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TALKS

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About these abstracts

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TALKS

Plenary Lectures

Saturday 29 June
16:00–17:00, Silver Room

Opening Plenary Lecture

PL-01-1
Cancer-resistant mice

B. Beutler
UT Southwestern, Dallas, Texas, United States of America

For many years we used random germline mutagenesis in mice to create new phenotypes, with an emphasis on immunity. Solving phenotypes – generally qualitative rather than quantitative traits – was achieved by positional cloning. Positional cloning depends on meiotic mapping: the use of meiotic recombination to confine the causative mutation to a small region of the genome. Generally, years were required to map a mutation to a critical region, although several mutations might be mapped in parallel in a large laboratory. Meiotic mapping was automated by statistical computation and machine learning. "Automated meiotic mapping" (AMM) allows us to establish cause and effect in real time: when a phenotype is detected, its mutational cause is now identified and brought to our attention simultaneously. Phenotypes need not be qualitative any longer: it is possible to unambiguously identify causative mutations even when there is considerable overlap between the phenotypes of heterozygous and homozygous populations. The degree of genome saturation is also monitored as a screen was pursued. AMM made forward genetics in mice thousands of times faster and allowed us to pursue screens that would otherwise have been impossible. Most notable among these are disease suppression screens. Using AMM, we have discovered numerous point mutations that suppress ordinarily lethal cancers in mice. Such mutations often point to targets for drug discovery.

Saturday 29 June
18:30–19:30, Silver Room

FEBS Datta Lecture

PL-02-1
Transcription of the genome: from molecular mechanism to cellular regulation

P. Cramer
Max Planck Society, Munich, Germany

Our laboratory uses a combination of structural biology, functional genomics and computational approaches to elucidate the molecular mechanisms and cellular regulation of transcription, the first step in gene expression that governs differentiation, development and cancerous cell growth. In my lecture I will provide an overview of our current understanding of the molecular mechanisms underlying gene transcription in eukaryotic cells. Amongst other things, I will discuss how chromatin remodelling leads to promoter accessibility, how the transcription preinitiation complex containing RNA polymerase II (Pol II) and the Mediator will form, and how Pol II elongation complexes can adopt paused, pre-termination and active states, and how the transcription machinery interacts with parts of the spliceosome and the DNA repair machinery. I will also provide data that suggest that transcription is regulated at three key steps, initiation, promoter-proximal pausing and early termination. Also, I will present unpublished work that elucidates the dynamic transitions between different transcription complex intermediates and provide an outline for future challenges.

Sunday 30 June
11:00–12:00, Silver Room

FEBS Sir Hans Krebs Lecture

PL-03-1
Innate immunity and inflammation: from molecules to cancer and COVID-19

A. Mantovani
Istituto Clinico Humanitas, Humanitas University, Milan Area, Italy

The immune system is an extremely complex orchestra. Alterations of immunity and inflammation represent a metanarrative of medicine, spanning from infectious diseases to cardiovascular pathology to cancer. In the context of a focus on innate immunity and inflammation we discovered genes (e.g. PTX3 and IL-1R8) and their functions. As a case in point, the effort on PTX3 included a pathway from gene identification, to structure and function, to bedside. More recently we engaged in elucidating the function of genes of unknown function, possibly involved in innate immunity, using genetic approaches. Genetic and structure/function studies have opened new vistas on innate immunity and inflammation in cancer and infection, including COVID-19. References: Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature 454(7203): 436-444, 2008. Garlanda C, Bottazzi B, Magrini E, Inforzato A, Mantovani A PTX3, a humoral pattern recognition molecule in innate immunity, tissue repair and cancer. Physiol. Rev. 98(2): 623-639 2018. Mattiola I. et al. The macrophage tetraspan MS4A4A enhances dectin-1-dependent NK cell-mediated resistance to metastasis. Nature Immunol. 20(8):1012-1022, 2019. Mantovani A, Dinarello CA, Molgora M, Garlanda C IL-1 and related cytokines in innate and adaptive immunity in health and disease. Immunity 50(4):778-795, 2019. Stravalaci M, et al. Recognition and inhibition of SARS-CoV-2 by humoral innate immunity pattern recognition molecules. Nat Immunol. 23(2):275-286, 2022. Mantovani A, Allavena P, Marchesi F, Garlanda C. Macrophages as tools and targets in cancer therapy. Nature Rev. Drug Discov. 21: 799-820, 2022. Mantovani A., Garlanda C. Humoral innate immunity and acute phase proteins. N. Eng. J. Med. 388(5):439-452, 2023.

Sunday 30 June
12:00–12:45, Silver Room

FEBS 2024 Special Plenary Lecture

PL-04-1
The century of vision in protein biochemistry

R. Huber
Max-Planck-Institut für Biochemie, Martinsried, Technische Universität München and Universität Duisburg-Essen, Germany

Abstract unavailable.

Sunday 30 June
15:30–16:00, Silver Room

The FEBS Journal Lecture

PL-05-1
Clathrin-mediated membrane traffic and mechanisms of tissue specialization

F. Brodsky
University College London, London, United Kingdom

Clathrin-coated vesicles selectively capture cargo proteins for transport between membranes. This intracellular membrane traffic enables cells to function in tissues by regulating plasma membrane composition and generating specialized intracellular organelles. Self-assembly of triskelion-shaped clathrin proteins drives vesicle coat formation, and differential binding to membrane-associated adaptors enables cargo transport at distinct cellular locations. We have defined the biochemical and evolutionary mechanisms that have diversified both the clathrin heavy chain (CHC) and light chain (CLC) subunits to drive clathrin specialization in tissues. In humans, two CHC isoforms form clathrin triskelia comprising CHC17 plus CLC subunits and CHC22 triskelia that do not bind CLCs *in vivo*. The more generalized CHC17 clathrin operates in receptor-mediated endocytosis and cargo sorting at endosomes and the trans-Golgi network. Recent studies of knock-out mice lacking genes encoding each vertebrate CLC isoform, CLCa or CLCb, demonstrate that a balance of CLC diversity is critical for tissue-specific CHC17 functions in neurons and epithelia. CHC22 operates in human muscle to traffic the GLUT4 glucose transporter to a holding compartment from which it is mobilized to the cell surface in response to insulin for post-prandial glucose clearance. CHC22 has diverged from CHC17 to enable its recruitment to the early secretory pathway for GLUT4 targeting. Biochemical and cellular studies have defined a bipartite mechanism for CHC22 binding to the early secretory tether p115, through direct binding at the CHC22 N-terminus and sorting nexin 5 binding at the C-terminus. In the muscle of species with insulin-responsive GLUT4 traffic that lack CHC22, convergent evolution has led to alternative splicing of CHC17. Preliminary studies indicate that the resulting CHC17 variant may function similarly to CHC22. The implications for diversification of membrane traffic pathways will be discussed.

Sunday 30 June
16:00–16:30, Silver Room

Molecular Oncology Lecture

PL-06-1
Loss of heterochromatin drives increased genome instability in aging and cancer

S.M. Gasser
ISREC Foundation, Agora Cancer Research Center, Lausanne, Switzerland

Genomic regions are segregated into accessible euchromatin and inaccessible heterochromatin. In *C. elegans*, the SETDB1 homolog MET-2 promotes heterochromatic silencing of satellite repeats, transposable elements and tissue-specific genes by histone H3 K9me. SET-25, a G9a homologue, trimethylates this same residue to repress retrotransposons. Animals lacking met-2 lose fertility, delay development, and have shortened lifespan, which correlate with promiscuous transcription of repeat DNA and the formation of RNA–DNA hybrids on these repeats. We previously demonstrated that MET2's ability to preserve heterochromatin requires its concentration in nuclear foci through physical interaction with an intrinsically disordered protein LIN-65. Intriguingly, the replacement of MET2 with a catalytic-dead allele supports heterochromatic focus formation, blocks inappropriate H3K9 and H3K27 hyperacetylation, and partially restores gene repression, which implicates focus formation in repression. Ablation of the H3K9 HMT MET2 drives significant gene derepression, as well as repeat element transcription, in both embryos and in differentiated tissues. To understand how H3K9me restricts gene expression, we depleted MET-2 in differentiated *C. elegans* muscle and hypoderm cells. The pattern of gene derepression was distinct in embryos, muscle and hypoderm, but in both differentiated tissues germline genes were derepressed. In normal muscle and hypoderm differentiation, we show that H3K9me is lost from tissue-specific genes that should be expressed, and is gained on genes that become repressed during differentiation. The binding of βHLH TFs is prevented by H3K9me in differentiated tissues, substantiating the longstanding hypothesis that heterochromatin controls a subset of promoters in differentiated tissues by restricting TF access to DNA. Thus, H3K9me both stabilizes the genome and drives appropriate gene expression in terminally differentiated cells.

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Monday 1 July
11:00–12:00, Silver Room

IUBMB Lecture

PL-07-1
Looking under the hood of cells: from whole cell organelle reconstructions to single molecule dynamics to atomic reconstructions of macromolecules

J. Lippincott-Schwartz
HHMI Janelia Research Campus, Ashburn, United States of America

Powerful new ways to image the internal structures and complex dynamics of cells are revolutionizing cell biology and bio-medical research. In my talk, I will focus on three emerging technologies capable of revealing new properties of cellular organization at scales ranging from nanometer to atomic resolution. Whole cell milling using Focused Ion Beam Electron Microscopy (FIB-SEM) was used to reconstruct the entire cell volume at 4-nm voxel resolution, revealing all membrane-bound organelles and their trafficking intermediates at isotropic resolution. Single particle tracking using Halo dyes revealed unexpected features of mRNA trafficking, including sites where secretory proteins are translated on ER and their regulation by lysosomes. Finally, High Resolution Template Matching (HRTM) of ribosome subunits in cryo-EM images of intact human cells afforded a look at ribosomes at different stages of peptide elongation at the atomic scale. Together, these new tools open-up a plethora of questions related to mechanisms of cell structure/function that can now be studied in intact cells at the nanometric/molecular level.

Monday 1 July
12:00–13:00, Silver Room

FEBS Theodor Bücher Lecture

PL-08-1
Initiation of translation by the ribosome

V. Ramakrishnan
MRC Laboratory of Molecular Biology, Cambridge, United Kingdom

Initiation of mRNA translation is a key regulatory step in gene expression in all eukaryotes. Canonical initiation of translation in eukaryotes involves recruitment of the 43S pre-initiation complex to the 5′ end of mRNA by the cap-binding complex eIF4F to form the 48S initiation complex (48S), followed by scanning along the mRNA until the start codon is selected. I will discuss our work on the structure of a 48S complex on mRNAs without and with a start codon. In addition to the eIF4A that is part of eIF4F, there is a second eIF4A helicase bound to the mRNA entry site. The entry channel bound eIF4A is positioned through interactions with eIF3 and the 40S subunit to enable its ATP-dependent helicase activity to directly unwind secondary structure located downstream of the scanning 48S complex. The structure also reveals universally conserved interactions between eIF4F and the 48S, likely explaining how this complex can promote mRNA recruitment in eukaryotes.

Tuesday 2 July
11:00–12:00, Silver Room

EMBO Lecture

PL-09-1
To degrade or not to degrade: molecular mechanisms of RNA homeostasis

E. Conti
Max Planck Institute of Biochemistry, Martinsried/Munich, Germany

In eukaryotes, the transcription of protein-coding genes is coupled to processing events (such as 5′ capping, splicing and 3′ polyA-tail addition) that modify the transcripts and coat them with proteins to form messenger ribonucleoprotein complexes (mRNPs). If all biogenesis steps occur correctly, the resulting mature mRNPs are transported through nuclear pore complexes by export factors. Failure in essentially any step of the biogenesis process can result in malformed mRNPs that are retained in the nucleus and eliminated by nuclear quality control pathways, primarily via the action of the RNA-degrading exosome. A large proportion of Pol II transcription indeed terminates before reaching the end of gene, resulting in degradation of the aborted transcripts. How do the quality control machineries recognize aberrant mRNAs/mRNPs and degrade them? Conversely, what are the features of mature, correctly packaged nuclear mRNPs that allows them to evade degradation? The talk will delve into our ongoing biochemical and structural studies, and discuss the current mechanistic understanding of RNA homeostasis.

Tuesday 2 July
12:00–12:30, Silver Room

FEBS Letters Lecture

PL-10-1
Signaling through the ubiquitin-proteasome system

B. Schulman
Max Planck Institute of Biochemistry, Martinsried, Germany

Ubiquitylation is a major eukaryotic post-translational modification that directs proteins for degradation by the proteasome. Regulation depends on hundreds of distinct E3 ligases, which ubiquitylate particular proteins in response to specific signals. Defects in E3 ligase pathways cause or contribute to pathologies across eukaryotes, including human diseases such as cancers, neurodegenerative disorders and viral infections. Meanwhile, harnessing the ubiquitin-proteasome system to degrade disease-causing proteins is an exciting opportunity for developing novel therapies. Our research group takes a multidisciplinary approach

to mechanistically dissect how cells employ ubiquitin-proteasome pathways to respond to extracellular signals. We recently illuminated how a family of 250 distinct but related E3 ubiquitin ligase complexes is regulated by systemwide multiprotein complex formation. Rather than synthesizing or degrading multiprotein complexes when they are needed or not, cells achieve regulation through recycling a limiting component from idling complexes for rapid incorporation into alternative complexes that are newly needed. This averts supply chain problems, obviates a need for producing new parts, prevents build-up of superfluous and potentially toxic molecular machines, and allows rapidly establishing new degradation pathways urgently needed for cellular regulation. We are building on this knowledge to investigate cellular ubiquitylation pathways that are transiently activated to respond to environmental perturbations such as shifts in metabolic conditions, or the presence of immune signals, pathogens, or therapeutic drugs.

Tuesday 2 July 12:30–13:00, Silver Room

FEBS Open Bio Lecture

PL-11-1 Membrane lipids and neural cell signaling: the role of gangliosides

S. Sonnino
Associated to the Department of Medical Biotechnology and Translational Medicine, University of Milan, Milano, Italy

Gangliosides are glycosphingolipids and are particularly abundant in the plasma membrane of mammalian neurons. Their presence in the human brain dates back to the end of nineteenth century but their structure was determined much later in the middle of the 1950s. From this time neurochemical studies rapidly suggested that gangliosides, and particularly GM1 ganglioside, display neurotrophic and neuroprotective properties. The involvement of GM1 in modulating neuronal processes has been studied in detail by *in vitro* experiments and the results indicated its direct role in modulating the activity of neurotrophin-dependent receptor signaling, the flux of calcium through the plasma membrane and stabilizing the correct conformation of proteins such as α-synuclein. In this lecture, we report on the current knowledge on the role played by GM1 ganglioside in regulating the biology of neurons, and on the therapeutic potential represented by the oligosaccharide chain of GM1 ganglioside for the treatment of neurodegenerative diseases.

Tuesday 2 July 15:30–16:30, Silver Room

PABMB Lecture

PL-12-1 New molecular and cellular insights in cardiovascular diseases

S. Lavandero
Advanced Center for Chronic Diseases (ACCDiS), Faculty of Medicine, Universidad de Chile, Santiago, Chile

Cardiovascular diseases, the leading cause of morbidity and mortality worldwide, are characterized by being incurable, slow progression, and resulting from a combination of genetic and environmental risk factors. Cardiomyocytes are the key protagonist cells in cardiac function. They are characterized by their number being defined for the rest of our existence a few weeks after birth, experiencing cell death by different mechanisms or increasing their size (cellular hypertrophy) in response to exposure to hemodynamic stress and/or catecholamines and/or angiotensin II. At the tissue level, it is known as cardiac hypertrophy, a process that precedes the development of heart failure, the terminal stage of different cardiovascular pathologies (high blood pressure, acute myocardial infarction, diabetes, obesity, etc.), characterized by the deterioration of systolic and /or diastolic blood pressure. In this presentation, I will describe two of our investigations that led to (a) the discovery of the anti-hypertrophic and cardioprotective actions of angiotensin-(1-9), a new peptide member of the non-canonical renin-angiotensin system with antagonistic actions to angiotensin II. (b) Establishment of the first experimental model of heart failure with preserved systolic (HFpEF) and the molecular mechanisms involved in its genesis and development. HFpEF is a cardiovascular syndrome with high morbidity and mortality for which there are no evidence-based therapies. We found that the combination of a high-fat diet and inhibition of constitutive nitric oxide synthase using Nω-nitro-L-arginine methyl ester (L-NAME) recapitulate the systemic and cardiovascular features of HFpEF in humans. Nitrosative stress, alterations in the function of key transcription factors (XBP1s and FoxO1), and mitochondrial dysfunction with deficiency of NAD^+ are the crucial mechanisms of cardiomyocyte dysfunction in HFpEF. ANID-Chile: FONDECYT 124044 & FONDAP 1523A0008.

Wednesday 3 July 11:00–12:00, Silver Room

FEBS 2024 Special Lecture

PL-13-1
The rotary mechanism of ATP synthase; how it is regulated and influences the assembly of the enzyme

J.E. Walker
MRC-Mitochondrial Biology Unit, University of Cambridge, Cambridge Biomedical Campus, Cambridge, UK

The ATP synthase in the mitochondria of each human being generates about 50 kg of ATP daily, maintaining a steady state level of about 10 g to sustain life. The enzyme has a rotary mechanism to transmit energy from a transmembrane proton motive force across the inner membrane of the organelle (derived by respiration) to the catalytic sites where ATP is formed from ADP and phosphate. The bovine enzyme is made of 29 protein subunits of 18 types, including the inhibitor protein IF1 [1,2]. They are organized into a rotor and a stator. The rotor consists of a membrane bound c8-ring attached to a central stalk (subunits γ, δ and ε) that protrudes into the mitochondrial matrix, and penetrates into the spherical catalytic domain (α3β3) of the stator. The stator is completed by a peripheral stalk (PS; subunits OSCP, F6, b and d), bound to the external surface of the catalytic domain and extending into the membrane domain (subunits ATP6 and ATP8 plus three small membrane subunits e, f and g, which form a wedge encapsulating lipid molecules). ATP6 is intimately associated with the c8-ring and provides two proton half channels involved in the generation of rotation. The wedges in two ATP synthases interact to form the characteristic dimers that sit on the tips of the cristae and subunit k links dimers together. The assembly of the human enzyme involves the formation of intermediate modules representing (i) the catalytic domain (α3β3γδε, or F1, plus IF1), (ii) the PS plus the membrane "wedge". [3,4], and (iii) the membrane bound c8-rotor ring [5]. They form the key intermediate F1-IF1-c8-PS [5] into which subunits ATP6 and ATP8 are inserted between the c8-ring and the wedge with subunit j bound to ATP6, forming the proton pathway. Two protein assembly factors are required to build the c8-ring and three others to assemble the catalytic domain. IF1 is another key assembly factor that intervenes to prevent partially formed complexes that are capable of ATP hydrolysis (but not synthesis) from doing so. The assembly pathway reflects the probable modular path of evolution of the enzyme. References [1] T. E. Spikes et al. Proc. Natl. Acad. Sci. USA 117 (2020), 23519-23526. [2] J. Carroll et al. J. Biol. Chem. 300 (2024), 105690 [3] J. He et al. Proc. Natl. Acad. Sci. USA 115 (2018), 2988-2993. [4] J. He et al. Proc. Natl. Acad. Sci. USA 117 (2020), 29602-29608. [5] J. Carroll et al. Proc. Natl. Acad. Sci. USA 118 (2021), e2100558118.

Wednesday 3 July 12:00–13:00, Silver Room

FEBS/EMBO Women in Science Award Lecture

PL-14-1
RNA transport in a large polarized cell

A. Ephrussi
European Molecular Biology Laboratory (EMBL), Heidelberg, Germany

Intracellular localization and local translation of mRNA is powerful strategy that allows precise regulation of protein synthesis in time and space and contributes importantly to the functional polarization of cells, from oocytes to neurons. Assembly of mRNAs into mRNPs competent for active transport by molecular motors on cytoskeletal tracks requires the sequential recruitment of proteins and dynamic remodeling of the mRNPs during different phases of the process. How the motors are recruited and their activities regulated is a central question.

Wednesday 3 July 16:30–17:30, Silver Room

FEBS 60th Anniversary Lecture

PL-15-1
Protein misfolding and aggregation: role in aging, neurodegeneration, non-neuropathic diseases and cancer

F. Chiti
Department of Experimental, Clinical Biomedical Sciences "Mario Serio", University of Florence, Italy, Firenze, Italy

An increasing number of human diseases that are typically associated with aging often originate from misfolding and aggregation of well-defined proteins. These include neurodegenerative conditions, such as Alzheimer, Parkinson and prion diseases, frontotemporal dementia, amyotrophic lateral sclerosis, as well as non-neuropathic diseases, such as systemic amyloidosis, type II diabetes and many others. It is also increasingly recognized that protein aggregation is a key process in some types of cancer. Many of the p53 mutations, for example, cause the misfolding and aggregation of the mutant p53 protein, with consequent loss-of-function of the nuclear protein and gain-of-toxic-function, wherein the fibrillar aggregates grow, sequester molecular chaperones and even spread from cell-to-cell seeding new wild-type p53 in surrounding cells. In this Closing Lecture, the main pathways leading a protein to misfold, oligomerize aberrantly and then form large and insoluble aggregates will be shown, along with the mechanisms of protection used by cells to prevent these deleterious events. The main mechanisms through which aging and mutations promote protein aggregation will be discussed. I will then show the recent progress in the field brought by solid-state nuclear magnetic resonance and cryogenic electron microscopy to elucidate the structure of the main protein aggregates that

accumulate in pathology, namely amyloid fibrils, and how our community has progressed substantially to the elucidation of their structure and polymorphism, with a resolution down to <3.0 Å. The Lecture will finally show the main contributions of our lab to elucidate the effect of mutations on amyloid fibril formation, the regions of the sequence determining fibril structure and their associated polymorphism. We will also show how aberrant protein oligomers forming during aggregation or released from mature fibrils cause cell dysfunction and prospective therapeutic strategies to neutralize them.

Symposia

Sunday 30 June
8:30–10:30, Silver Room

Methods and Progress in Structural Biology – Part A

S-01.1-1
Deciphering the molecular structure of muscle Z-disk assembly: an integrative structural biology approach

K. Djinovic Carugo[I,II]
[I]*Department of Structural and Computational Biology, Max Perutz Labs, University of Vienna, Vienna, Austria,* [II]*European Molecular Biology Laboratory (EMBL) Grenoble, 71 Avenue des Martyrs, Grenoble, France*

Sarcomeres are the smallest contractile units found in cardiac and skeletal muscle, where actin and myosin filaments move past each other to generate tension. This molecular machinery is supported by a subset of highly organised cytoskeletal proteins that perform architectural, mechanical, and signalling functions. The ultrastructure of a sarcomere is highly ordered and bordered by Z-disks, which play an essential role in mechanical stability and force transmission. The protein α-actinin-2 acts as a binding platform for other proteins and cross-links antiparallel actin filaments in Z-disks, the lateral borders of the sarcomere machinery. The Z-disk is a highly-organized three-dimensional assembly a highly organized three-dimensional assembly centred on α-actinin, with still a poorly three-dimensional interaction map. We are employing an integrative structural biology approach that melds molecular biophysics, structural, and biochemical methods to explore the Z-disk's structural framework, its assembly order, and the interplay between its structure and function. FATZ proteins, which interact with α-actinin and other key Z-disk proteins, play a central role in the formation and stability of myofibrils by serving as a nexus for protein interactions. In my presentation, I'll discuss our research on the interactions between the prominent Z-disk protein α-actinin-2, FATZ-1, and the Z-portion of titin. These interactions result in dynamic, fuzzy complexes, which have implications for the asymmetrical distribution of α-actinin, as well as the structure and formation of the sarcomeric Z-disk. Additionally, our latest discovery that FATZ-1 undergoes phase separation to create biomolecular condensates with α-actinin-2 and other Z-disk proteins introduces the fascinating concept that FATZ proteins might function as a central hub for Z-disk proteins during the development of myofibrils through a process of membrane-less compartmentalization.

S-01.1-2
Beyond structures: deciphering the regulatory interplay between cell-wall degrading machineries and bacterial cell division

J.A. Hermoso
Instituto de Química Física Blas Cabrera (IQF) CSIC, Calle Serrano 119, 28 006, Madrid, Spain

The bacterial cell wall is an essential gigantic macromolecule that defines the shape of the bacterium and enables the bacterium to resist lysis as a result of its high intracellular osmotic pressure. The main component of the cell wall is peptidoglycan (PG) which consists of repeating linear polymers of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) linked together via short oligopeptide chains. The steps involved in its regulation are the targets of antibiotics like beta-lactams that represent >50% of the available contemporary antibiotic arsenal. Growth, division, and morphogenesis, and in some cases antibiotic resistance, are intimately linked to the synthesis of this exoskeleton but also its hydrolysis. The enzymes that cleave the PG meshwork require careful control to prevent aberrant hydrolysis and loss of envelope integrity. Bacteria employ diverse mechanisms to control the activity, localization, and abundance of these potentially autolytic enzymes. A paradigmatic example is bacterial cell division, a central process that requires delicate regulation of PG hydrolases to prevent aberrant cell lysis and to allow the final separation of viable daughter cells. Recently [1–5] we provided structural insights on how this regulation is performed in bacterial cell division by the addition of modules capable of specific recognition of the septal PG, or by the interaction with regulatory protein complexes or with surface polymers. Remarkably, the regulation of these autolytic enzymes incorporates more than one of these control mechanisms to finely tune activity and provide spatial and temporal control during this essential process. Details of these regulatory mechanisms will be provided in the talk. References: [1] Alcorlo et al Nature Commun. (2019) 10: 5567 [2] Izquierdo-Martínez et al Nature Commun. (2023) 14:4095 [3] Xu et al PNAS (2023) V120, 21, 2301897 120 [4] Li et al Nature Commun. (2023) 14:7999 [5] Martínez-Caballero et al Cell Rep. (2023) 42, 112756.

ShT-01.1-1
Cryo-EM structure of the SAH-riboswitch involved in the virulence and survival of *Pseudomonas aeruginosa*

K. Woźniak[I], A. Ruszkowska[I], E. Kierzek[I], J. Nowak[II], M. Antczak[I], J. Szarzyńska[I], M. Popenda[I], M. Szachniuk[I], S. Glatt[II], M. Ruszkowski[I], K. Brzeziński[I]
[I]*Institute of Bioorganic Chemistry Polish Academy of Sciences, Noskowskiego 12/14, Poznan, Poland,* [II]*Malopolska Centre of Biotechnology, Jagiellonian University, Gronostajowa 7a, Krakow, Poland*

Infections caused by *Pseudomonas aeruginosa* are common medical problems in hospitals and are particularly dangerous for immunocompromised patients [Pang Z et al. (2019) Biotechnol Adv 37, 177-192]. In *P. aeruginosa* strains, *S*-adenosyl-L-

homocysteine (SAH)-responsive riboswitch (SAH-RS) is located at the 5'UTR of the mRNA that encodes five enzymes involved in different vital and virulent processes. The structural changes of the SAH-RS, upon ligand binding, activate the expression of downstream genes [Wang JX et al. (2008) Mol Cell 29, 691-702]. The genes within this operon encode crucial elements of bacterial metabolism: (1) SAH hydrolase (SAHase), regulating SAH concentration and, consequently, methylation reactions, (2) methylenetetrahydrofolate reductase, involved in L-homocysteine removal after SAH decomposition and methionine synthesis, (3) an alarmone hydrolase, responsible for a degradation of the guanosine-based second messenger ppGpp – a key participant in the stress response by inhibiting RNA synthesis, (4) a glycosyltransferase involved in a biofilm formation and (5) ATP-dependent RNA helicase, which play a crucial role in DNA replication and repair processes. The expression of this operon is tightly controlled by the concentration of SAH in the bacterial cells. The prevention of binding SAH to the riboswitch would disrupt the methylation reactions and the biosynthesis of five crucial proteins involved in *P. aeruginosa* metabolism at once. This aim requires structural studies of this riboswitch. Using single-particle cryo-EM analysis, we determined the native 3D structures of SAH-free RS (5.7 Å). These data will serve as the basis for future studies on developing innovative antimicrobial agents targeting SAH-RS. This project is supported in part by National Science Centre (Poland) 2018/30/E/NZ1/00729. Cryo-EM sample preparation and data collection took place at the SOLARIS National Synchrotron Radiation Centre (Poland), at the Cryo-EM facility infrastructure.

ShT-01.1-3
Centromeric protein M18BP1 is a condensin II loading factor

A. Borsellini[I,*], E. Cutts[II,*], D. Conti[III,*], B. Harrys[IV,*], K. Walstein[III], B. Rowland[IV], A. Musacchio[III], A. Vannini[I]
[I]Human Technopole, Milano, Italy, [II]MRC London Institute of Medical Sciences (LMS), London, United Kingdom, [III]Max Planck Institute of Molecular Physiology, Dortmund, Germany, [IV]The Netherlands Cancer Institute, Amsterdam, Netherlands

Condensin II is a multi-subunit protein complex that, together with Condensin I, Cohesin, Top2A and Kif4A, is responsible for the correct compaction and organization of the genome during cell division in humans. Condensin II belongs to the SMC family of proteins that use the energy from ATP in a process named loop extrusion, to generate loops of DNA that compact the disordered genome into chromosomes. Importantly, since Condensin II is always in the nucleus throughout the cell cycle, its activity must be tightly regulated to only start chromosome compaction during mitosis. Previous studies have demonstrated how the activity of Condensin II is negatively regulated by the MCPH1 protein, that prevents premature chromosome condensation in interphase. In our work we used cryo-EM, biochemistry and cell biology approaches to unveil the role of the centromeric protein M18BP1 as a novel regulator of Condensin II. M18BP1 directly binds Condensin II to recruit it to chromatin during G2-M phase. Binding is mediated by a conserved region of M18BP1, which contains a CDK1 phosphorylation site and a "central motif" of the NcapG2 subunit of Condensin II, previously found to be essential for MCPH1 binding. We found that while CDK1 phosphorylation of MCPH1 reduces its affinity for Condensin II, CDK1 phosphorylation of M18BP1 increases its affinity for Condensin II, suggesting a mechanism where phosphorylation mediates a switch between MCPH1 to M18BP1 binding, activating Condensin II at the start of mitosis. *The authors marked with an asterisk equally contributed to the work.

ShT-01.1-2
Towards an atomic-resolution functional study of the ClpXP machinery in action using cell-free and NMR technologies

A. Astrid, K. Giandoreggio-Barranco, A. Vermot, M. Trauchessec, L. Imbert, J. Boisbouvier
Institut de Biologie Structurale, Université Grenoble Alpes/CEA/CNRS, Grenoble, France

The assembly of caseinolytic proteases P and X (ClpXP) is a supramolecular machine involved in protein quality control in bacteria and mitochondria. ClpXP is required for a wide range of cellular processes, from protein maintenance to bacterial virulence. It also participates in the regulation of cell division by degrading its principal regulator, FtsZ. ClpX is an ATPase associated with a variety of cellular activities, and self-assembles to form a ring of six subunits capable of unfolding and translocating client proteins into ClpP's 14-mer barrel pore for degradation. The large size of this machine (800 kDa), its low stability, the complexity of its biological substrates and structural rearrangements, pose a series of logistical problems. Structural studies of such systems using X-ray crystallography or cryo-EM generally provide only a static image of the system, and rarely report the kinetic data needed for a complete, atomically-resolved understanding of the mode of action. In this project, we use state-of-the-art NMR spectroscopy, advanced isotope labeling and cell-free techniques to characterize the non-equilibrium dynamics of the ClpXP machinery. Cell-free technology is exploited to produce and stabilize the ClpX unfoldase by forming a homogeneous complex with the ClpP protease. Then, advanced isotope labeling methods are used to produce the perdeuterated particle, labeled with $^{13}CH_3$ probes on selected methyl groups of ClpP or ClpX. This strategy enabled the acquisition of high-quality 2D NMR spectra of the full-size ClpXP complex and the study of interaction with ATP and FtsZ. NMR allows us to detect the transient interaction with the client protein and the nucleotide-induced structural rearrangement of ClpXP as the molecular machine processes its substrate. These developments pave the way for future structural studies of the ClpXP mechanism.

ShT-01.1-4
Structural enzymology on EMBL beamlines at PETRA III

T.R. Schneider, M. Agthe, I. Bento, G. Bourenkov, Y. Bloch, K. Kovalev, S. Panneerselvam, D. von Stetten, S.L.S. Storm
European Molecular Biology Laboratory, EMBL Hamburg Unit, Hamburg, Germany

The Hamburg site of the European Molecular Biology Laboratory, EMBL, is operating three endstations – P13, P14, T-REXX – for macromolecular crystallography on the PETRA III synchrotron in Hamburg. T-REXX is operated in collaboration with HARBOR (https://www.cui.uni-hamburg.de/en/harbor.html). Instrumentation for sample characterization and crystallization is available in the adjacent Sample Preparation and Characterization Facility. We will present an overview of recent

applications of the facilities in structural enzymology including crystal structure determination at atomic resolution, time-resolved crystallography, structure determination from micro-crystals via serial crystallography and from large crystals of large complexes. New opportunities arising with the recently installed EIGER2 16 M CdTe detector and the planned fourth generation synchrotron PETRA IV will be discussed. We gratefully acknowledge funding from the BMBF and iNext-Discovery. For access to the facilities: smis.embl-hamburg.de.

Sunday 30 June
8:30–10:30, Red Room

Cutting Edge Approaches for Sustainable and Environmental Biotechnology

S-02.1-1
Enzyme discovery and engineering for sustainable applications in biocatalysis

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This lecture will cover recent achievements in the discovery, protein engineering and application of enzymes in biocatalysis [1]. Examples include the asymmetric synthesis of chiral amines using a sophisticated growth selection method. Thus, highly active and selective enzymes from three classes were improved to make important chiral precursors [2]. For the conversion of complex polysaccharides from marine algae, we discovered a new class of P450 monooxygenases from marine bacteria for the demethylation of porphyran [3]. We identified the entire degradation pathway of the complex algal carbohydrate ulvan involving >13 different enzymes [4]. For the recycling of PET, we have improved different esterases [5] and also established a protocol for a fair comparison of PETases reported in literature [6]. We also have identified the first urethanases in a metagenomic library able to degrade polyurethanes [7] and designed an enzyme cascade to degrade poly(vinylalcohols) [8]. References: 1. Buller, R. et al., Science, 382, eadh8615 (2023); Yi., D. et al., Chem. Soc. Rev., 50, 8003–8049 (2021); Wu, S. et al. Angew. Chem. Int. Ed., 60, 88-119 (2021); Badenhorst C.P.S., Bornscheuer, U.T., Trends Biochem. Sci., (2018), 43, 180-198; Bornscheuer, U.T. et al., Nature, 485, 185-194 (2012) 2. Wu, S. et al. Nature Commun., 13, 7458 (2022) 3. Reisky, L., et al., Nature Chem. Biol., 14, 342-344 (2018) 4. Bäumgen, M. et al., J. Biol. Chem., 297, 101 210 (2022); Reisky, L., et al., Nature Chem. Biol., 15, 803-812 (2019) 5. Wei, R. et al., ACS Catal., 12, 3382-3396 (2022); Wei, R. et al., Nature Catal., 3, 867-871 (2020); Bornscheuer, U.T., Science, 351, 1155-1156 (2016); Wei, R. et al., Nature Commun., 10, 558 (2019); Pfaff, L. et al., ACS Catal., 12, 9790-9800 (2022) 6. Arnal, G. et al., ACS Catal., 13, 13 156-13 166 (2023) 7. Branson, Y. et al., Angew. Chem. Int. Ed., 62, e202216220 (2023) 8. von Haugwitz, G. et al., Angew. Chem. Int. Ed., 62, e202216962 (2023).

S-02.5-2
Biotechnological processing of seaweed for value added products

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The increasing depletion of fossil fuels, rising greenhouse gas emissions, and a growing global population have increased the need for sustainable food and energy sources. Traditional industrial biotechnology, which relies on plant-based feedstocks, is competing with food production for arable land. Marine macroalgae, or seaweeds, are a promising alternative due to their rapid growth and high carbohydrate content, without requiring cultivable land, fresh water or fertilizers. In this presentation, the potential of brown macroalgae as a sustainable source to produce value-added chemicals, food products, and ingredients is shown. Extracted carbohydrate fractions from *Laminaria digitata* showed potential as carbohydrate source for cultivating thermophilic bacteria, demonstrating suitability of the extracts for replacing terrestrial carbohydrates for bacterial cultivation. Lactic acid bacteria showed the ability to ferment brown seaweeds e.g. *Alaria esculenta*, showing utilization possibilities in production of novel foods, enhancing the shelf life of the seaweed, serving as an alternative to current storage techniques. To valorize carbohydrate polymers from the seaweeds, laminarin, (a β-1,3 linked glucan with occasional β-1,6 linkages) that serves as a storage carbohydrate in brown seaweed, is an interesting example of a polymer, that can be was enzymatically modified to laminari-oligosaccharides using novel GH17 enzymes identified in the marine bacterium *Muricauda lutaonensis*, and produced β-1,3-linked oligosaccharides with DP above 5 with introduced β-1,6 linkages either in branched or kinked structure, resulting in potential bioactive properties. These findings collectively offer valuable insights into the potential of brown macroalgae as a renewable, sustainable source for biofuels and value-added compounds.

ShT-01.9-3
Optimization of biotechnological processes, using membranes, to recover different fraction in order to valorise industrial waste

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The main objective of this study regards the setup of an efficient small-scale platform for the conversion of renewable waste materials, specifically whey, into added-value products, thereby reducing environmental impact and costs deriving from the disposal of these liquid waste products. Recent examples of fermentation-based valorization strategies employ lactic acid bacteria (LAB) as probiotics to improve the nutritional value of different food matrices for the formulation of functional foods. Furthermore, the facultative anaerobic metabolism of LAB can be exploited to address the environmentally sustainable production of lactic acid (LA), which is a building block of degradable bioplastics and is widely applied in many biotechnological fields (Ahmad A.et al. 2020 Env Techn Innov). In this direction, the

present study focused on the optimization of a downstream process, using micro-ultra-nanofiltration membranes. From whey, different fractions will be recovered and tested as sources of bioactive compounds or as substrates for the growth of biotechnologically appealing strains. In particular, the carbohydrate-rich fraction will be evaluated as a substrate for the cultivation of diverse microorganisms to develop sustainable fermentation process to obtain added value products (LA, antimicrobial peptides and probiotics). The protein-rich fraction was tested for potential uses in the cosmeceutical field, for tissue hydration/regeneration and the assays took advantage of an experimental set up for scratch assays. Finally, the development of hydrogels based/containing these proteins with potential applications in regenerative medicine has been investigated. Dehydration tests on HaCat cells proved R100 was the most efficient fraction in preserving cell viability from this specific stress while R10 after extensive diafiltration, showed it was able to form transparent films with improved features when glycerol was added as a plasticizer (Alfano A. et al. 2022. Ferm).

ShT-02.1-2
Construction of engineered *Azotobacter vinelandii* for increased ammonia production in culture medium

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The ammonia synthesis process developed by Haber and Bosch has environmental sustainability issues. Therefore, researchers have been exploring alternative methods to address these problems. Among the biological methods, the nitrogenases of *Azotobacter* spp. and *Klebsiella* spp. have been attracting significant attention. However, ammonia production through heterologous expression of nitrogenase gene clusters in *E. coli* and yeast is challenging due to the complexity of the nitrogen fixation mechanism and the oxygen sensitivity of nitrogenase. In this study, we attempted to improve the ability of the nitrogen-fixing microorganism *Azotobacter vinelandii* to produce ammonia by modifying its function. To this end, we focused on the *nifL* gene of *A. vinelandii*, which is a negative regulator of nitrogen fixation in the presence of a nitrogen source, and the *amtB* gene, which encodes an ammonia accumulation channel. We created a strain by knocking out these two genes responsible for nitrogen fixation and ammonia release into the culture medium. The target gene was replaced with an antibiotic resistance gene using standard homologous recombination methods, resulting in the deletion of the target gene. The deletion of genes in *A. vinelandii* was confirmed through a series of steps, which included antibiotic selection, PCR confirmation, and DNA sequencing analysis. To evaluate ammonia production, the engineered strain was incubated for 48 h at 30°C and 300 rpm in the nitrogen-free modified Burk's medium. The ammonia concentration in the supernatant was measured using an Ammonia Assay Kit through a colorimetric reaction. The measurement of ammonia concentration in the culture supernatants of Δ*amtB*, Δ*nifL*, Δ*amtB*Δ*nifL*, and wildtype strains revealed a significant increase in ammonia concentration in the Δ*amtB*Δ*nifL* strain compared to the WT strain. These results suggest that *A. vinelandii* has the potential for ammonia production as a biological method.

ShT-02.1-1
Setting up a new enzymatic cocktail for the valorization of carbohydrates from lignocellulosic waste biomasses

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The increasing demand for recycling food waste products across multiple industrial sectors to advance the circular bioeconomy and convert lignocellulosic wastes has recently gained substantial attention. Among these biomasses, Spent Coffee Grounds (SCG), composed of galactomannan, arabinogalactan II, and cellulose[1], stand out as an excellent source of high-value saccharides. These include manno-oligosaccharides (MOSs), galacto-oligosaccharides (GOSs), mannose, galactose, arabinose, and glucose, which are well-suited for bioethanol production, fermentation, synthesis of biodegradable polymers and materials, and other high-value products[2]. In this context, (hyper)thermostable and thermoactive (hemi)cellulases, due to their stability, play a pivotal role in the saccharification of recalcitrant polysaccharides and oligosaccharides under harsh industrial conditions[3]. In this study, we effectively selected thermophilic and thermostable enzymes based on pH, thermal stability, and activity to set up an enzymatic cocktail for the hydrolysis of SCG. We used pod SCG from Italian Borbone coffee as the raw biomass for hydrolysis. Compositional analysis of this raw material revealed that it is composed of 50% carbohydrates, 40% lignin, and 10% other extractives. Various mild delignification pretreatments were applied on the raw SCG, resulting in SCG-derived biomass with reduced lignin content and enhanced accessibility for enzymatic hydrolysis. The enzymatic hydrolysis of the SCG-derived biomasses demonstrated the conversion of over 30% w/w of the biomass (equivalent to 50–60% w/w of cellulose and hemicellulose content) into reducing sugars. References: (1) Portillo OR et al. (2022) Revis Bionatura 7(3):11. (2) Nguyen QA et al. (2019) Bioresources Technology 272, 209–216. (3) Cobucci-Ponzano B et al. (2015) Enzyme Microb. Technol. 78, 63–73.

ShT-02.1–3
Bioprocessing of agri-food residues into value-added products

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Sustainable and resilient productions are based on a circular economy and minimal processing. In this frame, the valorization of agri-food residues (AFR) is a hot topic of research in zero-

waste global development and for the green production of biomaterials such as those based on bacterial cellulose (BC) and lactic acid (LA). Among AFR, citrus residues have been successfully used as carbon sources in culture media for microbial fermentation (1). However, existing methods for valorizing citrus waste often require chemical pretreatments of the biomass, which have a high environmental impact (2). In order to establish an ecofriendly and sustainable strategy for production of LA and BC we pointed to a moderately thermophilic lactic acid producer *Weizmannia coagulans* (3) strain and to a bacterial/yeast community (Kombucha) respectively, exploiting their degradative ability of complex biomasses. Our result show: (i) the feasibility of replacing the standard growth medium with less expensive carbon/nitrogen which are widely available at low-cost, and suitable for fermentation growth; (ii) the setting up of a green process for BC and LA production based on untreated biomass; (iii) the potential of citrus waste to release bioactive compounds with several health-promoting and therapeutic advantages upon microbial fermentation. References: 1. Aulitto M, Alfano A, Maresca E, et al. Thermophilic biocatalysts for one-step conversion of citrus waste into lactic acid. Appl Microbiol Biotechnol. 2024;108 (1):155 2. Huang, R et al. Enhanced ethanol production from pomelo peel waste by integrated hydrothermal treatment, multi-enzyme formulation, and fed-batch operation. J. Agric. Food Chem. 2014; 62(20): 4643-51 3. Aulitto M, Fusco S, Bartolucci S, et al. *Bacillus coagulans* MA-13: a promising thermophilic and cellulolytic strain for the production of lactic acid from lignocellulosic hydrolysate. Biotechnol. Biofuels 2017; 10: 210.

