Cancer Institute, Frederick, MD, United States of America

The power of modern molecular genetics together with other technological achievements has made possible a new era of therapeutic applications, which I call Interventional Genetics, i.e., the use of molecules carrying genetic information (DNA, RNA) for therapeutic purposes. Gene therapy is a large part of Interventional Genetics, but in recent years the use of nucleic acid technologies for therapeutic purposes have advanced beyond the classical definition of gene therapy. They include the use of native and artificial nucleic acid molecules as drugs and vaccines. My talk will focus on one aspect of Interventional Genetics, the use of DNA or RNA to produce new vaccines and immunotherapies. This is a brand-new application, but with a long history of preclinical development. Several technical and conceptual barriers had to be overcome before the acceptance of practical applications, which came famously with two new approved vaccines for coronavirus. Multiple delivery technologies will make practical applications reliable for multiple purposes, including vaccines and immunotherapies. I will discuss advantages and limitations of nucleic acid vaccines and several recent examples of these technologies.

Tuesday 12 July
16:00–18:00, Auditorium VIII

FEBS/IUBMB Special Session on Education – Where we go from here? Experiences, lessons learned and projections for hybrid post-COVID education

SS-Edu-2
The DJ-ification of education

C. Delgado Kloos
Universidad Carlos III de Madrid, Leganés (Madrid), Spain

There are live music concerts and recorded ones. But live and recorded performances are not the only way to enjoy music. A DJ

can mix and blend recorded music and introduce live elements into it, creating a unique experience. It is also too simplistic to classify teaching into pure face-to-face, online, and asynchronous modes of delivery. Multiple interesting modes can be found by combining where people (instructors, TAs, subgroups of students) are located, what is shown (live, predesigned/recorded, mixed), how interaction takes place (direct, cloud-based, mixed), and what devices are available and how spaces are equipped. Blended, hybrid, and HyFlex are examples of mixed modes of delivery. However, we are far away from exhausting the ample combinations that are possible. In this talk, I will present some illustrative examples of the educationally interesting combinations. After this talk, I hope you will be convinced that the traditional face-to-face mode of delivery is definitely not the optimal one and that you will use your imagination to identify new rich technology-enhanced ways to teach.

SS-Edu-1
Hybrid learning is here to stay and it is even more about student engagement and experiences

T. Kuit
University of Wollongong, Wollongong, Australia

As the COVID-19 pandemic continues globally, educators are facing the reality that hybrid learning is here to stay. In practical based subjects like the molecular life sciences this presents challenges, but also opportunities. The forced move to remote online delivery during the pandemic provided opportunities to reinvigorate and critique our teaching like never before. As we move forward, we are being tasked with deciding what aspects of our courses are essential to be delivered face-to-face and what can best be delivered online, and how do we ensure students have equitable learning opportunities. In the sciences we offer practical experiences as places for skill development and linking of theory with application, but also for social connection. Participation in these authentic activities provide opportunities for students to establish a scientific identity and a sense of belonging to the scientific community. What our students have shown us over the last 2 years is that engagement and social connection online is even more essential for their success, alongside the provision of authentic experiences that are meaningful and well-structured. Through the exploration of a case study from a research-intensive university in Australia, I will share insights from the delivery of a large first year molecular biology course (>850 students), a second year biochemistry course (>450 students), and a research-intensive third year capstone course (24 students), which were delivered completely online or in a hybrid model in 2020–2022. Students very clearly state that online learning is not preferred, however the flexibility with online learning can develop many key employability skills, particularly in regards to communication, teamwork, creativity and digital literacy. During this presentation, I welcome discussion on experiences of educators and shared insights in paving a new way forward in the hybrid delivery model of traditionally intense face-to-face courses.