Sunday 30 June
8:30–10:30, Blue Room

Biochemistry of Physical Activity and Health – Part A

S-03.1-2
Exercise as medicine in a translational perspective: focus on myokines

B.K. Pedersen
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Physical activity decreases the risk of a network of diseases, and exercise may be prescribed as medicine for lifestyle-related disorders such as type 2 diabetes, dementia, cardiovascular diseases, and cancer. During the past couple of decades, it has been apparent that skeletal muscle works as an endocrine organ, which can produce and secrete hundreds of myokines that exert their effects in either autocrine, paracrine, or endocrine manners. Recent advances show that skeletal muscle produces myokines in response to exercise, which allow for crosstalk between the muscle and other organs, including brain, adipose tissue, bone, liver, gut, pancreas, vascular bed, and skin, as well as communication within the muscle itself. Although only few myokines have been allocated to a specific function in humans, it has been identified that the biological roles of myokines include effects on, for example, cognition, lipid and glucose metabolism, browning of white fat, bone formation, endothelial cell function, hypertrophy, skin structure, and tumor growth. This suggests that myokines may be useful biomarkers for monitoring exercise prescription for people with, for example, cancer, diabetes, or neurodegenerative diseases.

S-03.1-1
The impact of immobilization and trauma/ inflammation on muscle mass and insulin resistance in humans

P.L. Greenhaff
School of Life Sciences, The Medical School, University of Nottingham, Nottingham, United Kingdom

Bed rest (BR) of only a few days duration reduces muscle protein synthesis and induces skeletal muscle atrophy and insulin resistance, but the scale and juxtaposition of these events have not been investigated concurrently in the same individuals. Furthermore, the concurrent impact of short-term exercise-supplemented remobilization following BR on muscle volume, protein turnover and insulin-stimulated glucose uptake in humans is relatively unknown, but nevertheless is of clinical importance. Muscle metabolic adaptation in the transition from acute (days) to chronic (weeks/months) BR and the mechanisms involved is also somewhat unresolved, and particularly under conditions where volunteers have been maintained in energy balance to minimize the confounding impact of excess energy intake on metabolic adaptation to BR. This presentation will therefore focus on these knowledge gaps, and will also consider the cumulative impact of combined immobilization and trauma related burden on muscle metabolic regulation in human volunteers. The talk will highlight that whilst muscle mass loss and insulin resistance are common end-points of BR and increased trauma related burden in humans, a gap in understanding of the pathophysiological mechanisms involved in each scenario exists.

ShT-03.1–2
Comprehensive multi-omics analysis of serum and fecal samples in a dietary intervention model with pectins for lipid transfer proteins (LTP) food allergy

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The gut microbiota is believed to play a crucial role in food allergen sensitization, with intestinal dysbiosis being linked to the risk of developing food allergy (FA). In this context, dietary interventions with prebiotics, such as pectin, might be beneficial. Therefore, this study aimed to explore the effects of a dietary intervention with pectin on lipid transfer proteins (LTP) allergic patients. To this end, 34 allergic patients to the peach LTP, Pru

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p 3, were included in this study. These patients were orally administered one of two pectin varieties or placebo twice a day for two months and were divided into three groups. Paired serum and fecal samples (Placebo, n = 4/9; Active 1, n = 13/6; Active 2, n = 12/6; respectively), obtained before and after the intervention, were analyzed to perform proteomics on a panel of 92 inflammation-related proteins; and targeted metabolomics on bile acids and short-chain fatty acids (SCFA) by liquid chromatography coupled to mass spectrometry (LC–MS). Lastly, oral food challenge with peach was performed after the intervention to assess tolerance. Following pectin dietary supplementation, specific serum proteomic changes indicating a downregulation of the Th2 response were observed, characterized by decreased levels of IL2, IL4, and IL13 compared to the placebo group. These findings were confirmed by metabolomics, which revealed alterations in the systemic and fecal levels of secondary microbial-derived bile acids (including isolithocholic, deoxycholic, and hyodeoxycholic acids) when comparing both pectin treatments to the placebo. Additionally, reduced levels of branched SCFA, such as isobutyric and 2-methyl butyric acids, were detected in the same comparisons. Taken together, our results provide evidence that dietary intervention with both pectins induced a differential metabolic and proteomic profile in LTP-allergic patients, giving rise to elucidate new potential immunomodulatory mechanisms and therapeutic targets in FA.

ShT-03.1-3
Pharmacological activation of the HIF-1α signaling pathway regulates satellite cells fate during aging through histone lactylation

F. Cirillo[I,II], L. Mornatti[I,II], I. Lavota[I,II], A. Tarantino[I,II], M. Piccoli[I,II], P. Rota[II,III], L. Mangiavini[IV,V], C. Pappone[II,VI,VII], L. Anastasia[I,II,VII]

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Sarcopenia is a multifactorial disease characterized by progressive loss of skeletal muscle mass and function in the elderly, associated with reduced muscle satellite cells (SCs) function and tissue oxygenation. The hypoxia-inducible factor-1α (HIF-1α) plays an essential role in the cellular response to oxygen levels by determining the switching of the glucose pathway from oxidative phosphorylation to glycolysis, thus acting as a regulator of cellular energy metabolism. In this context, we have previously reported that the HIF-1α pathway is strongly downregulated in human skeletal muscle biopsies from sarcopenic patients. This study aims to determine the role of pharmacological activation of HIF-1α, using the prolyl-hydroxylases inhibitor FG-4592, on the fate of mice satellite cells during sarcopenia and establish whether this treatment can counteract the switch of SCs metabolism to glycolysis. The results showed that treatment with FG-4592 increased the gene expression of all glycolytic enzymes, resulting in an enhancement in lactate production associated with the activation of anaerobic glycolysis. The main effect of this metabolic change is a decrease in the proliferation rate of treated SCs, reflecting an alteration of their cell cycle. Indeed, the progression of SCs in phase S of the cell cycle is severely impaired, which may be caused by an epigenetic modification in chromatin. The results revealed that lactate, a crucial metabolite mainly in muscle tissue, can also induce histone lactylation that alters the gene expression and fate of SCs. Indeed, treatment with FG-4592 promoted the expression of PAX7, the main marker of SCs, leading to the hypothesis that the treatment may increase the self-renewal power of SCs. In conclusion, these results support the notion that pharmacological activation of HIF-1α may counteract the development of sarcopenia by activating muscle regeneration.

ShT-03.1-1
Investigation of serum irisin, adropin and preptin values in obese and non-obese individuals

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Gaziosmanpasa University, Facult of Medicine, Department of Biochemistry, TOKAT, Türkiye

Obesity is one of the main public health issues growing rapidly all over the world and underlying many diseases especially Type 2 Diabetes Mellitus (T2DM). There have been few studies about newly discovered endocrine factors Irisin, adropin and preptin and their relation with T2DM. In this study, we aimed to evaluate these endocrine factors in normal, obese and obese diabetics thereby putting through their relation with T2DM. We received Ethics Committee approval on 29.04.2021 (21-KAEK-08). We created groups of obese, diabetic obese, and healthy controls, each including 60 participants. The endocrine factors levels were analyzed with Enzyme-Linked Immunosorbent Immuno-Assay (ELISA) commercial kits. The results were evaluated statistically by using SPSS 20 (IBM SPSS Statistics 20, SPSS inc., an IBM Co., Somers, NY). There was no significant difference between the groups for age and gender. While irisin was significantly lower in obese and diabetic obese than in controls ($p < 0.001$), no significant difference was observed between obese and diabetic obese. On the other hand, preptin was significantly higher in obese and diabetic obese than in controls ($p < 0.001$), but no significant difference was observed between obese and diabetic obese. In the control group, irisin and adropin showed strong positive correlation with each other (r: 0.849, $p < 0.001$). In obese, irisin and adropin showed strong positive correlation with each other (r: 0.986, $p < 0.001$) while adropin and preptin showed strong positive correlation with each other (r: 0.982, $p < 0.001$). In diabetic obese, irisin and adropin showed strong positive correlation with each other (r: 0.960, $p < 0.001$) while adropin and preptin showed strong positive correlation with each other (r: 0.927, $p < 0.001$). In conclusion, monitoring the levels of these new endocrine factors in obese and diabetic obese will be useful in preventing vital complications caused by. *The authors marked with an asterisk equally contributed to the work.

Sunday 30 June
8:30–10:30, Yellow Room

Genome Editing and Gene Therapy – Part A

S-04.1–2
Identification and evolution of novel CRISPR-Cas systems from the human microbiome

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CRISPR technologies are transforming the bio-medicine field by providing new therapeutic concepts for the treatment of diseases through genetic repairs and deployment of disease-protecting factors. Nonetheless, the currently available CRISPR nucleases and derived technologies do not address the hurdles related to genome modification in gene therapy applications. Challenges are imposed by specific properties of CRISPR tools which includes high molecular weight limiting their compatibility with most commonly delivery vectors including lipid nanoparticles, target sequence constraints, immunogenicity and heterogeneous efficiency and precision throughout the genome. We recently focused on the development of new technologies by retrieving CRISPR systems from a large databank of the human microbiome and through a directed evolution approach to enhance the activity of the prokaryotic enzymes to eukaryotic environment. This work led us to the discovery of new CRISPR systems and the enhancement of Cas nucleases with compelling features for genome editing applications.

S-04.1-1
Deciphering DNA–protein crosslink repair *in vivo* using CRISPR/Cas genome editing in a zebrafish model

M. Popovic, I. Anticevic, C. Otten, M. Kutnjak
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DNA–protein crosslinks (DPCs) are very common DNA lesions that interfere with all DNA transactions including replication and transcription. The consequences of impaired DNA–Protein Crosslink Repair (DPCR) are severe. At the cellular level, impaired DPCR leads to the formation of double strand breaks, genomic instability and/or cell death, while at the organismal level, deficiency in DPCR is associated with cancer, aging and neurodegeneration. Induction of DPCs is used in medicine to treat many cancers and understanding the repair at the organismal levels could provide an impetus for the development of new drugs and combination therapies with currently used chemotherapeutics. We use zebrafish (*Danio rerio*), an established vertebrate model to study cancer, neurodegenerative and cardiovascular diseases, and CRISPR/Cas gene editing to knock-out or mutate genes of interest in order to study the interplay of DPCR factors and sub-pathways including proteolysis-, and tyrosyl-DNA phosphodiesterase-dependent repair at the biochemical and cellular level. I will present our recent discoveries from three new zebrafish strains generated with the CRISPR-Cas system: a catalytic mutant and a C-terminal mutant of the ACRC protease involved in DPCR, as well as a transgenic strain with the inactive DPCR factor, tyrosyl-DNA phosphodiesterase 1 (TDP1). We have found that ACRC is an essential protease in vertebrate development, as a catalytic mutation leads to early embryonic lethality. By injecting ACRC (WT) mRNA constructs into mutant embryos, we were able to grow the transgenic line and perform DPCR analysis. We found that ACRC is a DPCR protease with many cellular substrates and that the SprtT domain is essential for repair, while the intrinsically disordered region is dispensable. We also show that TDP1 is required for the resolution of topoisomerase 1- and histone-DPCs at the organismal level and we further characterise a novel TDP1-mediated repair pathway for histone-DPC repair.

ShT-04.1-1
CRISPR CGBE1-based editing on CD34+ stem cells to grow Bombay blood group compatible red blood cells

M. Kurghinyan[I,*], M. Kurghinyan[I], J.A. Ng[II,*], S. Tusnady[III,*], C. Wang[IV]
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Bombay blood group is the result of a rare genetic disorder, where individuals with the Bombay phenotype do not express the H-antigen on their red blood cells (RBC); therefore, they cannot receive blood from any member of the ABO blood group system. The objective of this project is to create an artificial blood type which can be transfused to individuals with Bombay blood group in emergency situations. For this reason, the use of CRISPR based-editing technology to mutate the FUT1 gene is proposed, which is responsible for the formation of the H-antigen in hematopoietic CD34+ stem cells, and induce the cells to produce RBCs lacking the H-antigen. We propose an *in vitro* experiment, which is comprised of designing guide-RNA (gRNA) and base editor constructs, delivering base editor and gRNA to the CD34+ stem cells, verifying on- and off-target mutations by whole genome sequencing, growing RBCs in a G-Rex medium, validation of CRISPR editing using leukoreduction filters and preparing for transfusion by cryopreservation. The project was implemented within the frameworks of IBO Challenge 2020 International Group Project. *The authors marked with an asterisk equally contributed to the work.

ShT-04.1-2
Viral and non-viral vectors for the delivery of genome editing tools to mammalian cells

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While the discovery of CRISPR-Cas systems and their application to mammalian cells has rapidly expanded genome editing tools, their medical implementation remains constrained. A key

obstacle is the scarcity of suitable delivery tools for Cas nucleases and their sgRNA, particularly for advanced applications. Current adeno-associated viral vectors, being limited in size, struggle to accommodate both Cas and sgRNA. Addressing this limitation, liposomes and herpes viral (HSV) vectors emerge as promising alternatives, capable of overcoming size constraints and adaptable for *in vitro*, *ex vivo*, and *in vivo* applications. This study focuses on leveraging proprietary liposomes for Cas9 and sgRNA delivery to mammalian cells. Assessment involves the delivery of Cas9 RNPs encapsulated in liposomes, with Cas9-GFP fusion facilitating the visualization of protein localization in various cells. Variable Cas9 concentrations in liposomes and diverse incubation times are tested to optimize delivery efficiency. Additionally, we explore the potential of proprietary liposomes to traverse the blood-brain barrier using an *in vitro* BBB model. The second delivery approach involves HSV, targeting more intricate genome editing tools, such as prime-editors. A singular HSV vector, encompassing all prime-editor elements, was constructed using an extensible mammalian modular kit. Validation of this designed HSV vector was performed on an established reporter cell line within the laboratory, with the objective of editing genome-encoded GFP to BFP. These endeavours underscore the potential of liposomes and HSV vectors as robust delivery platforms for advancing CRISPR/Cas-based therapies, bringing us closer to overcoming existing barriers in genome editing applications.

ShT-04.1–3
Rad51-based editing of mitochondrial DNA via CRISPR approach

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Previously, we developed a CRISPR/Cas9-based method to excise the mitochondrial mutation 15059G > A. Subsequent mitochondrial DNA analysis revealed instances of double-strand break repair via homologous recombination. Exploiting this, we aimed to edit mitochondrial DNA by inserting a "barcode" sequence of either 20 or 100 nucleotides at the mutation site 1555A > G. This was achieved using two specific nickases, D10A and H840A, facilitating double-strand breaks by targeting the leading and lagging strands, respectively. Each nickase was paired with a guide RNA, one matched to the mutation and the other to a mitochondrial DNA conserved region. To enable efficient mitochondrial delivery of the nickases, the Cox8a signal peptide, a Mitochondrial Targeting Signal (MTS), was added to their domains, promoting cytoplasm-synthesized protein translocation into mitochondria. The delivery system comprised RNA encoding the nickases, the MTS, and a sequence for Streptavidin-SpyCatcher, forming a complex with biotinylated guide RNA for transport across mitochondrial membranes. The barcode insertion utilized an mRNA sequence of Rad51, tagged with an MTS, and a single-stranded oligonucleotide (ssODN) bearing unique sequence AAATTTAAA. Rad51's binding to a specific ssODN sequence facilitated its mitochondrial delivery. Our integrated system, involving mitochondrially targeted nickases and Rad51, successfully delivered guide RNA and ssODN into the mitochondrion, enabling precise genome editing. We verified the integration of both 20 and 100 nucleotide sequences into the mitochondrial genome, noting higher efficiency with the 20-nucleotide sequence. Integration was confirmed through PCR and sequencing, highlighting the system's potential for precise mitochondrial DNA mutation correction. This work was supported by the Russian Science Foundation (Grant #22–15-00064). *The authors marked with an asterisk equally contributed to the work.

Sunday 30 June
17:00–19:00, Silver Room

Translational Proteomics

S-01.3-2
Translational proteomics of rare diseases and the journey towards precision medicine

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INSERM US24, Paris, France

Rare diseases have their own diagnostic and therapeutic challenges that genetic information alone cannot solve entirely. Proteomics, the global study of proteins, offers dynamic insights into protein expression, modifications, and interactions hence contributing to the understanding of disease mechanism. Translational proteomics in particular, play a pivotal role in patients' stratification to enhance diagnostic accuracy and prognostic assessment. It also contributes to understanding the cellular pathways altered in the disease and to elucidate the relationship between phenotype and genotype. In this talk, through the results of translational proteomics studies in different rare diseases, cystinuria, cystic fibrosis, cystinosis and idiopathic nephrotic syndrome, I will highlight the significant contribution of translational proteomics to the long journey towards precision medicine. Despite challenges like data complexity and limited sample availability, translational proteomics holds promise in advancing precision medicine, contributing to personalized and targeted therapies for rare diseases.

S-01.3-1
Antivenomics: a translational venomics platform for the preclinical efficacy evaluation of antivenoms

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Snakebite envenoming is a WHO class A neglected tropical disease that claims over 100 000 human lives annually worldwide. Snakebite envenoming represents a major issue for impoverished populations living in rural areas of tropical and subtropical regions across sub-Saharan Africa, South to Southeast Asia, Latin America and Oceania. Antivenoms constitute the only scientifically validated therapy for snakebite envenomings, provided they are safe, effective, affordable, accessible and administered appropriately. The assessment of the capacity of antivenoms to neutralize the lethal activity of snake venoms is the gold standard in the preclinical analysis of antivenom efficacy. To aid in the preclinical testing of antivenoms, our group has developed "antivenomics", a venomics-guided affinity chromatography-based platform for the quantitative toxin-resolved assessment of the immunorecognition landscape of antivenoms towards homologous and heterologous venoms. Antivenomics is translational venomics. In this talk we will discuss the

operational principles of this omics platform and will show its practical application towards a knowledge-based improvement of antivenom design and efficacy.

ShT-01.3-2
Translational proteomic and metabolomic investigations to assess gene therapy/delivery efficacy in rare genetic disease

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Ataxia Telangiectasia (A-T) is a rare neurodegenerative disease caused by biallelic mutations in the Ataxia Telangiectasia Mutated gene (ATM). No cure is currently available for these patients apart the positive effects on neurologic features of A-T patients by dexamethasone administration through autologous erythrocytes. We recently developed a lentiviral system to deliver shorter but functional ATM variants, capable to restore some impaired biological processes of A-T cells. Here we performed proteomic and metabolomic analyses of an A-T cellular model, treated with two different ATM variants and compared to wild-type (WT) cells. We could demonstrate that omics sciences are suitable tools in choosing the most performant gene variant to be administered in a target cell line. By high specificity pruning statistic of proteomic data, we found that A-T cells exhibit (a) impaired RNA splicing, (b) weakened protein folding, (c) constitutive inflammation, and (d) impaired extracellular matrix organization. Alternative biological assays confirmed the observed deficiencies. Moreover, matched proteomic and metabolomic analyses revealed that glycolysis, mitochondrial metabolism, and redox homeostasis are impaired in A-T, also proven by alternative biochemical techniques. The same omics approaches achieved on treated A-T cells (by lentiviral transduction of ATM SINT and ATM 4-53, two shorter ATM variants) revealed that ATM SINT is the finest ATM variant to be administered for recovering the altered A-T phenotype, making the protein and metabolite levels similar to WT ones. Also, biochemical functional tests of administered ATM variants confirmed the observed restoration process. In conclusion, multi-omics approach represents a suitable tool in choosing the best biological therapy to be administered to achieve gene therapy or gene delivery strategy. *The authors marked with an asterisk equally contributed to the work.

ShT-01.3-3
The importance of saccharides in GAS1 function

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GAS1 (Growth Arrest-Specific protein 1) is a monomeric soluble protein, anchored to the plasma membrane, which carries out different functions, highlighting its role as coreceptor of Sonic Hedgehog ligand (SHH) [Wierbowski, BM et al. (2020) Dev. Cell 55 (4), 450-468]. In animal cells, this signaling pathway plays an essential role in embryogenesis and stem cells homeostasis in adult tissues. The alteration or absence of GAS1 has been related to the appearance and severity of a disease called holoprosencephaly, characterized by an incomplete division of the forebrain [Seppala M et al. (2007) J. Clin. Invest. 117 (6), 1575-1584]. One of its characteristics is the presence of a glycosylation at the residue Asn117. This modification has been revealed to be very important for GAS1 function, but, quite surprisingly, its role has not yet been studied in detail. So, the main purpose of our investigation is to characterize the glycosylation pattern of this protein and examine how different glycans introduced by host expression systems can affect its function. With this purpose, we have cloned and produced the soluble domain of GAS1 in the yeast recombinant expression system *Pichia pastoris* and in mammalian cells. The proteins were structurally characterized, and functional experiments were performed to evaluate how the different glycosylation patterns affect its function. We tested its ability to bind cholesterol, which is key for its function [Huang P et al (2022) Dev. Cell 57 (5), 670-685)]; and their ability to extract the ligand SHH from HEK293 membranes. We were able to determine that some of the differences observed between both recombinant proteins were very probably being caused by the different glycans added by the two host expression systems employed. More experiments are still required to confirm and quantify these already preliminary, though very promising, observations.

ShT-01.3-1
Multi-omic analyses of hiPSC-derived astrocytes

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Astrocytes have a key role in brain development and functions and contribute to neurodegenerative disorders by various mechanisms, including metabolic alterations. They are also the major source of L-serine (L-Ser) in the brain, which is synthesized from the glycolytic intermediate D-3-phosphoglycerate through the phosphorylated pathway (PP), which comprises three enzymes: 3-phosphoglycerate dehydrogenase (PHGDH), phosphoserine aminotransferase (PSAT), phosphoserine phosphatase

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(PSP). L-Ser is the precursor of the two main co-agonists of the N-methyl-D-aspartate receptors, glycine and D-serine. We generated human mature astrocytes from pluripotent stem cells (hiPSC) to study the changes occurring during astrocytes differentiation. By using an integrated multi-omics approach we studied differentiation from neural stem cells to 56-days-old astrocytes, showing that up to 30 days axon guidance processes, folate cycle, pyrimidine and amino acid metabolism were prevalent, along with sphingolipid synthesis and metabolites related to the serine pathway [published in Tripodi et al (2023) FEBS J 290 (18): 4440-4464]. We have recently reported that the levels of the enzymes of the PP are increased in Alzheimer's disease brains [previously published in Maffioli et al (2022) Cell Rep 40(10): 111271]. Following this observation, we overexpressed PHGDH, PSAT or PSP in the hiPSC-derived astrocyte model and investigated metabolomic and proteomic changes following PP enzymes overexpression. Strikingly, significant alterations were apparent in the pathways directly linked to serine metabolism as well as on folate and nucleotide metabolism, and TCA cycle, suggesting a complex rewiring of the metabolism. These results provide a valuable model for developing potential novel approaches to address brain diseases, especially those linked to NMDA receptor alterations due to modification of serine concentration. This work was supported by PRIN2017H4J3AS-Dissecting serine metabolism in the brain.

Sunday 30 June
17:00–19:00, Red Room

Cancer Epigenome and Transcriptome

S-04.3-1
Pancreatitis induces transcriptomic and epigenetic reprogramming of epithelial cells to elevate long-lasting cancer predisposition

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Acute pancreatitis (AP) is a relatively common inflammation of the pancreatic parenchyma that typically resolves without clinical complications. However, epidemiological evidence shows that individuals who suffered AP are at elevated risk of developing pancreatic cancer for several decades after the episode. We speculated that pancreatitis could represent a paradigm of long-lasting dyshomeostatic stress response that leads to the establishment of a pro-oncogenic memory of inflammation. Indeed, AP-primed epithelial cells show enhanced propensity to dysplasia *in vitro* and *in vivo*. We tested the hypothesis that AP events induce either permanent changes in the epigenome or skewing of subpopulations in the pancreatic ecosystem. To dissect molecular and cellular dynamics that outlast AP events, we performed single-nuclei multiomic (RNA + ATAC) sequencing in mouse pancreata after induction of and recovery from experimental pancreatitis. While immune-histological examination did not show any alteration post AP, granular analysis coupled with Bayesian modeling revealed extensive transcriptomic and epigenomic reprogramming in acinar cells, which are common cell-of-origin for pancreatic cancer. This is not linked to expansion of progenitor-like clones but is enforced on functionally-distinct ("idling") acinar cells. In detail, AP elevates cell-intrinsic unfolded protein response (UPR). In fact, AP-primed acinar cells show augmented spliced Xbp1 and cleaved ATF6 levels. We also observed that UPR inducers promote acinar cell plasticity, linking UPR stress to pancreatic cancer initiation. Mechanistically, AP induces an irreversible increase of chromatin accessibility in acinar cells. This leads to hypertranscription and protein dyshomeostasis, and to AP1-mediated poising of the epigenome. Together, these alterations set a phenotypic state in post-mitotic epithelial cells that makes them more susceptible to oncogenic transformation.

S-04.3-2
Highly multiplexed quantitative epigenome profiling for drug screening and biomarker discovery

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My laboratory aims to understand epigenetic mechanisms that govern gene expression during development and tumorigenesis. An emerging class of drugs – epigenetic modifiers, or 'epidrugs' – hold great promise in oncology. Designing epidrugs with high efficacy, while excluding potential side effects on the epigenome, remains a challenge. We have developed a highly multiplexed, quantitative method for systematically profiling the interaction of drugs with the epigenome. At the same time, we are interested in using epigenetic signatures in liquid biopsies as biomarkers for diagnostics and precision medicine. I will discuss the development of the methodology as well as emerging applications.

ShT-04.3-2
Hypoxia increases methylated histones to prevent histone clipping

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Hypoxia increases histone methylation by inhibiting O_2- and α-ketoglutarate-dependent histone lysine demethylases. This study is the first to demonstrate how the hypoxia-induced increase in methylated histone levels interacts with other epigenetic changes, such as histone clipping and heterochromatin redistribution (senescence-associated heterochromatin foci, SAHF) during oncogene-induced senescence (OIS). Raf activation in primary IMR90 human fibroblasts increased cathepsin L (CTSL)-mediated histone 3 (H3), H2B, and H4 clipping at H3 A21/T22, H2B T19/K20, and H4 G11/K12, respectively. Hypoxia protected H3 from CTSL by increasing histone methylation, especially at H3K23me3, without reducing CTSL activity. Maintaining methylated histones is sufficient for protecting histones from CTSL. However, these methylated histones are insufficient but necessary for inhibiting SAHFs. ATAC-seq analyses showed that Raf activation increased chromatin accessibility, which hypoxia prevented.

ShT-04.3-4
PARP1 regulates 3D structure and function of super-enhancers and hormone-control regions

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Poly (ADP-ribose) polymerase 1 (PARP1) has been linked to various genomic pathways and in the maintenance of genomic stability. PARP1 also regulates enhancers activity, but its role at super-enhancers (SE) remains unknown. Here, we employed genomic approaches to show how PARP1 occupies SE of T47D breast cancer cells and how PARP1 is also dynamically sequestered from SE by progesterone treatment to lodge into control regions regulated by the hormone. Disruption of PARP1 or inhibition of its PAR-ylation activity cause transcriptome reprograming altering the expression of both SE-associated and hormone-regulated genes. This is achieved through a coordinated action in the establishment of long-range chromatin loops by PARP1 which are lost upon PARP1 ablation and/or catalytic inhibition. Our results reveal PARP1 as a chromatin looper and SE-associated protein, gatekeeping genes linked to cell identity and hormonal response via 3D genome looping in breast cancer cells. *The authors marked with an asterisk equally contributed to the work.

ShT-04.3-1
HIF1A-EP300-BRG1 functional crosstalk on the chromatin defines transcription of ABC transporters in paclitaxel resistant breast and lung cancers

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Paclitaxel is a first-line drug for the treatment of advanced non-small cell lung cancers and metastatic breast cancers. However, the development of resistance to paclitaxel leads to treatment failure and tumor recurrence. One of the mechanisms of paclitaxel resistance is overexpression of ABC transporters, which may actively efflux anticancer drugs from the cell or trap these drugs in intracellular organelles. Analysis of TCGA Pan-Cancer dataset suggests the link between transcription of SMARCA4, which encodes chromatin remodeling complex SWI/SNF subunit BRG1, and ABC transporters. Using ChIP-Seq, RNA-Seq, co-immunoprecipitation, confocal microscopy, and other molecular biology methods we provide evidence on the occurrence and functional role of BRG1 at the promoters of ABC transporters, which are overexpressed in breast and lung cancer resistant phenotypes. Motif spacing analysis of BRG1 enriched regions indicate that BRG1 co-occurs on the chromatin on paclitaxel resistant cells with HIF1A, ISL1, MAF and ZNF76 transcription factors, but their functional impact on ABC overexpression revealed HIF1 as primary, effective BRG1 co-factor. TCGA Pan-Cancer data from drug untreated tumors suggest the existence of strong ABC gene co-repression between BRG1 and HIF, where the deficiency of both proteins boosts transcription of inter alia ABCC3, which remains repressed in paclitaxel resistant cells. Subsequently, we identify EP300 as co-activator of HIF1A. Transient silencing of either of these three factors considerably declined abundance of ABC proteins, which are overexpressed and enriched in lysosomes of paclitaxel-resistant cells, and intralysosomal accumulation of anticancer drugs. Our study provides first experimental evidence on the formation of BRG1-EP300-HIF1 functional complex at the promoters of ABC genes, which confer intralysosomal drug sequestration and, hence, paclitaxel resistance in breast and lung cancer cells. Grant: LIDER/22/0122/L-10/18/NCBR/2019.

ShT-04.3-3
Simultaneous targeting of LSD1 and PRMT5 through chimeric inhibitors as a novel therapeutic approach against leukemia

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The lysine-specific demethylase 1 (LSD1) catalyzes the removal of mono- and dimethyl modifications of Lys4 of histone H3 (H3K4me1/2), which are essential marks of transcriptional activation. LSD1 has been shown to play a central role in the insurgence of solid and blood cancers. In particular, it is highly expressed in acute myeloid leukemia (AML), a hematopoietic malignancy caused by abnormal proliferation and differentiation of blasts. In AML, LSD1 is crucial for the maintenance of cancer cell stemness, inhibition of cell differentiation, and prevention of apoptosis. Similar to LSD1, the protein arginine methyltransferase 5 (PRMT5), a methyltransferase that catalyzes the symmetric dimethylation of arginine residues, acts as an oncoprotein in AML. Indeed, PRMT5 activity was shown to support AML growth *in vitro* and *in vivo*. Given the involvement of both LSD1 and PRMT5 in AML, the simultaneous inhibition of these enzymes may represent a successful approach to treating this malignancy. Notably, we have identified a synergistic interaction between an LSD1 inhibitor and a PRMT5 inhibitor in multiple

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AML cell lines. The two inhibitors combined promote AML differentiation and eventually growth inhibition and apoptosis. To leverage this synthetic lethal interaction, we developed a series of dual-targeting LS*D1/PRMT5 inhibitors that could inhibit both enzymes *in vitro* in the submicromolar to nanomolar range while being selective over PRMT1 and PRMT7. Among, the prepared compounds, two of them impaired leukemic cell viability with higher potency compared to single-target inhibitors and induced apoptosis and myeloid differentiation. In addition, we were able to solve the X-ray co-crystal structure of one of the designed inhibitors with LSD1, thus elucidating its binding mode and providing a structural basis for the rational design of further inhibitors.

Sunday 30 June
17:00–19:00, Blue Room

Bio-based Polymers for Engineered 'Green' Materials

S-02.2-1
Tuning the properties of biopolymers: from biowaste towards more sustainable advanced materials

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Millions of tonnes of biowaste are generated annually by agricultural and industrial activities, leading to diverse environmental issues. The potential of biowaste to produce value-added products can help boost the circular economy and lead to a significant reduction in the amounts of waste generated and to the efficiency in the use of resources. In this context, alternative sources can be assessed to extract polysaccharides, such as agar or chitin. As for agar, red algae Rodophyta (*Gelidium sesquipedale*), collected in the Basque Country, can be used to extract agar in boiling water, a simple and sustainable process for agar production. Regarding chitin, this can also be obtained from marine waste, such as crustacean shells, following three processes: deproteinisation in an alkaline medium to separate proteins and polysaccharides, demineralisation to remove inorganic matter using an acid medium, and decolourisation to remove pigments. However, fruit fly pupae (*Ceratitis capitata*), which causes indirect economic losses in citrus production, or insects (*Tenebrio molitor*), used for food protein production, can be evaluated as alternative sources for chitin extraction since the demineralisation process can be avoided. Additionally, the use of squid pens as a source of chitin requires neither demineralisation nor decolourisation, thus reducing both production costs and environmental burden. This chitin can be used to improve the functional properties of other biopolymers, such as proteins (e.g. gelatin or soy protein), which can also be extracted from bio-residues, for the development of advanced materials with properties tailored to specific applications, such as tissue regeneration (e.g. wound healing).

S-02.2-2
Biopolymers as a tool for zero-waste materials

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We are entering a 'storm' of climate change and biological resource constraints. The earlier companies, cities, and countries plan ahead and prepare themselves for the predictable future, the better their chance of thriving. To slow down the impact, a step towards new logic including resilience and sustainability through exploring renewable biomass and waste streams not only to replace current practice but foremost pursue a way of delivering new value products and energy following the zero-waste concept by closing the loops, reducing the material and emission footprint and protecting the environment and resources for the future generations will be the inspiration for the presentation. The advances in biopolymer science and technology are aiming towards resilience, safe and sustainability by design approach and zero-waste. The successful showcase of natural biopolymers that have been utilized as a tool (cellulose nanomaterial, chitosan, alginate, and starch) towards the development of a business-ready application in the sectors of food, textile, cosmetics, and packaging will be demonstrated. The final note will be delivered towards the EU Green Deal Mission Ocean initiative, aiming to protect and restore our ocean, seas, and waters, where societal factors will be taken into account, thus giving a real future perspective for "green" bio-based polymers for an engineered future alternative to current material choice.

ShT-02.2-4
Fabrication of electrospun biofiber for biomedical applications

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Necmettin Erbakan University, Faculty of Science, Department of Molecular Biology and Genetics, Konya, Türkiye

Polyhydroxybutyrate (PHB) is a natural biopolymer synthesized by natural occurring microorganisms. In a natural environment, different bacterium such as purple non-sulfur bacteria (PNSB) can accumulate PHB as a carbon storage molecule under stress or nutrient limiting conditions. PHB polymer is alternative to conventional petroleum-based plastics and it can be used in the medical fields like tissue regeneration and wound healing due its biocompatibility and biodegradability properties. In this study, PHB extracted from various PNSB namely *Cereibacter sphaeroides*, *Rhodobacter capsulatus* and *Rhodopseudomonas palustris* was used to fabricate electrospun PHB/collagen biofibers to be used for wound healing and tissue regeneration. The bacterial strains were cultured under aerobic conditions with nitrogen supplied in scarce amounts, and the fermentation process was maintained at 30°C in shaker at 150 rpm. The solvent extraction method was utilized for recovery of intracellular PHB because it is simple, effective, fast, and produces high purity PHB. It was found that the PHB content of the bacteria was up to 11% (w/w). The extracted PHB was characterized by H-NMR and FTIR. Then, dried PHB was mixed with commercial collagen 1 to produce fibers by electrospinning. The PHB/collagen fibers were then developed using electrospinning and examined using field emission scanning electron microscopy (FE-SEM) to capture the nanofiber images. To conclude, the PHB/collagen fibers were successfully fabricated to be used in medical applications such as wound healing and tissue regeneration. The authors gratefully

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ShT-02.2-3
Enzyme-mediated tuning of cellulose surface reactivity for innovative compounding purposes

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In the last decades, biopolymers produced from natural sources (plants, animals, microorganisms) have gained popularity thanks to their outstanding properties. Cellulose, the most abundant biopolymer on Earth, is attracting attention because of its excellent physical and chemical properties, being also sustainable, renewable, and biodegradable. The intrinsic high crystallinity makes cellulose a promising reinforcing material in the formulation of elastomeric compounds. Nevertheless, modifying cellulose functional hydroxylic groups while preserving its crystalline structure is crucial for ensuring material compatibility. In this study, a lipase-mediated approach was exploited for the acylation of cellulose hydroxyl groups. The formation of the esteric bonds was confirmed through Attenuated Total Reflectance Fourier-Transform Infrared (FTIR-ATR) analysis and Thermodesorption-Gas Chromatography–Mass Spectrometry analysis (TD-GC-MS), whereas Wide-Angle X-Ray Diffraction (WAXRD) and Thermogravimetric Analysis (TGA) were used to verify the retention of crystallinity pattern and the polymer thermal stability, respectively. Subsequently, the modified cellulose was incorporated into a model elastomeric compound, with a commercial peroxide used as the vulcanizing agent. The mechanical and dynamic mechanical properties of the compound were tested, resulting in a general reinforcement of the system. In comparison to a control test using non-derivatized cellulose, the modified polymer exhibited increased stress at break and decreased tan delta, clearly indicating the compatibilization of cellulose through the tuning of functional group reactivity.

ShT-02.2-1
Exploring SCOBY bacterial cellulose interactions with graphene oxide in kombucha fermentation

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SCOBY bacterial cellulose (BC) is a biological macromolecule, deemed a by-product, that forms at the liquid–air interface during kombucha tea fermentation [1]. Given the extensive investigation on graphene hybrids and nanocomposite and biopolymer-based materials such as BC [2], this study explores the intricate interaction between the kombucha culture and graphene oxide (GO). The primary objective was to optimize the *in-situ* production of BC/GO hybrids during Kombucha cultivation, by monitoring the impact of GO on cellulose structure, mechanical properties as well as on consortium metabolism. Metagenomic analysis was performed to unveil the dynamic microbial consortium responsible for cellulose production of kombucha when grown in the presence of GO. Moreover, utilizing isolation techniques, specific cellulose-producing bacteria were identified and genomically characterized to gain insight into cellulose synthesis pathways and understand how GO-BC interactions shape the final material properties. Preliminary findings indicate that the proposed approach, after the thermal reduction of the GO phase to reduced GO (rGO), is able to produce porous BC/rGO hybrids with high electrical conductivity, with interesting applications in materials science. This comprehensive approach, combining metagenomic/genomic insights and BC/rGO production, provides new perspectives for developing innovative, sustainable materials with enhanced properties and multifunctional capabilities, thereby advancing biomaterials and applications across diverse scientific and industrial domains. References: [1] Laavanya D, Shirkole S and Balasubramanian P. "Current challenges, applications and future perspectives of SCOBY cellulose of Kombucha fermentation." Journal of Cleaner Production 295 (2021): 126454. [2] Kiangkitiwan N and Srikulkit K. "Preparation and properties of bacterial cellulose/graphene oxide composite films using dyeing method." Polymer Engineering & Science 61.6 (2021): 1854-1863.

ShT-02.2-2
From agrifood waste towards novel bio-based materials

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The organic fraction of urban waste is mainly composed of edible and non-edible discards from cereals and vegetables. Along with a strong effort to decrease food waste, the valorisation of non-edible biomass (peel, seeds, petals) is essential to decrease the problems connected to disposal while generating new value. In the logic of biorefinery, the initial biomass should be entirely valorised in an array of products. In this work, we aimed at applying this principle to a previously established production of novel leather-like material obtained from fruit peels, which generates a liquid waste that is currently not valorised, by introducing enzymatic hydrolysis and microbial fermentations. The first part of the study consisted in comparing the properties of the final leather-like material obtained by alternative processing of the biomass. The process involved (i) mechanical pre-treatment of the fruit peel; (ii) enzymatic *versus* chemical hydrolysis; (iii) blending of the solid fraction with plasticizers to obtain the desired film; (iv) characterization of the mechanical properties of the different final leather-like material. We proved that by changing the enzymatic cocktail or the conditions of the hydrolysis it is possible to vary the composition of the biomass and so to create bio-based materials with tuneable mechanical properties. In the second part of the study, we characterized the liquid fractions obtained from the chemical versus enzymatic hydrolysis and we tested them as growth media for yeast cell factories. The preliminary results showed that these liquid fractions can be used to grow model yeasts, with the possibility to produce high-value products such as carotenoids and organic acids. The work showed that it is possible to generate a process integrating the production of novel bio-based materials from agrifood waste

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with the production of biotechnological compounds through the valorisation of the liquid discards from the process by using yeast cell factories. *The authors marked with an asterisk equally contributed to the work.

Monday 1 July
8:30–10:30, Silver Room

Functional Foods and Human Health – Part A

S-03.3-1
Impact of dietary polyphenols on brain functions through the gut–brain axis modulation

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Accumulating evidence suggests that diet and lifestyle can play an important role in delaying the onset or halting the progression of age-related health disorders and to improve cognitive function. A growing number of dietary intervention studies in humans and animals and in particular those using polyphenols, have been proposed to exert a multiplicity of neuroprotective actions within the brain. Recently, tremendous progress has been made in characterising the bidirectional interactions between the central nervous system and the gastrointestinal tract. This concept of a microbiome–gut–brain axis suggests that modulation of the gut microbiota is a tractable approach for developing novel strategies for the regulation of overall brain function. Such findings argue in favour of an approach of modulating the microbiome and indirectly brain functions with dietary interventions containing defined nutrients and food bioactives designed to promote healthier ageing. Amongst those nutrients, polyphenols have been consistently reported to play a protective role against cognitive decline and have the ability to modify the microbiome composition and metabolism. Thus, the purpose of this presentation is to provide an overview the regulation of cognitive functions by dietary polyphenols and to present some of their molecular mechanisms of action, including a potential to modulate the gut microbiota, to protect neurons against injury induced by neurotoxins, an ability to suppress neuroinflammation. Altogether, these processes act to maintain brain homeostasis and play important roles in neuronal stress adaptation and thus polyphenols might have the potential to prevent the progression of neurodegenerative pathologies.

S-03.3-2
Microplastics and proteins interplay in food and environment

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The presence of tiny plastic particles (micro- and nanoplastics) in various environments pose a threat to food security, food safety, and human health. Solid data on the prevalence of microplastics particles in the environment are limited due to the analytical challenges of extraction, characterization, and quantification from complex environmental matrices. Microplastics found in the diet can be derived from food additives (salt, sugar), drinking water, microplastics incorporated into the food chain or released from plastic packaging during food processing. Reusable plastic bottles have also been identified as a source of microplastics. Apart from their physical presence as environmental pollutants, concerns have been raised regarding binding of the other components to microplastics, in which case, an interplay of contaminants can result in outcomes that are not easy to predict. For instance, there is a substantial lack of knowledge on binding of allergenic proteins to microplastics and influence on the development of allergy and processes relevant for allergen degradation and presentation to the immune system (i.e. digestibility and bioavailability). We aim to understand the effect of micro- and nanoplastics combined with allergens adhering to their surfaces throughout their way into the human body. Our findings support the impact of microplastics on digestion of (allergenic) food proteins and their degradation pattern. Interplay of digestive enzymes and proteins bound in corona (particularly high affinity binding partners) make a significant impact on the digestion profiles of allergenic proteins and profiles of epitopes to which we can be exposed and their survival in the digestive tract.

ShT-03.3-2
Inhibition of PUFA mitochondrial metabolism: a novel approach to elevate PUFA levels

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Omega-3 polyunsaturated fatty acids (PUFAs) are renowned for their health benefits in managing cardiometabolic diseases. However, the findings from epidemiological studies present a paradox, with some large-scale investigations failing to confirm the advantageous effects of omega-3 PUFA supplementation. One potential explanation lies in the rapid mitochondrial oxidation of PUFAs compared to saturated fatty acids (FAs), which may hinder dietary supplementation efforts from raising PUFA levels. Our study seeks to explore a novel strategy for enhancing PUFA levels by impeding PUFA breakdown in mitochondria. To investigate this hypothesis, we employed compounds such as methyl-GBB and meldonium, known to limit FA metabolism, alongside a knockout (KO) mouse model lacking the trimethyllysine hydroxylase epsilon (Tmlhe) gene, which results in lost activity of the first enzyme in the carnitine/acylcarnitine synthesis pathway. In Tmlhe KO mice, we observed a 30% reduction in FA metabolism, resulting in a significant increase in PUFA levels and unsaturated lipids by up to twofold. Pharmacological inhibition of FA metabolism with methyl-GBB similarly elevated plasma PUFA levels in obese Zucker rats. Furthermore, in a cohort of 30 healthy volunteers, we evaluated whether supplementation combined with meldonium-mediated inhibition of carnitine and acylcarnitine biosynthesis could selectively augment PUFA levels. Meldonium treatment notably decreased short- and medium-chain acylcarnitine levels. Combining PUFA supplementation with meldonium treatment led to a 1.5-2-fold increase in serum EPA and DHA levels compared to meldonium or PUFA supplements alone. In conclusion, inhibiting PUFA mitochondrial metabolism emerges as a promising approach for enhancing

PUFA levels, offering potential therapeutic implications for cardiometabolic health.

ShT-03.3-3
A chemical–biological study of bioactive compounds from propolis provides new details on the molecular basis of the anti-inflammatory effects of this multifunctional food

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Propolis is a very complex mixture of polyphenols, terpenoids, lipids and sugars, whose multiple beneficial effects on humans are widely reported. One of the major biological activities for which propolis is credited is anti-inflammatory, and its use for preventive or therapeutic treatments of rheumatoid arthritis (RA) has been suggested. As part of research on the functional characterization of propolis produced in the internal areas of the Italian region of Campania, we undertook a study aimed at shedding light on the biochemical mechanisms underlying their anti-inflammatory activity. As a first step, we subjected an ethanolic extract of propolis to chromatographic separation. We assayed the resulting fractions to evaluate their ability to reduce interleukin-6 secretion from human synoviocytes in which inflammation was induced, selected as an *in vitro* model of RA. The most active fraction was essentially composed by pinobanksin and phenethyl caffeate (CAPE). Therefore, we investigated the biological activity of these two compounds. Firstly, we analyzed their effect on the expression of inflammatory proteins in 2D cell model, thus revealing that pinobanksin and CAPE treatment induced down-regulation of COX-2, STAT-3 and phosphorylated-STAT-3. Conversely, CAPE seemed to affect marginally the levels of COX-2. Therefore, we used drug affinity responsive target assay, a chemical-proteomic approach, to identify the putative target(s) of this compound in synoviocytes. Among the protein emerged as potential targets of CAPE, we focused on transportin-2 and AP2 adaptor complex. Indeed, they are involved in nuclear-cytosolic shuttling of proteins, which plays a pivotal role in the inflammation processes. Finally, a 3D-synoviocytes culture was used to confirm the results in an innovative model of inflammation.

ShT-03.3-4
Trehalose effect in patients with Alzheimer's disease: focus on circulating microRNAs assessed by direct hybridization

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Trehalose is a non-reducing disaccharide constituted by two glucose molecules, and it is considered novel food. It is present naturally in some plants, microorganisms, and insects now included in human nutrition. It is worth noting that trehalose is not present in mammals. Recently, it has been suggested to delay the neurodegeneration process in Alzheimer's disease (AD), in which there is no cure at the moment. Trehalose could explicate its protective roles against the development of this chronic disease by modulating epigenetic molecules such as microRNAs. Herein, twenty AD patients were randomly assigned to two groups: one received 15 g per week of intravenous trehalose for 12 weeks, and a placebo receiving saline. The study was registered in the Iranian Registry of Clinical Trials (Code: IRCT20130829014521N15). Blood samples were obtained at the beginning and end of the treatment. Circulating microRNAs were assessed with the ncounter flex platform and differentially expressed to trehalose-treated group were identified: hsa-miR-1268a, hsa-miR-3605-3p, hsa-miR-555, and hsa-miR-6511a-3p were significantly downregulated, while hsa-miR-324-3p and hsa-miR-539-5p showed significant upregulation. Their gene targets were determined through bioinformatics approaches, revealing that trehalose treatment impacts critical AD-related pathways and proteins. * The authors marked with an asterisk equally contributed to the work.

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ShT-03.3-1
A step forward in understanding oleocanthal anti-inflammatory activity by proteomic analysis in microglial cells

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Neurodegenerative diseases are highly devastating disorders affecting millions of people worldwide. Although these diseases exhibit distinct pathogenetic mechanisms, including diverse protein aggregates and genetic variations, they all share a common characteristic: persistent neuroinflammation. Previous data obtained in our laboratory have demonstrated that oleocanthal (OL), a compound found in extra virgin olive oil, exhibits significant anti-inflammatory activity in microglial cells. To better characterize the underlying mechanism of this anti-inflammatory effect, we evaluate the proteomic profile of murine microglial BV-2 cells pre-treated with 10 μM OL and then exposed to 100 ng/mL LPS. Protein extracts were analyzed by 2D electrophoresis and LC/MS/MS was used to identify the spots of interest. About 111 differently expressed proteins were identified between LPS and control, 62 proteins between OL + LPS and LPS, and 31 proteins were present in both comparisons. Of note, the treatment with only OL did not alter the protein pattern compared to control cells. In a general overview of proteins modulated by LPS exposure, it emerges that OL pre-treatment is able to bring the levels of these proteins to values comparable to those of control cells, with an effect that could be defined as signal dampening. Among these proteins, aconitate decarboxylase 1, cytochrome b5 type B, ubiquitin C-terminal hydrolase L3, ATP synthase F1 subunit alpha, tyrosine 3- /tryptophan 5-monooxygenase activation protein beta and theta, interferon-induced protein with tetratricopeptide repeats 3, cathepsin A were strongly up-regulated by LPS and down-regulated by OL pre-treatment; clathrin light chain A was strongly down-regulated by LPS and up-regulated by OL pre-treatment. Our findings indicate novel molecular targets of LPS, and since OL alone did not modify protein levels, it leads us to hypothesize that it could act through an antagonistic mechanism on TLR4, blocking the LPS signal. This work was supported by MUR-PRIN 2022 (Prot. 20222W7P7S) to Cristina Angeloni.

Monday 1 July
8:30–10:30, Red Room

Enzymes and Cell Therapies

S-04.4-2
Liver directed gene therapy for inherited metabolic diseases

N. Brunetti-Pirtti
Telethon Institute of Genetics and Medicine, Pozzuoli (NA), Italy

Inherited metabolic disorders (IMDs) are a growing group of genetic diseases caused by defects in enzymes that mediate cellular metabolism, often resulting in the accumulation of toxic substrates. The liver is a highly metabolically active organ that hosts several thousand chemical reactions. As such, it is an organ frequently associated with IMDs. In my talk, I will discuss current approaches and unresolved challenges for liverdirected gene-based therapy aimed at metabolite detoxification in a variety of IMDs.

S-04.4-1
Enzyme enhancing strategies: reshaping the treatment landscape of a galactose disorder?

E. Rubio-Gozalbo
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Classic galactosemia is an inherited metabolic disorder with thousands of patients affected in Europe (prevalence 1:16 000 to 1:50 000 live births in Western countries). It is caused by severe deficiency of galactose-1-phosphate:uridylyltransferase activity, pivotal to galactose metabolism. Substrate reduction using a galactose-restricted diet does not prevent brain and ovarian complications and poses a high burden to patients and families. To address the unmet treatment need, enhancing or restoring GALT activity seems promising. Our group studies chaperones and nucleic acid therapies. Many of the pathogenic variants lead to conformational abnormalities that make GALT amenable to chaperones. The high prevalent variant in the Caucasian population, p.Gln188Arg, gives rise to a protein with lower stability and catalytic activity. In a prokaryotic model, the chemical chaperone arginine showed beneficial effect. A study with arginine administration to patients with the p.Gln188Arg/p.Gln188Arg variant, as well as *in vitro* studies, failed however to exhibit a positive effect. Other variants and other chaperones need to be explored yet. Nucleic acid therapy is another approach to restore enzyme activity. In our *galt* knockout zebrafish model, lipid nanoparticle (LNP)-packaged *hGALT* mRNA was effectively translated and processed without signs of toxicity. One cell-stage and intravenous single-dose injections resulted in hGALT protein expression and enzyme activity at 5 days post fertilization. In adult fish, the used LNP primarily targeted the intestine and pancreas, with absent signal in the ovaries. Our next step is to facilitate direct delivery to the female gonads by using nanoparticles that specifically target ovarian follicle receptors. The results encourage and challenge us, as each modality comes with advantages and limitations. For all of them, there are many unknowns that need to be tackled in our journey to reshape the treatment landscape.

ShT-04.4-1
Drug and genetic material co-loaded liposomes for repairing chondrocytes in osteoarthritic conditions

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Chronic knee and lower back pain due to osteoarthritis (OA) and intervertebral disc (IVD) degeneration (IVDD) have a global prevalence and impact human wellbeing by impairing mobility. Oxidative stress is a key factor in OA and IVDD pathogenesis. Non-viral gene therapy is a promising approach for safe and precise joint and disc restoration. Our study focuses on developing a liposomal formulation for efficient co-delivery of curcumin and therapeutic siRNA. Curcumin downregulates many inflammatory cytokines along with free radicals and upregulates collagen and aggrecan, therefore reducing pain and helping in regeneration. Luciferase siRNA was utilized for screening and prototyping, demonstrating effective transfection into luciferase-expressing chondrocytes (C28/I2 cells). Quality by Design principles guided formulation development, employing risk assessment, Design of Experiments (DoE), and optimization. Responses evaluated included particle size, polydispersity index, zeta potential, curcumin encapsulation efficiency, cell viability, siRNA complexation capacity, and luciferase activity. Statistical analysis using MODDE software helped us formulate optimum liposomes. The primary chondrocytes were then induced with oxidative stress and inflammatory conditions and treated with optimum liposomes. The effect of curcumin was analyzed through numerous biochemical tests like total antioxidant capacity, malondialdehyde levels, and qRTPCR for the cytokines. The optimum liposomes complexed with therapeutic siRNA like the IL-6 and IL-8 siRNA were evaluated by qRTPCR and ELISA on inflamed primary chondrocyte cells. These co-loaded liposomes effectively transfected chondrocytes with no toxicity and could be successfully carried forward for testing *in vitro* in OA and IVDD models. This study has yielded not only an optimum formulation but also serves as a platform for the incorporation of other lipophilic drugs and any negatively charged genetic material for various ailments.

ShT-04.4-3
Red blood cells-derived extracellular vesicles for the loading and delivery of RNA molecules

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The recent advent of biologics needs increasingly sophisticated drug delivery systems. Red blood cells (RBCs) have long been used as cell therapy thanks to their unique properties. Lately, extracellular vesicles (EVs) are attracting much more attention for the delivery of therapeutic cargoes. RBC-derived EVs (RBCEVs) are one of the most promising. However, their production is still challenging for several reasons (e.g., yield of production, loading efficiency and translatability to the clinics). Here, we propose a new way for the generation of RBCEVs loaded with RNA molecules that overcomes most of these issues. Our strategy is based on the preloading of the cargo into RBCs followed by vesiculation. The first is carried out by an already established technology, while the latter by "soft extrusion", a newly developed and patented physical vesiculation method. We showed that RBCs could be efficiently preloaded with several kinds of molecules and further used to generate RBCEVs. Indeed, our method was able to produce a very high yield of cargo-loaded RBCEVs. Preloading of fluorescent dextran-conjugates enabled to demonstrate that the cargo was definitely retained in RBCEVs. Moreover, the obtained RBCEVs population has been deeply characterized by DLS, NTA, TEM and flow cytometry, showing great homogeneity in terms of size, biological features and cargo. *In vitro* results demonstrated that RBCEVs were abundantly internalized by cells. Finally, proof-of-concept studies proved that a miRNA could be efficiently loaded into RBCEVs, effectively delivered to HUVEC and able to exert its biological effect. Briefly, miR-210 was capable of inhibiting its target PTP1B and lowering mitochondrial metabolism. Studies with long mRNAs to treat metabolic diseases are ongoing. Of note, the bench-scale process might be easily scaled-up and translated into the clinics. Hence, this investigation could lay the foundation for the development of a new biomimetic platform for RNA-based therapies.

ShT-04.4-2
Mycobacterium tuberculosis methionine aminopeptidase a new target for the development of novel antitubercular compounds

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Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis, is a major global health challenge in particular due to Multi-Drug Resistant (MDR) strains, and eXtensively Drug Resistant (XDR) strains. Methionine aminopeptidase (MetAP) is essential for the N-terminal methionine excision of peptides during protein synthesis and hence regulates protein activity by

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determining and enabling additional modifications, including acetylation and others. This enzyme is indispensable for all life forms, including eukaryotes and prokaryotes and is particularly vital for bacterial replication. As such, MetAP represents a potential target for the development of antibacterial and anti-tuberculosis therapies. In Mtb, the mapB gene encodes the MetAP1c isoform that is of major significance. To this purpose, the enzyme was produced in recombinant form and purified. The enzyme activity assay was set up, using a synthetic fluorescent substrate, allowing the investigation of its steady-state kinetic properties, and divalent metal ions dependence. The enzyme was then proved to be suitable for inhibition studies, thus was used for the *in silico* and *in vitro* screening of compounds library, to identify scaffolds suitable to be developed as significant inhibitors. The identification and validation of inhibitors could pave the way for novel drugs to fight tuberculosis, particularly against drug-resistant Mtb strains. Our findings contribute valuable knowledge to the ongoing efforts to address the global tuberculosis burden and underscore MetAP1c as a promising target for future anti-tuberculosis drug development, this will be supported by a study in Mtb cells to further confirm the anti-mycobacterial activity of the developed compound.

Monday 1 July
8:30–10:30, Blue Room

New Insights into Cellular Organelles

S-01.4-2
Mitochondrial signaling – touch me, don't touch me, just be sweet

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Organelle communication is a key feature or eukaryotic cells. Physical contact sites between organelles provide an excellent platform for cross-organelle molecule transfer. The mitochondria-endoplasmic reticulum contact sites (MERCS) are conserved structures from yeast to higher eukaryotes. Many tether proteins keeping MERCS have been identified in mammals. However, it remains unclear how MERCS integrate with the cellular signaling environment. AMP-dependent protein kinase (AMPK) is a key regulator of cellular metabolism, with anti-anabolic and pro-catabolic roles, including promotion of mitochondrial biogenesis and autophagy. Here, we use electron microscopy, confocal imaging and biochemical approaches to show that AMPK is also involved in the regulation of MERCS. Cells lacking AMPK activity have increased number of MERCS, and restoring AMPK activity or its downstream target mitochondrial fission factor also restores the number of MERCS. Reciprocally, hyperactivation of AMPK reduces the number of MERCS. This study shows that MERCS are regulated by a key cellular signaling hub, AMPK, and dynamically regulated in response to the signaling environment.

S-01.4-1
Unbiased screenings illuminate regulators of mitochondria-endoplasmic reticulum contact sites

L. Scorrano
University of Padova, Padova, Italy

Mitochondria and endoplasmic reticulum (ER) are physically linked and appropriately spaced at specific points known as Mitochondria-Endoplasmic Reticulum (ER) contacts (MERCs) through partially understood protein bridges. To establish a comprehensive molecular atlas of MERCs, we conducted a genome-wide screening using short hairpin RNA (shRNA) and combined it with high-content, ratiometric, quantitative microscopy of a FRET ER-mitochondria proximity probe (FEMP) and iBAQ proteomic analysis of MERCs. Through automated image analysis, statistical evaluations, and iterative screening, we identified 107 gene candidates classified as tethers (genes whose removal increases the distance between ER and mitochondria) which included well-known mammalian tethers like Mfn2. Additionally, we identified 97 Spacer genes (genes whose removal decreases the distance between ER and mitochondria). These gene candidates were found to be enriched in calcium signaling, lipid biosynthesis, and metabolism processes known to localize at this interface. By cross-referencing the gene list with the proteome of MERCs determined by iBAQ analysis, we refined our findings to 25 Spacers and 18 Tethers. Orthogonal assays validating mitochondria-ER juxtaposition further highlighted the effectiveness of our screening approach in identifying and functionally characterizing MERCs components.

ShT-01.4-2
Voltage dependent anion-selective channel (VDAC), the gatekeeper of mitochondria, as a signaling hub for diseases

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VDACs are outer membrane mitochondrial proteins, allowing cross-talk between the organelle and cytosol and the transport of anions, cations, ATP, Ca^{2+} and metabolites. VDAC is a critical player in mitochondria mediated apoptosis and its role in cytochrome c release is mediated by interactions with pro−/anti-apoptotic proteins (1). In addition, VDAC1 is now considered a general hub on the surface of mitochondria, since it was demonstrated to interact with over 154 proteins including metabolic, cytoskeleton, apoptogenic, intra-organelle receptors (2). Most pathological conditions lead to dysfunction of the mitochondria. Striking evidence exist of the generalized overexpression of VDAC1 at the onset of tumors, of neurodegenerative diseases but also of cardiovascular ones and even Type 2 Diabetes, and with age. This indicates that VDAC1 overexpression is a hallmark of disease (2). VDAC1 gene can thus be seen as a signaling hub where the overexpression of the protein is decided. The decision will contribute to the on/off apoptotic pathway switch. In this work we will report unpublished data about the regulatory pathways involved in the expression of VDAC1 gene and the impact on them of anti-cancer drugs like cisplatin and JNK inhibitor SP600125. Another approach will also be delivered, showing that a new group of small molecules, partially selected by a drug screening aimed to discover new inhibitors and others formed by RNA, impact on the VDAC1 activity and on the interaction between VDAC and hexokinase.

These data will show that VDAC1 has a crucial role in diseases since it is involved in cell processes at the beginning of the pathological cascade. References: 1) De Pinto V. Biomolecules (2021) 11:107. 2) Shoshan-Barmatz V, Shteinfer-Kuzmine A, Verma A. Biomolecules (2020) 10: 1485. Ackn: PNRR M4C2-Investment 1.4- CN000041, PRIN 2022NLLTRJ_001.

ShT-01.4-1
Sweet Golgi – the role of vesicular tethers and SNAREs in Golgi glycosylation

F.T. Sumya, A. Khakurel, Z. D'Souza, I. Pokrovskaya, **V. Lupashin**
University of Arkansas for Medical Sciences, Little Rock, United States of America

The Golgi apparatus plays a pivotal role in the secretory pathway by facilitating the sorting and modification of macromolecules through glycosylation. Maintaining Golgi function requires a coordinated effort of recycling and retrieval mechanisms. These mechanisms involve recycling Golgi enzymes through transport vesicles originating at the trans-Golgi network (TGN) and trans-Golgi. These carriers are then tethered and fused to early Golgi cisternae by vesicular tethers and SNAREs. The exact mechanism of intra-Golgi trafficking is an enigma. In our study, we employed a comprehensive array of techniques, including gene editing, degron-assisted rapid degradation, TurboID proximal biotinylation, mass-spectrometry analysis, EM and superresolution microscopy. Our aim is to unravel the role of COG and GARP vesicle tethering complexes, as well as SNAREs, in Golgi. Our findings highlight the crucial role of the GARP in facilitating proper Golgi glycosylation. Acute depletion of the unique GARP Vps54 subunit resulted in rapid defects in O-glycosylation, suggesting the existence of a regulated pathway responsible for recycling enzymes beyond the Golgi stack. We also discovered that all COG subunits are essential for recycling of the Golgi glycosylation machinery. Acute degradation of COG4 led to the accumulation of distinct vesicle populations carrying Golgi enzymes and sugar transporters. Proximity labeling of endogenously expressed TurboID-fused tethers identified coats, cargo, and SNAREs that utilize specific recycling pathways. Furthermore, the mass-spectrometry analysis of Golgi SNAREs uncovered two previously unidentified SNARE complexes, revealing unexpected flexibility in intra-Golgi trafficking in response to failures in the canonical tethering and fusion machinery. Our findings provide novel insights into the regulation of Golgi glycosylation machinery and have strong implications for the development of therapeutic approaches targeting diseases arising from disruptions in Golgi functions.

ShT-01.4-3
Impact of lysosomal nutrient sensing on metabolic dysregulation

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Rabconnectin-3α (Rbcn-3α) is an essential protein encoded by the DMXL2 gene, and it is believed to regulate the eukaryotic vacuolar-type proton pumping ATPase (v-ATPase), a key component of organelles that acidify. Patients with DMXL2 mutations exhibit growth retardation and impaired glucose metabolism. However, the molecular links between Rbcn-3α and cell metabolism, as well as its effects on cell proliferation and glucose metabolism, remain largely unknown. In this study, we utilized a DMXL2 knockout (DMXL2KO) model using mouse fibroblasts to investigate the significance of Rabconnectin-3α in lysosomal biology. We evaluated lysosomal gene expression and assessed membrane acidification as indicators of lysosomal function. Additionally, we explored the impact of Rbcn-3α deficiency on v-ATPase expression and examined the response of the DMXL2KO model to a v-ATPase inhibitor, Bafilomycin A1. Our findings reveal several key insights. Firstly, we observed decreased v-ATPase activity in the absence of Rbcn-3α. Furthermore, alterations in lysosomal size and morphology were evident, suggesting impaired lysosomal function and autophagy defects. Interestingly, nutrient deprivation led to increased lysosomal biogenesis in DMXL2KO MEFs. In context to cell metabolism, we found altered glucose flux, increased uptake of glucose and decreased glycolysis enzyme protein expression in DMXL2KO MEFs. Overall, our study provides novel insights into the role of Rabconnectin-3α in lysosomal biology, cell metabolism, and its potential implications for human health. Understanding the molecular mechanisms underlying Rbcn-3α function may pave the way for future therapeutic interventions targeting lysosomal dysfunction and metabolic disorders associated with DMXL2 mutations.

Monday 1 July
8:30–10:30, Yellow Room

Biochemical Strategies for Cultural Heritage

S-02.3-1
Chasing spectral shadows into the dark proteome. How cultural heritage is making us question our assumptions about the analysis of old proteins

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In the realm of cultural heritage – parchments, glues, and binders – spectral shadows haunt the dark proteome. A profound challenge exists: a significant portion of fragment ion spectra – up to 94% – fail to be matched through conventional database searches. Identification is impeded by the databases selected, the modifications considered, missed cleavages, inconsistent digestion, and almost certainly many other factors. The complexities encountered in palaeoproteomics are not isolated to this field. They must also be relevant in areas such as metaproteomics, studies involving non-model organisms, low-abundance and/or highly priced proteins. The complex composition of historical materials, from the types of animal skins used in parchments to the diverse range of natural adhesives and binders, adds layers of complexity to the identification process, mirroring the challenges faced in these other proteomic disciplines. Such advances are pivotal not only for uncovering the untold stories hidden within the "dark matter" of ancient proteomic datasets but also for enriching our understanding of historical and cultural heritage.

Improvements in data sharing and search methodologies will help the study of ancient manuscripts and artifacts, offering new insights into the materials and techniques employed by past civilizations. This collaborative endeavor has the potential to shed light on the legacy of human creativity and ingenuity, revealing connections across time.

S-02.3-2
Decoding biomolecules and their networks in artists' materials using layer-by-layer high resolution MALDI imaging and crosslinking mass spectrometry

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Recent technical advances in mass spectrometry (MS) have allowed the emergence of new methods to study ancient biomolecules constitutive of objects of cultural heritage. One major breakthrough is the reliable structural elucidation of proteins, oils and sugars using omics techniques. Spectacular progress has been made throughout the years, reducing sample amounts and increasing the quality of output information, producing a deeper knowledge of the artwork and facilitating conservation or resolving treatment issues. The biggest challenge faced today in heritage MS is the chemical decoding of the networks through the characterization of crosslinked biopolymers, their modifications, degradation and interaction with other components. The newest MS-driven methods such as top-down proteomics and hydrogen deuterium exchange MS developed to address this challenge will be presented. We will discuss how their combined use contribute to explaining the molecular complexity of these networks and accessing the original, transformed or degraded forms of the biomolecules in their environment. Among the topics that will be discussed are the protein conformational changes and interactions with pigments that occur during paint manufacture, drying and aging. Furthermore, several case studies will show how chemical signatures can distinguish a particular restoration procedure, focusing on induced chemical crosslinking. Finally, the presentation will show how high resolution MALDI imaging can decode the biomolecular organization of paint layers by identifying and mapping both organic and inorganic compounds. We will demonstrate the suitability of the technique to detect intact materials, by-products and complexes within samples, at the surface, within the layers and at layer interfaces of artworks. The informative power of this technique applied for the first time to historic artworks will be illustrated by the study of several master pieces from the Metropolitan Museum of Art's collection.

ShT-02.3-1
Bioactive preservation: chitosan/poly-γ-glutamic acid for cultural heritage restoration

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This research delves into innovative approaches for preserving cultural heritage by prioritizing sustainability and environmental consciousness. The study explores the transformative potential of chitosan (CH) and poly-γ-glutamic acid (γ-PGA) in developing hydrogel materials through non-covalent, electrostatic interactions using a physical blending technique. The investigation employs CH derived from crustacean shrimp shells and γ-PGA synthesized by Bacillus species, focusing on specific molecular weights (CH: 285 kDa, γ-PGA fractions: R1 = 55 kDa, R2 = 20 kDa). Blended materials are created under acidic conditions (pH 3.5) with different ratios of CHR1 to CHR2. The resulting hydrogels exhibit saloplastic and thermoplastic characteristics, transforming into a dry, solid state at room temperature. Various characterizations confirm complexation and robust crosslinking between biopolymers, emphasizing the gelation properties of CHR1 with a smooth and compact structure and excellent thermal stability. The study extends to bioadhesive applications on wood and aluminium surfaces, comparing them to a commercial polyvinyl acetate glue. CHR1 and CHR2 demonstrate comparable adhesive strength on wood, exhibiting adhesive/cohesive failure and stress endurance. On aluminium substrates, CHR2 displays the highest adhesive strength and adhesive/cohesive failure, while CHR1 shows similar adhesion strength with adhesive failure. Beyond their adhesive properties, the versatility of these materials opens avenues for applications in heritage formulations, including paint, coatings, and adhesives. *The authors marked with an asterisk equally contributed to the work.

ShT-02.3-2
Ancient proteins: current challenges in identification and characterization

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Lessons from the past. Materials used in the past in artworks and crafts have been the subject of numerous investigations. The recent technical advances in bioanalytical chemistry and mass spectrometry (MS) allowed the emergence of methods fully adapted to the study of ancient proteins in works and objects of our cultural heritage, and specifically are central to several paleoproteomic projects aimed to develop knowledge as well as to provide molecular details useful for conscious restoring interventions. While, early in this century, the big question was whether it was possible to identify proteins in degraded and complex environments such as those of artistic objects and archaeological finds, the biggest challenges we are facing today in relation to proteins in cultural heritage materials, relate to the

characterization of their modifications and degradation profile, their networks and interaction with other components (organic and inorganic material). The molecular signatures impressed in the primary structure reflect the environment and the age the objects lived in, but also the conformational changes the proteins underwent upon interacting with the other chemical component the objects were made of. Study cases will be presented ranging from proteins within the pictorial matrices of tempera paintings to bone proteins exposed to peculiar burial environments such as those experienced by victims of volcanic eruption. On the other side, methodology development is now in the direction of facing the compelling request for less invasive and more sensitive analyses that can meet the needs of the world of cultural heritage. The development and implementation of innovative tools for sampling proteins in ancient objects will be presented.

Monday 1 July
16:30–18:30, Silver Room

Targeting Metabolism in Cancer

S-04.5-1
Modulating amino acid cross-talk between the tumor and the host to improve cancer diagnosis and therapy

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Cancer-associated cachexia (CAC) is an incurable, pervasive clinical challenge. All cancer types can present with CAC, which contributes to up to 30% of cancer-related deaths, either directly or by fostering therapy resistance. Addressing CAC requires the dissection of intricate interactions between multiple physiological systems contributing to CAC pathogenesis. Despite extensive investigations into how tumors rewire their metabolism and that of their microenvironment, the broader impact of tumors on whole-body metabolism, i.e. tumor MACRO-Environment, and conversely, remains largely unexplored. We recently demonstrated that cancer-induced inflammation alters liver metabolism by reducing levels of HNF4a, impacting tumor proliferation and weight loss in human cancer patients and animal models. Interestingly, we now find that HNF4a is also regulated by the autonomic nervous system (ANS), which innervates the liver. Furthermore, we find that neuromodulation of the ANS preserves liver and muscle amino acid metabolism and alleviates CAC manifestations independent of tumor burden. We hence hypothesize that liver metabolism is pivotal in promoting CAC in extrahepatic cancers. Undoubtedly, gaining knowledge on the contribution of host metabolism to tumor progression to CAC holds immense potential to interrupt the cascade of deteriorating events and ultimately enhance outcomes for cancer patients.

S-04.5-3
Targeting cancer metabolism by ketogenic diet

D.D. Weber[I], S. Aminzadeh-Gohari[I,II,III], J. Tevini[I], J. Barret[III,IV], C. Herzog[II,III], L. Catalano[I], V. Stefan[I], E. Redl[II,III], R. Lang[V], T.K. Felder[VI,VII], M. Widschwendter[II,III,VIII,IX], **B. Kofler[I]**
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Cancer metabolism is frequently characterized by low mitochondrial OXPHOS activity due to partially dysfunctional mitochondria and elevated aerobic glycolysis, which is known as the Warburg effect. Dietary interventions, such as a low-carbohydrate, high-fat ketogenic diet (KD), are highly attractive approaches to target these metabolic vulnerabilities of tumor cells. We have used several preclinical cancer models, including the childhood cancer neuroblastoma, melanoma, renal cell carcinoma and breast cancer, to evaluate the effect of the KD on tumor proliferation, mainly in combination with other anti-cancer therapies. Apart from the renal cancer model, which is associated with a paraneoplastic syndrome of the liver, we observed an anti-tumor and a pro- survival effect in the remaining cancer models (1, 2). In addition, KD enhanced the effect of established anti-tumor therapies (3). Interestingly, ketone bodies have been shown to directly inhibit proliferation of only certain types of tumor cells *in vitro*. Therefore, also other mechanisms seem to be responsible for the anti-proliferative effect of the KD. We and others have observed that certain tumor types being responsive to KD lack OXCT1, a key enzyme responsible for ketone body metabolism. Furthermore, integrated multi-omics analysis including transcriptomics, metabolomics and epigenetic analysis indicate tumor type specific effects. Our data support the anti-tumor effect of KD in immunocompromised and immunocompetent preclinical cancer models and demonstrate that KD can sensitize tumors to other targeted anti-cancer therapies. References: (1) Vidali et al. (2017) Oncotarget 8(34):57201–57215 (2) Weber DD (2022) Cancer Metab. 18;10(1):12 (3) Catalano L (2023) Metabolites 13(8):910.

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S-04.5-2
The interplay of oxygen, diet and microbiota in inflammation and cancer: lessons from fruit flies

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Diet and microbiota impinge on inflammatory disease and cancer, and oxygen availability plays a key role in angiogenesis and tumorigenesis. To uncover the basic mechanisms contributing to inflammation and cancer *in vivo*, we are using the fruit fly, *Drosophila melanogaster*, as a powerful genetic model. Focusing on the adult fly intestine, which is structurally, molecularly and cellularly similar with the human gut, we have addressed the role of oxygenation, diet and microbiota in intestinal homeostasis, infection-induced inflammation and tumorigenesis. We have shown that the fly gut is oxygenated by a complex vascular network, the tracheae, which expands extensively upon infection-induced inflammation and in tumors. This neoangiogenesis-like phenotype depends on the conserved FGFR/FGF/Hif-1a pathway and promotes inflammation and cancer. In addition, oxygen is necessary for intestinal stem cell (ISC) mitosis during regeneration and tumor growth, and neither can proceed in hypoxic conditions [Published in: Tamamouna V et al. (2021) Nature Cell Biology 23(5): 497–510]. Furthermore, we have shown that ISCs depend on the absorption of the essential vitamin B7 (or biotin), and the recycling of cholesterol: when these nutrients are not available, ISC mitosis is abolished and cancer is reduced. Interestingly, we have found that silencing of the biotin internalization receptor Smvt or the cholesterol recycling receptor Npc2c causes microbial dysbiosis. This altered microbiota composition impinges on dietary nutrient availability and affects animal physiology [Published in: Neophytou C & Pitsouli C (2022) Cell Reports 38(10): 110505; Neophytou C et al. (2023) Metabolites 13(10) 1084]. In conclusion, we have shown that oxygen, biotin, cholesterol, and microbiota control ISC regeneration and cancer in flies. Expanding these studies in mammals may lead to the development of chemicals targeting intestinal disease and cancer.

ShT-04.5-1
Deciphering the role of cytokine-induced glutamate dynamics in breast cancer brain metastasis

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Metastasis is a critical stage in cancer progression, where tumor cells spread from their original site to distant organs, often leading to dire consequences. Our research delves into the intricacies of the "extravasation" process, wherein tumor cells exit the bloodstream to infiltrate the parenchyma of target organs. In particular, we try to understand the proclivity of breast cancer cells to form metastases within the brain. Our data indicate that "brain-seeking" cells possess the ability to disrupt the integrity of the blood–brain barrier by releasing a suite of inflammatory cytokines. This indication adds a layer of complexity to the already acknowledged correlation between cytokine activity and metastasis formation. Our data indicate that specific metabolic alterations are induced by cancer-released cytokines on brain resident cells like astrocytes, notably the accumulation and subsequent release of glutamate within the brain. This glutamate enhances the migratory and invasive capabilities of tumor cells that are predisposed to targeting the brain. We finally demonstrated that inhibiting pathways used by metastatic cells, particularly those involving glutamate and cytokines, could prevent brain metastases. Our research provides new insights into the tumor ecosystem in breast cancer and identifies novel therapeutic targets for brain metastases, paving the way for innovative treatment strategies to improve outcomes for patients at risk of brain metastasis.

ShT-04.5-2
Unraveling metabolic vulnerabilities to counteract sorafenib resistance in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the most common type of primary liver malignancy worldwide often diagnosed in late stages with a median survival of less than a year. Current treatments, including the oral kinase inhibitor sorafenib (SOR), for advanced-stage HCC are unsatisfactory due to the onset of resistance. However, the biochemical and molecular mechanisms of SOR resistance remain unclear. Here, we show that murine hepatoblasts with myc oncogene overexpression and $p53^{-/-}$ are resistant to SOR. We defined that SOR negatively affects mitochondrial function by acting as an uncoupling agent, disrupting electron transport chain (ETC) complexes, and altering ETC super-complex assembly. This causes reactive oxygen species (ROS) production within mitochondria. To counteract ROS production and continue to proliferate, SOR-resistant cells rewire their metabolism by activating the glyoxalase system to promote glutathione (GSH) shuttling into the mitochondria and the production of D-lactate. Integrated data from transcriptomic and metabolomic profiles, along with metabolic tracing experiments, demonstrate that SOR-resistant cells increase glucose uptake for serine production. This serine is used in mitochondria by the one-carbon metabolism to generate enough NADPH for GSH regeneration and to promote purine biosynthesis. Moreover, SOR-resistant hepatoblasts rely on cysteine for L-lactate generation, as the contribution of glucose, glutamine, and alanine was only marginal. Chemical and genetic inhibition of these key metabolic pathways engaged by SOR-resistant cells identify metabolic vulnerabilities that enhance sorafenib efficacy. Furthermore, analysis of SOR-resistant human hepatocyte cells (Huh7) and SOR-resistant patient samples revealed superimposable metabolic gene expression profiles identified in our SOR-resistant hepatoblasts, highlighting the role of these metabolic pathways in drug resistance.

Monday 1 July
16:30–18:30, Red Room

Enzyme Engineering: The Future is Now

S-02.4-1
Engineering polymerases for designed glycans and glycoconjugates

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An impressive diversity of glycopolymers and glycoconjugates exists in nature. They present biological, chemical and physicochemical properties that open a vast application field in health, nutrition, cosmetic and biomaterial sectors. In addition, their native structures can also be subsequently transformed or can serve as model in biomimetic approaches. With this in mind, identifying glycan-synthesizing enzymes is a key step to access new structures and this is largely facilitated by the flow of carbohydrate-active enzymes sequences arising in databases. Combining enzyme discovery to enzyme engineering and protein design further expands tremendously the scope of accessible catalytic reactions allowing to better control glycan-based architectures. As an illustration of the power of these approaches, we will focus the presentation on glucansucrases a class of naturally very efficient transglucosylases, which use sucrose as a simple substrate to catalyze the formation of a broad variety of polymers[1]. We will show how sequence mining allowed the isolation of atypical enzymes with new product specificities and how structure function investigations combined with computer-assisted engineering enabled us to better understand and engineer glucansucrase processivity and specificity to open access to well-controlled architectures of polymers, glycosurfactants, new prebiotic and antigenic glycoligosaccharides and glycoconjugates. References: 1- Claire Moulis, David Guieysse, Sandrine Morel, Etienne Séverac, Magali Remaud-Siméon (2021) Natural and engineered transglycosylases: Green tools for the enzyme-based synthesis of glycoproducts. Curr. Opin. Chem. Biol. 61: 96-106.

S-02.4-2
Development of a highly optimized engineered PETase enzyme for plastic degradation

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The uncontrolled accumulation of plastic waste in the environment has long begun to impact on the natural ecosystems and to pose an existential threat to all forms of life on our planet. Advanced technical solutions to the plastic waste management problem are therefore in urgent demand. To this end, enzymatic approaches to plastic degradation hold great promise as novel and more efficient enzymes are constantly being developed. Leaf-branch compost cutinase (LCC) is a naturally occurring PETase that has been reported to outperform all other PET-degrading enzymes known to date. This enzyme has been noticeably engineered in 2020[1], leading to the so-called ICCG variant (Tm = 94.0°C), the current gold standard. We have engineered a LCC named DRK3 that features enhanced PETase activity and thermal stability relative to gold standard ICCG.[2] The DRK3 mutant shows a Tm > 98°C and remarkable activity on amorphous PET films beyond 6 days at 68°C. The high-resolution crystal structure of DRK3 shows no significant changes in its folding relative to both the wild type LCC (r.m.s.d. = 0.257 Å) and the ICCG variant (r.m.s.d. = 0.152 Å). The catalytic triad (D210, H242, and S265) overlaps perfectly with the catalytic triad in the parent enzymes. The mutations introduced in the design process are found mostly on the surface of the enzyme and away from the catalytic triad. We hypothesize that the increase in stability provided by the additional surface charges may help to keep the surface-exposed catalytic site in place even at high temperatures. This is confirmed by MD simulations, which show that at increasing temperatures DRK3 features a lower r.m.s.f., particularly near the key catalytic residue H242. The enzymatic degradation of PET holds immense promises as a sustainable solution to the escalating crisis of plastic waste accumulation. References: 1. Tournier, V. et al. Nature 2020, 580 (7802), 216; 2. Bhattacharya S. et al. Submitted.

ShT-02.4-3
Novel GH109 enzymes for bioconversion of group a red blood cells to the universal donor group O

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The ABO blood type classification is based on the composition of oligosaccharides on the surface of red blood cells (RBCs). Incorrect blood transfusions can have fatal consequences, emphasizing the critical importance of correct blood group availability. In this regard, it has been demonstrated that some glycoside hydrolases (GHs) may be helpful in the conversion of groups A and B blood types to produce group O universal donor blood. GHs belonging to the GH109 family, could have the ability to convert blood from group A to group O. Here, we report the biochemical characterization of three novel GH109 enzymes: NAg68, NAg69, and NAg71. These enzymes showed higher specificity on pNP-α-N-acetylgalactosamine compared to previously reported GH109 enzymes. In addition, the enzymes were able to act on purified antigen-A trisaccharides and convert human donor blood type A to group O. NAg71 converted type A RBC to group O with increased efficiency in the presence of dextran compared to a commercially available GH109. These data demonstrated that this enzyme could represent a powerful tool for

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biomedical applications. Reference: Curci N, Iacono R, Segura Raventos D, Cillo M, Cobucci-Ponzano B, Strazzulli A, Leonardi A, Giger L, Moracci M. New Biotechnology, 2023, 77, pp. 130–138 * The authors marked with an asterisk equally contributed to the work.

ShT-02.4-1
Divergent genetic evolution employs the same molecular mechanisms

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Epistasis, defined as the non-additive impact of mutational effects, profoundly influences the adaptive landscapes of antimicrobial resistance (AMR) enzymes. Consequently, comprehending the molecular mechanisms governing epistasis and the evolutionary conservation of these effects across various adaptive pathways is imperative for predicting evolutionary outcomes and the resulting resistance phenotypes. Using directed evolution, we investigated the extent to which the evolution of the β-lactamase OXA-48 toward the β-lactam ceftazidime is reproducible. We unveil that by repeating evolution three times, adaptation proceeded through completely distinct genotypic trajectories while reaching similar phenotypic maxima. Characterization of constructed fitness landscapes, comprising more than 150 mutational combinations, demonstrated that resistance development predominantly occurred through positive pairwise epistasis. We showed that epistasis was driven by changes in catalytic efficiency with the selection of an enzymatic burst phase. While ceftazidime binding is rate-limiting in the wild-type enzyme, the molecular mechanisms driving resistance through pairwise epistasis were highly conserved within each trajectory and driven by the interplay of improvements in substrate binding and catalysis. To assess cross-trajectorial compatibility, we combined mutations that improved either binding or catalysis from all trajectories. Combining only catalysis or binding enhancers did not lead to a boost in resistance. In contrast, 75% of the combinations that positively stimulated both binding and catalysis acted highly synergistically, significantly driving AMR. While AMR can evolve through entirely distinct trajectories, we demonstrate that the molecular mechanisms by which epistasis leads to changes in susceptibility can be highly conserved. Our work emphasizes the dire need to understand the driving forces of epistasis, as this knowledge is crucial for predicting AMR development.

ShT-02.4-4
Enhancing the thermal stability and activity of the artificial self-sufficient $P450_{SP\alpha}$-SOX by switching the domains linker

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Fusion proteins are powerful tools to facilitate the combination of two or more enzyme functionalities in a cascade reaction, to increase the protein stability and to enhance the catalytic performance of a multi-enzyme system. Our laboratory recently developed an artificial multi-enzyme fusion protein to increase the peroxidase activity of the fatty acids α-hydroxylase $P450_{SP\alpha}$ (CYP152B1) by fusing it to a H_2O_2-donor sarcosine oxidase (SOX) through a flexible poly-glycine linker. In order to investigate the effect of the linker on the biophysical and catalytic properties of the multi-enzyme system we modified the amino acid sequence of the linker between $P450_{SP\alpha}$ and SOX, obtaining a new fusion protein characterized by a high structural rigidity. The $P450_{SP\alpha}$-rigid-SOX displayed a higher energy barrier to thermal denaturation compared to the flexible construct, as shown by an increase in the T_{onset} of 10°C and an increase of 227 cal/mol of the unfolding enthalpy measured by differential scanning calorimetry (DSC). We investigated the effect of thermal inactivation on the heme-thiolate ligand by UV–VIS spectroscopy for both flexible and rigid $P450_{SP\alpha}$-SOX constructs. The data indicates that the rigid linker has a positive effect on the stabilization of a P420 semi-folded state. Furthermore, residual CO-binding experiments also demonstrated a 5.7°C increase of the T_{50} for the $P450_{SP\alpha}$-rigid-SOX. Most importantly, $P450_{SP\alpha}$-rigid-SOX showed an increased total turnover for the oxidation of p-nitrophenol to p-nitrocatechol, herein reported for the first time as $P450_{SP\alpha}$ substrate. Finally, $P450_{SP\alpha}$-rigid-SOX was used for the turnover of styrene and also in this case it outperformed $P450_{SP\alpha}$-flexible-SOX. Overall, we demonstrated that the rigid linker improved the fusion enzyme thermal stability and its catalytic performance.