SS-Edu3
Digital strategies for blended and interdisciplinary learning

R. Clyne
Queen Mary University of London, London, United Kingdom

The pandemic abruptly disrupted teaching and learning approaches and swiftly steered attention toward blended delivery models. Blended learning refers to educational approaches that integrate digital learning tools with more traditional teaching methods. In molecular biosciences, video and virtual labs are examples of blended learning tools that can be employed to increase student engagement and solidify learning. A major area of my teaching practice has been fostering laboratory, research and cross-disciplinary skills using such blended learning approaches. In this talk, I will reflect on my experience delivering remote teaching for a transnational education programme in China during lockdown. I will describe my collection of biomedical technique demonstration videos and an online virtual lab to support engagement and learning of experimental skills, and present feedback collected from multiple cohorts that highlights the importance of videos and why students value them. I will also outline a cross-discipline student video design project in China and a leadership development programme in London for collaborative learning, and discuss the solutions implemented to minimise the impact on these initiatives that was brought on by the onset of lockdown. These approaches offer resources and promising digital alternatives for learning and skills development in future hybrid environments.

Wednesday 13 July
16:00–18:00, Auditorium VIII

FEBS/IUBMB Special Session – Unlocking SARS-CoV-2

SS-SC-2
Computational microscopy of SARS-CoV-2 *in situ*

R. Amaro
University of California, San Diego, La Jolla, United States of America

I will discuss our lab's efforts, together with collaborators, to use computational microscopy to understand the SARS-CoV-2 virus in atomic detail, with the goals to better understand molecular recognition of the virus and host cell receptors, antibody binding and design, and the search for novel therapeutics. I will focus on our studies of the spike protein, its glycan shield, its interactions with the human ACE2 receptor, our ACM Gordon Bell Special Prize winning efforts to model the SARS-CoV-2 virion, and escape variants. I will also discuss our efforts to completely revise current models of airborne transmission of respiratory viruses by providing never-before-seen atomic level views of the SARS-CoV-2 virus within a respiratory aerosol.

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SS-SC-1
Structural biology at Sirius and the SARS-CoV-2 pandemic – challenges and opportunities for global collaborations

A. Zeri
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The SARS-CoV-2 pandemic, waning now after over two years, generated a global response from the structural biology community. The first experiments at the 4th generation Synchrotron source SIRIUS, in Brazil, were focused on the structural studies of the viral proteases, including those encoded by SARS-CoV-2, their transition states and potential ligands. In this talk, we will present some of the findings concerning SARS-CoV-2 proteases and the status of MANACA beamline, as well as the latest developments in phasing (native SAD), multi-crystal, serial and room-temperature data collection. The MX beamline, MANACA, (MAcromolecular micro and Nano Serial CrystAllography), was commissioned during 2020, and the initial results helped to assess not only important features of the proteins and ligands, but also the quality and potential of the new beamline. Natural products and fragment libraries have been used by our users and collaborators [1], in academic and industrial settings. MANACA is optimised for high flux, micro-beam size and small beam divergence (0.44 mrad). Setups for serial crystallography data collection and analyses, as well as automation procedures, are being prepared [2]. The great beam characteristics provided by Sirius [3] and the high stability and precision of the optics and experimental station allows the diffraction of challenging samples such as viruses (and other crystals with large unit cells), membrane proteins and complexes, which commonly yield small crystals. The experiment control uses a user-friendly graphical interface (MXCuBE) [4], and automatic data processing (from data reduction to initial modelling) is available. The MANACA beamline is also prepared for remote access and has already performed remote experiments with foreign scientists. [1] Noske, G.D. *et al*., *Journal of Molecular Biology*, Volume 433, Issue 18 (2021). [2] Nascimento, A.F.Z. *et al*. *Synchrotron Radiation News* 34, 3-10 (2021). [3] Liu, L., Milas, N., Mukai, A. H. C., Resende, X. R. & Sa, F. H. de. *J Synchrotron Rad* 21, 904-911 (2014). [4] Oscarsson, M. *et al*. *J Synchrotron Rad* 26, 393-405 (2019).