ShT-02.4-2
Development of an enzymatic treatment for valorizing (recycling and upcycling) PET-textile waste

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Since 2000, the textile industry has experienced significant growth, leading to overproduction of waste and environmental concerns. Worldwide textile production currently relies on synthetic fibers, with polyethylene terephthalate (PET) fibers representing 54% of the total annual output, with a production of 61 million tons in 2021. Our work aims to develop a multienzymatic treatment for the degradation of mixed textile waste, with a specific focus on recycling and upcycling PET fibers. While PET-degrading enzymes can selectively convert PET into its monomers, they face considerable challenges, particularly in breaking down substrates with high crystallinity. In this study, we tested the efficacy of an engineered variant of LC-cutinase (S101N/F243T LCC) (previously published in: Pirillo V. *et al.* (2023) The FEBS Journal 290, 3185–3202), on pre- and post-consumer textile wastes at different composition (100% PET or blended, and dyed or undyed). Given the negligible hydrolytic activity in the direct enzymatic treatment of 100% PET textiles, we explored the use of physical pretreatments (thermal, microwave, ultrasound, ball-milling) of the substrate to enhance PET depolymerization. Thermal and ball-milling pretreatments emerged as the most effective, enabling the enzyme to produce a 60% and 6% TPA yield, respectively, after a 4-day incubation at 55°C in 100 mM phosphate buffer pH 8.0. DSC analysis of the pretreated textiles shows a significant decrease in crystallinity after thermal treatment and no significant changes after ball-milling, suggesting that the improvement in enzymatic depolymerization could be attributed to a reduction in sample crystallinity and an increase in substrate-enzyme interface area. Furthermore,

we observed that the end of the depolymerization process seems associated with the unproductive adsorption of the enzyme onto the substrate. In conclusion, the enzymatic degradation of PET-textile waste is close to being a reality.

Monday 1 July
16:30–18:30, Blue Room

Long ncRNA and microRNA Networks

S-01.5-1
A male-essential microRNA is key for avian sex chromosome dosage compensation

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Birds have a sex chromosome system in which females are heterogametic (ZW) and males are homogametic (ZZ). The differentiation of avian sex chromosomes from ancestral autosomes entailed the loss of most genes from the W chromosome during evolution. However, to what extent mechanisms evolved that counterbalance the consequences of this extensive gene dosage reduction in female birds has remained unclear. Here we report functional *in vivo* and evolutionary analyses of a Z-chromosome-linked microRNA (miR-2954) with strongly male-biased expression that was previously proposed to play a key role in sex chromosome dosage compensation. We knocked out miR-2954 in chicken, which resulted in early embryonic lethality of homozygous knockout males, likely due to the highly specific upregulation of dosage-sensitive Z-linked target genes of miR-2954. Our evolutionary gene expression analyses further revealed that these dosage-sensitive target genes have become upregulated on the single Z in female birds during evolution. Altogether, our work unveils a scenario where evolutionary pressures on females following W gene loss led to the evolution of transcriptional upregulation of dosage-sensitive genes on the Z not only in female but also in male birds. The resulting overabundance of transcripts in males resulting from the combined activity of two dosage-sensitive Z gene copies was in turn offset by the emergence of a highly targeted miR-2954-mediated transcript degradation mechanism during avian evolution. Our findings demonstrate that birds have evolved a unique sex chromosome dosage compensation system in which a microRNA has become essential for male survival.

S-01.5-2
Regulatory RNAs in the brain and neuroendocrine system

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RNA molecules exhibit plentiful regulatory functions during development, in homeostatic states and disease. We are particularly interested in microRNA (miRNA), circular RNA (circRNA) and long non-coding RNA (lncRNA) functions and their contribution to regulating protein-coding genes. Our previous work showed that circRNA Cdr1as is highly expressed in glutamatergic neurons in the mammalian brain where it interacts and stabilizes miR-7 and affects miR-7 target mRNAs [Piwecka et al., 2017]. That regulatory RNA circuit impacts the synaptic output and influences the behavior is evidenced by studying Cdr1as knockout mice and their phenotype. In the first part of my talk, I will comment on the most recent advances in understanding the Cdr1as-miR-7 network in glutamatergic neurons. In the second part, I will turn to our newest results from studies on the Cdr1as-miR-7 network in the part of the neuroendocrine system, a pituitary gland, where miR-7 and Cdr1as ratio is opposite as compared to the brain tissue. Additionally, I will present the preliminary results on the dynamic regulation of circRNA, lncRNA and miRNA profiles in the murine pituitary in the course of postnatal development. Piwecka et al. (2017) Loss of a mammalian circular RNA locus causes miRNA deregulation and affects brain function. Science 357(6357): eaam8526.

ShT-01.5-2
Spatially clustered piRNA genes promote the transcription of piRNAs via condensate formation of the H3K27me3 reader UAD-2

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PIWI-interacting RNAs (piRNAs) are essential for maintaining genome integrity and fertility in various organisms. In flies and nematodes, piRNA genes are encoded in heterochromatinized genomic clusters. The molecular mechanisms of piRNA transcription remain intriguing. Through unique molecular indexed-small RNA sequencing and chromosome editing, we discovered that spatial aggregation of piRNA genes enhances their transcription in nematodes. The heterochromatinized piRNA genome recruits the piRNA transcription complex USTC (including PRDE-1, SNPC-4, TOFU-4, and TOFU-5) and the H3K27me3 reader UAD-2, which phase separate into droplets to initiate piRNA transcription. We searched for factors that regulate piRNA condensate formation and isolated the SUMO E3 ligase GEI-17 as inhibiting and the SUMO protease TOFU-3 as promoting condensate formation, thereby regulating piRNA production. Our study revealed that spatial aggregation of piRNA genes, phase separation and deSUMOylation may benefit the organization of functional biomolecular condensates to direct piRNA transcription in the heterochromatinized genome.

ShT-01.5-1
Identification of cholestasis-induced long non-coding RNAs in liver cells

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A significant increase in the concentration of bile acid in hepatic tissue is commonly observed in many liver pathologies. This cholestasis represents a health threat for patients with chronic liver damage and could lay the basis for cancer formation. However, markers for detecting liver cholestasis lack sensitivity and specificity. The aim of the study was to identify long non-coding

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RNAs (lncRNAs) that could serve as biomarkers for cholestasis. To recapitulate cholestatic conditions *in vitro*, hepatocellular HepG2 cells were treated with the bile salt chenodesoxycholic acid (CDCA) and the farnesoid X nuclear receptor (FXR) agonist GW4064. Viability assays and mRNA expression of the FXR target genes organic solute transporter α/β (OSTα, OSTβ) were used to define efficient and non-toxic induction of a cholestatic cell condition. Subsequently, quadruplicates of untreated, CDCA-, and GW4064-treated cells were subjected to next-generation sequencing. As expected, bioinformatic analysis of mRNA data revealed the regulation of processes involved in bile acid synthesis and transport. Moreover, we identified and confirmed a four lncRNA gene signature that is consistently regulated by CDCA and GW4064. The *in vitro* analysis of bile acid-treated hepatocyte-derived cells unveiled a four lncRNA signature associated with liver cholestasis. We hypothesize that lnRNAs could serve as an alternative diagnostic tool for the early and robust detection of acute or chronic liver damage. * The authors marked with an asterisk equally contributed to the work.

ShT-01.5-4
Identification of prognostic biomarkers in hepatocellular carcinoma using a combined quantitative mass spectrometry and *in silico* analysis approach.

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Hepatocellular carcinoma (HCC) represents a formidable challenge in oncology, necessitating a deeper understanding of the molecular drivers implicated in its pathogenesis. Among these, microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) have emerged as critical regulators of cancer development and progression. Previously, we elucidated the miR-423-5p capability to bind, and consequently downregulate Metastasis-Associated Lung Adenocarcinoma Transcript 1 (MALAT1) expression thus acting as oncosuppressor. In this study, we have investigated the proteomic profiling of the miR-423-5p-transduced HCC SNU-387 cell line, aiming to understand its involvement in pathways and gene ontology processes and clinical relevance in TCGA-LIHC patients. An integrated analysis was performed to identify differentially expressed proteins (DEPs) from miR-423-5p experimental and predicted targets. Using the TCGA-LIHC dataset, these targets were assessed for correlation with HCC by performing stage-wise expression profiling, overall (OS) and disease-free survival (DFS) analysis. Mass spectrometry analysis identified 698 DEPs in miR-423-5p-transduced HCC cells when compared to controls. Pathway analysis showed that these proteins are involved in metabolic pathways modulating purine and pyrimidine metabolism and gluconeogenesis. Seven miR-423-5p targets were significantly associated with TCGA-LIHC patients (OS and DFS, log-rank p-value <0.05). Comparative expression profiling analysis revealed that these targets are downregulated in our dataset; in contrast, they were specifically upregulated in stage III of the TCGA-LIHC dataset. Our analysis suggests a putative role of miR-423-5p in modulating significant metabolic processes and downregulating the expression of several targets significantly associated with TCGA-LIHC patient survival, suggesting their role as potential therapeutic and prognostic targets in HCC patients. * The authors marked with an asterisk equally contributed to the work.

ShT-01.5-3
Systematic experimental and computational strategies to identify and functionally characterize lncRNAs and RNA modifications in development and disease

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The mammalian genome is transcribed into several thousand long noncoding RNAs (lncRNAs) whose properties and functions remain largely elusive. Our group has developed a combination of genome-wide *in silico* and functional strategies to explore their regulation and biological roles. Using a computational pipeline[1] to analyze RNA-seq datasets from human and mouse tissues, tumors and cell lines, we identified thousands of lncRNAs in syntenic locations, whose promoters overlap chromatin loop anchor points containing conserved CTCF sites, dubbed tapRNAs (topological anchor point RNAs). These RNAs are associated with developmental genes with which they: (a) are co-expressed and able to regulate each other's expression, (b) are similarly misregulated in cancers, and (c) influence differentiation of embryonic stem cells and metastatic characteristics of cancer cells *in vitro*. We find novel associations of ncRNAs with specific chromatin compartments and evidence for the role of tapRNAs in chromatin organization. To also explore the potential impact of RNA post-transcriptional modifications (PTMs), we are using Nanopore RNA-seq and a robust statistical framework to determine the distribution of PTMs in lncRNAs and mRNAs. We do so by comparing native RNAs isolated from biological samples of interest (including mammalian disease models) against modification-depleted RNA controls, also mapping known and novel modification sites in conserved polyA-noncoding RNAs. We are extending similar experimental and guilt-by-association expression network analyses to study lncRNAs in different systems, identifying lncRNAs functionally associated with key biological pathways, such as with immune protection in EBOLA vaccine cohorts. Altogether, we find support for widespread roles of RNAs and their modifications in the control of gene expression in developmental and disease models, as well as in other biological systems. Reference 1. Amaral PP et al. (2018) Genome Biol 19, 32.

Monday 1 July
16:30–18:30, Yellow Room

Biochemistry of Physical Activity and Health – Part B

S-03.2-2
Mining skeletal muscle for optimal metabolic health and well-being in type 2 diabetes

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People with type 2 diabetes are characterized by skeletal muscle insulin resistance and exercise can counteract this defect. Thus, there is great therapeutic potential in prescribing exercise to prevent and treat insulin resistance. Type 2 diabetes shares many features of accelerated/secondary aging including insulin resistance, defective oxidative metabolism/mitochondrial function, and loss of muscle mass. Strikingly, long-term participation in vigorous exercise programs mitigates secondary aging and reduces disability and mortality. Here I will present a multi-faceted approach to validate exercise-responsive treatment targets to mitigate secondary aging and prevent metabolic disease. This work leverages a deep dive into exercise biology to discover new inroads into prevention and treatment of type 2 diabetes. The overarching goal of our research is to identify and validate molecules, pathways, and ultimately new treatments that confer the benefits of exercise to improve insulin sensitivity, preserve mitochondrial energetics and attenuate muscle function and metabolism.

S-03.2-1
Compartmentalization in cardiomyocytes modulates kinase activities

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Intracellular molecules are transported by motor proteins or diffusion. Whereas the former is targeted, the latter is the result of random molecular motion. For molecules existing in low concentrations, confinement of their diffusional space is a way the cells can regulate their signals to make them more rapid and targeted. Such confined spaces form compartments in which local concentrations are different from the overall, average concentrations. Calcium and cyclic AMP are well-known examples of compartmentalized molecules. Energetic compartmentalization means that in some compartments, the ratios of AMP and ADP to ATP are different from the average ratios. This modulates the activity of kinases, which are enzymes catalysing the transfer of a phosphate group typically from ATP or GTP to a specific substrate. Kinases are important in metabolism and signalling. A recent study demonstrated that energetic compartmentalization modulates the activity of creatine kinase and adenylate kinase *in situ*. This suggests that the diffusional space around these kinases is restricted. It highlights the importance of developing new methods to assess local concentrations and taking compartmentalization into account when interpreting experimental results.

ShT-03.2-2
Understanding ribosomal protein distribution and expression in neurons and their compartments using single-cell RNA sequencing and single molecule microscopy

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Given neurons' diversity and polarization, understanding local translation mechanisms and ribosomal regulation in different neuronal subtypes and their compartments is crucial for unraveling novel functions. Interestingly, recent studies have challenged the traditional view of the ribosome as a uniform translation molecular machine, revealing evidence of ribosomal heterogeneity and extra-ribosomal roles for ribosomal proteins (RPs) in translation regulation. We investigate RP gene expression and distribution in neuronal subtypes and axons using single-cell RNAseq data re-analysis and RP proteins in the axon compartment. Several RP genes exhibit differential expression among 13 neuronal subtypes in mouse cortex, notably with Rpl21 and Rps27 overexpressed in GABAergic Lamp5 and Vip neurons, respectively, compared to glutamatergic neurons. Preliminary results suggest Rps27 overexpression conservation in Vip GABAergic neurons across species. Moreover, we examine RP mRNA and protein localization in axons using advanced and super resolution microscopy, revealing their presence and neosynthesis in axons. We also find evidence of ribosome-RNA associations in axon wholemounts and study ribosome organization in mature myelinated axons with super resolution microscopy. These findings underscore variability in RP gene expression among neuronal somas and shed light on local synthesis processes in axons, raising questions about regulatory functionality and potential protein abundance differences as properties of the translational machinery.

ShT-03.2-1
Inflammatory activity of extracellular vesicles induced by different exercise regimens

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Regular physical exercise promotes systemic adaptations that positively affect the cardiovascular, nervous and immune systems, promotes weight loss and counteracts sarcopenia. Moreover, the level of physical activity is the major modifiable risk factor for metabolic and cancerous diseases [Fiuza-Luces C et al. (2018) Nat. Rev. Cardiol 15, 731–743]. Exercise-induced benefits have been linked to the secretion of myokines. However, recent evidence suggests extracellular vesicles (EVs) as new players through which muscle communicates with other tissues or organs [Guescini M et al. (2015) PLoS ONE 10, e0125094]. Our study focused on the secretion of EVs in response to different physical exercise regimens. We conducted our research on healthy individuals who were subjected to various exercise protocols: acute aerobic exercise (AAE) and training, acute maximal aerobic exercise and

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altitude aerobic training. The most significant finding was the increase in EV secretion in the sedentary condition compared to trained participants following AAE. This result underscores the role of exercise in modulating EV secretion and its potential implications for health and disease. Specifically, we observed an upregulation of EV-associated miR-206, miR-133b and miR-146a following AAE, and this trend appeared to be intensity-dependent. Furthermore, our findings showed that THP-1 macrophage treatment with exercise-derived EVs led to an increase in the mRNAs encoding for IL-1b, IL-6 and CD163 using baseline and immediately post-exercise EVs [Maggio S et al. (2023) Int. J. Mol. Sci. 24, 3039]. These findings provide a deeper understanding of the release of circulating EVs and their role as mediators of the inflammatory processes associated with exercise. Importantly, we also highlighted that the release of EV-miRNAs into the bloodstream depends on the subject's fitness condition; the more trained the subjects are, the less circulating EV-miRNAs increase following an exercise bout.

Tuesday 2 July
8:30–10:30, Silver Room

Post-translational Modification of Membrane Proteins

S-01.6-2
Mono-ADP-ribosylation in the control of intracellular membrane traffic

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ADP-ribosylation, a pivotal post-translational modification catalyzed by mono- and poly-ADP-ribosyltransferases (ARTs), modifies key protein residues, impacting various cellular functions. Originating from an ancient enzyme superfamily, ARTs have evolved within bacterial defense and offense systems and have been integrated through lateral gene transfer during eukaryotic evolution. As a result, ARTs target proteins essential for numerous cellular processes, revealing their potential role in physiological and pathological conditions. The mechanism of mono-ADP-ribosylation was first revealed through the action of diphtheria toxin, a mono-ART that hinders protein synthesis by modifying the GTPase elongation factor 2. Similar ART activities have been identified in other toxins that target proteins essential for cellular functions, like the small GTPases of the Rab family. Our studies show that PARP12, a mono-ART localized at the Golgi complex, is key in controlling some intracellular membrane traffic pathways. In response to oxidative stress, PARP12 relocates to stress granules, thereby disrupting traffic from the Golgi to the plasma membrane. Importantly, PARP12 modifies Golgin-97 and Rab14, proteins that are central to exocytic and endocytic pathways, respectively. This action on the Golgin and Rab families underlines the significance of mono-ADP-ribosylation in membrane traffic regulation. Furthermore, the modification of Rab5 and Rab1A by mono-ARTs points to the broader impact of this post-translational modification in cellular functions. PARPs are also active in other contexts like cytosol-to-nucleus transport and in the development of cancer invasiveness. In summary, the role of mono-ARTs underlines that mono-ADP-ribosylation is a significant modifier of intracellular trafficking and numerous other cellular mechanisms, with profound implications for both health and disease.

S-01.6-1
Regulation of organic cation transporters

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Organic cation transporters (OCTs) are membrane proteins that translocate organic cations across the cellular plasma membrane. Organic cations are positively charged molecules of endogenous (important neurotransmitters such as serotonin and histamine) or exogenous (drugs such as doxorubicin and metformin) origin. OCTs are highly expressed in excretory organs such as the liver and kidneys, where they play an important role in drug excretion, and in the brain, where they are thought to be involved in the cellular reuptake of neurotransmitters. Regulation of OCT activity may therefore have important physiological, pharmacological, and toxicological consequences. OCT activity can be rapidly regulated by several different protein kinases by altering their affinity for substrates or by influencing their trafficking to/from the plasma membrane. The trafficking of OCTs may also be determined by interaction with specific partners, such as the tetraspanin CD63, which appears to contribute to the specific basolateral expression of OCTs in the cells of the renal proximal tubules. Post-translational modifications resulting from a direct interaction of OCTs with kinases such as dual-specificity tyrosine (Y)-phosphorylation-regulated kinase 1A (DYRK1A) can alter the cellular distribution of transporters, thereby modifying their activity. As DYRK1A is a druggable kinase, which is highly expressed in some neurodegenerative diseases, this protein-protein interaction may alter the cerebral neurotransmitter balance in these conditions. Therefore, regulation of OCT activity may be an important tool to restore OCT function under pathological conditions. The studies of the author on this topic are supported by the DFG (CI 107/14-1).

ShT-01.6-3
Post-translational modification of SLC3A2 plays a crucial role in the membrane trafficking of some amino acid transporters belonging to the SLC7 family

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Human proteome complexity is not only due to genetic processes, such as alternative mRNA splicing, but is also enriched by post-translational modifications (PTM), pushing the total number of proteoforms to at least several millions. Moreover, PTM can profoundly affect different protein aspects, such as function regulation, stability, and cellular localization, playing a crucial role in cell homeostasis and disease development. Among other PTM, N-glycosylation of some membrane proteins was

found to be critical for their trafficking and stability. However, some amino acid transporters of the SLC7 family do not have N-glycosylation sites. These transporters need ancillary proteins such as SLC3A2, also known as CD98, to reach the plasma membrane. In this scenario, the role of the four N-glycosylation sites of SLC3A2, a single-pass membrane protein that can form heterodimers with SLC7 members but has no transport function, was investigated. A combined approach that includes bioinformatics, site-directed mutagenesis, and cell biology was used. Single or multiple mutants of the four glycosylated sites were used to evaluate the stability and the trafficking of SLC3A2 to the plasma membrane by a biotinylation assay and a brefeldin assay. Results highlighted that the ablation of all the glycosylation sites severely affected the stability and the abundance of SLC3A2 at the plasma membrane. The impairment of SLC3A2 trafficking correlated with a lower presence of its interactions, belonging to the SLC7 family, at the cell surface. SLC7 members, such as SLC7A5, are considered hot targets for cancer therapy. Hence, targeting SLC3A2 may act synergistically for potential anticancer treatment.

ShT-01.6-1
Farnesyl-GFP as a promising fluorescent tag for exosomes labelling

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Extracellular vesicles (EVs) are cell-derived nanoparticles essential in cell-to-cell communication. Among EVs, exosomes represent membrane vesicles formed by the inward budding of endosomal membrane and secreted by fusion with the cell membrane, thus containing thousands of different macromolecules originating from the parent cell and delivering to the host cell. Therefore, exosome bioengineering represents an appealing approach to mainly sort the cargo as well as modify the exosome surface to target desired recipient cells, thus producing bio-inspired nanovesicles with specific loading and/or targeting features[1]. Consequently, monitoring exosomes' journey by staining with fluorescent dyes is a current appealing approach, although challenging. Therefore, herein HEK293T cell-derived exosomes were isolated by size exclusion chromatography and characterized by dynamic light scattering, tuneable resistive pulse sensing, and transmission electron microscopy for physical properties assessment as well as western blot for biological evaluation; also, micro BCA™ assay was performed to evaluate the protein concentration. The exosome labelling was performed by using different methods. Particularly, transient transfections with a DNA plasmid encoding for green fluorescent protein (GFP), a well-known tag protein, or farnesyl GFP (f-GFP), facilitating the GFP anchoring to the cell membrane, were performed in HEK293T cells. Also, exosomes were labelled post-isolation by the addition of a fluorescent lipophilic dye (Vybrant™ DiD). Then, the tagged exosomes' uptake into recipient cells was monitored by confocal microscopy. The obtained preliminary results highlighted that f-GFP allows to efficiently tag exosomes and track their delivery into recipient cells. Overall, the current work aims to suggest a simple and systematic workflow to optimize a method to track the exosome route from biogenesis to delivery.

[1]Previously published in: Teng F et al. (2020) Adv Sci (Weinh). 8,2003505.

ShT-01.6-2
Structural insights into vesicular monoamine transport and neurological drug interactions

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Biogenic monoamines, crucial transmitters orchestrating neurological, endocrinal, and immunological functions, are stored in secretory vesicles by vesicular monoamine transporters (VMATs) for controlled quantal release. VMATs harness proton antiport to enrich monoamines ~10 000-fold and sequester neurotoxicants. VMATs are targeted by an arsenal of therapeutic drugs and imaging agents to treat and monitor neurodegenerative disorders and drug addiction. However, the structural mechanisms underlying VMAT function and drug interaction remain largely unknown. Here, we report a series of cryo-electron microscopy structures of human VMAT1 and VMAT2 with monoamines and drugs in multiple conformations (Ye et al, *Nature*, in press). VMAT1 structures are determined with four monoamines, the Parkinsonism-inducing MPP+, the psychostimulant amphetamine, and the antihypertensive drug reserpine. The structures reveal that a favored transition to lumenal-open state contributes to monoamine accumulation, while protonation facilitates the cytoplasmic-open transition and prevents monoamine binding to avoid unintended depletion. Monoamines and neurotoxicants share a binding pocket possessing polar sites for specificity and a wrist-and-fist shape for versatility. VMAT2 structures highlight a partially occluded, unbound state that blocks reverse transport to facilitate monoamine accumulation, and a fully occluded state for tetrabenazine binding. Amphetamine induces VMAT2 into a conformation that enables monoamine competition for psychostimulatory release. Structural comparisons between VMAT1 and VMAT2 reveal similarities in their mechanisms of monoamine accumulation and differences in substrate preferences and conformational flexibility. These structural and functional insights provide a deep understanding to the mechanisms of vesicular monoamine transport, and lay the foundation for developing novel therapeutics for neurodegenerative diseases and substance abuse.

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Tuesday 2 July
8:30–10:30, Red Room

Towards Sustainable Use of Natural and Renewable Resources

S-02.5-1
Synthetic biology for a sustainable recycling and upcycling of PET

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Despite increasing recycling efforts, 65% of plastic waste collected in Europe (amounting to 30 million tons/year) is still incinerated or released into the environment, posing a significant threat to human health. In the context of a circular bioeconomy, newly discovered enzymes capable of depolymerizing polyethylene terephthalate (PET) represent invaluable tools for novel processes aimed at sustainable and environmentally friendly plastic life cycle management. Within our research group, we set up a modular workflow for the evolution of the PET hydrolyzing enzyme (PHEs) which was utilized in the engineering of the two most promising enzymes of this family: the *Ideonella sakaiensis* PET hydrolase (IsPET) and the thermostable leaf-branch compost cutinase (LCC). An improved variant of IsPET, exhibiting enhanced stability and affinity for PET, was generated by a directed evolution approach [previously published in Pirillo et al. (2022), Int. J. Mol. Sci. 2022, 23, 264]. Subsequently, we produced variants of LCC, the most promising PHE for industrial applications due to its thermostability and high hydrolyzing activity. These variants showed significantly superior performances for the biodegradation of PET even at moderate temperatures. Indeed, 1.25 mg of the S101N/F243T-ΔLCC variant were able to fully depolymerize 1.3 g of untreated postconsumer PET in less than 3 days at 55°C. These evolved PHE variants represent the ideal biocatalyst for the initial step of novel synthetic metabolic pathways exploited both *in vitro* and in engineered prokaryotic (*E. coli*) and eukaryotic (black soldier fly larvae) organisms. In detail, novel integrated and transversal biotechnological processes for the simultaneous removal of PET from the environment (e.g. bioremediation from solid organic urban wastes or freshwaters) and the upcycling of degradation products (terephthalic acid and ethylene glycol) into high added-value compounds (e.g. amino acids) were designed.

S-02.1-2
The development of biorefineries and prospects for the future

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The noun biorefinery, and the associated gerund biorefining, first began to appear in scientific literature in the 1980s. This was a result of the oil crisis provoked by the Iran–Iraq war. However, until the first decade of the present century, these terms remained relatively unused. The term biorefinery has been defined many times and in many ways, but it according to the IEA task 42 definition it is the "the sustainable processing of biomass into a spectrum of marketable products and energy". Although this definition is rather simple, it includes key notions related to sustainability and multiproduct outputs. In my presentation I will provide a personal view of how biorefineries have developed so far, considering how sustainability and multiproduct criteria have been tackled. Moreover, I will reflect upon the oil refinery paradigm and its limited usefulness to frame the development of biorefining. I will also consider future prospects, focusing on those that can be driven by biotechnology and biomanufacturing. In this regard, I will provide a brief appraisal of the European situation and make proposals on how to move biotechnology forward, especially at the preindustrial R&D level.

ShT-02.5-2
Sustainable production of cis,cis-muconic acid production from vanillin by an engineered *E. coli* strain

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The efficient valorization of lignocellulosic biomass components, and particularly the lignin fraction, could serve as a starting point for the establishment of a circular bioeconomy model aimed at recycling and reusing industrial by-products over the exploitation of virgin feedstock. In a previous work, we developed an engineered *E. coli* strain co-expressing 4 different recombinant enzymes (the dehydrogenase LigV, the demethylase VanAB, the decarboxylase AroY and the dioxygenase C12O) to convert lignin-derived vanillin (Van) into cis,cis-muconic acid (ccMA), a valuable precursor for the production of plastic materials [Previously published in: Molinari et al. (2023) ACS Sustainable Chem. Eng. 11, 6, 2476–2485]. The whole-cell biocatalyst used with a resting cells approach converted >95% of 10 mM Van in ccMA in 2 h. In this work, the scaled-up production of ccMA from Van using engineered *E. coli* growing cells was performed. The bioconversion reaction was carried out in a bioreactor, providing improved control of reaction conditions such as pH, dissolved oxygen, and substrate pulse-feed rate, streamlining the biocatalytic process and enhancing scalability. The optimized growth medium composition (0.5 g/L glucose and 2 g/L lactose) and the substrate addition strategy (1 mmol/h pulse-feed) enabled the engineered strain to produce 5.2 ± 0.4 g/L of ccMA in 48 h, corresponding to 0.86 g_{ccMA}/g_{Van}. The purification of the produced ccMA from the fermentation broth was achieved through crystallization, yielding 2.6 ± 0.1 g_{ccMA}/L of broth, corresponding to a ≈ 50% purification yield. Noteworthy, the proposed process proved to be more efficient and environmentally sustainable compared to the previous resting cells approach. To our best knowledge, this is the first reported production of ccMA from Van with an engineered *E. coli* strain using a growing cells approach in a bioreactor.

ShT-02.5-1
Sustainable production of carbon nanodots with controlled properties via a green Maillard reaction approach

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Carbon nanodots (CNDs) are highly desirable due to their unique optical properties and diverse potential applications. However, conventional synthesis methods often rely on harsh chemicals and high temperatures, raising concerns about their sustainability and food safety risks. This study explores a novel green approach for CNDs synthesis using the Maillard reaction at low temperature (120°C) and long time (12 hours), a well-known natural process, and investigates the optimization of reaction conditions. The Maillard reaction was employed using various combinations of amino acids and sucrose as precursors, with reaction temperature and extraction solvent composition optimized for CNDs yield and fluorescence intensity. The synthesized CNDs (M-CNDs) were characterized using fluorescence, UV–Vis, Raman, and transmission electron microscopy (TEM). Glycine, combined with sucrose in a 1:1 molar ratio, resulted in the most effective formation of CNDs. Utilizing 40% ethanol as the extraction solvent significantly enhanced fluorescence intensity, while higher ethanol concentrations negatively impacted CND formation, likely due to hindered Schiff base formation. The purified M-CNDs exhibited desirable characteristics, confirming the success of the green synthesis approach, which was used as a standard to detect CNDs in bakery products. This study demonstrates a novel and sustainable method for synthesizing CNDs with tailored properties using the Maillard reaction under optimized conditions. Readily available precursors, green solvents, and low temperatures make this approach environmentally friendly and cost-effective. The findings pave the way for developing CNDs with various bioimaging, food, and environmental monitoring applications while promoting sustainable and safe nanomaterial development. Previously published in: Nguyen Huu Huong, D et al. (2024). Materials Advances. https://doi.org/10.1039/D4MA00037D

ShT-02.5-4
Posidonia oceanica egagropili as renewable source for melanin production by *Streptomyces nashvillensis*

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Melanin is isolated from the ink sac of *Sepia officinalis* with expensive procedures that depends on the availability of the supplies. Biotechnological production processes of melanin are considered more environmentally friendly and easy to be scaled up. However, the possibility to biotechnologically produce melanin by *Streptomycetes* has been poorly investigated so far, as well as the employment of lignocellulose substrates for its biosynthesis. *Posidonia oceanica* egagropili are considered a new, possible, unexpensive biomass to valorize as it accumulates as waste along the Mediterranean Sea coasts as dry ball-shaped material. Therefore, their lignocellulose content might support bacterial growth and melanin production if used as substrate for *Streptomyces* strains. In this work the possibility to produce melanin by *S. nashvillensis* DSM 40314 was first investigated on a glucose, yeast extract, and malt extract-based medium, by testing the influence of different temperature (26, 28 and 30°C) and pH (6.0 and 7.0) values on bacterial growth and melanin production. At 28°C and pH 7.0, a maximum biomass of 8.4 ± 0.5 gcdw·L^{-1} and a melanin concentration of 0.74 ± 0.01 g·L^{-1} (with a yield on biomass of 0.09 ± 0.01 g·gcdw^{-1} and a productivity of 0.008 ± 0.001 g·L^{-1}·h^{-1}) were reached in 96 h. Then, different concentration of the egagropili (1.0, 2.5 and 5.0 g·L^{-1}) were supplemented to the growth medium, and, a maximum biomass of 10.1 ± 0.1 gcdw·L^{-1} and a four-time higher melanin production, up 3.0 ± 0.2 g·L^{-1} (with a yield on biomass of 0.3 ± 0.05 g·gcdw^{-1} and a productivity of 0.031 ± 0.01 g·L^{-1}·h^{-1}) were obtained in 96 h by adding to the medium 5.0 g·L^{-1} of egagropili, just in the form of powder, without any pre-treatment. The pigment was purified by acidic precipitation and characterized by UV, FT-IR, mono e bi-dimensional NMR and elemental analyses. Reference: Restaino OF et al. Microorganisms, 2024, 12 (2), 297. doi.10.3390/microorganisms12020297.

ShT-02.5-3

Abstract withdrawn

ShT-02.5-5

See Abstract SpT-09-2 on p. 67

Tuesday 2 July
8:30–10:30, Blue Room

Young Scientists Session A: Biosensors

S-05.1-2
AspSnFR: a genetically-encoded biosensor for real-time monitoring of aspartate in live cells

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Aspartate plays a pivotal role in nucleotide synthesis, ammonia detoxification, and redox balance through the Malate–Aspartate Shuttle (MAS). To unravel the complex roles of aspartate metabolism, real-time and live cell measurement tools are essential. We present AspSnFR, a green fluorescent, genetically-encoded biosensor for aspartate inside cells, developed by screening and testing libraries of biosensors in mammalian cells. AspSnFR accurately measures cytosolic aspartate levels and shows how it is synthesized from glutamine. By integrating high-content imaging of AspSnFR with drug interventions, we identify how aspartate metabolism responds under different nutrient environments. Additionally, AspSnFR helps monitor aspartate efflux from mitochondria via SLC25A12, a key transporter in the MAS. In live cells, we demonstrate that SLC25A12 rapidly links Ca^{2+} signaling with the export of mitochondrial aspartate, highlighting its essential role in connecting cellular signaling, mitochondrial function, and overall metabolism.

S-05.1-1
Metabolite and peptide nanotechnology: implications for materials science and biosensing

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The formation of ordered nanostructures by molecular self-assembly of proteins and peptides represents one of the principal directions in nanotechnology. A systematic reductionist approach allowed us to identify extremely short peptide sequences, as short as dipeptides (e.g. diphenylalanine), which could form well-ordered amyloid-like β-sheet-rich assemblies comparable to supramolecular structures made of much larger proteins. The unique physical properties of these assemblies (mechanical, optical, electronic, piezoelectric) allowed their utilization in various technological applications, including the fabrication of ultrasensitive sensors. The ability to increase the surface area of electrodes in orders of magnitude, as well as its functionality, is especially useful for specific and sensitive detection. The use of exceptional helix inducer motifs allowed the fabrication of single heptad repeats used in various bio-interfaces, including their use as surfactants and DNA-binding agents. Two additional directions of the reductionist approach include the use of peptide nucleic acids and co-assembly techniques. In recent years, we have become interested in metabolite self-assembly. Metabolites are immensely important for the routine function of every cell and take part in numerous physiological processes. We discovered that metabolites (including amino acids, nucleobases, sphingolipids, and vitamins) could form ordered assemblies with supramolecular β-sheet-like organization. Metabolite self-assembly has several advantages, including exceptional biocompatibility, inexpensive production, facile modeling, and biodegradability *in vivo*. Co-assembly of metabolites resulting in high rigidity can be further used in different biomedical and nanotechnological applications. Metal-coordinated metabolite assemblies were also found as exceptional catalysts serving as nano-mimics. We currently explore the utilization of metabolite assemblies for various sensing applications.

ShT-05.1-1
Aggregate-binding aptamers for Parkinson's disease diagnostics

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Parkinson's disease (PD) affects over 10 million people worldwide. The aberrant aggregation of alpha-synuclein, a protein involved in synaptic transmission, is one of the early events in the pathogenesis of PD, and the one most closely linked to pathology. My project investigates alpha-synuclein aggregate-binding aptamers as potential biomarkers. Aptamers are small nucleic acid molecules that can specifically bind cellular targets. There are three goals of my project, which all should come together at the end. *(1) Developing aggregate-binding synthetic Xeno Nucleic Acid (XNA) aptamers using Systematic Evolution of Ligands by Exponential Enrichment (SELEX)*. There is currently no aggregate-specific aptamer for alpha-synuclein. By using SELEX we are able to screen libraries of aptamers, and to select for those that bind the aggregates with high specificity. Moreover, XNAs are synthetic nucleic acid analogues with a backbone that is not recognized by the cellular quality control mechanisms, and are more stable as a result. (Previously published in: Taylor A.I. et al. (2018) Curr. Protoc. Chem. Biol. 10, e44) *(2) Developing aptamer-based biosensors for facile detection of alpha-synuclein aggregates in patient biofluids*. We are developing a sensor based on strand-displacement. This mechanism involves competitive binding between two aptamers and the target aggregates. This approach will enable us to use an aptamer in a biosensor to detect the level of alpha-synuclein aggregates in PD patients' biofluids (Ye C. et al. (2023) Nat. Nanotechnol, 1-8). *(3) Multimerization of aptamers*. To increase the binding avidity, aptamers are multimerized to increase the sensitivity of the diagnostic sensors, allowing detection of the target earlier in disease progression. In conclusion, we are developing XNA aptamers for alpha-synuclein aggregates using SELEX, and then multimerizing them to increase their binding avidity so that they can be used in electrochemical sensors.

ShT-05.1-2
Development of paper-based origami biosensor platform for microorganism monitoring

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The global burden of bacterial pathogens underscores the critical need for accessible and efficient diagnostic tools. While current methods offer high sensitivity, they are often economically demanding and practically complex, which particularly impacts resource-limited regions. Point-of-care (POC) approaches that meet ASSURED (Affordable, Sensitive, Specific, User-friendly, Rapid, Equipment-free, Deliverable) criteria are essential for rapid detection and intervention. Incorporating origami into paper-based biosensing platforms presents an innovative solution, offering affordability, portability, and ease of disposal. Herein, a colorimetric paper-based origami biosensing platform suitable for use in POC applications was developed. The platform was constructed via laser ablation utilizing polyvinylidene fluoride (PVDF) and cellulose membranes that constitute specific primary and secondary antibodies. In order to develop the biosensor platform, optimization of fabrication parameters and PVDF hydrophilization was carried out first. Imaging of the fabricated platforms via light microscopy, wettability analyses, protein adsorption assay, and contact angle measurements were done for characterizing throughout these optimization steps. Then, optimization of reagent amounts was carried out in terms of improving sensory characteristics, utilizing Box-Behnken experimental design. The responses generated by the biosensor in form of visible color development were then analyzed using image processing via MATLAB 2018b. The developed platform was validated against *E. coli* B21 strain and was calculated to have a limit of detection (LOD) of 2 CFU/mL and a dynamic working range up to 10^6 CFU/mL. Overall, developed biosensor platform offers a detection method that has an economic advantage compared to conventional methods, and provides rapid and sensitive results without the requirement of expertise or complex equipment.

ShT-05.1-3
Exploring the potential of yeast cells as biosensors for environmental monitoring

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Biosensors are among the emerging sensitive and cost-effective methods for detection of chemicals in the environment. Due to their amenability to genetic manipulation and sensitivity to different analytes, microorganisms are suitable for biosensor development. Recently, there is a growing interest in yeast-based biosensors due to their stability, higher tolerance to harsh environment and possession of advanced chemical receptors. In our laboratory, we aimed to design and develop *Saccharomyces cerevisiae*-based biosensors to be used in environmental applications (1). In this regard, we followed a multidisciplinary approach, combining biological, chemical, and electrical engineering knowledge. Thus, a polydopamine-yeast biohybrid system was developed and characterized for the detection of copper (2). We also found that different growth conditions, resulting in fermentative or respiratory metabolism, could affect the electrochemical performance of the cells and copper sensing. Procedures of cell immobilization, which is a critical step for the mechanical and chemical stability of the sensor assembly, were compared for different matrices with respect to manipulation time, cell survival and metabolic features. Obtained data indicated that polydopamine-yeast biohybrid showed a concentration dependent electrocatalytic response to copper sulphate and sodium alginate has a better effect on cell viability and metabolism when compared to agarose. Overall, we achieved a proof of concept for the development of a prototype in the form of an electronic device. References: 1) Wahid E. et al. (2023) Biological and technical challenges for implementation of yeast-based biosensors. Microb Biotechnol. 16(1):54-66. 2) Ocheja O. B. et al. (2024) Polydopamine-immobilized yeast cells for portable electrochemical biosensors applied in environmental copper sensing. Bioelectrochemistry 157:108658.

Tuesday 2 July
8:30–10:30, Yellow Room

Digital Twins for Precision Medicine

S-04.6-2
Cell level simulations and digital twins

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In this presentation, I will address the area of simulations of cell behaviour that include molecular networks, pathways and cellular interactions, covering the space between atomistic simulations, based on molecular dynamics and system level simulations of organs, based on fluid dynamics equations. PerMedCoE (HPC/Exascale Centre of Excellence in Personalised Medicine, [1] has developed a framework (PhysiBoSS [2]) that combines agent based simulations of cell interactions in the context of a given environment (PhysiCell, [3]) with Boolean simulations of biological pathways (MaBoSS, probabilistic Boolean framework [4]) implemented in each cell. We have tested the capacity of these models to use as input genomic information, from bulk to single cell data, to provide mechanistic molecular models and testable hypothesis on different biomedical scenarios. Use cases include simulations of COVID infection on layers of epithelial cells or the simulations of temporal evolution of tumours and their micro-environments in response to genomic alterations or drug treatments. In the final part, I will discuss the possible avenues for the integration of cell level simulations in the context of larger systems including digital twins of human organs and the potential application in areas of personalised medicine and preclinical trials [5].

S-04.6-1
Digital twins for complex human diseases: accelerating diagnostics, prognostics and personalized care

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Digital twins, virtual copies of physical objects that have long been employed in industry and manufacturing, have emerged as a promising technology for personalised care. In biomedicine, digital twins include computational models that can simulate a patient's health state over time, enabling predictions regarding prognosis, diagnosis and treatment optimisation. Successful examples include the artificial pancreas and numerical simulation models used in cardiovascular diagnostics. Building digital twins for human pathologies is a complex endeavour requiring interdisciplinary approaches and collaboration among different stakeholders. For instance, the immune system is central to many health conditions, yet no detailed blueprint of its function exists. The immune response is complex and heterogeneous across diseases and patients, and its modelling requires the collective expertise of the international clinical, immunology, and computational modelling communities. On May 2023, a three-week workshop on Building Immune Digital Twins (IDT) was hosted at the Institute Pascal at the University of Paris Saclay in the outskirts of Paris, France, which brought together more than 100 scientists from 19 countries to set the basis of an international IDT Working Group. The primary focus of the IDT Working Group is to create a framework that will lead to IDT implementation in both clinical and preclinical settings. We aim to develop the necessary infrastructure in a community-driven collaborative fashion, leveraging prior knowledge and achievements to accelerate development. We want to support and coordinate efforts with similarly oriented communities and foster interdisciplinary exchanges. Our community was recently selected to become a Research Data Alliance Working Group, and we are moving forward in creating an international IDT community, sending an open invitation for others to join us in this challenging yet formidable journey.

ShT-04.6-3
Spatial flux balance analysis reveals spatially resolved metabolic heterogeneity in renal cancer

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Understanding spatial metabolism is crucial for unraveling the intricacies of cellular functions in complex biological systems. Despite its significance, spatial metabolomics remains in its infancy. Current pathway enrichment frameworks leveraging spatial gene expression data exhibit limitations, lacking expressivity in flux direction, proliferation rate indication, and exhibiting biases towards pathways with numerous isoforms or subunits. In this study, we propose spatial flux balance analysis (SpatialFBA) to harness spatial transcriptomic RNA-sequencing data, thereby identifying spatially resolved metabolic phenotypes. Our method couples constraints on flux boundaries based on differential gene expression with flux sampling. We applied spatialFBA to a public renal cancer dataset. SpatialFBA successfully recapitulates tissue architecture by clustering feasible flux distributions. SpatialFBA accurately distinguishes between tumor core and adjacent normal tissue. Our spatial metabolic analysis unravels the Warburg effect in subpopulations of cancer cells, shedding light on the metabolic reprogramming occurring in specific cellular contexts. By incorporating real-time spatial transcriptomic RNA-sequencing data, spatialFBA addresses the existing limitations in spatial metabolomics, aligning with the evolving paradigm of digital twin technology. Indeed spatialFBA enhances our ability to create digital representations of cellular environments, obtaining digital replicas that mirror the intricacies of cellular metabolism within tissues, providing a virtual environment where the effects of different perturbations and interventions can be simulated and studied. SpatialFBA may facilitate the identification of spatially specific vulnerabilities in pathological conditions, guiding the development of precision medicine approaches tailored to individualized spatial metabolic profiles.

ShT-04.6-4
Learning and applying general principles of cancer cell drug sensitivity

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High-throughput screening platforms for the profiling of sensitivity of hundreds of cancer cell lines (CCLs) to drugs have generated large datasets that hold the potential to unlock new targeted, anti-tumor therapies. In this study, we leveraged these datasets to train explainable machine learning algorithms by employing cell line transcriptomics to predict the growth inhibitory potential (e.g. IC50) of drugs. We used large language models (LLMs) to expand descriptions of the mechanisms of action (MOA) for each drug starting from available annotations, which were matched to the semantically closest pathways from reference knowledge bases. Through this AI-curated resource, and thanks to the inherent interpretability of our model, we demonstrated that pathways enriched for genes most important for predictiveness often match known drug-MOAs and essential genes, suggesting that most of the models learned the molecular determinants of drug response. We then showed that by using the LLM-curated MOA-genes, we were able to further improve the predictive performances of drug models. To enhance translatability to clinical samples, we employed a pipeline to align bulk RNAseq from CCLs, used for training the models, to the ones from patient samples, used for inference. We showcased the utility of our approach on transcriptomics data from TCGA samples, for which patients samples that are best scored for each drug are consistent with therapies prescribed for the corresponding cancer type. We further demonstrated the applicability of our method by inferring effective drugs on samples from pancreatic cancer and glioblastoma patients. Predictions were experimentally validated on commercial as well as primary cell lines, respectively, confirming the reliability of our model. In summary, we demonstrated that our method facilitates the inference and interpretation of cancer cell line drug sensitivity, and holds potential to effectively translate them into new cancer therapeutics.

ShT-04.6-2
An adaptable *in silico* model of the arachidonic acid cascade

G. Horne[I,*], M. Uttley[I,*], A. Tsigkinopoulou[I], F. Del Carratore[I,II], A. Hawari[I], M. Kiezel-Tsugunova[I], A.C. Kendall[I], J. Jones[III], D. Messenger[IV], R.K. Bhogal[IV], R. Breitling[I], A. Nicolaou[I]
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Eicosanoids are a family of bioactive lipids, derivatives of the polyunsaturated fatty acid arachidonic acid. The intimate involvement of eicosanoids in inflammation motivates the development of predictive *in silico* models for a systems-level exploration of disease mechanisms, drug development and replacement of animal models. Using an ensemble modelling strategy, we developed a computational model of the arachidonic acid cascade. This approach allows the visualisation of plausible and thermodynamically feasible predictions, overcoming the limitations of fixed parameter modelling. A quality scoring method was developed to quantify the accuracy of ensemble predictions relative to experimental data, measuring the overall uncertainty of the process. Monte Carlo ensemble modelling was used to quantify the prediction confidence levels. Model applicability was demonstrated using mass spectrometry mediator lipidomics to measure eicosanoids produced by HaCaT epidermal keratinocytes and 46BR.1 N dermal fibroblasts, treated with stimuli (calcium ionophore A23187, ultraviolet radiation, adenosine triphosphate) and a cyclooxygenase inhibitor (indomethacin). Experimentation and predictions were in good qualitative agreement, demonstrating the ability of the model to be adapted to cell types exhibiting differences in arachidonic acid release and enzyme concentration profiles. The quantitative agreement between experimental and predicted outputs could be improved by expanding network topology to include additional reactions. Overall, our approach generated an adaptable, tuneable ensemble model of the arachidonic acid cascade that can be tailored to represent different cell types and demonstrated that the integration of *in silico* and *in vitro* methods can facilitate a greater

understanding of complex biological networks. *The authors marked with an asterisk equally contributed to the work.

ShT-04.6-1
Integrative discovery of SARS-CoV-2 Mpro inhibitors through virtual screening and *in vitro* validation

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In this comprehensive study, we delineate an integrative strategy to inhibit the SARS-CoV-2 main protease (Mpro), a critical enzyme in the viral replication cycle, using a synergy of computational and experimental techniques. Initially, we developed and validated ligand-based pharmacophore models, which served as a basis for the virtual screening of extensive chemical libraries, intending to identify potent Mpro inhibitors. The screening process yielded promising candidates which were subjected to rigorous molecular dynamics simulations. These simulations provided deep insights into the binding dynamics and stability of the inhibitor-Mpro interactions, enabling the refinement of compound selection based on their predicted efficacy and binding affinity. Following computational analyses, compounds with the highest potential were advanced for *in vitro* validation. These assays confirmed the inhibitory activity of selected compounds against the SARS-CoV-2 Mpro, with some showing significant efficacy in impeding viral replication. Moreover, the study also explored the structureactivity relationships (SAR) of these compounds, providing valuable insights into the molecular determinants of inhibitor efficacy and laying the groundwork for further optimization. This research embodies a paradigm shift in antiviral drug discovery, highlighting the power of combining virtual screening and molecular dynamics simulations with empirical validation to expedite the identification of therapeutic candidates. By offering a detailed exploration of the molecular interactions between inhibitors and the SARS-CoV-2 Mpro, our study not only identifies potential leads for COVID-19 treatment but also sets a precedent for the rapid development of antiviral therapies against future pandemic threats.

Tuesday 2 July
17:00–19:00, Silver Room

Functional Foods and Human Health – Part B

S-03.4-1
Bioactive lipids supporting the functionality of the epidermal barrier

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The skin presents a multifaceted barrier that protects the body from external threats including exposure to ultraviolet radiation, pathogens and chemicals, regulates temperature and prevents excessive water loss. To support these diverse activities, skin relies upon a vast array of lipids that contribute to the structure and function of the permeability barrier, as well as the related chemical, microbiological and immunological barriers. The epidermal physical barrier is dependent on a specific mixture of free fatty acids, cholesterol and ceramides, which form intercellular lipid lamellae in the stratum corneum. Sebum- and keratinocyte-derived acylglycerols, fatty acids and oxylipins cover the skin's surface, contributing to the acid mantle, and supporting and regulating the skin microbiome. Fatty acid-derived lipid mediators comprising eicosanoids, octadecanoids and endocannabinoids, and various sphingolipids, drive cutaneous processes, immune reactions, skin inflammation and its resolution. These bioactive lipids are not only necessary for maintaining homeostasis in healthy skin, but are also implicated in many cutaneous diseases, systemic conditions with cutaneous involvement, and natural processes including ageing. As the long chain polyunsaturated fatty acids (PUFA) that are important for the epidermal barrier homeostasis are provided systemically, nutritional supplementation has been explored as a means of supporting the cutaneous lipid microenvironment. PUFA have also been considered as potent nutritional supplements with anti-inflammatory activities, important for reducing cutaneous inflammation and, potentially, facilitating its efficient and timely resolution.

S-03.4-2
Clinical relevance and potential of functional foods to improve human health

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Functional foods are similar in appearance to or may be conventional foods, are consumed as part of a usual diet, and have been demonstrated to possess physiological benefits and/or reduce the risk of chronic diseases beyond basic nutritional function. The clinical relevance and potential of these foods becomes apparent when comparing the magnitude of effects with that of standard drugs used to treat common conditions like arterial hypertension. Despite in parts strong evidence for physiological and health benefits, functional foods are not widely used by clinicians. This presentation will discuss the example of cardiovascular benefits of flavanol-rich foods and how functional foods could be part of clinical practice.

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ShT-03.4-3
Systemic metabolic effects of medium-chain fatty acids supplementation: insights from murine models

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In the wake of surging interest and widespread acclaim for medium-chain triglyceride (MCT) supplementation for health optimization, scrutinizing the scientific foundation behind such functional food additives becomes imperative. MCT-derived medium-chain fatty acids (FAs) with chain lengths ≤C10 are known for their ketogenic potential in humans, along with pronounced anti-inflammatory and metabolic effects in preclinical rodent models. Our prior investigations, revealing cellular cardiac adaptations when MCTs were incorporated into a long-chain triglyceride (LCT)-based ketogenic diet (KD), led us to hypothesize that an increase in C8 and C10 FA levels in tissues following MCT supplementation in mice would be accompanied by a corresponding transcriptional response in nutrient sensing and metabolism. Male C57BL/6NRJ mice were fed a standard diet (SD), LCT-KD, or a combination of LCTs and MCTs (LCT/MCT, 70/30) for eight weeks, with an interval fasting (IF) group (24 h/24 h, SD) serving as a fasting control. Tissue aliquots were analyzed using untargeted LC-MS-based metabolomics and lipidomics, as well as quantitative PCR. MCT addition showed no significant alteration in C8 FA levels in brown adipose tissue, heart, cortex, and striatum, but relative concentration levels were significantly elevated in the liver and skeletal muscle. C10 FA levels were significantly increased in LCT/MCT vs. SD in all tissues except the brain in line with absolute quantitation via targeted LC-MS/MS of free C10 FA in, e.g., heart: 0.112 ± 0.011 vs. 0.075 ± 0.022 μg/mg, cortex: 0.073 ± 0.021 vs. 0.092 ± 0.015 μg/mg. Interestingly, both LCT/MCT and IF feeding induced similar metabolism-specific gene expression. Given the overlap of IF and LCT/MCT gene expression, along with C8/C10 FA independent tissue responses, the current analysis aims to reveal the similarities in the tissue metabolome profiles, enabling us to discern the specific effects attributable to C8/C10 FAs from those that are not.

ShT-03.4-2
Nutraceutical supplementation on behavioral and biochemical alterations in fibromyalgia

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Fibromyalgia (FM) is a chronic condition that affects 1–5% of the population. FM is marked by widespread musculoskeletal pain, exhaustion, cognitive problems, and mood swings and it is also characterized by neuropathic/neurodegenerative modifications, oxidative and nitrosative stress. An appropriate therapy is hard to find and the currently used treatments are able to target only one of these aspects. Strong antioxidant, antinociceptive, and neuroprotective properties have been documented in spirulina. The aim of this study was to assess spirulina's beneficial abilities in protecting rats against reserpine-induced FM. Wistar rats were injected with reserpine (1 mg/kg; subcutaneous; once daily for three days) to induce FM, inducing a significant increase in pain pro-inflammatory mediators and in neuroinflammation. The rats received food supplementation with spirulina (orally at the dose of 400 mg/kg) for 21 days. Behavioral analyses were conducted at the beginning of the experiment and at 3, 5, 7, 14 and 21 days from the first reserpine injection. Numerous biochemical and behavioral characteristics were assessed from the brain tissue. Important cytokines such as IL-1β, IL-2, IL-4, IL-6, IL-10, TNF-α, and IFN-γ are all regulated by spirulina. This protein extract exhibits chelating capabilities, lowers lipid peroxidation and DNA damage, and scavenges free radicals. Different behavioral changes were evaluated in addition to the neuroinflammation, oxidative stress, and decrease in biogenic amine levels that were induced by the administration of reserpine. These analyses revealed that the daily food supplementation of spirulina was able to limit biochemical impairment and oxidative stress, improve the physiological antioxidant system and biogenic amines, as well as limit behavioral alterations. In conclusion, the results suggest that spirulina could be used as an additional dietary supplementation food to help prevent and treat FM.

ShT-03.4-1
Antioxidant, anti-inflammatory, and antibacterial activities of pomegranate peel and tomato skin extracts in human primary gingival epithelial cells

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Agricultural food waste and by-products boast a wealth of high-added-value compounds that positively affect human health.

Thus, the waste matrices hold great promise as a source of active ingredients to be included in nutraceutical and cosmeceutical products, benefiting both human and environmental health. This study explores the biological activities of tomato skin (T) and pomegranate peel (P) extracts on oral mucosa to evaluate their possible use in mouthwash formulations. The biological activities of extracts at different concentrations [0.5–3%] and a mouthwash formulation (F) containing both extracts at 3% were evaluated in human primary gingival epithelial cells (GECs). Once ensure safety, the antioxidant and anti-inflammatory activities were analyzed by a cell-based chemiluminescent assay for detecting intracellular H_2O_2 production and real-time PCR, in GECs injured with lipopolysaccharide [25 μg/mL]. After 24 h of treatment with T, P, and F, a significant antioxidant activity (IC50P: 0.51 ± 0.01 μg/mL; IC50T: 0.57 ± 0.02 μg/mL, IC50F: 0.04 ± 0.02 μg/mL) and an increased superoxide dismutase-1 expression ($p < 0.0001$) were observed. Additionally, the extracts significantly reduced the expression of tumor necrosis factor α ($p < 0.001$) and monocyte chemoattractant protein-1 ($p < 0.0001$), suggesting an anti-inflammatory role. Lastly, the antibacterial activity was assessed against *S. mutans* and *S. sanguinis* by broth microdilution method and agar diffusion test for the extracts and the mouthwash, respectively. These treatments evidenced an antibacterial activity on the reference strains (MICP,T = 10% ∅F = 24 ± 1 mm for *S. mutans* and MICP, T = 5% ∅F = 18 ± 1 mm for *S. sanguinis*). Results show the beneficial effects of tomato skin and pomegranate peel in mitigating oxidative stress, inflammation, and bacterial plaque within the oral mucosa, and emphasize the potential of upcycling in promoting human and environmental health. This work was supported by MIUR-PRIN 2022 (Prot. 2022LW54KC) to SH, CC, AP.

Tuesday 2 July
17:00–19:00, Blue Room

G protein Coupled Receptor-mediated Nutrient Sensing

S-01.7-1
Diurnal cues metabolically regulate hematopoietic stem cell maintenance and function

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Primitive hematopoietic stem cells (pHSC) are mostly maintained quiescent in the bone marrow (BM), preserving the stem cell reservoir. In response to hematopoietic stress, pHSC which are the only chemotherapy resistant HSC population, rapidly proliferate and differentiate to replenish the blood with new mature and immune cells in order to prevent lethal hematology failure and infections. For these tasks, pHSC require new and tested mitochondria in order to accommodate the enhanced metabolic needs to fuel the emergency stressed hematopoiesis. How quiescent pHSC are metabolically regulated to rapidly be ready on demand and when their mitochondrial remodeling and turnover takes place is not fully understood. In the current study we examined diurnal changes in BM retained pHSC and their metabolic regulation. We report that five hours following light onset the pHSC mitochondria reach peak activity as evident from higher mitochondria membrane potential (MMP). In contrast, five hours following darkness onset there were peak local BM melatonin levels; as a result pHSC mitochondria was least active with lowest MMP levels. At this time point there were high levels of HIF-1alpha, Wnt signaling, Glut-1 expression, leading to significantly higher glucose uptake in pHSC, providing them with higher long -erm competitive repopulation potential (LT-HSC). Our study suggests higher mitochondrial activity in BM retained pHSC during daylight priming pHSC for migration and development in order to replenish the blood with new mature blood and immune cells. Higher glycolysis at night time suggests mitochondrial remodeling and turnover at night, which is part of pHSC BM maintenance accompanied by increased LT-HSC competitive repopulation potential.

S-01.7-2
The potential of orphan G protein coupled receptors as novel therapeutic targets

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Although G protein coupled receptors (GPCRs) are the most successful drag targets, 30% of the FDA-approved drugs targeting GPCRs act on only 10% of the entire human GPCRome. In humans, around 160 receptors have unknown ligands and functions and therefore are termed orphan GPCRs. The expression of GPCRs in the pancreatic beta cells is critical in the regulation of glucose-stimulated insulin secretion (GSIS) therefore the characterization of novel metabolic pathways involving the functions of orphan GPCRs remains a key target in academia and the pharma industry for promoting novel therapeutic strategies for type-2 diabetes. Our previous work has demonstrated the existence of negative and positive metabolic loops that inhibit and enhance GSIS by involving two major metabolites, acetate and 20-hydroxyeicosatetraenoic acid (20-HETE) acting on the FFAR1, FFAR2, and FFAR3 (1-2) receptors, respectively. Next studies were focused on the role of orphan GPCRs that are expressed in both the pancreatic beta cells and neurons in the regulation of GSIS. GPR27 and GPR75 receptors are expressed in the pancreatic beta-cells and various neuronal populations. By employing a combination of reverse pharmacology, mouse genetics and cell signaling techniques, we demonstrated that GPR27 is an atypical receptor that can recognize a major metabolite and plays a significant role in the regulation of GSIS. In addition, GPR27 expressed in insulinoma cells has significant effects on cellular proliferation. GPR75 is also expressed in the pancreatic beta-cells. *In vivo* studies using a mouse model of *Gpr75* deficiency showed its implication in metabolism, particularly in regulating body weight. References: [1]Tunaru S, Bonnavion R, Brandenburger I, Preussner J, Thomas D, Scholich K, Offermanns S. Nat Commun 2018 Jan 12;9(1):177. [2]Tang C, Ahmed K, Gille A, Lu S, Gröne HJ, Tunaru S, Offermanns S. Nat Med 2015 Feb;21(2):173-7.

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ShT-01.7-1
Emerging role of the succinate/SUCNR1 axis in microglial metabolism: from physiology to pathology

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Obesity, characterized by excess adiposity and systemic inflammation, has emerged as a global health concern. Hypothalamic inflammation induced by overnutrition has been implicated in the disruption of neuronal regulatory pathways controlling energy homeostasis. Microglia, as key players in maintaining brain homeostasis and orchestrating immune responses, have gained attention in this context. Recent studies have shed light on the emerging role of succinate/SUCNR1 axis as a signaling pathway involved in energy metabolism and immune regulation both in physiology and pathology. However, the specific interplay between microglia and succinate remains poorly understood. In this study, we newly generated a mouse model with conditional knock-out of SUCNR1 specifically in microglia to investigate the connection between succinate and microglia functionality. Our results revealed that under physiology, mice displayed higher body weight with no significant differences in food intake, but exhibited increased satiety and circulating leptin, along with alterations in the glucose metabolism and increased fat depots. Remarkably, in the context of diet-induced obesity, mice presented diminished weight gain, decreased satiety, lower circulating leptin, and improved glucose metabolism homeostasis. These findings suggest that the absence of SUCNR1 in microglia may disrupt feeding behavior associated to leptin alterations, leading to imbalanced peripheral metabolism in physiological conditions. However, in response to a nutritional challenge, the absence of microglial succinate/SUCNR1 axis appears to confer protection against metabolic derangements. In conclusion, our study provides novel insights into the functional relevance of succinate/SUCNR1 axis in establishing a crosstalk between the brain and the peripheral tissues mediated by microglia.

ShT-01.7-3
Dissecting the sequence and structure determinants of GPCR–G protein selectivity via structural bioinformatics and machine learning

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GPCRs are crucial in cellular signaling, interacting variably with G proteins. To elucidate these interactions at the sequence level, we have developed PRECOGx, a novel machine learning framework utilizing ESM1b embeddings from protein language models. It can predict GPCR–G protein coupling, enhancing our understanding by encompassing all GPCR classes. We are also able to examine GPCR mutation variants and splice forms. [Previously published in: Matic M et al. (2022) J Nucleic Acids Research, 50(W1), W598–W610]. Complementing PRECOGx, our analysis of the GPCRome involved examining 362 GPCR–G protein 3D structures from the PDB database. This study identified interaction networks for Gs and Gi/o binding, highlighting crucial interaction fingerprints and secondary structure elements. Additionally, we analyzed the conformational differences between Gs and Gi/o by calculating the root-mean-square deviation (RMSD) of the G protein part of the structures showing a more flexible mode of binding of Gi/o. We also compute interface binding energies (Rosetta) and observe a greater stability in Gs vs Gi/o couplings, with class A receptors exhibiting more stability than class B1 for Gs couplings [Previously published in: Matic M et al. (2023) Nat Commun 14, 4361]. Expanding our research, we employed AlphaFold Multimer to predict tetramer complexes of 827 GPCR–G protein structures, including those involving Gq11 and G1213 proteins. This comprehensive study offered new insights into G protein binding modes across all four families. We also compared these structures to inactive states of GPCRs (predicted by AlphaFold and stored in GPCRDb), investigating the potential of GPCR intramolecular contacts to indicate varying G protein couplings. Our approach underscores the importance of conformational shifts within the transmembrane (TM) bundle in dictating GPCR–G protein interactions, shedding light on potential avenues for drug design and therapeutic strategies. *The authors marked with an asterisk equally contributed to the work.

ShT-01.7-2
Molecular insights into G protein specificity and biased agonism at the β2-adrenergic receptor

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G protein coupled receptors (GPCRs) activated by their native hormone or neurotransmitter exhibit varying degrees of selectivity for different G protein isoforms. Despite the abundant structures of different GPCR–G protein complexes, little is known about the mechanism of G protein coupling specificity. There are a growing number of examples of pathway-selective or biased synthetic agonists that alter the G protein coupling preference for specific GPCRs. The β2AR is an example of a GPCR with high selectivity for coupling to Gαs, the stimulatory G protein for adenylyl cyclase, and much weaker for the Gi family of G proteins that inhibit adenylyl cyclase. While the Gαs pathway is the major therapeutic target for β2AR agonists, β2ARs have been shown to couple to Gαi isoforms in the heart, and this Gαi signaling may have relevance in the pathogenesis of heart failure. Here we present a new Gαi-biased agonist (LM189) for Gαi activation by the β2AR. We provide structural and biophysical evidence that the Gαi bias of LM189 can be attributed to an alteration in the structure and dynamics of ICL2 and TM6.

Tuesday 2 July
17:00–19:00, Yellow Room

Genome Editing and Gene Therapy – Part B

S-04.2-2
Precision genetic engineering of hematopoiesis to treat human disease

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Genetic engineering of hematopoietic stem cells (HSC) with lentiviral vectors has been providing substantial benefit to growing numbers of patients affected by primary immunodeficiencies, hemoglobinopathies and storage disorders. Long-term follow up shows stable hematopoietic reconstitution by high numbers of corrected HSC without signs of clonal expansion or exhaustion. Precise engineering by gene editing may further improve the reach and safety of HSC gene therapy by achieving in situ gene correction or targeted transgene integration. Homology-driven editing, however, remains limiting in long-term HSC and the genetic outcome at target sites heterogenous and, for some by-products, potentially genotoxic. Template delivery by Integrase-defective lentiviral vectors rather than AAV6 and the use of lipid nanoparticles instead of electroporation may increase safety and efficiency of the procedure. Coupling selection for the intended edit and purging adverse outcomes may provide a preferred path towards clinical application of this currently unique modality enabling long-range edits. On the other hand, the emergence of base and prime editors that bypass the requirement for DNA double-strand breaks (DSB) allows editing single/few mutant nucleotides with limited activation of DNA damage response. We have shown, however, that DSBs are significantly lowered but not abrogated. Moreover, the expression of constitutive deaminase domains within the editors may impact the mutagenic load of treated cells. While these potentially genotoxic outcomes can be mitigated by optimizing expression and culture conditions, they should be better investigated and monitored in emerging clinical applications. Overall, our work should advance HSC gene therapy by a combination of transformative approaches leveraging on precision genetic engineering while alleviating the morbidity of the procedure, broadening application to several diseases and patients worldwide.

S-04.2-1
Gene editing for the treatment of inherited liver disorders

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The deliberate and precise modification of the host genome using engineered nucleases represents a ground-breaking advancement in modern medicine. The initiation of several clinical trials employing these approaches to address metabolic liver disorders, along with the recent remarkable outcomes observed in patients with transthyretin amyloidosis, underscores the progress in this domain and highlights the potential of these innovative therapies to safely and effectively treat such diseases. These advancements have been facilitated by significant recent technological improvements, particularly the emergence of CRISPR Cas9-based technology, which has revolutionized the field of gene editing and enabled *in vivo* modification of the cellular genome for therapeutic purposes. These modifications can encompass gene supplementation, correction, or silencing, opening up a plethora of therapeutic possibilities. Moving forward, it is likely that we will witness the therapeutic potential of these strategies unfold in the coming years. In this presentation, we aim to summarize some of the preclinical data gleaned from animal models and explore the various gene editing strategies employed in the treatment of liver diseases. Finally, we will focus on the latest results obtained by our group using gene editing for the treatment of primary hyperoxaluria type I. The therapeutic efficacy of nucleases and nickases delivered by adeno-associated vectors will be showcased, along with interesting data on the nature of the genome modification, vector integration, and safety implications.

ShT-04.2-3
Fine-tuning site-directed RNA editing: controlled gRNA synthesis with T7 RNA polymerase

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Efficient synthesis of components for site-directed RNA editing is crucial, yet existing approaches lack modulation. To address this, we devised a system for controlled guide RNA (gRNA) synthesis in the cytosol, achieved through transcription under the control of the T7 promoter. This approach allows modulation of gRNA expression only in presence of exogenous T7 RNA polymerase. To prevent read-through transcription, we have also incorporated an engineered termination sequence with high termination efficiency. We tested our system using the LEAPER RNA editing system and demonstrated controlled induction of gRNA expression without leakage. In conclusion, development of our system could provide an easy solution to modulate site-directed RNA editing.

ShT-04.2-1
Moonlighting role for dUTPase: establishing a viable mammalian cell line with high uracil-DNA levels

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The dUTPase enzyme has a prominent role in the preventive repair by the hydrolysis of dUTP to dUMP [Vertessy BG et al. (2009) Acc Chem Res 42, 97–106]. The dUTP nucleotide can be incorporated into the DNA, however uracil bases are excised mainly by UNG and SMUG1, which are enzymes of the base

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excision repair mechanism. Elevated dUTP level can generate a recurring cycle of uracil incorporation and excision leading to cell death. Interestingly, the *Ung* and *Smug1* knockout mice are viable and fertile [Alsøe L et al. (2017) Sci Rep 7, 7199]. However, knockout of the dUTPase in mice leads to early embryonic lethality [Palinkas HL et al. (2019) Biomolecules 9, 136]. To consider the importance of dUTPase in cellular physiology, it is paramount to address the essential function of the enzyme, therefore our aim is to generate catalytically inactive dUTPase in MEF cells. Change of the conserved aspartic acid to an asparagine leads to an inactive enzyme which is unable to coordinate the catalytic water for the hydrolysis of the dUTP. To date, using CRISPR cytosine base editing technology we have successfully created cell lines that are either heterozygous or inactive for the dUTPase encoding gene in *Ung* −/− *Smug1* −/− MEF cells. We have found that the genomic uracil content in the inactive mutant cells is highly increased. We determined via western blotting that in the inactive mutants the expression of dUTPase is decreased. Normally, dUTPase localises to the nucleus, but in the inactive mutant cells the nuclear localisation is altered as characterised with immunocytochemistry. In conclusion, we have successfully generated a viable *Ung* −/− *Smug1* −/− MEF cell line that is inactive for dUTPase and represents a highly uracilated mammalian genome. As the inactive dUTPase mutant cells are viable with high genomic uracil content, our results suggest that the catalytic activity may not be the essential function of dUTPase, but the enzyme may have a moonlighting function.

ShT-04.2-2
Exploiting the role of miR-155 in triple negative breast cancer via CRISPR/Cas9 based genome editing

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Breast cancer is a global health concern, with around 2.3 million diagnosed cases annually. Triple negative breast cancer (TNBC) is a heterogeneous disease, constituting 10-20% of all new breast tumors and lacking the biomarkers that can be effectively targeted in the clinical practice (estrogen receptor (ER), progesterone receptor (PR), and HER2). Most TNBC exhibits aggressive behavior and poor prognosis due to high rates of distant metastases, leading to elevated mortality rates. Despite the efforts to explore alternative strategies, the absence of effective targeted therapies makes TNBC treatment a remaining challenging task for clinicians. The discovery that dysregulation of microRNAs (miRNAs) and epigenetic factors play a role in carcinogenesis and cancer progression has led to the suggestion that miRNAs could be a potential target for cancer treatment. Among the various miRNAs linked to cancer, miR-155 is one of the most frequently overexpressed miRNAs in solid tumors, as well as in hematological malignancies, including breast cancer. Herein, we elucidated the role of miR-155 in TNBC by demonstrating its upregulation in various TNBC cell lines. A clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 genome editing system targeting miR-155 was designed and validated. Disruption of miR-155 expression in TNBC cells impaired cell proliferation, induced a G2/M cell cycle arrest, inhibited cell migration, and triggered the intrinsic apoptosis signal pathway through the downregulation of anti-apoptotic Bcl-2 and Bcl-XL proteins, and upregulation of pro-apoptotic Bax protein and apoptotic protease Caspase-3. Moreover, extracellular vesicles (EVs) derived from epithelial cells demonstrated the ability to encapsulate and deliver CRISPR/Cas9 DNA plasmid into TNBC cell lines. Altogether, these findings provide a proof-of-principle for a targeted, precise, and effective delivery approach for *in vitro* CRISPR genome editing in TNBC tumors.

Wednesday 3 July
8:30–10:30, Silver Room

Methods and Progress in Structural Biology – Part B

S-01.2-2
Mechanisms of posttranslational modification of microtubules by two distinct families of specific detyrosinases

A. Perrakis
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Microtubules are a major component of the cytoskeleton and can accumulate a plethora of modifications. The microtubule detyrosination cycle is one of these modifications it involves the enzymatic removal of the C-terminal tyrosine of α-tubulin on assembled microtubules and the re-ligation of tyrosine on detyrosinated tubulin dimers. This modification cycle has been implicated in cardiac disease, neuronal development, and mitotic defects. The enzymes responsible for microtubule detyrosination remains a long-sought mystery for well-over four decades since the biochemical process of detyrosination was described. The vasohibins and their cofactor Small Vasohibin Binding Protein (SVBP), are cysteine peptidases which were originally known as extracellular angiogenesis regulators, and were the first tubulin detyrosinases we discovered. Later, the MicrotubuleAssociated Tyrosine CArboxyPeptidase (MATCAP) and a close homologue (TMCP2) were identified as zinc metalloproteases that can cleave α (and β)tubulin tails. The structures of the catalytic domains of members of the two evolutionary unrelated enzyme families were determined by X-ray crystallography, establishing the corresponding cleavage mechanisms. Both families act preferentially on microtubules rather than on tubulin dimers, and the cryoelectron microscopy structures of vasohibins and MATCAP bound to microtubules, were key to understand fundamental differences: the vasohibins catalytic domain interacts both with the α-tubulin monomer whose tail will be cleaved and with α-tubulin of the adjacent protofilament, but MATCAP binds to adjacent tubulin dimers along a single protofilament. The current information is key to further study how these enzymes interplay with other modifications and define the tubulin code. Previously published in: Nieuwenhuis J et al (2018) Science 58(6369):1453–6 Adamopoulos A et al (2019) Nature Struct Mol Biol 26(7):567–70 and Landskron L et al. (2022) Science 376(6595)

S-01.2-1
Predictions and experiments in structure determination

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Accurate protein structure prediction from sequence has been a game-changer and not just in the structural biology community; researchers in every branch of the life sciences are consulting such models[1]. The current success of artificial intelligence on a long-standing quest, showcases a power for harnessing prior knowledge that will go deeper in the next years as we learn how to steer it into particular questions. While AlphaFold predictions are useful hypotheses about protein structures, experimental information remains essential for creating an accurate model[2]. Predictions have been effectively incorporated in our structural determination methods[3]. The new balance demands a redefinition of how we integrate prior knowledge in experimental interpretation[4] and verification of the information gained beyond the starting model. Conversely, conditioning predictions with experimental knowledge opens new opportunities to gain a dynamic view by using experimental structures to establish the background of a prediction or impose boundary conditions. The broad scale search for structural and genomic information preceding a prediction simultaneously retrieves knowledge on all possible contacts and states a sequence may fulfil in time or under different conditions. Our method systematically deconvolves the signal from multiple states. VAIRO informs AF predictions with experimental structures, setting boundary conditions to target particular states within a dynamic or recover weak interactions. References: 1. Jumper, J., et al. Nature 2021 596, 583. 2. Terwilliger, T. C. et al. Nat Meth 2024. 21, 110. 3. Simpkin, A. J.; Caballero, I.; et al. Acta Crystallogr. 2023, D79, 806. 4. Medina, A; Jiménez, E., et al. Acta Crystallogr. 2022, D78, 1283. * The authors marked with an asterisk equally contributed to the work.

ShT-01.2-3
Tracking transcription-translation coupling in real-time

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A central question in biology is how macromolecular machines function cooperatively. In bacteria, transcription and translation occur in the same cellular compartment, and can be physically and functionally coupled. While several recently published high-resolution structures of the ribosome-RNA polymerase (RNAP) complex provided first mechanistic insights into the coupling process, we lack knowledge of how these structural snapshots are placed along a dynamic reaction trajectory. Here, we reconstitute a complete and active transcription-translation system and develop multi-color single-molecule fluorescence microscopy experiments to directly and simultaneously track transcription elongation, translation elongation and the physical and functional coupling between the ribosome and the RNAP in real-time (Qureshi & Duss, bioRxiv, 2023, 10.1101/2023.12.07.570708). Our data show that physical coupling between ribosome and RNAP can occur over hundreds of nucleotides of intervening mRNA, by mRNA looping, a process facilitated by transcription factor NusG. We detect active transcription elongation during mRNA looping and show that transcription factor NusA-paused RNAPs can be activated by the ribosome by long-range physical coupling. On the other hand, the ribosome slows down while colliding with the RNAP, a state with no intervening mRNA between both machines and physical coupling between both machineries becomes more transient once the RNAP escapes from a collision. We hereby provide an alternative explanation on how the ribosome can efficiently rescue RNAP from frequent pausing without requiring collisions by a closely trailing ribosome. Overall, our dynamic data mechanistically highlight an example of how two central macromolecular machines, the ribosome and RNAP, can physically and functionally cooperate to optimize gene expression.

ShT-01.2-4
An integrative structural biology and molecular biophysics approach towards SOS response characterization: from mechanistic insights to inhibitor screening and design

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The bacterial SOS response to genotoxic stress is orchestrated by the interplay between the DNA damage sensor RecA (which oligomerizes on ssDNA) and the transcriptional repressor LexA. Upon binding to oligomeric RecA, LexA undergoes autoproteolysis and loses the ability to repress effector SOS genes, including many involved in hyper mutagenesis, antibiotic resistance and virulence. To foster the development of new antagonists of the SOS response, deep investigations on its structural and functional features are needed. In particular, the RecA-LexA interaction model has long remained elusive. To fill this gap, an integrative structural biology approach was applied to the *Pseudomonas aeruginosa* SOS response. The structure of LexA autoproteolytic domain was obtained by X-ray crystallography, while the RecA/ssDNA complex was solved by electron microscopy. Cryo-EM was successfully applied to the LexA-RecA/ssDNA complex as well, revealing a peculiar interaction mechanism that locks LexA in the conformation needed for self-cleavage. Building on these structures, molecular dynamics simulations and biophysical assays further sustained the proposed model for RecA-induced LexA autoproteolysis. In a parallel project, anti-LexA nanobodies have been developed by llama immunization and phage display selections. Autoproteolysis assays revealed that these nanobodies are the most potent LexA inhibitors discovered so

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far, while SOS genes expression profiling assessed their efficiency as SOS suppressors in bacterial cells stressed by antibiotics [published in Maso et al., 2022, *Structure* 30, 1479-1493]. X-ray structures of LexA-nanobody complexes unveiled that these molecular tools stabilize the uncleavable LexA conformation and have sustained nanobody improvement by computer-aided design. Besides answering long-standing questions in the study of the SOS response, work summarized here paves the way to innovative anti-evolutive and anti-virulence strategies in antimicrobial warfare.

ShT-01.2-2
Structural characterisation and protein tool generation for investigating SLF1 complexes in replication-coupled DNA repair

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Understanding how cells repair damaged DNA is highly relevant to cancer biology. DNA damage, such as interstrand crosslinks, can stall the replication fork, prompting the engagement of DNA damage response (DDR) proteins, such as SMC5/6 Localisation Factor (SLF) 1. SLF1 interacts with RAD18 (a ubiquitin ligase required for post-replication repair of damaged DNA), a specific histone marker present in replicated DNA and SLF2, to regulate recruitment of the structural maintenance of chromatin (SMC) complex, SMC5/6. However, the structural mechanism of the SLF1–SLF2 interaction network remains unclear, with a lack of protein tools to modulate their function within the DDR. Our study reveals that SLF1's tandem BRCA1 C–Terminal (tBRCT) domain interacts directly with phosphorylated RAD18 (S442, S444) via a conserved tBRCT phospho-recognition mechanism. Additionally, we determined the crystal structure of the ankyrin repeat domain (ARD) of SLF1 bound to histone H4 peptide unmethylated at lysine 20 (H4K20me0). Isolation of high-affinity Affimers, small non-antibody binding proteins against the tBRCT and ARD of SLF1, also provides tools to probe the significance of SLF1 complexes *in vivo*. This research enhances our understanding of SLF1 function during replication-dependent repair and offers potential for developing DNA repair inhibitors for sensitising cancer cells to existing chemotherapeutics.

ShT-01.2-1
Elucidating the activation mechanism for GBP1 oligomerization – a key player in innate immunity against microbial pathogens

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The dynamin-related human guanylate-binding protein 1 (GBP1) mediates host defense against microbial pathogens. Upon GTP binding and hydrolysis, auto-inhibited GBP1 monomers dimerize and assemble into soluble and membrane-bound oligomers, which are crucial for innate immune responses. How higher-order GBP1 oligomers are built from dimers and how assembly is coordinated with nucleotide-dependent conformational changes has remained elusive. Here, we use cryo-electron microscopy and a detailed biochemical analysis to elucidate the activation mechanism of GBP1 leading to oligomerization and encapsulation of bacterial pathogens. We present cryo-electron microscopy-based structural data of the soluble and membrane-bound GBP1 oligomers demonstrating that GBP1 assembles in an outstretched dimeric conformation. By combining new and published structural insights with biochemical, mechanistic, and pathogen-based data, our study provides the molecular basis for understanding GBP-mediated antimicrobial functions. We identify a surface-exposed helix in the large GTPase domain, which contributes to the oligomerization interface, and probe its nucleotide- and dimerization-dependent movements facilitating the formation of an antimicrobial protein coat on a Gram-negative bacterial pathogen. Our results reveal a sophisticated activation mechanism for GBP1 in which nucleotide-dependent structural changes coordinate dimerization, oligomerization, and membrane binding to allow encapsulation of pathogens with an antimicrobial protein coat. In this way, our structure-function study deepens our understanding of the underlying molecular coupling of the GTPase cycle and oligomerization within the GBP protein family which is crucial for its antimicrobial functions.

Wednesday 3 July
8:30–10:30, Red Room

Redox Biochemistry

S-01.8-1
Exploiting redox-active molecules against mitochondrial diseases

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Mitochondrial diseases result from a decreased oxidative phosphorylation (OXPHOS) that leads to a broad spectrum of incurable pathologies [1,2]. Our goal was to understand whether membrane-permeant small molecule(s) can be exploited to treat OXPHOS-related diseases as an alternative to gene therapy. Therefore, we selected some molecules for their ability to replace the redox functions of complex III and among them identified a few promising agents. Sub-μM dose of these drugs is harmless, restores respiration and increases ATP production in Ttc19−/− mouse embryonic fibroblasts as well as in fibroblasts from patients harboring pathogenic mutations in three different assembly/stabilization factors of complex III, including TTC19. The drugs normalized the mitochondrial membrane potential, mildly

increased ROS production, and triggered mitochondrial biogenesis. These *in vitro* effects were confirmed also *in vivo*, in both *Drosophila melanogaster* TTC19KO, in *Danio rerio* TTC19KD [3]. Here we show that redox cyclers and their derivatives with enhanced life-time and tissue distribution exhibited a benefit in Ttc19 KO mouse model. Administration of low, non-toxic concentrations of the drugs significantly ameliorated movement and coordination proficiency, without inducing toxicity. Likewise, drugs able to receive electrons from NADH, showed a beneficial effect also in the case of cells and mice with complex I disease. Our results point to exploitation of redox cyclers for therapy against diseases due to OXPHOS dysfunction. References: [1] Suomalainen A, Battersby BJ. Nat Rev Mol Cell Biol. (2018) 19, 77. [2] Viscomi C, Zeviani M. J Intern Med. (2020) 287(6):665-684. [3] Peruzzo R et al. Nat Commun (2021) 12, 2103.

S-01.8-2
Hydrogen peroxide diffusion in the extracellular space: redox volume signaling in the brain

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Hydrogen peroxide (H_2O_2) is a major redox signaling molecule underlying a novel paradigm of cell function and communication. A role for H_2O_2 as an intercellular signaling molecule and neuromodulator in the brain has become apparent from studies showing that it can to regulate neuronal polarity, connectivity, synaptic transmission and tuning of neuronal networks. Furthermore, H_2O_2 can travel in the extracellular space, from source of production to target, suggesting it may act as a volume signaling molecule. This activity requires H_2O_2 to have the ability to diffuse in the extracellular space, from the source of production to the target. Using a novel electrochemical microsensor, we have investigated H_2O_2 concentration dynamics in the living brain and the factors which shape its diffusion pattern and half-life in the brain extracellular matrix both in an *ex vivo* model using rodent brain slices and *in vivo*. We found that exogenously applied H_2O_2 is rapidly removed, with an average half-life in the extracellular space of $t_{1/2}$ = 2.2 s *in vivo*. We determined the *in vivo* effective diffusion coefficient of H_2O_2 to be $D^* = 2.5 \times 10^{-5}$ cm^2 s^{-1}, which allows it to diffuse over 100 μm in the extracellular space within its half-life. These quantitative details allow us to interpret the physiology of the redox signal, tentatively placing H_2O_2 within the class of volume transmitters connecting all cell types with the complex network of brain tissue, regardless of whether the cells are physically connected.

ShT-01.8-3
Structure-function studies of human MICAL1, the multidomain flavoenzyme participating in actin cytoskeleton dynamics

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Human MICAL1 belongs to the MICAL family of multidomain, mainly cytoplasmic, enzymes, which are conserved from insects to humans and participate in the control of the dynamics of the actin cytoskeleton through their redox activity. MICALs are involved in cell migration, differentiation, division, cell-cell contacts, cell invasion and even gene expression regulation. Interfering with their activity has been proposed to be beneficial to treat cancer, neurodegeneration and pathogen (viral) invasion [see Esposito A et al. (2019) Protein Sci. 28, 150-166 for a recent review]. MICAL1 contains an N-terminal FAD-containing catalytic domain followed by a calponin homology (CH) and a LIM domain, and a region that may mediate the interaction with regulating proteins and ends with a Rab-binding domain. Current evidence indicates that MICAL1 exists in an autoinhibited catalytically inactive form in equilibrium with an active one. The N-terminal flavoprotein domain catalyzes a NADPH oxidase activity producing H_2O_2, which may serve as a second messenger in the cell. In the presence of F-actin, the NADPH oxidase activity is greatly enhanced due to both k_{cat} and $K_{m,\ NADPH}$ effects. Local production of H_2O_2 leads to oxidation of actin residues close to the interface between monomers with destabilization of the filament. We are currently investigating the mechanism of self-inhibition of human MICAL1 and its conformational flexibility by combining enzyme kinetics, limited proteolysis and cryoEM. Available data are fully consistent with the proposal that the catalytic domain is a bona fide NADPH oxidase that exploits the conformational flexibility of the p-hydroxybenzoate hydroxylase fold to allow for the strict control of its activity. The NADPH oxidase reaction appears to be controlled by the C-terminal Rab binding domain, which interferes with the conformational changes of the flavoprotein domain that are integral part of the catalytic cycle.

ShT-01.8-4
NADPH regeneration via renewable hydrogen: exploiting oxygen-resistant [FeFe]-hydrogenase and BMR reductase in a non-physiological cascade

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NADPH cofactor plays a pivotal role in biocatalysis, facilitating the production of high-value compounds like regio- and stereoselective intermediates utilized in fine chemicals and pharmaceuticals synthesis. Many proposed cofactor regeneration systems encounter challenges such as the accumulation of by-products,

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acidification, and the need for purifying the desired products for which the cofactor regeneration is ancillary. Alternatively, employing H_2 enables efficient NADPH recycling without by-products that could disrupt pH balance or complicate product recovery. Additionally, the availability of inexpensive H_2 derived from renewables like solar and wind-powered electrolysis or dark fermentation of waste materials ensures sustainability of the process and aligns with the principles of circular economy. The system proposed here is the first of its kind, based on a modular combination of non-physiological partners already successfully employed in the "Molecular Lego"[1,2]. We exploited the very robust, highly active and oxygen resilient [FeFe]-hydrogenase CbA5H from *C. beijerinckii*, previously identified in our group[3,4], combined with a reductase (BMR) from *P. megaterium*. The system showed a good stability as evaluated by DSC and it reached up to 28 ± 2 nmol NADPH regenerated s^{-1} mg of hydrogenase^{-1} (TOF: 126 ± 9 min^{-1}). To demonstrate feasibility in biotechnological applications, a cascade reaction employing [FeFe]hydrogenase, BMR, and a mutated Baeyer-Villiger monooxygenase[5] confirmed efficient indigo production across multiple reaction cycles. References: 1. Sadeghi and Gilardi (2013) Biotechnol Appl Biochem 60(1):102-10. 2. Giuriato et al. (2022) Protein Sci 31(12):e4501. 3. Morra et al. (2016) Biochemistry, 55(42), 5897-5900. 4. Winkler et al. (2021) Nat Commun 12:756. 5. Catucci et al. (2022) Biocatal Agric Biotechnol 44, 102 458. LB contribution is produced while attending PhD SDC at IUSS Pavia and funded by NextgenerationEU - PNRR. DM118/2023, M4C1, Inv. 3.4 - Transizioni digitali e ambientali.