SS-SC-3
From crystallographic fragment screen to preclinical candidate: Open science discovery of SARS-CoV-2 antivirals

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The development of novel, low cost and globally available antiviral therapeutics remains an essential goal for the current SARS-CoV-2 pandemic. Furthermore, future pandemics could be prevented with easily deployable broad-spectrum oral antivirals and open knowledge bases that de-risk and accelerate novel antiviral discovery and development. To identify starting points for the development of such therapeutics, the XChem team at Diamond Light Source, in collaboration with various international colleagues, performed large crystallographic fragment screens against 8 key SARS-CoV-2 protein targets including the Main protease[1], the Nsp3 macrodomain[2] and the helicase Nsp13[3]. The expeditious collection and open dissemination of the data from these fragment screening campaigns was enabled by the well-established platform at Diamond Light Source and by the implementation of various experimental and computational tools. This work identified numerous starting points for the development of potent anti-viral therapeutics as exemplified by the COVID Moonshot – a fully open-science structure-enabled drug discovery campaign targeting the SARS-CoV-2 main protease.[4] By leveraging crowdsourced medicinal chemistry design, high throughput structural biology, machine learning and exascale molecular simulations, we discovered a novel chemical scaffold that is differentiated to current clinical candidates in terms of toxicity and pharmacokinetics liabilities, and developed it into orally-bioavailable inhibitors with clinical potential within 2 years. All compound designs, structural data, assay data and synthesized molecules have been shared rapidly and openly, creating a rich, IP-free knowledgebase for future anti-coronavirus drug discovery. 1. Douangamath, A. *et al*., Nat Commun., 11, 5047 (2020). 2. Schuller, M. *et al.,* Science Advances, 7 (2021). 3. Newman, J. A., *et al.,* Nat Commun., 12, 4848 (2021) 4. The COVID Moonshot Consortium, *bioRxiv,* 10.1101/2020.10.29.339317 (2020).

ShT-SC-2
High-resolution interaction prediction in the CAPRI covid-19 open science initiative

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SARS-CoV-2 has been deregulating society for over two years. Whereas targeted interactions allow the virus to recruit signaling pathways in order to facilitate replication, fortuitous host-pathogen interactions lead to the emergence of covid-19 at various levels of severity. The CAPRI community has been mobilizing its resources and expertise to model the structures and interaction interfaces of SARS-CoV-2 to human protein complexes. A comprehensive list of putatively interacting protein pairs has been prioritized from a Y2H-determined protein–protein interaction map, out of which five targets were offered for prediction in a uniquely open initiative, sharing data, analyses and results as they were being produced. Close to 30 predictor groups produced close to 30 GB of data, representing more than 50,000 three-dimensional interaction models. These have been processed and filtered by approximately the same amount of scorer groups using a plethora of scoring functions to produce an enriched subset of some 1000 structures. We have further reduced this set through clustering and contact overlap scoring to produce a high-resolution community consensus prediction for the host-pathogen interactions offered. The results show a varying degree of reliability for the targets, with generally a better consensus for the interaction surface on the human protein than on the SARS protein. The results also show the feasibility of such large-scale approaches and the added value of using several, distinctly different, prediction methodologies to reach a consensus prediction.

ShT-SC-1
Cysteine peptidase inhibition: The way to impair SARS-CoV-2 infection and replication

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Since its outbreak, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) caused one of the most severe pandemics in recent history and had enormous effects on the lives of millions of people worldwide. Peptidases, both viral and host, have been described as critical enzymes in mechanisms underlying SARS-CoV-2 infection and replication. Among host peptidases, the involvement in activation of viral glycoproteins and processing of the SARS-CoV spike protein has been described for endosomal/lysosomal cysteine peptidases cathepsins B and L, which therefore represent promising targets for development of effective drugs for treatment of COVID-19. To date, a large number of cathepsin B and L inhibitors have been identified and evaluated for treatment of various pathological processes. In this study, we have evaluated well-established known potent selective and reversible cathepsin B inhibitors for their potential to act against SARS-CoV-2. Cathepsin B inhibitors showed significant activity in preventing viral entry and replication of SARS-CoV-2. Next, we observed that antiviral activity of compounds was dependent on the cell type and correlated well with the intracellular amount of the targeted cathepsin. Taken together, we have demonstrated the important role of host cysteine peptidase cathepsin B during SARS-CoV-2 infection and identified its inhibitors as potential new therapeutic agents for treatment of SARS-CoV-2 infection.

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