ShT-01.8-1
Biochemical portrait of the inner-membrane associated cytochrome CbcA: insights into its role in extracellular electron transfer

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Extracellular electron transfer (EET) is a respiratory mechanism that allows electrogenic bacteria to sustain their growth by using exterior electron acceptors, including electrode surfaces. This key metabolic feature involves the transfer of electrons through consecutive redox partners connecting the cell's interior to its exterior and can be explored in several biotechnological fields, namely bioremediation, bioenergy production and microbial electrosynthesis [1]. CbcBA is an inner-membrane oxidoreductase from *Geobacter sulfurreducens* that was shown to be essential for the reduction of extracellular metal oxides and electrodes with a redox potential below –210 mV [2]. The complex is formed by CbcA that binds 7 *c*-type heme groups and is anchored to the membrane by a C-terminal α-helix, and CbcB, an integral membrane di-heme *b*-type cytochrome. The periplasmic domain of CbcA (37 kDa) was heterologously produced in *E. coli* and different spectroscopic techniques were used to characterize it at the structural and functional level. The crystal structure of CbcA was determined by the multi-wavelength anomalous dispersion (MAD) technique, and the crystals diffracted up to 1.9 Å resolution. The apparent midpoint reduction potential value of CbcA was determined by potentiometric redox titrations and is the most negative ever reported for *G. sulfurreducens*' inner-membrane oxidoreductases. Finally, nuclear magnetic resonance was used to monitor electron transfer reactions and probe biomolecular interactions with putative redox partners in the periplasm, shedding light on the complex networks for EET in *G. sulfurreducens*. References: [1] Kumar R et al. (2015) Int J Energy Res 39, 1048–1067. [2] Joshi K et al.(2021) Mol Microbiol 116, 1124–1139. This work was supported by Fundação para a Ciência e a Tecnologia through grants 2022.11900.BD (JMAA), PTDC/BIA-BQM/4967/2020 (CAS), EXPL/BIA-BQM/0770/2021 (LM), UIDP/04378/2020 and UIDB/04378/2020 (UCIBIO), and LA/P/0140/2020 (i4HB).

ShT-01.8-2
The antioxidant function of coenzyme A: a renaissance of a key metabolic cofactor

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Reactive oxygen species (ROS) are cellular metabolites that participate in various signaling and metabolic pathways. However, imbalanced levels of ROS can lead to oxidative stress and damage to cellular macromolecules: DNA, proteins and lipids. Cells overcome oxidative stress by using different enzymatic and non-enzymatic antioxidant systems. In recent years we discovered novel antioxidant function of a key cellular metabolite, coenzyme A (CoA). We demonstrated that during oxidative stress CoA can form a mixed-disulfide bond with protein cysteine thiols termed as protein CoAlation. Using a combination of anti-CoA monoclonal antibodies and LC-MS/MS methodology we showed that established cell lines, single cell and multicellular organisms contain increased level of CoAlated proteins upon exposure to oxidative or metabolic stress. Our research on protein CoAlation has shown that it is a widespread and reversible post-translational modification. More than 2200 prokaryotic and eukaryotic proteins have been found to be CoAlated. To date, we found that under oxidative stress protein CoAlation protect cysteine residues from hyperoxidation and in addition modulate the activity of modified proteins by inducing significant conformational changes. CoAlation of S. aureus GAPDH, aurora kinase A, peroxiredoxin 5 and metastasis suppressor protein NME1, were shown to reversibly inhibit their function, mediated by covalent modification of their catalytic cysteines or cysteine residues located close to catalytic sites. A study performed on the transcription factor accessory gene regulator AgrA (*S. aureus*), showed that CoAlation at its Cys199 interfered with its binding to DNA. Overall, these studies show the important cellular role of CoA in regulating the function of proteins and their downstream interacting partners under different cellular stress conditions and uncover CoA as a major antioxidant in both prokaryotic and eukaryotic cells.

Wednesday 3 July
8:30–10:30, Blue Room

Young Scientists Session B: Biochemistry for Drug Repurposing

S-05.2-2
Drug repurposing in neurodegenerative disease: potential and pitfalls

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The pharmacologic pipeline of drugs in neurogenerative diseases has been slow to propose new treatment in this disease area. Drug repurposing has much potential to identify new therapeutic options, alongside traditional routes of design and development, and can accelerate the identification of pharmacotherapy for persons with neurodegenerative diseases such as Alzheimer's disease. Developing a framework for drug repurposing is challenging, combining knowledge of pathology, pharmacology, pharmaceutics, drug safety and evaluation of evidence. The aim of this framework is ultimately to identify drug candidates that have the potential to show clinical benefit in trials and subsequently be licensed for a new indication. One such framework to approach repurposing will be proposed. However, the drug repurposing route also has its share of pitfalls. These include limited evidence being available at the time of assessment. Ultimately, the evaluation of evidence and expert opinion indicating whether a drug is a good or bad candidate for repurposing is a nuanced process which must be developed *ad hoc*. This in itself can be considered a methodological limitation. However, in view of the difficulty in identifying new drugs for neurodegenerative diseases through traditional methods, repurposing must be taken seriously as an alternative, economical and feasible route to drug discovery, and optimized, to make the process of drug discovery more efficient.

S-05.2-1
Heterogeneity in tumors: a real-time and single-cell perspective

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Cells within a cancer are highly heterogeneous with respect to their phenotype and can manifest distinct morphological, molecular and functional features. As a consequence, it is challenging to design treatment therapies that effectively target all cancer cells. Using human samples of colorectal cancers, the Snippert lab studies the causes and consequences of heterogeneity in cellular behavior during tumor growth, tumor evolution and the emergence of therapy resistance. Primarily, we study tumor cells using cancer organoids and live-cell imaging experiments to assess changing cell states, real-time signaling dynamics and evolving genomes, all with single-cell resolution. From a cancer cell signaling point of view, I will discuss our work on drug response measurements in patient-derived organoids where real-time and single-cell resolution helps us to understand their mode of response and our drug screen efforts to improve therapies.

ShT-05.2-1
Synthetic antimalarial peptides to combat drug resistance in malaria

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Malaria is a deadly infectious disease caused by the *Plasmodium falciparum* (*Pf*) parasite, the most virulent *Plasmodium* species. The lack of an effective vaccine and the emergence of drug-resistant parasites increase the challenge of eradicating malaria worldwide. Antimicrobial peptides (AMPs) offer a promising therapeutic avenue for developing new treatments based on their broad activity against the membranes of pathogens. Here, we identified a set of synthetic AMPs capable of inhibiting *Pf* proliferation while the parasite is growing within its host human red blood cells (RBCs). Importantly, these AMPs exhibit a non-toxic effect on uninfected human RBCs. Fluorophore-tagged AMP demonstrated stronger binding affinity to the infected RBCs (*Pf*-iRBCs) in contrast to uninfected ones. We subsequently employed atomic force microscopy to investigate the mode of action of these peptides. We revealed mechanical alterations in the plasma membrane of the treated *Pf*-RBCs, indicating the presence of a distinct membranal factor that mediates the peptide interaction. To further identify the key components involved in the AMP-membrane interaction, we used unilamellar liposomes to model the plasma membrane of *Pf*-iRBCs versus uninfected RBCs and measured the peptide binding. Remarkably, we found that the presence of cholesterol in the RBC membrane inhibits the peptide binding, whereas its absence, as in *Pf*-iRBCs' membranes, enhances its activity. Overall, our data show that synthetic AMPs could serve as new antimalarial drugs, capitalizing on the membrane alterations induced by the parasite during its blood stage. Furthermore, the broad mode of action of these AMPs may reduce the likelihood of drug resistance. *The authors marked with an asterisk equally contributed to the work.

ShT-05.2-2
High-density lipoprotein engineering for treatment of age-related macular degeneration

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Age-related macular degeneration (AMD) is a leading cause of blindness in people aged 60 years or older worldwide. The current major treatment for AMD is intravitreal injection of biopharmaceuticals that inhibit neovascularization. While they have revolutionized AMD therapy, the route of their administration is highly invasive. Eye drops have been desired, but none have been

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clinically approved yet. The main pathological mechanisms of AMD are dyslipidemia, chronic inflammation, oxidative stress, and neovascularization, which are similar to those of atherosclerosis. In contrast, high-density lipoprotein (HDL) is well known for its anti-atherosclerotic effects. This coincidence led us to hypothesize that HDL could also show anti-AMD activities. Although reconstituted HDL (rHDL) nanoparticles have been clinically tested as drugs, their eye drops had never been tested for any purposes at that time. Considering the poor corneal/conjunctival absorption of rHDL due to rapid tear clearance, we decided to develop an engineered rHDL library with various types of cell-penetrating peptides and phospholipids and by changing the size from 10 to 25 nm and to screen it for the efficiency of posterior delivery of a fluorescent cargo molecule via eye drop instillation. The best rHDL, designated as engineered lipoprotein 1 (eLP1), showed therapeutic efficacy in a mouse model of AMD (Suda, K. et al., J. Control. Release, 2017). Recently, we reported that this efficacy was dramatically improved by attaching the AsnGlyArg tripeptide to eLP1 (Fukuda, R. et al., Adv. Therap., 2023). In this congress, we will present the structure, HDL biological activities, and the therapeutic activity of this second-generation eye drop, eLP2. Our study demonstrates that eLP2 is a novel and promising eye drop for AMD treatment, which potentially overcomes the limitations of current therapies and provides a non-invasive and effective option for patients.

ShT-05.2-3
The antipsychotic drug penfluridol displays cytotoxicity in breast cancer cells by inducing mitochondrial damage and activating the endoplasmic reticulum stress response

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Breast cancer (BC) is the most diagnosed cancer and the second leading cause of cancer mortality. Brain metastasis is detrimental since most of the available treatments fail to reach the central nervous system (CNS). Drug repurposing by reducing the time and costs required for *de novo* drug discovery, represents a promising approach in cancer therapy. Psychotropic drugs have been widely exploited owing to their safety and long clinical use. Herein, based on our previous screening, we investigated in BC cells the cytotoxic activity of the commercially available psychotropic drug penfluridol (PF). Notably, PF displayed a 24 h IC_{50} of 5 μM in human estrogen receptor-positive MCF7 cells and estrogen receptor-negative MDA-MB-321 cells. Also, 0.5 μM PF treatment significantly inhibited the clonogenicity of stem-like BC cells. Besides, 16 h pretreatment with PF 5 μM sensitized by 50% BC cells to doxorubicin. Because of the cationic amphiphilic nature of PF, we investigated the mitochondrial activity of BC cells in response to PF treatment. We observed a significant decrease in the mitochondrial membrane potential, while oxygen consumption rate results revealed a compromised mitochondrial activity and the inhibition of nearly all mitochondrial complexes. Investigation of the integrated stress response – as a response to mitochondrial dysfunction – disclosed the phosphorylation of IRE1α and eIF2α already after 3 h of treatment with PF 5 μM along with an increase in ATF4 protein levels. mRNA analysis unveiled increased DDIT3/CHOP expression while immunofluorescence displayed nuclear localization. Proteomic data showed cell cycle block, DNA damage, and altered cell metabolism. Ongoing metabolomic analysis aims for a comprehensive understanding of PF activity. In conclusion, our data support the repurposing of PF for BC treatment, and, considering PF localization in the CNS, our findings are a promising starting point for the management of BC patients undergoing brain metastasis.

ShT-05.2-4
Tacrolimus, a topical calcineurin inhibitor, could be used as a candidate compound for chondrosarcoma treatment

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A rare kind of malignant bone tumor called a chondrosarcoma is typically located in the spine. These forms of cartilage-forming bone sarcomas are known for their resistance to traditional chemotherapy and radiation therapy. Chondrosarcomas are heterogeneous, and the molecular landscape may vary among different cases. Understanding the basic basis of chondrosarcoma is crucial, as it could facilitate the identification of possible therapeutic targets. The advancement of technology *in silico* studies shows promise for discovering potential targeted treatments for cancer. Gene2Drug, a pathway-based rational drug repositioning website, was used to find compounds that target the PBRM1 (polybromo-1) gene. Drug Set Enrichment Analysis (DSEA) was used to assist in *in silico* screening. The anti-tumor activities of candidate drugs were extracted from DepMap via a PRISM viability assay on nine chordoma cell lines. A total of 665 compounds were analyzed using the DRUG Sensitivity [Drug Sensitivity AUC (CTD^2)] Tool. Tacrolimus is a topical calcineurin inhibitor that is used to treat moderate-to-severe atopic dermatitis and prevent organ transplant rejection. This study showed tacrolimus could be used as a potential therapeutic effect on chondrosarcoma cell lines (8.29E–9). Therefore, tacrolimus showed the most promising potential as a PBRM1-targeted treatment for candidate in chondrosarcoma. If further validated, this approach could offer a personalized treatment option based on the molecular characteristics of the tumor.

Wednesday 3 July
8:30–10:30, Yellow Room

The D-side in Health: D-amino acids in Pathological States

S-04.7-1
Disentangling the many roles of D-serine/ NMDA receptor system in the enteric nervous system in health and disease

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While the functions of the N-methyl D-aspartate receptors (NMDARs) in the brain have been extensively studied, their roles in the enteric nervous system (ENS) remain paradoxically poorly defined and highly controversial. In addition, emblematic studies during the last two decades have demonstrated that D-amino acids are produced by mammals to support important functions. In particular, D-serine has gained traction as a key signaling molecule for synaptic circuits and memory encoding by binding to NMDARs. Despite important progress, whether D-serine and more generally D-amino acids could be produced in the mammalian gut by the host cells and if it could regulate the activity of the ENS and gastrointestinal (GI) functions has remained unexplored. Here, we uncover a new role for D-serine and non-conventional GluN1-GluN3 NMDARs in regulating ENS functions. We demonstrate that D-Ser is produced by serine racemase (SR) expressed in enteric neurons. By using both *in situ* patch clamp recording and calcium imaging, we show that D-serine alone acts as an excitatory neurotransmitter in the ENS independently of the conventional GluN1-GluN2 NMDARs. Instead, D-serine directly gates the non-conventional GluN1-GluN3 NMDARs in enteric neurons from both mouse and guinea pig. Pharmacological inhibition or potentiation of GluN1-GluN3 NMDARs had opposite effects on mouse colonic motor activities, while genetically driven loss of SR impairs gut transit and fluid content of pellet output. Our results demonstrate the existence of native GluN1-GluN3 NMDARs in enteric neurons and open new perspectives on the exploration of excitatory D-serine receptors in gut function and diseases.

S-04.7-2
The D-side of neurodevelopmental disorders

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Dysfunction of glutamatergic NMDA receptors (NMDARs) contributes to the motor and non-motor symptoms of Parkinson's Disease (PD), and to L-DOPA-induced dyskinesia. Besides the main excitatory L-amino acids, also D-serine (D-Ser) activates NMDARs as a co-agonist at the glycine binding site of GluN1 subunit. We performed an extensive characterization of the levels of the endogenous ligands of NMDARs, including D-Ser, L-glutamate, D-aspartate, L-aspartate, glycine and their precursors, in PD patients and animal models of the disease. HPLC determinations highlighted abnormally higher D-Ser and L-Ser levels in the striatum of monkeys with severe MPTP-mediated denervation of nigrostriatal dopaminergic fibers (~75%), while no changes were found in the striatum of MPTP-treated mice, which showed mild dopaminergic degeneration (~30%). In line with results in MPTP-treated monkeys, we found greater D-Ser and L-Ser levels in the post-mortem caudate putamen of PD patients. We also put forward a significant elevation of both Ser enantiomers in the CSF of *de novo* PD patients but not in patients with other neurodegenerative disorders, including Alzheimer's disease and amyotrophic lateral sclerosis. Beyond CSF and post-mortem brains, we reported that also the blood serum D-Ser levels were selectively upregulated in PD compared to controls. Overall, our biochemical analyses carried out in humans, monkeys and mice identify Ser metabolism variations as biochemical signatures of nigrostriatal dopaminergic degeneration in PD brain and let us hypothesize that such changes occur as a secondary response to support the metabolic and neurotransmission demands imposed by dopaminergic neuron degeneration.

ShT-04.7-3
Biological effects of the loss of homochirality in a multicellular organism

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Homochirality is a fundamental principle of all living organisms. Accordingly, biomolecules occur in only one chiral form, L-amino-acids in proteins and D-sugars in DNA/RNA. While D-amino acids are thought to be non-proteinogenic, pieces of evidence demonstrate that proteins can undergo spontaneous chiral post-translational modifications under conditions of stress and ageing, leading to homochirality loss. Despite its pivotal importance for life, the biological and pathological consequences of homochirality loss remain to be elucidated. Combining interdisciplinary chiral-selective techniques with genetic, cellular and biochemical approaches we identified novel heterochiral protein motifs that accumulate in Protein-L-isoaspartate (D-aspartate) O-methyltransferase mutant (*Pimt KO*) chiral-deficient animals. We show a direct link between D-amino acids and protein dysfunction *in vivo* which, in turn, promotes a progressive 'heterochirality syndrome' through a cascading effect across biological scales, spanning from loss of molecular homochirality to increased resistance to caspase activity, increased tumour susceptibility, and shortened lifespan [Previously published in: Banreti et al. (2022) Nat Commun 18, 13(1):7059]. Our recent advances broaden our understanding of the underlying molecular and cellular mechanisms connecting heterochirality to biological sequelae.

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ShT-04.7-2
Synthesis and biological evaluation of olanzapine-based PROTACs targeting human D-aspartate oxidase (hDASPO)

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D-Aspartate oxidase (DASPO) is one of the most relevant enzymes involved in the degradation of D-amino acids, especially D-aspartate (D-Asp), which has the potential to activate *N*-methyl-D-aspartate (NMDA) receptors. Dysfunction of NMDA receptor-mediated neurotransmission is associated with various mental disorders, such as schizophrenia. Therefore, developing inhibitors for DASPO could increase brain levels of D-Asp, potentially enhancing NMDA receptor function and offering therapeutic benefits. Recently, the antipsychotic drug olanzapine was reported acting as a potent binder of DASPO (IC_{50} = 23.4 μM) increasing extracellular levels of D-Asp in prefrontal cortex [previously published in Sacchi *et al*. (2017) Sci. Rep. 7(1), 46 288]. Aware of this result, we have decided to employ proteolysis targeting chimeras technology (PROTAC) to target DASPO with bifunctional compounds based on olanzapine structure [previously published in Wang *et al*. (2022) Eur. J. Med. Chem. 235, 114 290]. The primary goal of PROTACs is to trigger the degradation of enzymes within cells via the proteasomal pathway, consequently enhancing cellular levels of D-Asp. This innovative strategy could offer a potential treatment for diseases connected to a deficiency of D-Asp. A panel of new bifunctional molecules were designed starting from the olanzapine core structure using aliphatic, amide, or PEGylated linkers, with lenalidomide and VHL ligand serving as E3 ligase binders. Straightforward "click" chemistry strategies were employed to efficiently synthesize the compounds and a preliminary biological assessment showed that the first PROTAC MGA26 exhibited significant DASPO inhibition (IC50 = 4.13 μM) and affected the cellular DASPO level.

ShT-04.7-1
New insights in the function and regulation of the phosphorylated pathway for serine biosynthesis in the human brain

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L-serine (L-Ser) is the precursor of D-Ser, the dominant co-agonist of N-methyl-D-aspartate receptors in mammalian CNS. In the human brain the L-Ser pool is maintained by *de novo* biosynthesis through the phosphorylated pathway (PP). 3-Phosphoglycerate dehydrogenase (PHGDH), 3-phosphoserine aminotransferase (PSAT) and 3-phosphoserine phosphatase (PSP) catalyse the three steps of the pathway, and defects in any of the three enzymes cause a group of diseases known as serine deficiency disorders. We have investigated the functional and structural properties of the three enzymes, both *in vitro* and in human astrocytes where they were found to form a metabolic assembly we named serinosome. Through the *in vitro* reconstruction of the PP we concluded that PSAT and PSP are the main players in shifting the flux towards L-Ser synthesis [previously published in: Rabattoni, V et al. (2023) FEBS J 290, 3877-3895]. Several known pathogenic variants of PSAT and PSP were analysed, and the molecular basis of their defective function was assessed. In the case of PSP, two new variants were characterized: the N133S, found in two siblings with intellectual deficiency and spastic paraparesis, was proven to destabilize the protein and to affect serinosome assembly; the R27S/D32G, identified in Alzheimer's disease patients, showed a deeply impaired catalytic efficiency because of a 20-fold reduction in k_{cat}. One interesting feature that emerged from the *in vitro* reconstructed PP is that PSP hypofunctional variants are less likely to affect the flux through the PP as compared to PHGDH or PSAT variants [previously published in: Marchesani, F et al. (2024) BBA Mol Basis Dis 1870, 167 034; Marchesani, F et al. (2023) Biomolecules 13, 1219]. Moreover, inhibition by L-Ser, albeit at play on the isolated PSP, only affects the flux through the PP when the enzyme activity is severely impaired by inactivating substitutions. Project funded by" PRIN-2017 - Dissecting serine metabolism in the brain".

Wednesday 3 July

14:00–16:00, Silver Room

From Brain Molecules to Brain Functions and Diseases

S-04.8-2
Studying lipid metabolism in the human brain

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Signaling lipids, such as the endocannabinoids, play an important role in the brain. They regulate synaptic transmission and control various neurophysiological processes, including pain sensation, neuroinflammation, stress and anxiety. Unlike classical neurotransmitters, lipid messengers are produced on demand and degraded by metabolic enzymes to control their lifespan and signaling actions. Chemical biology approaches have become one of the main driving forces to study and unravel the physiological role of lipid messengers in the brain. In this presentation, I will discuss our program to study lipid metabolism in the brain of multiple sclerosis patients using chemical probes. Punt JM, van der Vliet D, van der Stelt M. Chemical Probes to Control and Visualize Lipid Metabolism in the Brain. Acc Chem Res. 2022;55 (22):3205-3217.

S-04.8-1
Deciphering the human blood–brain barrier with *in vitro* physiological models

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The brain, the most enigmatic organ of the human body, is effectively protected by the blood–brain barrier (BBB), which functions as its administrative center. This complex and dynamic interface of the central nervous system (CNS) separates the brain from the circulatory system and other organs, playing a crucial role in safeguarding brain health. However, the BBB also presents challenges by impeding drug transport to the CNS, complicating the treatment of brain diseases. Despite the BBB's significance, our understanding of the fundamental processes underlying its development, maintenance, and interaction with other bodily systems remains limited. Impaired BBB function has been associated with various neurodegenerative and neurodevelopmental disorders, as well as traumatic brain injury, stroke, and brain malignancies. One major obstacle to effectively treating neurological disorders is the inability of many drugs to penetrate the BBB. This challenge is compounded by the fact that many of these disorders are not yet curable, and our current knowledge about them is incomplete. At the Mustafaoglu Lab, we are addressing these challenges by developing innovative microfluidic systems that mimic the BBB under physiological conditions, both in healthy and diseased states such as epilepsy, brain cancer, and fetal familial insomnia. Our research involves creating new protocols for differentiating stem cells into neurons and brain endothelial cells. Additionally, we design and fabricate novel microfluidic devices capable of applying shear and tensile stresses to cells, simulating physiological vascular movements in the brain. By leveraging these state-of-the-art bioengineering platforms, we aim to deepen our understanding of the human BBB and its implications for health and disease. Ultimately, our goal is to develop unique nano-shuttle systems that can effectively deliver drugs to the brain, offering new avenues for treating and potentially curing various brain diseases.

ShT-04.8-3
Aβ1-6A2V(D): a bio-inspired peptide as potential multitarget treatment for tauopathies

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Alzheimer's disease (AD), the most common form of tauopathy, is a double proteinopathy characterized, in addition to tau deposition, also by amyloid-β (Aβ) misfolding, resulting in the formation of neurofibrillary tangles and amyloid plaques in specific regions of the brain. Despite enormous efforts in the last decades, no effective therapies are available for the treatment of AD. The approaches followed so far focused solely on Aβ or tau protein. This study aimed to investigate the mechanism of action of a bio-inspired all-D-isomer synthetic peptide Aβ1-6_{A2V}(D). This peptide stems from the clinical discovery that the presence of the A2V mutation in the N-terminal region of Aβ plays a protective role against amyloidogenesis in heterozygous carriers suffering from AD [Di Fede G et al. (2009) Science, 3 23,1 473–1477]. Previous observations from our group demonstrated that $A\beta_{1\text{-}6}$A2V(D) interacts with Aβ, reducing oligomer generation and fibril formation, and interferes with Aβ-dependent neurotoxicity *in vitro* and *in vivo* [Di Fede G et al. (2012) Prog Neurobiol 99, 281-292]. We here investigated if $A\beta_{1\text{-}6}$A2V(D) could also interact with tau. In-depth biochemical studies involving thioflavin T fluorescence assay, circular dichroism, and structural studies demonstrated that $A\beta_{1\text{-}6}$A2V(D) peptide can interfere with the aggregation and stability of tau. Additional analysis indicates that the peptide increased the susceptibility of tau to degradation by proteases, significantly reducing the tau protein level without affecting the fraction of insoluble tau [Diomede L et al. (2023) Mol Psychiatry 28, 2433-2444]. These findings confirm that this peptide interferes with Aβ and tau aggregation propensity and proteotoxicity, providing proof of concept for developing and optimizing multitarget treatments for tauopathies.

ShT-04.8-2
Optimizing performance characteristics of antibodies for single-molecule quantitative bioimaging

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Antibody use is ubiquitous throughout biomedical research. Antibodies are applied to probe for proteins of interest, often in complex samples. Many of these techniques implicitly assume sufficient levels of antibody selectivity and specificity. Single-molecule fluorescence microscopy (SMFM) is a highly sensitive method of visualizing proteins *in situ*, capable of detecting single antibodies in high-background samples. SMFM is especially vulnerable to false positives due to its unparalleled sensitivity: any off-target binding event will produce a false positive signal. Recently, we employed SMFM to quantify disease-relevant alpha-synuclein oligomers in human brain tissue. Especially in this size domain (<250 nm) antibodies with low selectivity produce many biological false-positives due SMFM's sensitivity. Here, we present a novel, high-resolution quantitative fluorescence microscopy bioimaging pipeline. This method quantifies and optimizes the selectivity and specificity of antibodies for use in SMFM, with rapid acquisition, processing and quantification of (false) positive antibody binding events. We identify thousands of oligomers of alpha-synuclein (<250 nm) in mouse brain. Here, we find significant levels of off-target interactions of multiple commercially available alpha-synuclein antibodies with other proteins which yield false positive signal in SMFM. We are able to reliably quantify antibody selectivity and sensitivity, specifically for the application of SMFM. Our findings showcase a pipeline for the optimization of antibody selectivity and specificity for the purpose of SMFM which will be beneficial for the quickly evolving field of high-resolution microscopy with applications such as super-resolution microscopy techniques, SiMPull and, more generally, any technique reliant on antibodies.

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ShT-04.8-1
Molecular basis of coupling Ca^{2+}-sensing to fast membrane fusion by Synaptotagmin-1 in neurotransmitter release

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Neuronal communication relies on rapid neurotransmitter release through Ca^{2+}-evoked synaptic vesicle exocytosis. Synaptotagmin-1 (Syt1) acts as the calcium sensor for fast, synchronous neurotransmitter release. However, the molecular mechanisms underlying Syt1 action and how Ca^{2+}-sensing is coupled to membrane fusion remain unknown. To address these questions, it is crucial to understand the cooperation between Syt1 and SNARE proteins, which drive membrane fusion by forming a tight four-helix bundle that brings the membranes together. In the primed state of synaptic vesicles, Syt1 binds to a partially assembled SNARE complex through a primary interface [described in Zhou et al. (2015) Nature 525, 7567], and to the plasma membrane through a polybasic region, inhibiting complete helical zippering and hence membrane fusion. The primary interface consists of two key regions involving interactions of an arginine cluster of Syt1 with a polyacidic patch on the SNARE complex (region II), and interactions of a tyrosine of Syt1 with another surface of the SNARE complex (region I). Using NMR spectroscopy, we show that mutation of the region II arginines completely abolishes Syt1-SNARE binding, whereas mutation of a key tyrosine abrogates binding at region I while region II remains intact. These data, together with fluorescence experiments, suggest a dissociation of the primary interface region II upon Ca^{2+}-binding, while the Syt1 C_2B domain remains persistently bound through the arginine cluster in region II. Our results lead us to propose a lever model for Syt1 action whereby a Ca^{2+}-induced re-orientation of Syt1 at the plasma membrane pulls the SNARE complex, enabling complete helical zippering that induces fast membrane fusion and subsequent neurotransmitter release.

Wednesday 3 July
14:00–16:00, Red Room

Impacts of Climate Change on Nutrition and Health

S-03.5-1
Impacts of climate change on food production and human nutrition

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Food systems contribute a third of all human-induced greenhouse gas emissions but, at the same time, climate change is causing major challenges to food production. Currently, over 735 million people suffer from hunger, and over 40% of the people globally are not able to access healthy diets. Changes in temperature, precipitation patterns and carbon dioxide concentration in the atmosphere affect both the crop yields and nutritional quality of crops. Climate change has been predicted to reduce yields especially in regions where the prevalence of hunger is already currently high, such as in Africa, South-East Asia and South America. Even though increased carbon dioxide content can improve crop growth, the concentration of nutrients in the crops can decrease [Mayers et al. (2015) Nature 510, 139-142]. Climate change also increases issues with pests, weeds and crop/animal diseases. To mitigate the impacts of climate change, the whole food system needs to be addressed [as shown in Tuomisto et al. (2017) Wellcome Open Res. 2, 21]. Climate-smart agriculture can help adapt to changing conditions through improvements in the soil properties, for instance, through versatile crop rotations, reducing tillage and increasing organic matter inputs into soils. Crop breeding can improve the resilience of crops to changes in temperatures and precipitation. The new ways of producing food through indoor farming and cellular agriculture (e.g. cultivated meat, microbial proteins and precision fermented foods) are less affected by climatic conditions. Dietary changes towards plant-based diets reduce the climate impacts of food systems and also improve the resilience due to requiring less land for food production [Willet et al. (2019) The Lancet 393, 447-492]. To reduce the negative consequences of climate change on human nutrition and health, efforts need to focus both on reducing the greenhouse gas emissions from food systems and improving the resilience of food systems to climate change.

S-03.5-2
The biochemistry behind quality and sustainability of plant-based food

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Human health is closely linked to food and the environment. All over the world, we are facing the sustainability of food chains. On the other hand, people are pushed to consume more plant-based foods to reduce the negative impacts of the modern food supply on human health. Climate change directly impacts cellular cycles and metabolism The increasing atmospheric carbon dioxide and the rising temperatures may reduce the overall yield of major staple crops and cause plants to react, leading to the formation of new compounds that can have repercussions on the safety and nutritional features of plant food. Changes can cause compositional variations which in turn lead to variations in nutritional, bioavailability and techno-functional potential. Biochemistry can contribute to fighting the negative impacts. The molecular and physicochemical properties of plant-derived ingredients are peculiar. It is therefore critical to understand the fundamental attributes of plant-derived ingredients. Although the primary production and the quality of the plant products are influenced by the atmospheric conditions and the quality must be preserved, on the other the recovery and valorisation of what is still contained in the waste and by-products, which in some matrices amounts up to 40-50% of the content of the original raw material, is of equally primary importance. Today the set-up of eco-sustainable technologies based on biological approaches, including the use of enzymes, microorganisms, and insects/larvae for the recovery and reuse of compounds to be used as such or as food ingredients is a need. New technologies have been developed or are under development in this respect. The present communications aim to focus on these emerging aspects to review and critically discuss the possible application of biomolecular approaches to guarantee the quality and sustainability of plant-

based food to the entire current population and future generations.

ShT-03.5-4
Improving salt tolerance in tomato plants through the use of biofertilizers: a biochemical approach

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Salinity figures among the most harmful abiotic stresses affecting tomato development and metabolism. As an environmentally friendly practice, the aim of this study was to evaluate the potential effects of biostimulants, in particular arbuscular mycorrhizal fungi (AMF) and compost, in improving tomato tolerance to salt stress. A greenhouse experiment was carried out using a Cambell33 tomato variety. Tomato seeds were treated with a native AMF consortium and compost under non-saline (0 mM) and high-saline (150 mM) conditions. The research included an in-depth examination of key biochemical parameters such as chlorophyll, protein, sugar, and malondialdehyde (MDA) content as well as catalase (CAT), peroxidase (POX), and polyphenol oxidase activities (PPO). The results showed that compost had a positive effect on protein content and PPO activity. In addition, AMF reduced MDA content, indicating a potential for mitigating oxidative stress under salinity. The combined application of compost and AMF showed a positive effect on chlorophyll content and CAT activity suggesting an improvement in antioxidant capacity and photosynthetic processes under salt stress. This research provides valuable information on sustainable agricultural practices that promote advances in the application of compost and AMF to improve tomato performance under adverse environmental conditions of salt stress.

ShT-03.5-3
Biochemical and structural characterization of a newly identified lipase in hazelnut (*Corylus avellana*)

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Rancidity is common in oilseeds. Although the molecular basis of this process have not been clarified in full, lipases play essential role in the release of free fatty acids (FFA) that reportedly turn rancid much faster than triacylglycerols (TAG)[1]. From the industrial standpoint, the identification and characterization of lipases as potential "predictive" markers of raw material shelf-life seems relevant to making food processing and storage more sustainable and efficient[2] as well as for climate change effect on seed development. A new protein purification protocol, combined with lipolytic enzymatic assay, was set up to isolate lipase(s) from hazelnut, and led to the identification of a monomeric 9 kDa protein active towards both synthetic and natural substrates, with optimal activity at 37°C and pH 8.0. Surprisingly, this novel enzyme shares little if any sequence or structure homology with canonical lipases. Structural characterization through circular dichroism reveals a predominantly α-helical structure. It should be noted that 40% of the signature spectroscopic features of the protein's secondary structure were still present after a temperature-ramp treatment from 20 to 90°C. The unusual stability of the secondary structure of the enzyme may stem from the intrinsic rigidity conferred by the fact that it contains 8 cysteine residues paired to form 4 disulfide bridges (thermal stability is drastically reduced in the presence of reductants), similarly to what observed for other plant proteins involved in lipid transfer and metabolism in plants. References: [1] Rosso MC et al. (2018) Anal Bioanal Chem 410,15: 3491-3506. [2] Li, Bo et al. (2016) PloS One 11,12 e0167330.

ShT-03.5-1
Biochemical insights into sustainable plant-based food products: the buckwheat case

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This study explores the micro- and macro-molecular features of food products enriched with sprouted buckwheat. Interest in buckwheat is driven by its value in the food chain and by its adaptability to drought and poor soil conditions, that make it a resilient and sustainable crop. Buckwheat's biochemical traits, including its high protein content – with no coeliac-toxic sequences – and its richness in functional and nutraceutical components make it a versatile and health-promoting staple. However, the presence of various anti-nutritional factors in buckwheat may hamper nutrient bioavailability. Sprouting, a sustainable and easily scalable process, holds the potential to address these limitations. Buckwheat couscous, already available on the market, was chosen in this study as prototype of a natural and sustainable food. Molecular characterization of sprouted buckwheat reveals changes induced by endogenous enzymes during the sprouting process. Sprouting for a short period (48–72 h) modifies the protein profile and the protein-protein interactions, changes the ratio between soluble and insoluble polyphenols, and lowers the content of anti-nutritional factors. Adding 50% sprouted grains flour did not impair couscous production processes, and the product retained strong similarities with the non-enriched one. The molecular impact of the couscous-making process involves changes in the overall protein organization and in the interactions among micro- and macro-molecules. However, these modifications did not affect the improvement in nutritional traits brought forward by sprouting-related enzymes. These results offer valuable insights into ways of exploiting the potential of buckwheat as a sustainable functional food through simple and environment-friendly processes. This work is supported by

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the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 - Call for tender No. 341 of 15/03/2022, MIUR project "ON Foods".

ShT-03.5-2
The biochemistry for the valorization of agri-food waste and by-products: the case of Okara for sustainable Agri-tech applications in circular economy

L. Pericciolli[I], B. Prinsi[II], G. Ceravolo[I], D. Emide[I], C. Magni[1], S. De Benedetti[I], A. Scarafoni[I]
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Food chains play a significant environmental and economic role for their consequences regarding the large amounts of waste and by-products produced. Recently, the monetization of matrices obtained from agri-foods wastes has been a crucial step forward for the circular economy concept. Biochemically, such by-products are very interesting because of their content in proteins, peptides, and other high-value molecules like polyphenols. Therefore, these matrices will have high biotechnological potential for different fields and applications. Okara, the by-product of soya bean (*Glycine max*) [1], has garnered agro- and biotechnological interest because of its versatility, sustainability, and abundance [2]. In the present work, we developed different approaches for the recovery and purification of Okara's peptides, which will be valorized for their agrotechnological potential (e.g. biodefense activity). Sustainable extraction methodologies and hydrolytic enzymes were used to overcome the matrix effects that hinder extraction yields. Biochemical methodologies including sequencing and spectroscopy were used for the molecular characterizations of the peptides purified in the different fractions. The selected fractions were tested with different *in vivo* systems to assess their biological activities. References: [1] Belobrajdic, D.P. et al. Soy and Gastrointestinal Health: A Review. Nutrients 2023, 15, 1959. https://doi.org/10.3390/nu15081959. [2] De Benedetti, S. et al.. Molecules 2021, 26, 4858. https://doi.org/10.3390/molecules26164858 This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022).

Wednesday 3 July
14:00–16:00, Blue Room

Liquid–Liquid Phase Separation

S-01.9-1
Biomolecular condensates in cellular stress and disease

S. Alberti
TU Dresden, Dresden, Germany

Stress-inducible biomolecular condensates such as ribonucleoprotein (RNP) granules and DNA damage sites play major roles in cellular organization and physiology. In this talk, I will discuss how the concept of biomolecular condensates has expanded our view of stress responses and has revealed important links to aging-related diseases. I will introduce quantitative bottom-up biochemistry approaches that now allow us to reconstruct multicomponent, active condensates such as RNP granules and DNA damage sites in the test tube. Using these approaches as well as innovative imaging and biophysics, we have gained important insights into the molecular rules underlying condensate assembly, such as the molecular driving forces that govern condensation, the conformational changes underlying assembly and the molecular mechanisms of condensate regulation. I will further discuss how the concept of biomolecular condensates has allowed us to dissect the functions of stress inducible condensates, and I will demonstrate how condensates can be used to sense and respond to changes in the environment and regulate fundamental cellular processes such as protein synthesis and DNA damage repair.

S-01.9-2
Redox-dependent phase separation and cytoplasmic granulation by human single-stranded DNA binding protein 1 (hSSB1) delineate new mechanisms of cellular stress response

G.M. Harami[I,II], Z.J. Kovacs[I,III], J. Palinkas[I], R. Pancsa[IV], K. Tarnok[V], H. Harami-Papp[I,II], J. Hegedus[I], L. Mahmudova[VI], E. Hazy[I,III], B. Jezso[I], P. Ecsedi[I], N. Kucsma[VII], S. Toth[VII], G. Szakacs[VII], **M. Kovacs**[I,III]
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Single-stranded DNA binding (SSB) proteins are present in all domains of life, and are essential in protecting single-stranded (ss) DNA segments and organizing protein complexes during DNA replication, recombination, and repair. Our recent discovery on the liquid-liquid phase separation (LLPS) propensity of *E. coli* SSB highlighted a novel role for macromolecular condensation in genome maintenance (Harami et al. 2020 PNAS 117:26206). Two recently discovered human SSB homologs (hSSB1, hSSB2), structurally resembling bacterial SSBs, have been found central to preserving genome stability. In our current work (bioRxiv 2023.07.25.550517) we define an unprecedented cytoplasmic stress response role for hSSB1, demonstrated in human cell culture and *in vivo* rodent models for ischemia-reperfusion. This function scales in proportion to stress exposure, is linked to stress granules, and is brought about via redox-dependent phase separation of hSSB1 mediated by cysteine/methionine switches. As these mechanisms are probably central to tissue development and regeneration, immune cell function, and cancer

cell survival under chronic stress, pharmacological targeting of SSB condensation appears as a promising route for both suppressing drug resistance and enhancing regenerative therapy. Accordingly, we have established a screening and functional evaluation pipeline for substances affecting condensation by SSB and other nucleoprotein complexes.

S-01.9-3
Regulation of gene expression through transcriptional condensates in *Caenorhabditis elegans* embryonic development

A. Klosin, A. Grabowska, N. Stec
Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

The assembly of transcriptional machinery into dense, submicrometer-sized, liquid-like condensates is emerging as a key feature of transcriptional regulation. However, the mechanism of formation and function of these assemblies has not been thoroughly explored in a developing organism. We show, that in the nuclei of early *Caenorhabditis elegans* embryos, RNA polymerase II (Pol II) is organized into dynamic condensates. Quantitative microscopic analysis revealed that these condensates could be classified into two classes based on size, as most nuclei contained two major foci and several smaller ones. Using combined DNA FISH and immunohistochemistry, we found that the two major Pol II condensates form at the sites of a highly repetitive SL1 locus, which encodes an essential splice leader used in most *C. elegans* transcripts. Through time-lapse microscopy, we found that the Pol II condensates were sensitive to stress, as they dissolved in elevated temperatures. This disappearance coincided temporally, but not spatially, with the formation of nuclear stress bodies formed by Heat Shock Factor 1. Using genetics and genomic approaches, we are investigating the impact of these structures on animal physiology. The structures described here will serve as a model system to understand the role of biomolecular condensation in transcriptional regulation during development and stress response.

ShT-01.9-1
Metal-induced liquid–liquid phase separation of heterochromatin protein 1a

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During liquid–liquid phase separation (LLPS) proteins or nucleic acids form a dense, droplet-like phase which is surrounded by a diluted phase. This phenomenon, occurring in living cells, is responsible for the formation of many membraneless compartments and structures. One of the most important processes associated with LLPS is the formation of heterochromatin. One of the proteins involved in this process is heterochromatin protein 1a (HP1a), itself able to undergo LLPS after the phosphorylation of four serine residues in its N-terminal disordered region. Since LLPS is particularly sensitive to environmental conditions including temperature, ionic strength and pH, we decided to study the effects of divalent metal ions, such as magnesium and calcium, on the ability of HP1a to form droplets *in vitro* at different temperatures. By means of the solution nuclear magnetic resonance (NMR) spectroscopy, using recombinant human HP1a phosphorylated *in vitro* by casein kinase II we demonstrate that biometals are specifically coordinated by phosphoserine residues. Moreover, as measured by a spin-down centrifuge assay metal binding promotes LLPS. We determined saturation concentrations as a function of metal concentration and show that calcium and manganese strongly promote droplets formation even at the elevated temperatures. Our research shows that indeed divalent metals interact with phosphorylated residues on human HP1a, and induce LLPS of HP1a, although the phase diagrams obtained differ for each metal. Our results shed light on the possible regulation of the chromatin condensation by biometals.

ShT-01.9-2
Unveiling research frontiers: coherent anti-stokes Raman spectroscopy insights

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The study of proteins, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) and their interactions is a cornerstone of modern biochemistry and molecular biology. They drive biological phase separation processes leading to the formation of membrane-less organelles (MLOs) within cells with distinct material properties and which structure will be directly linked to their final behavior.[1] Thus, understanding and controlling them is key in any biological process, as demonstrated in degenerative diseases such as amyotrophic lateral sclerosis (ALS) or frontotemporal dementia (FTD).[2] However, although their competing molecular interactions are known to play a key role in regulating condensate composition and structure, they remain poorly understood. Here, we propose the use of Coherent anti-Stokes Raman spectroscopy (CARS) as a suitable technique to unravel what changes are taking place over time in any biological system, free from dyes, markers or nanoparticles, as in surface-enhanced Raman spectroscopy, that could induce secondary interactions. Focusing on nucleic acids, their fingerprint region will allow us to determine the internal and external interactions taking place. Using DNA biomolecular condensate model systems, ssDNA to dsDNA hybridization or salt effects in the final assembled conformation can be followed. For proteins, their secondary structure can be also elucidated from an ordered α-helix or β-sheet to a disordered random coil. Furthermore, we employ this technique as a reliable source for the characterization of protein–RNA interactions. While adding another layer of complexity, the Raman shifts observed in specific regions of the formed condensate can give an indication of its characteristics, such as its structure or viscoelastic state. Furthermore, they can be related to its ultimate behavior, as observed in living systems. References: [1]Garcia-Jove Navarro, *et al.* Nat Commun 10, 3230 (2019). [2]Arseni, D., *et al.* Nature 601, 139–143 (2022).

Wednesday 3 July
14:00–16:00, Yellow Room

Marine Biochemistry

S-02.6-2
Revealing the biotechnological potential of marine extremophilic bacteria for the production of novel biosurfactans and other bioactive compounds

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Due to a wide range of household, biotechnological and pharmaceutical applications, surfactants are extremely important from an industrial perspective. In this scenario, biosurfactants (surfactants from natural sources) have raised significant attention, as being less toxic, active at lower concentrations, and more resistant to extreme conditions as compared to the synthetic ones. Moreover, biosurfactants are endowed with several pharmacological properties, such as antimicrobial, antitumoral, antiviral and antiinflammatory activities. So far, marine extremophilic bacteria have been acknowledged as a prolific factory of bioactive natural products, including biosurfactants, which feature unusual chemical structures with no counterpart in the terrestrial environment. In the framework of the EU funded project SECRETED (H2020), about 300 extremophilic bacteria from the SZN microbial collection were subjected to primary and secondary screening selecting promising strains for biosurfactants production. A bottomup (bioactivityguided) and a topdown approach (based on genome mining) allowed the selection of two marine bacteria belonging to *Bacillus* and *Rhodococcus* genera capable to produce different biosurfactants (along with other bioactive compounds) displaying several biological activities. More specifically, scaleup and fractionation, HPLCMS/MSbased metabolomics combined with automated and manual annotation approaches (molecular networking and *in silico* dereplication), allowed the identification of novel lipopeptide biosurfactants. Both approaches confirmed the high potential of marine bacteria as source of novel molecules with relevant biotechnological applications.

S-02.6-1
Anticancer compounds isolated from Arctic marine organisms

J. Andersen, E. Hansen, K. Hansen
UiT-The Arctic University of Norway, Tromsø, Norway

The significance of natural products in drug discovery, particularly for anticancer therapeutics, is unparalleled. Nearly half of the currently available anticancer drugs are derived from natural sources. The marine environment comprises the majority of the global biodiversity. As the marine environment and its organisms have become more accessible over the last decades, it is expected that the ocean will be the next great source of novel chemistry. Our research group, Marbio, UiT explore Arctic and sub-Arctic marine organisms, searching for compounds with activities against cancer, bacteria and diabetes as well as compounds with immunomodulatory and antioxidative effects. We are screening a unique collection of cold-water invertebrates and marine microorganisms, and we have identified several novel bioactive molecules. Through the screening and chemical investigation of extracts from the Arctic marine hydrozoan *Thuiaria breitfussi*, our efforts led to the isolation of breitfussin A–H. Notably, breitfussin C and D displayed selective inhibition of the survival of various cancer cell lines. These findings underscore the potential of Arctic marine biodiversity to yield novel chemistry.

ShT-02.6-2
The effect of antimicrobial peptides from Antarctic fishes against some viral and bacterial fish pathogens

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The aquaculture industry represents an essential food source worldwide, accounting for about 52% of fish production. Antimicrobials are extensively used in aquaculture for growth promotion and to maintain good health of animals. Unfortunately, this resulted in the development of the antimicrobial resistance. Therefore, there is a high pressure to find alternatives that could help in manage microbial infections in aquaculture. Antimicrobial peptides are a promising solution, due to their broad spectrum of action against viruses, bacteria, fungi and parasites and their immunomodulatory properties. They are widely distributed in all organisms and primarily target the plasma membrane of pathogens with their selectivity depending on both sequence composition and membrane charge density. In this work, we investigated the capability of peptides, previously isolated from Antarctic teleosts, to target viral and bacterial fish pathogens. Specifically, virological investigations focused on betanodavirus (NNV), viral haemorrhagic septicemia virus (VHSV) and spring viraemia of carp virus (SVCV). The Trematocine peptide was effective in reducing viral titer for both VHSV and SVCV but had no effect on NNV. It is interesting to highlight that both VHSV and SVCV are surrounded by a viral envelope, that is absent in the case of NNV. Moreover, the Chionodracine peptide, and to an even greater extent its mutant, demonstrated bactericidal and bacteriostatic activity against various bacterial strains, including *Lactococcus garvieae* and *Vibrio harveyi*, as evidenced by the obtained MIC and MBC values. TEM analysis confirmed the action of the chionodracine mutant peptide on bacterial plasma membrane. Finally, the cytotoxicity of both peptides against a fish cell line and their hemolytic activity against fish erythrocytes were evaluated. Overall, these data represent a fundamental preliminary set of information necessary to assess their possible application as novel drugs in aquaculture.

ShT-02.6-3
In vitro bisphenol A impairs testicular energy metabolism and spermatogenesis through nuclear estrogen receptors activation in zebrafish

H. Batista-Silva[I], F.R. Mena Barreto Silva[II], C. Delalande[III]
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Energy metabolism and the availability of energy substrates play a critical role in spermatogenesis. However, aquatic organisms, particularly fish, are highly vulnerable to xenobiotics such as bisphenol A (BPA), which is closely related to the impairment of physiological and biochemical processes. Therefore, the aim of this study was to investigate the *in vitro* effects of BPA and the involvement of nuclear estrogen receptors (ESR) on testicular energy metabolism and spermatogenesis in zebrafish. Testes were incubated with DMSO, 10 pM or 10 μM BPA for 72 h through an organotypic culture. Additionally, testes were pre-incubated in the presence or absence of ESRα/β antagonist, ICI 182780. Then reverse transcription, followed by real-time polymerase chain reaction was performed to analyze gene expression. Moreover, the proportion of the surface of testicular cells was analyzed using Ilastik software. The results revealed that the relative expression of pyruvate kinase M1/2a (*pkma*) and outer dense fiber protein 3b (*odf3b*), a spermatids gene marker, was reduced by 10 pM BPA. The reduced expression of odf3b and the reduced proportion of spermatids and spermatozoa by BPA were through ESRα/β activation. In addition, the relative expression of alanine aminotransferase (*gpt2*), 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (*pfkfb2a*), lactate dehydrogenase (*ldhba*) and estrogen-related receptor expression was reduced by 10 μM BPA. In contrast, the relative expression of glycogen phosphorylase (*pygl*), monocarboxylate transporter 4, synaptonemal complex protein 3, estrogen receptors β1 and β2 was increased by 10 μM BPA. The reduced relative expression of *pfkfb2a* and ldhba, as well as increased expression of *pygl* by 10 μM BPA were through ESRα/β activation. Overall, these results indicate that exposure of male fish to environmental concentrations of BPA may impair testicular energy metabolism by altering gene expression and spermatogenesis through ESRα/β activation.

SPECIAL SESSIONS

Sunday 30 June
17:00–19:00, Yellow Room

Science and Society: Private and Public Funds in Translational Research

SS-01-2
CDP venture Capital's technology transfer model

C. Pingue
CDP Venture Capital, Italy

Abstract unavailable.

SS-01-1
Maintaining a long breath: building a long-term vision to advance translational research

W. Ellmeier[I,II]
[I]*Medical University of Vienna, Institute of Immunology, Vienna, Austria,* [II]*Biomedical Alliance in Europe, Brussels, Belgium*

The translational road from a great basic research discovery to a potential clinical benefit for the patients is a bumpy one. Nevertheless, there are many success stories from which millions of patients are profiting. But we must aim to do better, and Europe must take a lead. We know some of the essential ingredients required for this, such as providing sufficient and sustainable funding for biomedical health research, fostering interdisciplinary transnational collaborations, connecting "silos" along the translational value chain, and implementing proper regulatory frameworks for supporting translational and clinical research for the benefit of the patients, to name just a few. On a bigger picture, Europe also has to have a forward-looking and pro-active approach to identify current and anticipate future health issues, also taking into account the increasing incidence of chronic diseases in an ageing population. But how can we achieve all this? New structures and funding programs have been established in the EU in response to the COVID-19 pandemic. What is required to ensure that all instruments work smoothly together and that synergies are fully exploited? And how can one develop a strategic and insightful long-term view within the EU that integrates the views of many stakeholders? This year are EU elections and EU health research needs to remain a funding priority. These topics will be discussed.

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Tuesday 2 July

17:00–19:00, Red Room

From Passive Students to Active Learners: Reimagining Education with Student-Centred Tools and Unlocking Engagement in the Digital

SS-02-1
Enhancing learning through student-centered teaching methods

S. Petchey
University of Zurich, Kantonsschulstrasse 3, 8001 Zurich, Switzerland

Student-centered teaching is a pivotal strategy for enhancing learning outcomes and fostering a more inclusive and responsive educational environment. But what does this kind of teaching look like? And how can we learn to teach in this manner? The presentation and workshop will introduce structured tools that facilitate student-centered teaching by helping us adequately consider student prior knowledge and recognize what makes certain content difficult to learn. Participants will gain insights into creating and using powerful analogies that engage students and facilitate their understanding of complex subjects. In addition, the session will provide strategies to promote conceptual change, equipping educators to guide students in revising misconceptions and building an accurate knowledge base.

SS-02-2
Design a learning community to catalyze active learning

F.M. Fung
Department of Chemistry, National University of Singapore, Singapore, Singapore

Because of today's interconnected social landscape, the application of technology-enabled education has become widely recognized. Surprisingly, the primary challenge of digital teaching and learning lies in its social dimension. The current methods of digital instruction have resulted in diminished student–faculty interactions due to the absence of non-visual cues and a longer response time for inquiries. Moreover, the reduced physical connections among peers have hindered collaborative learning and raised concerns about students' mental well-being. There is mounting evidence indicating that students from different socioeconomic backgrounds are disproportionately affected, exacerbating educational inequalities. In this context, we propose a framework to build learning communities with the thoughtful utilization of popular digital platforms as a simple and cost-effective method.

SPEED TALKS

Sunday 30 June
13:10–13:30, Silver Room

Advanced Methods of Structural Biology

SpT-01-4
How to self assemble: oligomeric structures of septin complexes and sub-complexes from *Ciona intestinalis*

D. Mendonça[I], S. Morais[I], A. Pinto[I], D. Leonardo[I], N. Valadares[II], R. Portugal[III], B. Klaholz[IV], **R. Garratt**[I], A.P. Araujo[I]
[I]Institute of Physics of São Carlos, University of São Paulo, São Carlos, Brazil, [II]Institute of Biological Sciences, University of Brasilia, DF, Brazil, Brasília, Brazil, [III]Brazilian Nanotechnology National Laboratory, CNPEM, Campinas, SP, Brazil, Campinas, Brazil, [IV]Centre for Integrative Biology (CBI), IGBMC, 67404 Illkirch, France, Illkirsch, France

Over recent years much has been learnt about septin filament assembly and the specific interfaces that must spontaneously form in order to correctly build oligomers, filaments and higher-order assemblies. The latter seem to be essential for the vast majority of septin functions in membrane remodeling, bacterial entrapment and barrier formation. Most of this information has derived from mammalian systems although much of our current understanding appears to be transferable to other species, including schistosomes, fruit flies and fungi. In the current work we investigate the structures of septin complexes and sub-complexes from the sea squirt *Ciona intestinalis*, an interesting model system possessing only one member of each of the four animal septin subgroups (SEPT2, SEPT6, SEPT7 and SEPT9), thereby eliminating the redundancy seen in many species. We obtained single-particle cryo-EM structures for the octameric, hexameric and central tetrameric particles at 9, 3.3 and 2.7 Å, respectively. This has enabled us to better understand the features of the inter-subunit interfaces, essential for spontaneous assembly. In the upper part of the G-interface we rationalize the need for an Asx residue within switch II based on its unusual φ/ψ angles and in the lower part, the cluster of intercalated aromatic residues which has been largely unappreciated until now. At the NC-interface we fully describe the Hook-Loop region for this first time. This appears to be essential for each subunit to embrace its neighbor, thereby, together with a polybasic helix ($\alpha 0$), lending stability to the NC-interface and determining inter-subunit separation. Taken together, this study gives novel insights into the assembly of the septin oligomers, which is an elegant example of subtle molecular recognition. This work was supported by FAPESP through grants 2020/02897-1, 2021/08158-9, 2018/20209-5, 2023/06866-1.

SpT-01-3
The unique allosteric regulation of crocodilian hemoglobin revealed by cryo-EM

K. Takahashi[I], Y. Lee[I], A. Fago[II], N.M. Bautista[III], J.F. Storz[III], T. Nishizawa[I], J.R.H. Tame[I]
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Among all vertebrates, only crocodilian hemoglobin (Hb) has acquired a mechanism of allosteric regulation by bicarbonate ions (HCO_3^-), which allows the animal to stay underwater for extended periods. More than 40 years ago, it was discovered that bicarbonate ions strongly reduce the oxygen affinity of crocodilian Hb, but the molecular mechanism has remained unexplained because of difficulties with crystallization. Using cryo-EM, we have solved the structures of crocodilian Hb in the deoxy, oxy, and carbonmonoxy states at 2.2–2.3 Å resolution. Hemoglobin has a molecular weight of only 64 kDa, still a challenging size for cryo-EM, and past cryo-EM studies of human Hb have reached a maximum resolution around 3 Å. Thanks to recent improvements in hardware and software, and the use of fresh native Hb samples from living animals, we achieved high enough resolution to observe details of the allosteric mechanism. The maps of bicarbonate ions are clearly visible, and we have unveiled the interactions between bicarbonate ions and polar or positive-charged sidechains of deoxy crocodilian Hb, at a site where no ligands are found to bind other animal Hbs. Two unique amino acid replacements are essential to form the binding site for bicarbonate ions in the T-state (deoxy) Hb, while this site is lost on the protein switching to the R-state. Moreover, our models show some significant differences from earlier X-ray models of liganded (R-state) human Hb, indicating possible effects of crystal packing.

SpT-01-1
Damage recognition process in DNA repair pathway: cryo-EM-based analysis of the UvrA-UvrB complex in *Mycobacterium tuberculosis*

M. Genta[I], G. Ferrara[I], M. Bolognesi[II], F. Rossi[I], M. Rizzi[I], A. Chaves[II,*], R. Miggiano[I,*]
[I]*University of Piemonte Orientale, Department of Pharmaceutical Sciences, via Bovio 6, Novara, Italy,* [II]*University of Milan, Department of Biosciences, via Celoria 26, Milan, Italy*

Nucleotide excision repair (NER) pathway represents one of the major molecular machineries that control chromosome stability in all living species. In Eubacteria, this pathway includes the three components of the UvrABC excinuclease complex, namely the UvrA, UvrB and UvrC proteins. These proteins act in a multi-step pathway in which the dynamic assembling of protein complexes is required for the lesion sensing and removal activities in an ATP-dependent manner[1]. Specifically, UvrA and UvrB are the first actors of NER and they have been reported as interacting proteins for the recognition of the damage across the DNA double helix. Interestingly, there is evidence in the literature of the formation of UvrA-UvrB complex[2], but both the stoichiometry and the function of the complex is still under debate and many molecular aspects of this process remain unsolved. NER pathway is extremely essential for *Mycobacterium tuberculosis* (MTB) which, being an intracellular pathogen, must face toxic agents and oxidative stress, altering its genomic stability[3]. Here we present a cryo-EM-based structural investigation of the recombinant UvrAUvrB complex from MTB, as well as of the UvrA dimer, both in complex with damaged DNA. Our analyses reveal new insights in the DNA binding mode of UvrA, with an alternative conformation of some crucial regions involved in DNA coordination. Moreover, at supramolecular level, we obtained a structural snapshot of the different oligomers which alternate during the early stages of the damage recognition process, adding details to the scientific debate regarding the stoichiometry of the protein assemblies that lead the system to the formation of the UvrB-DNA pre-incision complex and to the repairing of DNA damages. References: 1 Goosen N et al. (2001) Res Microbiol 152(3-4):401-9. 2 Pakotiprapha D et al. (2012) Nat Struct Mol Biol 19, 291–298. 3 Miggiano R et al. (2020) Molecules 25(5):1205. * The authors marked with an asterisk equally contributed to the work.

SpT-01-2
Structural description of the multivalent interaction of the post-synaptic scaffold protein GKAP and dynein motor molecule

E. Nagy-Kanta[I], Z. Dobson-Kálmán[I], H. Tossavainen[II], Z. Gáspári[I], P. Permi[II,III], B. Péterfia[I]
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GKAP (guanylate-kinase associated protein) is an important scaffold protein in the post-synaptic density, accumulating essential signal transmission proteins like the NMDA receptors-PSD-95-Shank-Homer scaffold complex, but also connecting to the dynein motor via dynein light chain 2 (DLC2, a.k.a. LC8) molecule. It is mostly disordered therefore the structural properties and binding mechanisms can most accurately be described via NMR. We have performed NMR titration measurements and SAXS analysis of the GKAP-DLC2 complex and executed molecular dynamics calculations to describe the multivalent complex structure in atomic detail proposed in the literature. Backbone and sidechain assignments were completed for both GKAP and DLC2 dimer and chemical shift perturbance (CSP) datasets were acquired for both members of the complex. Characterization of the binding kinetics is also performed. Our results indicate that GKAP retains much of its flexibility in the bound state, although the multivalent interactions between the partners might lead to the formation of an elaborate complex structure. This complex of GKAP and DLC2 is reported to go through liquid–liquid phase separation (LLPS) and describing the atomic details of their connection will lead to a better understanding of molecular organization of the post-synaptic density, thus the fundamentals of learning, memory, and synaptic plasticity.

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Proteomics and Metabolomics

SpT-03-1
Investigation of maternal diabetes effects on metabolomic profile of umbilical cord blood plasma by IVDr NMR spectroscopy

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Diabetes mellitus (DM) is a systemic metabolic disease characterised by increased insulin resistance and/or β-cell defects. DM is one of the most common complications of pregnancy and, if left uncontrolled, often leads to negative short-term or long-term consequences for the newborn. Preeclampsia, macrosomia, low Apgar score, hypoglycaemia, hyperbilirubinaemia and cardiac anomalies are among the serious adverse effects of maternal diabetes (MD). In this study, we aimed to elucidate the effects of MD on neonatal metabolism and to identify potential biomarker metabolites. Accordingly, we performed targeted metabolomic analysis of umbilical cord blood (UCB) serum samples of newborns MD-affected using a nuclear magnetic resonance (NMR) spectrometer in the *in-vitro* diagnostic (IVDr) system category specially developed for clinical screening. The study consisted of 20 newborns, MD-affected ($n = 5$) and healthy newborns ($n = 15$). UCB was collected within 5 minutes after birth and plasma isolation was performed. A total of 144 metabolites, including lipid derivatives, were quantitatively analysed in UCB plasma samples by IVDr NMR spectrometry. Moreover, differences in clinical and demographic data between the MD and control groups and their correlation with the metabolites showing variation were also examined. According to the results of NMR-based metabolomics analysis; the level of the 2-hydroxybutyric acid was found to be higher in the MD group compared to the control group ($p < 0.001$); the levels of alanine, lactic acid and acetoacetic acid were found to be lower in the MD group compared to the control group ($p < 0.05$). In clinical parameters, the capillary bilirubin level higher in the MD group ($p < 0.05$). Further studies are needed to elucidate the effects of maternal diabetes on neonatal metabolism, to identify metabolites with biomarker potential and to integrate IVDr NMR technology into neonatal clinical screening.

SpT-03-3
Exploring potential biomarkers for the correlation between inflammation and age-related hearing loss (ARHL)

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Hearing loss affects more than 5% of the global population and is projected to reach 10% by 2050. It mainly affects individuals over 65, with 33% undergoing its effects, and is known as age-related hearing loss (ARHL). ARHL is linked to cognitive decline, serving as a risk factor for dementia. It involves auditory neurodegeneration, related to apoptosis, inflammation, and metabolic dysfunction in the organ of Corti. Moreover, genetic, environmental, and nutritional factors influence the development of ARHL. Recent findings link changes in the gut microbiota to systemic inflammation impacting various organ systems, including the brain and inner ear. This suggests a potential correlation between hearing loss and microbiota changes. Our goal is to investigate genetic and molecular mechanisms of the gutbraincochlea axis, exploring antiinflammation molecules via comprehensive gut metabolic profiling in a premature ARHL Dusp1 knockout mice model. Faecal samples from mice of different genotype, sex, and age underwent metabolomics analysis via liquid chromatography and capillary electrophoresis coupled to mass spectrometry. Untargeted and targeted strategies were used to analyse the entire metabolome, emphasizing on short-chain fatty acids, vital in the intestinal microbiota paradigm. Metabolic pathways mainly affected include fatty acids, amino acids, glutathione, and lipid metabolism. Compounds, such as sphingosine-1-phosphate, several lysophospholipids, and butyric acid are noted for their roles in inflammation signaling. These metabolites and proinflammatory cytokines may contribute to an inflammatory scenario that links changes in microbiota with the onset and progression of ARHL. Currently, there are no robust faecal biomarkers for evaluating ARHL. Our study data, combined with other omics platforms, will aid in identifying characteristic ARHL biomarkers and potential antiinflammatory molecules for this condition. Funding: THEARPY PID2020-115274RB-I00, FEDER/MICIN.

Sunday 30 June
13:10–13:30, Red Room

Cutting Edge Approaches for Sustainable and Environmental Biotechnology

SpT-07-2
PETzyme: towards a novel enzymatic immobilization strategy for biorecycling of plastic waste

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The accumulation of plastic garbage in the environment has become one of the biggest challenges facing the world in the 21st century, with imminent consequences not only for wildlife but also for human well-being. Polyethylene terephthalate (PET) is the most abundant polyester plastic in the world, with an annual production of 70 million tons. However, less than 20% of PET is recycled, with most of the volume being released into landfills and oceans [Diao, J et al. (2023) Cell Rep 42.1]. Although mechanical and chemical recycling methods have been explored, their negative impact on the biosphere and the requirement for extreme operational conditions have limited their potential benefits. In response, enzyme-based plastic biodegradation has recently emerged as an eco-friendly and cost-effective strategy for managing PET waste. In this work, we propose the use of our own technology, the IC-Tagging system [Brandariz-Nuñez, A et al. (2010) PLoS One 5.11 e13961], as a new platform for the immobilization and stabilization of PET-degrading enzymes in order to overcome some of their constraints, such as efficiency, reusability or thermal stability. IC-Tagging allows us to load any enzyme of interest into protein nanospheres, maintaining its correct folding and catalytic activity. Our results demonstrate the capability of this method for the stabilization of active LCC-ICCG and *dura*PETase, two of the most promising enzymes for PET degradation. Immobilized enzymes are highly resistant to temperature and pH, and can be reuse up to 10 cycles without losing activity. Furthermore, both enzymes have proved to depolymerize PET beads to different monomers; particularly, LCC-ICCG lead to a weight loss of 30% in 70 hours. These results, in combination with the successful scale-up of the production of immobilized enzymes, lay the foundations for the use of IC-Tagging for recycling or upcycling PET residues to value-added products, contributing towards a circular plastic economy.

SpT-07-3
Exploring the copper-detoxification mechanisms of wild-type *Pseudomonas* strains as a potential tool for metal-polluted environments remediation

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Understanding the physiological adaptation of metal-resistant bacteria and the mechanisms involved in metal detoxification can help in the development of new biotechnologies for environmental bioremediation. The copper-resistant *Pseudomonas lactis* UKR1 strain, previously isolated from Ukrainian soil (Kyiv region), exhibits great potential for use in the remediation of metal-polluted environments. In a previous study, we found that the UKR1 genome contains several genes related to copper (Cu) resistance and metal detoxification [Previously published in Havryliuk O et al. (2020) Curr Res Microb Sci 1, 44-52]. Therefore, the aim of this work is to describe the biochemical, physiological, and molecular adaptation of UKR1 exposed to moderate levels of Cu (250 ppm) by analyzing the growth rate, biofilm-forming ability, cellular levels of reactive oxygen species, and metal-resistance gene expression. Strain UKR1 showed no growth difference in the presence of Cu compared to the control condition. In addition, the copA, copB, copC, and copD genes were detected in the genome of this bacteria by end-point PCR. Later, the expression of copA and copB genes was quantitated by RT-qPCR as a response to metal stress. Obtained results show that Cu presence induced the expression of copA gene, which is related to the physiological adaptation of the bacteria to metal toxicity. CopA groups of genes encode for copper-resistant proteins that mediate metal sequestration in the bacterial periplasm, thus playing an important role in the resistance of UKR1 to Cu. Moreover, the level of intracellular ROS in the Cu-exposed bacteria as well as the biofilm-forming ability increased at 250 ppm Cu presence. Further studies including other metal resistance genes and their products, as well as a more comprehensive analysis by using advanced high-throughput methods (e.g., RNAseq), will deepen knowledge of the molecular mechanisms underlying Cu resistance in the wild-type UKR1 strain.

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Bio-based Polymers for Engineered "Green" Materials

SpT-08-1
Use of anthocyanin enriched fraction of *C. citrinus* for the functionalization of PVA-based bioplastic

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Today, attempts are being made to overcome the economic model based on the linear economy and to apply the circular economy model, favouring environmentally friendly and green industrial and chemical processes. As a result, molecules from the plant world are becoming increasingly valuable and attempts are being made to construct new materials. This work focuses on the first part, on the production and optimization of bioplastics based on poly(vinyl) alcohol (PVA) that has been modified, and after its functionalisation with an anthocyanin-enriched fraction obtained from the flower of *C. citrinus* (EAC), a by-product of the horticultural industry. Besides being an ornamental plant, *C. citrinus* is also used for its therapeutic potential in drug therapies. We obtained the EAC fraction via a protocol we developed and then prepared bioplastics with and without different concentrations of EAC to provide new properties to the bioplastics in a dose-dependent manner. In addition, morpho-functional analysis was conducted by spectroscopy techniques. From the data obtained, the functionalised films acquire antioxidant and antimicrobial activity, while the mechanical properties are almost unchanged, making them a good candidate as biodegradable packaging for preserving the shelf-life of different fruits and vegetables. Further investigations into their effect on real food will also be evaluated. * The authors marked with an asterisk equally contributed to the work.

SpT-08-2
Mechanical, barrier and thermal properties of hydrocolloid-based bioplastics from amylose and argan proteins prepared in the presence of transglutaminase and magnetic nanoparticles

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The work exploited amylose, obtained by RNA interference technique from barley plants, and proteins, derived from argan oilcake, to produce novel blended bioplastics. Amylose is a biopolymer that provides added-value functionalities to the normal starch for bioplastics production, reducing the need for subsequent chemical modification or blending with synthetic polymers [1]. Argan protein-based oilcake is a byproduct of argan oil extraction that is currently used for animal feeding or disgarded. In this work, proteins were recovered from this high-added-value product with the aim to prepare novel hydrocolloid bioplastics blending amylose (AM) and argan proteins (APs). Three different kinds of film were prepared, AM-based films, APs-based films and AM-APs-blended films, and characterized for their mechanical, barrier, and thermal properties. Moreover, additives were used to further enhance the performance of the films, such as the enzyme microbial transglutaminase (mTGase) as reticulating agent for protein components, and ferromagnetic nanoparticles (NPs) as fillers [2,3]. The films showed suitable properties for application in the bioplastic industry. The presence of APs influences the water vapor permeability in composite films, providing a higher barrier effect which notably increases when mTGase is used as a reticulating agent, while the presence of NPs seems to increase the thermal stability of the films. Finally, the degradation of the films was studied by the burial test method using three different soils: a loamy textured soil of volcanic origin; a flood soil with a sandy clay loam texture; an alkaline flood soil. The performed tests have underlined that the novel bioplastics can be easily degraded in all the soils tested. References: [1] Xu, J. et al. (2021) Carbohydr. Polym. 253, 117 277. [2] Famiglietti, M. et al. (2023) Sustainable Food Science - A Comprehensive Approach: Volumes 1-4, pp. V4-110–V4-128. [3] Liu, S. et al. (2020) Adv. Colloid Interface Sci. 281, 102 165.

Towards Sustainable Use of Natural and Renewable Resources

SpT-09-2
Towards sustainable processes: use of agro-food waste biomasses to produce recombinant proteins

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Escherichia coli cells are the most widely used microbial cell factories for the production of recombinant proteins and enzymes. Typically, recombinant production involves the use of well-defined media and expensive inducers of gene expression, such as isopropyl β-D-thiogalactopyranoside (IPTG). Agro-food waste biomasses can be valuable growth substrates for microbial cultures due to their organic matter content, offering potential solutions to reduce costs and improve process sustainability. This study focuses on the valorization of cheese whey permeate (CWP) and crude glycerol, collected from two Italian plants. CWP is derived from the extraction of whey protein, while crude glycerol is obtained from the hydrolysis of waste oils to produce fatty acids. We exploited both biomasses for the production of various recombinant proteins in *E. coli* BL21 (DE3) cells. Our results indicated that CWP is a cost-effective alternative inducer for recombinant protein production. In addition, the micronutrients present in CWP, such as vitamins and coenzymes, reduce the cellular stress caused by heterologous expression[1]. To improve recombinant protein production and maximize the use of crude glycerol and CWP, a 1.5 L fed-batch cultivation process was developed. This process used 250 mL crude glycerol (125 g)

and 300 mL CWP (52.5 g lactose) to produce 52.9 ± 2.2 g dry cell weight and 1980 ± 135.9 kU of a recombinant β-galactosidase with biotechnological potential. In conclusion, crude glycerol and CWP can serve as alternative carbon sources and inducers in high-density cell cultivations, resulting in water conservation and a sustainable and economical recombinant protein production process. Reference: de Divitiis et al. (2023). Biotechnol Biofuels and Bioprod, 16(1), 1-19. This work has been supported by Fondazione Cariplo, grant n° 2020-0838.

SpT-09-1
Enhancement of H_2 production by *Chlorella vulgaris* using potato peel waste

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Waste utilization with green algae can be coupled with possible use of algal biomass for clean and renewable energy production, which is even more important, considering the current global environmental challenges. This work aims to investigate the hydrogen (H_2) production activity in *Chlorella vulgaris* Pa-023, isolated in Armenia, during cultivation in potato peel waste (PPW)-containing media. PPW is generated in households and food industry; 15-40% of a potato peel is removed as waste depending on the peeling methods [Gebrechristos HY, Chen W (2018) Food Sci Nutr. 6, 1352-1356]. Thus, it was shown that *C. vulgaris* Pa-023 demonstrated the ability to intensively generate H_2 during cultivation in PPW-containing media. H_2 production in PPW media started after 24 h and was detected at least during 96 h, while the control culture cultivated in Tamiya standard media produced H_2 during 72 h of growth. During growth in PPW media H_2 yield in *C. vulgaris* reached the maximal levels at 24–48 h, which were 2 times higher than the H_2 yield in the control. It is known that two pathways, photosystem (PS) 2-dependent and PS 2-independent, provide H_2 generation in green algae [Manoyan J et al. (2022) Int J Hydrogen Energy 47, 16 815-16 823]. Diuron, a specific inhibitor of the photosynthetic electron transfer, has been applied to determine the H_2 generation pathway in *C. vulgaris* Pa-023 under cultivation in PPW-containing media. Diuron inhibited the H_2 yield by 60% in *C. vulgaris* grown in PPW media, which emphasize that water is the main donor of electrons for the H_2-producing key enzyme – hydrogenase in tested algae, and up to 60% of electrons for H_2 generation are provided by PS 2. Thus, PPW can be used as a cheap and valuable source for promoting H_2 generation in green algae due to its high biodegradability and carbon-rich composition. The work was supported by the Higher Education and Science Committee of RA, in the frames of the research project 23AA-1F003.

Sunday 30 June
13:10–13:30, Blue Room

Immunobiochemistry

SpT-31-2
Structural and biochemical studies of the hepatitis C virus envelope proteins to promote germline-targeting vaccine design

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Hepatitis is a serious liver disease that can be caused by the hepatitis C virus (HCV) and is a growing health concern, despite improvements in antiviral medication. An effective prophylactic vaccine is crucial to eliminating global HCV. Thus far, vaccination trials have failed to elicit an effective broadly neutralising immune response due to inefficient vaccine antigens. HCV E2 envelope (Env) glycoprotein is the main target for neutralising antibodies (nAbs) and thus the primary candidate for B-cells based vaccine design. A molecular-level understanding of HCV neutralisation by broadly nAbs (bnAbs) is imperative for the design of cross-reactive vaccine antigens to elicit high levels of bnAbs. Previous studies indicated that the broad neutralisation response against HCV is biased towards VH1-69-encoded bnAbs that target the E2 main neutralisation epitope. These results suggest the potential for vaccine strategies that aim to amplify VH1-69-encoded antibody responses by targeting the corresponding antibody germline precursors to elicit a potent, broadly neutralising response. Therefore, a better understanding of the recognition of HCV E2 by VH1-69-encoded germline B-cells can facilitate designing a more effective vaccine. To this aim we expressed and characterised the interfaces of two germline reverted antibodies, AR3A 1-69 and U1 GL, along with the antigen E2. We crystallised antibody-antigen complexes for X-ray crystallography and succeeded in solving their structures to 2.05 and 2.7 Å respectively. We proceeded to compare them to their mature counterparts showing the difference in binding affinities based on biolayer interferometry analysis and structural observations. Utilising functional studies, we characterised the cross-binding and neutralisation potency of the reverted antibodies. These results will be used to design and improve antigens and develop a more effective vaccine against HCV.

SpT-31-1
Development of new humanized monoclonal antibodies for the treatment of fungal infections

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Fungal infections represent a serious global health threat. The new emerging pathogens and the spread of different forms of resistance are now hardly challenging the tools available in therapy and diagnostics. The urgent need for new strategies led to

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the use of biological drugs and, in particular, monoclonal antibodies as innovative approach. 2G8 is the perfect example of this trend hence it is a murine mAb that resulted efficient in controlling fungal infections *in vitro* and *in vivo* by selectively recognizing β-1,3 glucans, vital components of the fungal cell wall. Nevertheless, its murine nature represents an immunogenicity risk, therefore two humanized mAbs were derived and characterized *in vitro*. It is the case of the full-length mAb Dia-T51, and the single chain-fragment variable scFv-3 T. Dia-T51 showed a higher affinity compared to the murine parental (IC_{50} 0.06 vs 0.12 and Kd in a subnanomolar range) for β-1,3 glucans, and proved to be extremely stable (melting point: 82.1°C). In a dose-dependent manner, Dia-T51 inhibits *Candida auris* growth and adhesion to suitable biological substrates, and Dia-T51-opsonized *C. auris* cells are better eradicated by the immune system effectors. ScFv-3 T also demonstrated to bind β-1,3 glucans and to be stable when stored at different temperatures for different timepoints. Nevertheless, the greatest potential of both the humanized mAbs was proved in combination with commercially available antifungal drugs. Additivity was shown with echinocandins and synergy with amphotericin B against *C. auris* and *Candida glabrata* (including resistant strains). The antibodies must be further investigated but can already be considered promising drug candidates for the treatment of fungal infections, especially candidiasis. *The authors marked with an asterisk equally contributed to the work.

SpT-31-3
Phage display-derived monoclonal antibodies against the staphylococcal collagen adhesin CNA

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Staphylococcus aureus is a human commensal bacterium; however, its pathogenic form is responsible for infections including endocarditis, osteomyelitis, and bacteremia. Since the phenomenon of antibiotic resistance has been estimated to cause 10 million per year of deaths by 2050, the aim of this study was to select human monoclonal antibodies (mAbs) through antibody-phage display as potential vaccines against staphylococcal infections to use in clinics. The mAbs were *in vitro* selected against the collagen-binding adhesin (CNA) expressed by *S. aureus* and they were biochemical, biophysical and biological characterized. 18 unique mAbs were selected and characterized with enzyme linked immunosorbent assay (ELISA) and surface plasmon resonance (SPR) techniques to determine the binding and the affinity for the antigen. The activity was evaluated on recombinant purified adhesins and on adhesin-expressing bacteria. Finally, the epitopes of the most interesting mAbs were experimental and *in silico* mapped. The results on the antibody-antigen binding demonstrated that they not only bind to CNA, but one can also recognize an adhesin expressed from another Gram-positive bacterium. We discovered that two mAbs were able to neutralize the *in vitro* infection (either by inhibition or displacement) of adhesins-expressing bacteria. In conclusion, completely human mAbs have been selected for the first time against the staphylococcal CNA and 2 antibodies showed an interesting neutralization activity against *Staphylococcus aureus* and *Enterococcus faecium* bacteria. A 3D model with eukaryotic cells will be introduced to study the mAbs activity before animal models studies for assessing the potential of mAbs as therapeutic agents for human applications.

Other Topics

SpT-35-1
Pleiotrophin enhances mTORC1 activity and protein synthesis in endothelial cells through crosstalk of multiple receptors

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Pleiotrophin (PTN) is a secreted factor that enhances endothelial cell migration through its PTPRZ1 and $\alpha_v\beta_3$ integrin receptors. PTN also binds to vascular endothelial growth factor receptor 2 (VEGFR2), although the effect of this binding on endothelial cell activation is unclear. In the present work, we aim to elucidate the PTN signaling pathway in endothelial cells isolated from human umbilical veins (HUVEC) or the lungs (LMVEC) of *Ptprz1*$^{+/+}$ and *Ptprz1*$^{-/-}$ mice. PTN increases mTORC1 activity and protein synthesis, both eliminated by the mTORC1 inhibitor, rapamycin. PTPRZ1 is involved in this effect since *Ptprz1*$^{-/-}$ LMVEC have increased mTORC1 activity and protein synthesis, sensitive to rapamycin. Given the inhibitory effect of crizotinib on the enhanced angiogenic phenotype of *Ptprz1*$^{-/-}$ LMVEC, we tested the involvement of cMet in PTN activities. The cMet inhibitor crizotinib abolished the PTN-induced mTORC1 activation or the enhanced mTORC1 activity in *Ptprz1*$^{-/-}$ LMVEC, and PTN enhances cMet tyrosine phosphorylation through inhibition of the PTPRZ1. Considering the direct interaction between PTN and VEGFR2, we studied the role of VEGFR2 on the activation of mTORC1 by PTN. PTN enhances VEGFR2 tyrosine phosphorylation, while the selective VEGFR2 tyrosine kinase inhibitor nullified the PTN-induced mTORC1 activation and protein synthesis. Finally, the involvement of $\alpha_v\beta_3$ integrin was studied by using the selective anti-$\alpha_v\beta_3$ LM609 antibody or a PTN peptide that inhibits the PTN-$\alpha_v\beta_3$ interaction; both abolish the effect of PTN in endothelial cell migration. Surprisingly, LM609 and the PTN peptide induced cMet tyrosine phosphorylation and mTORC1 activation, providing a potential explanation for integrin inhibitors' failure in clinical trials. Our data suggest that PTN activates mTORC1 and protein synthesis in endothelial cells downstream of PTPRZ1, $\alpha_v\beta_3$, VEGFR2, and cMet, and give insights into the complex signaling regulating endothelial cell functions.

SpT-35-2
Probing the nucleobase selectivity of RNA polymerases with C-nucleoside antibiotics

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Formycin A (FOR) and Pyrazofurin A (PYR) are nucleoside analogues produced by actinobacteria. FOR and PYR are known to exhibit antiviral and antitumor properties by interfering with nucleic acid metabolism, yet their direct influence on transcription and viral RNA replication remains less understood. In this study, we explored the utilization of triphosphorylated FOR, PYR and oxidized purine nucleosides by three distinct RNA polymerases (RNAPs): the multi-subunit bacterial RNAP from *Escherichia coli*, the human mitochondrial RNAP, and the viral RNA-dependent RNAP (RdRP) from coxsackievirus B3. All tested polymerases incorporated FOR in place of adenine and 8-oxoguanine in place of guanine. In contrast, PYR and 8-oxoadenine were primarily incorporated in place of uridine. Remarkably, the bacterial RNAP and viral RdRP also incorporated FOR as a cytosine substitute, with RdRP demonstrating efficient utilization during processive RNA synthesis. In the latter case, FOR dual coded as a substrate and as an acceptor base in the template RNA. Comparison of the incorporation specificity of FOR, PYR, 8-oxoguanine, and 8-oxoadenine suggested that *syn* conformers are responsible for C-coding of FOR and U-coding of PYR. By examining base pairings that led to substrate incorporation and the entire spectrum of geometrically compatible pairings, we have deepened our understanding of the nucleobase selection mechanisms of structurally diverse RNAPs.

SpT-35-4
Osmoadaptation in yeast cells with mitochondrial dysfunction: gaining insight into stress signaling networks

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Alterations in mitochondrial function have been linked to a variety of cellular and organismal stress responses including apoptosis, aging, neurodegeneration and tumorigenesis. However, adaptation to mitochondrial dysfunction can occur through the activation of survival pathways, with the mechanisms behind still poorly understood. Yeast *Saccharomyces cerevisiae* is an invaluable model organism for studying how mitochondrial dysfunction can affect stress response and adaptation processes. We analyzed and compared wild type cells with cells lacking mitochondrial DNA (ρ^0) or a mitochondrial pyrimidine nucleotide transporter (ΔRIM2) or the catalytic subunit of the transcriptional complex Hap (ΔHAP4) in the absence and in the presence of osmostress. Our results revealed that mitochondrial dysfunction confers an advantage in the kinetics of stress response. CIT2, encoding the peroxisomal isoform of citrate synthase and whose up-regulation is prototypical of RTG pathway activation, appeared up-regulated in all the mutants. Interestingly, selected TCA cycle genes, CIT1 and ACO1, whose expression depends on RTG signaling upon stress, showed a different regulation in ρ^0 cells and ΔRIM2, although both strains lack mitochondrial DNA. These data suggest that osmoadaptation can occur through different mechanisms in the presence of mitochondrial dysfunction. All mitochondrial mutants showed an increased glycerol level required in the early phase of the response. In order to gain insight into the molecular mechanisms of improved osmoadaptation, an untargeted metabolomics analysis was performed. Multivariate data analysis and comparison of the metabolomes allowed us to identify relevant metabolites that might be involved in the stress signaling. Understanding the interplay between stress mediators and environmental context will help to identify key points of reconfiguration in the dynamics of cell stress adaptation.

Sunday 30 June
13:10–13:30, Yellow Room

Enzyme and Cell Therapies (Medicinal Biochemistry)

SpT-21-2
Sialidase Neu3: a novel cardioprotective target against ischemia and reperfusion injury

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Coronary reperfusion procedures are crucial for restoring blood flow to the heart tissue after an acute myocardial infarction. However, they also lead to ischemia and reperfusion injury (IRI), which exacerbate the damage and promote heart failure. Despite the urgent need for cardioprotective strategies against IRI, none have been clinically implemented, emphasizing the need for a comprehensive understanding of the pathophysiology of IRI. Our research team found that sialidase Neu3, an enzyme responsible for the removal of sialic acid from glycosphingolipids, is modulated during IRI *in vivo*. Constitutive overexpression of Neu3 in rat cardiomyoblasts was shown to attenuate IRI through activation of the reperfusion injury salvage kinase (RISK) and HIF-1α signaling pathways. To further investigate the role of Neu3 in promoting cardioprotection, in this study we developed an inducible Neu3 overexpression model in human cardiac cells and mice. Inducible upregulation of Neu3 significantly improved cell resistance and reduced oxidative stress in AC16 cells exposed to IRI *in vitro*. These effects were attributed to Neu3-mediated maintenance of mitochondrial membrane potential, which was impaired in control cells. When looking at the upregulated genes exclusively in cells overexpressing the sialidase Neu3, RNAseq analysis revealed the predominant activation of MAP kinases

typical of the RISK pathway. We also generated, for the first time, αMHC-Cre/LSL-Neu3 mice overexpressing Neu3 in an inducible and cardiomyocyte-specific manner. These mice showed no significant differences in cardiac morphology and functionality compared to wild-type animals. However, the Neu3-overexpressing mice exhibited a significant reduction in infarct size and improved cardiac functionality after surgical induction of IRI. These results suggest that Neu3 is a promising target for improving cardioprotection and attenuating the incidence and severity of myocardial infarction.

SpT-21-1
Applying autophagy as a treatment for Huntington's disease

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Huntington's disease, a fatal genetic disorder, leads to the breakdown of brain nerve cells due to mutated Htt protein aggregation, with no existing cure. This study proposes enhancing the degradation of both soluble and aggregated forms of mutant Htt (mHtt) through chaperone-mediated and ubiquitin-mediated autophagy, employing adeno-associated viruses (AAV) to deliver mRNA coding for autophagy-regulating proteins. Chaperone-mediated autophagy (CMA) selectively degrades proteins via the HSC70 chaperone and LAMP-2A lysosomal protein. For insoluble mHtt aggregates, optineurin (OPTN), recognizing ubiquitinated proteins, is suggested. Enhancements include synthetic dimer QBP1 for HSC70 targeting mHtt, and AAV-delivered mRNA for producing HSC70, QBP1, and OPTN, aimed at crossing the blood–brain barrier (BBB). Early-stage Huntington's may benefit from CA77.1 to boost LAMP-2A, with later stages requiring AAV-mediated OPTN mRNA for degrading polyQ aggregates. The approach targets mHtt specifically, minimising wild-type HTT degradation. Repeated AAV treatments necessitate alternative vectors due to immune response risks. While focusing on mHtt degradation, gene editing is highlighted for a more permanent solution, with the need to investigate interactions between methods to mitigate potential side effects. The project was implemented within the frameworks of IBO 2022 International Group Project. * The authors marked with an asterisk equally contributed to the work.

Epigenome and Transcriptome

SpT-25-1
Inhibiting the escape: unveiling the potential of type I PRMT inhibitors in targeting lysosomal exocytosis for improved cancer therapy

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Epigenetic modifications exert a profound influence on gene expression, directing oncogenesis and resistance to drugs. Lysosomes, crucial for cellular signaling and component management, have recently emerged as pivotal players in cancer cell survival through lysosomal sequestration and exocytosis to evade programmed cell death. This study focuses on the epigenetic coordination of these processes, hypothesizing that epigenetic modifier drugs capable of suppressing lysosomal exocytosis could serve as effective therapeutics, thereby enhancing the efficacy of cisplatin. Our study entailed screening a library of epidrugs, evaluating their impact on lysosomal exocytosis (β-hex assay), lysosomal content (LysoTracker staining), and combined cytotoxicity with cisplatin (cell viability assay). Remarkably, MS023, a type I PRMT inhibitor, emerged as a promising candidate, demonstrating reduction in exocytosis and enhancing cisplatin cytotoxicity. Furthermore, silencing each PRMT targets (PRMT1, 6, and 8) was less effective in reducing exocytosis compared to the effect of MS023, suggesting the need for concurrent inhibition of multiple PRMTs or the involvement of an unidentified target. RNA-seq and gene ontology studies unveiled potential biological alterations and among the differentially expressed genes, three notable candidates – ABCA1, ABCA3, and SerpinE1 – previously linked to drug resistance were identified. Focusing on ABCA3, which exhibited the highest fold change and lysosomal localization, we found that knocking down all MS023 target PRMTs led to a reduction in ABCA3 expression. Further investigation into other Type I PRMT inhibitors revealed that GSK3368715 also reduces exocytosis in addition to synergizing with cisplatin. In summary, Type I PRMT inhibitors emerge as promising epidrugs impacting secretory pathways and drug efflux processes, with the aim of establishing future targets for cancer intervention and ultimately enhancing drug efficacy.

SpT-25-2
Alterations in the expression and subcellular localization of the SWI/SNF chromatin remodeling complexes subunits and their potential clinical significance in clear cell renal cell carcinoma

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The SWI/SNF chromatin remodeling complexes (CRCs) play a critical role in gene expression control by modifying the nucleosome structure of chromatin, thereby enabling access to genomic DNA for processes such as transcription and DNA repair. The functional diversity and specificity of SWI/SNF CRCs arise from the complex assembly of multiple subunits, post-translational modifications of certain subunits, and interactions with various proteins. Up to 25% of cancers are linked to mutations in genes encoding SWI/SNF subunits, even minor alterations in the levels of individual SWI/SNF subunits can impact the overall complex activity. In 40% of clear cell renal cell carcinoma (ccRCC) cases, mutations in the PBRM1 gene, which encodes the BAF180 SWI/SNF subunit, have been identified. Additionally, ccRCC is characterized by a multitude of epigenetic alterations. The investigation utilized ccRCC cell lines along with a non-cancer control. The expression levels of genes encoding SWI/SNF subunits and their subcellular distribution were evaluated through qPCR, cell fractionation, and western blot analyses. Epigenetic alterations were investigated using ChIP-qPCR, treatment of cells with DNA methyltransferase inhibitors and bioinformatic analysis. IHC staining of clinical samples was conducted to determine the abundance of the proteins. Changes in the gene expression patterns of SWI/SNF CRCs subunits were noted, with an increase in BAF60A and BAF60B levels observed in the metastatic cell line. Additionally, certain SWI/SNF subunits, typically localized in the nucleus, were found in the cytoplasm. The presence of the BAF60B subunit in the cytoplasm appears to be associated with tumor recurrence. The upregulation of SWI/SNF subunits in metastasis may become a target for new therapy. The increased cytoplasmic abundance of BAF60B linked to cancer recurrence may be viewed as a potential novel biomarker. Foundation: Polpharma Scientific Foundation 5/XVII/18 (TJS), FBW-SD-05/2024 (MW).

Cancer and Metabolism

SpT-26-3
Mutant p53 (mutp53)-driven HMGA1 secretion promotes pancreatic ductal adenocarcinoma (PDAC) proliferation and chemoresistance

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Pancreatic ductal adenocarcinoma (PDAC) is among the most lethal cancers. In this tumor type mutant p53 (mutp53) has a key role in altering the secretion of many signaling molecules, thus manipulating the tumor microenvironment (TME) to drive tumorigenesis. Since an extensive characterization of cancer secretome may lead to the identification of druggable targets for tumor treatments, we focused our study on the roles of mutp53-dependent secretome in PDAC cells. Through mass-spectrometry analysis, we detected secreted proteins modulated by mutp53 and, among them, we selected the nuclear high mobility group A1 (HMGA1) for further studies. HMGA1 is an architectural transcription factor involved in several cellular processes and found to be upregulated in several tumors, but its function in cancer remains unclear. We demonstrated that mutp53-dependent secretion of HMGA1 promotes PDAC cells hyperproliferation and resistance to gemcitabine (GEM) treatment *in vitro* suggesting a critical role of this protein in tumor aggressiveness. This observation is also confirmed by our *in vivo* data showing that HMGA1 deficiency significantly affects tumor progression. Moreover, we showed that chemotherapy increases HMGA1 secretion only in cells harboring mutp53 with a mechanism that fully relies on the activity of the Casein kinase 2 (CK2). Lastly, since our preliminary data suggest that mutp53-driven secretion of HMGA1 may act in an autocrine/paracrine manner stimulating crucial anabolic and oncogenic pathways, we analyzed which pathways are activated by the secreted HMGA1 by performing phosphoproteomic analysis. Overall, our study links the secretome of PDAC cells to hyperproliferation and chemoresistance highlighting HMGA1 as a promising secreted target in aggressive PDAC with mutations in TP53 gene thus confirming that the alteration of TME might provide new therapeutic opportunities counteracting chemoresistance in mutp53-PDAC patients.

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SpT-26-1
Serine-glycine metabolism drives VEGFR2 modulated tumorigenesis

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Vascular endothelial growth factor receptor 2 (VEGFR2) is a classical receptor tyrosine kinase (RTK) expressed in several tumors where it regulates proliferation, migration and metabolism. Despite VEGFR2 representing an attractive therapeutic target, the clinical application of tyrosine kinase inhibitors has shown limited responses and rapid development of resistance. Recently, our laboratory demonstrated that inactive VEGFR2 leads to tumor growth and rewires metabolism. To elucidate this behavior, we expressed inactive VEGFR2R1032Q and VEGFR2S1100F mutants in human melanoma cells. Seahorse experiments revealed that the expression of inactive VEGFR2 induces to changes in energy metabolism. Moreover, metabolomics analyses on tumor xenografts of melanoma cells expressing wild-type and mutated VEGFR2 highlighted alterations in one-carbon metabolism. Transcriptomic analyses and qPCR confirmed the differential regulation of SHMT1, SHMT2, TYMS, ATIC and MTHFD2 in this metabolic pathway. Preliminary results indicate that serine supplementation promotes the proliferation and migration of cells expressing both VEGFR2R1032Q and VEGFR2S1100F mutants. Building upon these findings, we utilized SHIN1 as a potent inhibitor of serine hydroxymethyltransferase. The inhibition of SHMT decrease the proliferation and migration of the Sk-Mel-31 cells expressing inactive VEGFR2 mutants. This study underscore serine-glycine-one-carbon metabolism as a downstream target of VEGFR2 dysregulation in melanoma cells, suggesting its involvement in tumor progression and positioning it as a promising therapeutic target.

Monday 1 July
13:10–13:30, Silver Room

Membrane Biochemistry

SpT-17-3
Calcium transport by sarcoplasmic reticulum Ca^{2+}-ATPase: stimulatory and inhibitory effects investigated on a solid supported membrane

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The sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) belongs to the P-type ATPase family of membrane transporters. In muscle cells SERCA couples ATP hydrolysis to the transport of two Ca^{2+} ions against their electrochemical gradient from the cytoplasm into the lumen of the sarcoplasmic reticulum (SR), thus promoting muscle cell relaxation. SERCA maintains a low cytoplasmic Ca^{2+} concentration and contributes to intracellular calcium homeostasis which is essential for cell signaling and survival. SERCA dysfunction has been related to several pathologies, such as cardiovascular diseases, diabetes and cancer. Thus, SERCA represents an important target for the development of novel therapeutic compounds. A bioelectrochemical approach based on a solid supported membrane (SSM), which consists of a gold-supported hybrid alkanethiol/phospholipid bilayer, was used to investigate the transport activity of SERCA. SR vesicles containing SERCA were adsorbed on the SSM and were activated by an ATP concentration jump. Following SERCA activation, an electrical current was detected which was attributed to movement of Ca^{2+} ions across the vesicular membrane. We used the SSM method to characterize the effects of different compounds, e.g. natural products or compounds of synthetic origin, on SERCA activity. Our results indicated that such compounds affect Ca^{2+} transport by SERCA and behave like activators or inhibitors of the SERCA enzyme. In particular, we examined the effect of a novel photoactivable ruthenium complex, which can be used as a molecular photosensitizer in photodynamic therapy. We found that photoactivation of the ruthenium complex induced the synthesis of the potent singlet oxygen which markedly affected SERCA transport activity with an inhibitory effect dependent on the duration of light exposure. Regione Toscana (Bando Ricerca Salute 2018, RESEARCH project n. D78D20000870002) is acknowledged for financial support.

SpT-17-2
Aquaporin-3 and aquaporin-5 modulate cell biomechanical properties and influence cell migration

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Aquaporins (AQPs) are transmembrane proteins that mediate the transport of water, glycerol, and small neutral solutes across cell membranes. AQPs are overexpressed in different types of cancer, being involved in cancer cell proliferation, migration, angiogenesis, and metastasis. Our previous study with AQP3-, AQP5-, and double-silenced human pancreatic ductal adenocarcinoma cells showed morphological alterations and lower cell-cell adhesion, with AQP5 influencing cell stiffness and membrane fluidity. These findings suggest that these AQPs can impact tumor progression by modulating cell biomechanical properties. With this work, we developed a cell-based platform of HEK-293 T (HEKT) cells overexpressing individually the isoforms most associated with cancer: AQP3 (water and glycerol channel) and AQP5 (water channel) to investigate how their overexpression can modulate biological processes and cell membrane features. After cell model validation, we assessed their impact on morphological properties through atomic force microscopy (AFM) imaging. AQP5-overexpressing cells exhibited a higher roughness and area, with no differences observed for AQP3-HEKT cells. Using AFM-based force spectroscopy, we evaluated the influence of these AQPs on cell stiffness and cell-cell adhesion. AQP3-

HEKT cells showed higher cell stiffness and lower cell-cell adhesion. In contrast, AQP5-HEKT cells demonstrated higher elasticity and cell-cell adhesion. Afterwards, we investigated the effect of AQP3 and AQP5 overexpression on cell migration, proliferation and adhesion. Both AQPs promoted cell migration and impaired cell adhesion to matrix, while no differences were observed for cell proliferation. Further studies are needed to understand the mechanisms underlying the roles of AQP3 and AQP5 on cell membrane properties and their impact on biological processes crucial for tumorigenesis.

SpT-17-1
Sec24D-positive ER exit sites sort raft-preferring proteins for rapid ER export

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The specific determinants of subcellular trafficking are unknown for many membrane proteins. Although motifs responsible for cargo integration into envelope/adapter-mediated sorting schemes have been identified, these are insufficient to explain the kinetics of protein movement between organelles. Another potential factor in the organization and traffic of membrane proteins is the membrane nanodomains known as lipid rafts. These domains are small, dynamic assemblies of lipids and proteins that preferentially interact with each other. They are often associated with the plasma membrane but are probably also present in various endomembranes. To assess the role of membrane nanodomains in the early secretory pathway, we use a robust tool for synchronized protein traffic known as RUSH (Retention Using Selective Hooks), in which tagged proteins can be retained by a resident "hook" in specific organelles and then rapidly released. We applied RUSH to a library of transmembrane domains (TMDs) to investigate the role of raft affinity in ER exit kinetics and the machinery involved. We found that raft-preferring TMD probes exit the ER faster than those without raft affinity and that they have different preferences for ER exit sites characterized by specific isoforms of sec24, the cargo adaptors that form the inner envelope of ER export vesicles. Namely, probes with raft affinity tend to localize to sec24D-positive exit sites, whereas probes without raft affinity tend to localize to sec24A-positive sites. Surprisingly, probes with identical COPII recognition motifs are sorted differently into the sec24D or sec24A exit sites based on the lipid raft affinity of their transmembrane domains. Consistent with this, sec24D, but not sec24A, ER exit sites accumulate a fluorescent cholesterol analog. These observations suggest that despite relatively low cholesterol concentrations in the ER, cholesterol-rich domains can sort proteins for rapid export from the ER.

SpT-17-4
Regulation of human Akt1 by phosphatidylinositol (3,4,5)-trisphosphate lipid species

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Akt1 is a membrane-associated serine/threonine-protein kinase known to mediate the phosphorylation of more than 100 metabolic and mitogenic substrates regulating growth, proliferation, protein synthesis, or glucose metabolism. Akt1 activation is coupled to the accumulation of phosphatidylinositol (3,4,5)-trisphosphate ($PI(3,4,5)P_3$) in the cytosolic leaflet of the plasma membrane. Akt1 binds to $PI(3,4,5)P_3$ through its lipid binding pleckstrin homology (PH) domain. However, $PI(3,4,5)P_3$ is actually a class of lipids consisting, in fact, of species of various acyl chain compositions, which differ in terms of length and saturation. Thus, a question arises if this variability plays a role in modulating the interactions between the Akt1 and $PI(3,4,5)P_3$ and, therefore, could influence the signaling outcomes. To address this question, a bottom-up approach with purified components was applied. Purified full-length Akt1 interactions with membrane systems containing different $PI(3,4,5)P_3$ species was assessed by complementary biochemical and biophysical techniques. Our results show that Akt1 membrane translocation is associated with significant conformational change. We also show that Akt1-membrane binding affinity is $PI(3,4,5)P_3$ species dependent. Additionally, Akt1 binding to different $PI(3,4,5)P_3$ species influences its kinase activity. Thus, our work demonstrates that the $PI(3,4,5)P_3$ acyl chain composition can modulate Akt1 membrane binding and, consequently, its kinase activity. It, therefore, suggests an additional layer of complexity regarding the ability of cells to manipulate enzymatic activities and selective downstream signaling pathways.

Cellular Organelles

SpT-18-2
Nonmuscle myosin II participates in controlling the fission of cellular organelles

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The fission events of various organelles require the merging of two lipid bilayers, but how the two lipid bilayers can come close together during the fission is not well explored. Here, we show that knocking down the actin-binding motor proteins, nonmuscle myosin IIs (NM II) by siRNA or inhibition of their activities by blebbistatin causes the formation of a ring-like assembly of early endosomes (raEE). Inhibition of NM II assembly by an inhibitor of myosin light chain kinase results in the formation of raEE whereas inhibition of NM II disassembly by inhibitors of heavy chain kinases, PKC and CK2 causes the dispersion of early endosomes. The raEEs retain EEA1, Rab7 and LAMP2 markers. Overexpression of an assembly incompetent form, RLC-AA and

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disassembly incompetent form, NMHC IIB-S6A or NMHC IIA-1916A, induces such defects, respectively. In parallel, we find the fission of mitochondria is similarly regulated by NM II assembly and disassembly dynamics. Altogether, these data support a model in which NM II activity provides force in regulating the fission events to maintain the size of various organelles in cells.

SpT-18-1
T-cell-restricted intracellular antigen-1 directs cellular senescence by regulating mitochondrial dynamics

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Mitochondrial homeostasis is critical for various cellular processes and mitochondrial dysfunction is involved in the pathophysiology of cells. Senescent cells exhibit a diverse spectrum of changes in their morphology, proliferative capacity, senescence-associated secretory phenotype (SASP) production, and mitochondrial homeostasis. These cells often manifest with elongated mitochondria, a hallmark of cellular senescence. However, the precise regulatory mechanisms orchestrating this phenomenon remain predominantly unexplored. In this study, we provide compelling evidence for decreases in the expression of T-cell-restricted intracellular antigen-1 (TIA-1), a pivotal regulator of mitochondrial dynamics, in models of both replicative senescence and ionizing radiation (IR)-induced senescence. The downregulation of TIA-1 was determined to trigger mitochondrial elongation and enhance the expression of senescence-associated β-galactosidase, a marker of cellular senescence, in human fibroblasts and keratinocytes. Conversely, the overexpression of TIA-1 mitigated IR-induced cellular senescence. Taken together, our findings underscore the significance of TIA-1 in governing mitochondrial dynamics and cellular senescence.

Monday 1 July
13:10–13:30, Red Room

Proteomics and Metabolomics

SpT-03-2
Integrative multi-omics approach in subjects affected by glycogen storage disease type Ia

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Glycogen storage disease type Ia (GSDIa) is a genetic metabolic disorder caused by the deficiency of glucose-6-phosphatase-α that affects carbohydrate metabolism by impairing the final step of gluconeogenesis and glycogenolysis. The most common biochemical features include hypoglycemia, lactic acidosis, hypertriglyceridemia, hypercholesterolemia and hyperuricemia. Over time, patients with GSDIa accumulate glycogen and fat storage in the liver and kidneys, leading to hepatomegaly and kidney disease, respectively. Currently, there are no approved drug therapies for the treatment of GSDIa, but highly personalized diets to prevent hypoglycemia and secondary metabolic perturbations. Although GSDIa patients show a good compliance with the specific dietary treatments, over time they may develop long-term complications such as hepatocellular adenomas and carcinomas, as well as chronic kidney disease. To unravel the wide-ranging metabolic perturbations that occur in GSDIa and to elucidate the molecular basis of the known long-term complications, an integrative multi-omics approach was adopted including data from mass spectrometry-based serum proteomics, metabolomics and lipidomics in 12 GSDIa patients. Patients showed a unique multi-omics serum signature compared to age- and sex-matched healthy controls. Multi-omics data integration indicated liver injury and lipid metabolism dysfunction, highlighting the pivotal role of the liver in both early and late stages of the disease. In conclusion, our findings provided new insights into the field of GSDIa highlighting the primary role of the liver in the progression of the disease and the need for prognostic biomarkers discovery in GSDIa.

SpT-03-4
Effect of nutrient stress on protein stability in the bioplastic producer *Cupriavidus necator* – monitoring conformation at proteome level using thermal proteomics

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Cells can respond to environmental insult through coordinated expression changes (mRNA, protein) or via structural modifications to individual proteins that confer functional change (e.g. conformation, allostery, molecular interactions). The former can be assessed using approaches like transcriptomics and expression proteomics, while until recently the latter have been difficult to study en masse. Thermal proteome profiling (TPP) assays the thermostability of proteins on a proteome-wide level by measuring the progressive loss to aggregation of proteins subjected to a temperature gradient. The melting behaviour of each protein can be compared under different conditions, permitting the identification of conformation response to the condition. Furthermore, studies have demonstrated that proteins participating in shared pathways and protein complexes can show correlated melting pattern changes. TPP is therefore a promising approach to understanding the global response to environmental changes that may be independent of, or supplementary to, gene or protein expression programmes. Here, we apply the TPP method to a nutrient stress insult in the bioplastic (PHA, poly-hydroxyalkanoate) producing chemolithoautotroph *Cupriavidus necator*. *C. necator* is of intense interest in sustainability studies since it can consume carbon dioxide to produce biofuel. Over 90% of the dry cell weight of *C. necator* can comprise of PHA granules. *C. necator* cells, grown in complete media and in media with reduced nitrogen, were harvested. TPP analysis was implemented using tandem mass tag (TMT) labelling to encode the soluble proteome surviving each of 10 temperature steps (30–100°C). Trypsin-digested samples were then analysed by Orbitrap mass spectrometry and melting curves calculated for >1000 proteins. Proteins showing evidence of conformation change in response to low nitrogen included enzymes involved nitrogen metabolism, as well as central metabolism pathways like the TCA cycle.

Protein Phase Separation and New Organelles

SpT-06-2
The hyperactivity of estrogen receptor fusion proteins in breast cancer

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Estrogen receptor-positive (ER+) cancers represent 70% of all breast cancer cases. Despite their initial response to anti-hormonal treatments, acquired resistance eventually arises, allowing the disease to progress into an aggressive, metastatic form. An emerging mechanism of therapeutic resistance is through chromosomal translocations that fuse a diverse set of genes with the *ESR1* gene in a way that eliminates the ligand-binding domain of the ER. These chimeric proteins possess constitutive, ligand-independent activity that leads to increased cell growth, proliferation and initiation of the metastatic cascade. A growing body of research suggests that liquid-liquid phase separation (LLPS) may be a mechanism driving aberrant gene transcription and genome organisation. Research in the Toseland Lab has revealed that *ESR1* fusion proteins recurrently form nuclear condensates in various breast cancer cell lines. Considering the transcriptional role of ER, we hypothesise that *ESR1* fusion condensates represent transcriptional hubs that drive gene expression programmes associated with breast cancer. To test this, the ER+ epithelial cell line MCF7 was transiently transfected with the patient-derived fusions *ESR1-DAB2* and *ESR1-SOX9* to test their LLPS capacity and contribution to the cancer phenotype. We have thus far demonstrated that *ESR1* fusion condensates display various LLPS characteristics including spherical morphology, reversibility, and fusion upon contact. Furthermore, these hubs are associated with elevated levels of RNA Polymerase II, indicating enhanced transcriptional activity. Additionally, their localisation in areas of active transcription and the altered levels of chromatin condensation provide evidence for chromatin reorganisation. Unravelling condensate formation, dynamics, and their functional relevance should provide new insights on oncofusion-driven cancers and may open new therapeutic avenues for treating advanced, therapy-resistant breast cancers.

SpT-06-1
Chemotactic interactions drive migration of membraneless active droplets

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In nature, chemotactic interactions are ubiquitous and play a critical role in driving the collective behaviour of living organisms. Reproducing these interactions *in vitro* is still a paramount challenge due to the complexity of mimicking and controlling cellular features, such as metabolic density, cytosolic macromolecular crowding and cellular migration, on a microorganism size scale. Here, we generate enzymatically active cell-size droplets able to move freely and, by following a chemical gradient, able to interact with the surrounding droplets in a collective manner. The enzyme within the droplets generates a pH gradient that extends outside the edge of the droplets. We discovered that the external pH gradient triggers droplet migration and controls its directionality, which is selectively towards the neighbouring droplets. Hence, by changing the enzyme activity inside the droplet we tuned the droplet migration speed. Further, we showed that these cellular-like features can facilitate the reconstitution of a simple and linear protometabolic pathway with improved overall activity. Our work suggests that simple and stable membraneless droplets can be applied to reproduce complex biological phenomena opening new perspectives as bioinspired materials and synthetic biology tools. * The authors marked with an asterisk equally contributed to the work.

Redox Biochemistry

SpT-19-1
Exploring the redox properties of flavocytochrome c from *Shewanella* sp. DSM9451 – Comparison between pathogenic and non-pathogenic *Shewanella* species

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Biotechnological applications are considered a viable solution for several environmental challenges. *Shewanella* are ubiquitous Gram-negative bacteria, that are capable of reducing several inorganic and organic compounds by a process designated extracellular electron transfer (EET). This capacity allows the development of several biotechnological applications, such as microbial fuel cells. The most studied organism for this purpose is *Shewanella oneidensis* MR-1. However, there are *Shewanella* species that were isolated from clinical samples and their EET wasn't explored. Flavocytochrome *c* (FccA) is a tetraheme cytochrome *c* involved in the EET process in *Shewanella* spp., with a key role in controlling the flux of electron transfer within the periplasmatic space in anaerobic conditions towards EET or fumarate reduction. The FccA from *S. oneidensis* MR-1 and *Shewanella frigidimarina* NCIMB400, two environmental species, were already characterized, showing distinct properties. To compare the redox properties of FccA from environmental and clinical isolates, the FccA from *Shewanella* sp. DSM9451, isolated from the cerebrospinal fluid of a 1-year-old child, was purified from the native organism grown aerobically. The structure was determined by X-ray crystallography and showed the same overall fold. Paramagnetic ^{1}H-NOESY and ^{1}H-^{13}C HMQC NMR spectra were used to discriminate spectral fingerprints of the individual hemes and determine their order of reduction. Protein film voltammetry was used to characterize the electrochemical properties of the protein. The results revealed that the order of oxidation changes in all the three proteins studied, but, in contrast to the other FccA, the hemes of FccA from *Shewanella* sp. DSM9451 revealed a redox-Bohr effect and a different orientation of the heme axial ligands. These differences will be discussed in the context of the different ecosystems where the host organisms were isolated.

SpT-19-2
Exploring the antimicrobial potential of natural compounds inducing oxidative stress on *Rhodococcus fascians*

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Mycoredoxins (Mrxs), a type of thiol disulphide oxidoreductases, have been demonstrated to play a crucial role in redox homeostasis in different species of *Actinobacteria*. Our research group previously showed that mycoredoxins are very important for maintaining redox homeostasis during macrophage infection in the animal pathogen *Rhodococcus equi* [Previously published in: Mourenza Á et al. (2019) Antibiotics (Basel) 8(11), 558]. Subsequently, to explore the implications of mycoredoxins in other actinobacterial phytopathogens, genes coding for mycoredoxins were identified in *Rhodococcus fascians* through gene homology. Using optimized protocols for unmarked gene deletion, we generated several *R. fascians* mutants lacking genes coding for mycoredoxins (*mrx*). Interestingly, mutants lacking the three identified genes displayed phenotypic differences under oxidative stress conditions compared to the wild-type strain. Capitalizing on this finding, both strains were utilized to identify natural compounds that potentially exert their antimicrobial activity by inducing oxidative stress. We screened a commercial library comprising more than 3000 natural compounds (MedChemExpress). The identified compounds ideally have the potential to replace conventional pesticides in a sustainable and environmentally friendly manner. Furthermore, we are not only investigating the individual activity of the compounds but also exploring their efficiency in combinations, seeking synergies. In this study, we present the antimicrobial activity of oxidative stress-inducing natural compounds alone and in combination, tested against different *R. fascians* mycoredoxin mutants. As a result, we discern the antimicrobial potential of the selected natural compounds, and concurrently, the distinct role of individual mycoredoxins in regulating intracellular oxidative stress in *R. fascians* and potentially in other phytopathogens. * The authors marked with an asterisk equally contributed to the work.

Monday 1 July
13:10–13:30, Blue Room

Molecular Mechanisms of Functional Foods and Their Bioactive Compounds

SpT-14-2
Opuntia ficus-indica fruit ameliorates glucose dysmetabolism in a murine model of metabolic syndrome: a comparative study with chromium picolinate

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Opuntia ficus-indica fruit (OFF) contains relevant amounts of fibres, prebiotics and phytochemicals with anti-oxidative and anti-inflammatory properties as previously published in: Silva MA et al. (2021) Molecules 26, 951. In the light of the self-feeding, vicious cycle between low-grade inflammation, oxidative stress and insulin resistance (IR), we here explored whether and how OFF administration counteracts IR generated after 10 weeks in high-fat diet (HFD) fed mice as previously published in: Terzo S et al. (2022) Antioxidants 11, 80. Due to the limited seasonal availability and short shelf-life of OFF, we employed a lyophilised OFF, given per os at 40 mg/kg/day for 4 weeks, alone or with chromium picolinate (CrPi), one of the most used supplements against IR, at 2.6 μg/kg/day. Our results show that

OFF ameliorated HFD-induced IR to the same extent of CrPi, as evaluated by HOMA-IR, glucose and insulin tolerance tests. Furthermore, OFF co-administrated with CrPi does not potentiate its effects on the above-mentioned parameters. From a mechanistic perspective, OFF-mediated anti-dysmetabolic effects were associated with the reduction of hepatic oxidative stress and inflammation. Indeed, OFF treatment inhibited the HFD-induced production of reactive oxygen species and malondialdehyde, evaluated by fluorimetric assays. Coherently, western blotting analysis (WB) revealed an increase of Nfr-2 nuclear translocation and SOD-2 expression levels by OFF. Accordingly, OFF administration counteracted inflammation evaluated, by WB, as NF-kB nuclear translocation and i-NOS and COX-2 expression levels. Relevantly, the OFF dose employed, if extrapolated from mice to humans, could be administrable as food supplement, while that of CrPi is the one contained in multimineral supplements. As a whole, our results show that OFF counteracts IR by modulating the expression of crucial proteins involved in the oxidative stress-dependent, inflammatory reaction underlying the HFD-induced IR.

SpT-14-1
The study of the interaction between ampelopsin, myricetin, and their sulfate conjugates and multispecific organic anion-transporting polypeptides (OATP1B1, OATP2B1)

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Organic anion-transporting polypeptides (OATPs) are membrane transporters that facilitate the cellular uptake of various organic substances. Among them, OATP1B1 is a key uptake transporter in hepatic detoxification. Hence, OATP1B1 inhibition through food-drug and drug-drug interactions could lead to adverse effects. Additionally, the ubiquitously expressed OATP2B1 is essential for the intestinal absorption of many orally administered drugs. Flavonoids, known for their beneficial biological effects, such as ampelopsin and myricetin are commonly present in various foods and beverages. Yet, except for myricetin, no data have been reported about the potential inhibitory effect of these flavonoids on OATPs. Therefore, in the current study, we investigated the interactions between OATP1B1 and OATP2B1 and ampelopsin, myricetin, and their sulfate metabolites using the fluorescence-based indirect assay developed earlier in our laboratory. Our research revealed that most of the flavonoids tested are strong inhibitors of OATP1B1 and OATP2B1 transport activity with low micromolar or even nanomolar IC50 values. In addition, we investigated potential flavonoid uptake with the help of 2-aminoethoxydiphenyl borate (2-APB), a cell-permeable molecule that, upon forming a complex with flavonoids, leads to enhanced fluorescence and allows fluorescence-based detection of certain flavonoids. Based on this method, we identify myricetin-3′-sulfate as a transported substrate of OATP1B1 and OATP2B1 for the first time. Our findings show that not only the original flavonoids but also some of their conjugates can interact with OATPs. Consequently, high intake of ampelopsin, myricetin, and their sulfate metabolites may disrupt the pharmacokinetic profiles of OATP substrate medicines. Furthermore, OATP1B1 and OATP2B1 can promote transcellular movement of the otherwise poorly cell-permeable myricetin-3′-sulfate.

Nutraceuticals Effects on Cell Metabolism and Chronic Diseases

SpT-15-1
In vitro effects of resveratrol supplementation of plasma from patients affected by Alzheimer's disease on cultured human endothelial cells

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An increasing body of evidence suggests that Alzheimer's disease (AD) is associated with an endothelial dysfunction. Given the absence of absolute cure for AD, the aim of the present study was to evaluate the effects of a short incubation with plasma from AD patients on cultured human aortic endothelial cells (HAECs) and to repeat the determinations following the supplementation of plasma with resveratrol (RSV). In particular, nitric oxide (NO) and peroxynitrite production, superoxide dismutase (SOD) and Na+/K+ -ATPase activities, membrane fluidity and thiobarbituric acid-reactive substances (TBARS) levels were analyzed. Our results show a decrease in NO levels, enzymatic activities and membrane fluidity and an enhanced peroxynitrite and TBARS production in HAECs exposed to AD plasma, compared to cells incubated with plasma from healthy subjects. The exposure to RSV supplemented plasma, conversely, lowers ROS levels and stimulates the activity of the antioxidant enzyme SOD. Furthermore, resveratrol may improve the endothelial function by in-creasing membrane fluidity and Na+,K+ -ATPase activity, and ameliorate cerebral perfusion through an enhanced NO formation and bioavailability. In conclusion, our study suggests the use of AD therapies based on dietary natural compounds able to reduce oxidative stress and prevent or reverse vascular endothelium dysfunction.

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Impacts of Climate Change on Nutrition and Health

SpT-16-2
Biochemical aspects of food sustainability: molecular basis of bread improvement through enrichment with sprouted legumes flour

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Legumes offer balanced nutritional profile, but presence of anti-nutrients and digestibility issues hinder their staple food status. Biotechnological approaches, including germination, aim to modify the macromolecular composition of legumes, lowering the levels of anti-nutritional factors and modifying relevant macromolecular traits. After 72-hour, germinated bean flour showed a marked decrease in anti-nutritional factors (phytates and protease inhibitors) and an increased protein hydrolysis. The flour from germinated cowpea was used to prepare bread up to a 25% addition to wheat flour, with non-sprouted bean flour as a control. In addition to protein profiling and quantitative analysis of anti-nutritional factors, bread samples underwent *in vitro* static digestion following the INFOGEST protocol, with protein hydrolysis monitored at the end of the gastric phase, as well as at the middle and final steps of the intestinal phase. Protein profiling suggests the absence of interactions between wheat proteins and cowpea proteins regardless of the germination step. Also, cowpea proteins appeared to be thoroughly degraded to small-sized peptides – after duodenal digestion –in both bread types. Peptide profiling is currently under way, in order to pinpoint the possible formation of bioactive species. Finally, from a nutritional standpoint, bread containing sprouted bean flour had slightly higher levels of resistant starch than both the non-enriched bread and the bread enriched with non-sprouted cowpea flour. This investigation is partially supported by National Recovery and Resilience Plan (NRRP), Mission4 Component 2 Investment 1.3 -Call for tender No. 341 of 15/03/2022 of Italian Ministry of University and Research funded by the European Union–NextGenerationEU, in the frame of the project: Research and innovation network on food and nutrition Sustainability, Safety and Security (ON Foods). * The authors marked with an asterisk equally contributed to the work.

SpT-16-1
Cadmium-induced growth and physiological disruptions in wheat

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The escalating environmental concern over heavy metal pollution necessitates a comprehensive understanding of its impact on vital crops like wheat. This study investigates the influence of cadmium (Cd) on wheat growth, addressing a critical gap in our understanding of heavy metal-induced phytotoxicity. Employing a controlled greenhouse environment, wheat plants were subjected to varying concentrations of Cd through soil application. Meticulous monitoring of growth and physiological parameters revealed a negative induced impact by Cd exposure. The results reveal a dose-dependent reduction in wheat growth metrics, indicating a discernible inhibitory effect of Cd on both above-ground and below-ground biomass, which was complemented by in-depth biochemical analyses to unravel molecular changes induced by Cd exposure Further analysis highlights alterations in physiological processes, such as photosynthesis and nutrient uptake, underscoring the intricate mechanisms underlying Cd-induced toxicity in wheat. These findings provide valuable insights into the potential risks associated with Cd contamination in agricultural soils and emphasize the need for sustainable management practices to mitigate adverse effects on crop productivity. In conclusion, this study highlights the Cd dependent-dose adverse alterations on wheat growth and physiological performances and underscores the urgency of developing strategies to safeguard wheat cultivation from Cd-induced stress, ensuring global food security in the face of mounting environmental challenges.

Other Topics

SpT-35-3
Substrate recognition mechanisms and cleavage modes by human separase

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Accurate propagation of the genetic material is key for successful cell division – the process in which one parental cell divides into two identical daughter cells. To do so, chromosomes are duplicated, and the resulting sister chromatids are co-transcriptionally entrapped in a ring-shaped protein complex named cohesin. Dissolution of the cohesin ring initiates the segregation of the paired sister chromatids, and the resulting chromosomes migrate towards the opposite cell poles. The cohesin ring (specifically the subunit SCC1/RAD21) is cleaved by an evolutionarily conserved cysteine protease called separase. Separase's task is to initiate metaphase-to-anaphase transition in mitosis. Because of its central role in cell division, it has been extensively studied and, not surprisingly, has been found to be overexpressed in various human cancer types. It is therefore only consequent that separase has emerged as an interesting candidate for structure-based drug design studies. My project aims at understanding the molecular mechanisms of SCC1 substrate binding to human separase and to define its consequent cleavage modes. To address these open research questions, we made use of cryo-EM and biochemical assays. In specific, we investigated the different separase cleavage sites of SCC1 through gel-based assays, anisotropy and real time fluorescent-based assays and correlate these results with separase-substrate cryoEM structures. The aim of the project is to discover how this crucial protein performs the irreversible and decisive action of substrate cleavage. Besides the fundamental nature of this biological process, we aim at developing novel anti-cancer drugs that target separase cleavage activity.

Monday 1 July
13:10–13:30, Yellow Room

Cancer Biochemistry

SpT-27-3
Semaphorin3A switches cancer associated fibroblasts towards an anti-tumor phenotype in pancreatic ductal adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) is characterized by a typical tumor microenvironment (TME) ruled by abundant desmoplastic stroma and overactivated cancer-associated fibroblasts (CAFs). To date, the highest challenges in PDAC are to identify novel therapeutic targets in the TME in order to efficiently hamper tumor growth. We investigated the changes concerning the TME of a PDAC mouse model upon semaphorin3A (Sema3A) treatment. It has been shown that, following binding to its receptor PlexinA4, Sema3A, regulates immunity, angiogenesis and tumor progression. We performed single cell RNA-sequencing (scRNA-seq) and spatial transcriptomics (ST) to identify the molecular changes in the PDAC TME induced by a mutated form of Sema3A (mut-Sema3A) able to bind PlexinA4 with high affinity. We observed that mut-Sema3A rearranged the percentage of pro-tumoral myofibroblastic, inflammatory and antigen-presenting CAFs reprogramming them to a novel CAF subtype with cancer restraining features. We therefore established a novel gene signature defining this unconventional class, called sema-associated fibroblasts (SemAFs), that was enriched for genes such as PDGFRA, ISLR, MMP2, CYGB, THBS2, CDH11. Many of them, such as ISLR, coding for the meflin protein, have been correlated with improved survival and prognosis of PDAC patients. Notably, mut-Sema3A treatment of *ex-vivo* isolated and activated fibroblasts, enhanced the expression of several SemAF genes, and inhibited CAFs chemo-invasion potential. Moreover, mut-Sema3A enhanced plexinA4 expression, suggesting a positive autocrine loop feeding the upregulation of its own receptor. Remarkably, the SemAF genetic profile, and in particular ISLR and plexinA4, have been also identified by ST, in mut-Sema3A-treated samples. These data suggest that Sema3A is a powerful agent able of reshaping the CAF populations and probably representing a good candidate to be coupled with pre-existing therapies to more efficiently hamper PDAC progression.

Cancer and Metabolism

SpT-26-5
Systematic identification of druggable PKA substrates involved in colon cancer progression

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Deregulation of G protein coupled receptor (GPCR) controlled kinase pathways contributes to the development and progression of cancer. Examples are activating mutations in the AC-stimulatory Gαs proteins (GNAS), which occur in 4,2% of all tumors. These lead to constitutive downstream activation of the cAMP-dependent protein kinase A (PKA) pathway. In order to identify druggable PKA-effector proteins, we determined the phosphoproteomic composition of macromolecular PKA complexes from a collection of Gαs-mutated cancer cells and human glioblastoma biopsies. Using a substractive phospho-proteomic approach, we identified a multitude of proliferation-relevant PKA substrates and selected two druggable and cancer-implicated candidates for closer examination, namely the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) and Tripartite motif 28 (TRIM28) respectively. PFKFB3 is a key modulator of glycolysis, implicated in maintaining cancer cell metabolism. We showed that nuclear PFKFB3 acts as a PKA substrate. Moreover, small molecule mediated inhibition of PFKFB3 reduced proliferation of Gαs-mutated colon cancer cells. Further, besides quantitative metabolite analyses of the cellular glycolytic flux, we revealed a nuclear function of PFKFB3. Using the RNAseq technology TUCseq we recorded immediate transcriptome changes upon PFKFB3 inhibition. Thus, we gained evidence for a possible link of PFKFB3 to p53 function in the studied colon cancer cell setting. TRIM28, the second novel PKA substrate, supports tumor progession through ubiquitination of the tumor suppressor p53. We investigated changes in protein stability of known anti-oncogenic TRIM28 ubiquitination substrates upon kinase activation. Currently, we explore a polypharmacology approach by inhibiting nuclear TRIM28 and PFKFB3 functions which may hamper proliferation of selected colon cancer cells. *The authors marked with an asterisk equally contributed to the work.

SpT-26-2
Mechanisms regulating lipid storage in breast cancer cells

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Alteration of lipid metabolism is a hallmark of breast cancer (BC). However, the underlying mechanisms are still largely elusive. We performed screenings for metabolic modifiers in BC cells focusing on a gene family encoding forty-five membrane

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trafficking proteins. In parallel, the prognostic value of these genes in BC was assessed by interrogating the Metabric dataset. Among the genes identified in the screenings, whose upregulation also predicted worse prognosis in BC, we selected TBC1D7 for high-resolution studies. TBC1D7 is highly expressed in glucose-avid Triple-negative tumors (TNBC) and correlates with reduced patient survival in univariate and multivariate analyses in the TNBC patient subpopulation. The best characterized function of TBC1D17 is to bind and to stabilize the TSC1/TSC2 complex, which negatively regulates mTORC1. However, additional TSC-independent functions have also been proposed. By performing metabolic flux analyses we found that cells overexpressing TBC1D7, or a TBC1D7 point mutant that is impaired in its binding to TSC1, elevate glucose metabolism to generate fatty acids, while glutamine is mostly employed to sustain the Tricarboxylic Acid cycle. Accordingly, in the overexpressing cells the size of lipid droplets is increased and inhibition of the lipid enzymes ACSL3 and SCD1 impairs their formation suggesting that TBC1D7 promotes *de novo* lipogenesis. Moreover, overexpression of TBC1D7, or of its mutant, stimulates the growth of tumor cell spheroids, when cultured in 3D Matrigel, a proneoplastic feature that is blocked by treatment with an SCD1 inhibitor. Finally, we found that TBC1D7 inhibits lipophagy further contributing to the accumulation of intracellular lipids. Taken together, these data suggest a novel mechanism that integrates lipogenesis and lipid consumption in BC cells.

SpT-26-6
Hexosamine biosynthetic pathway inhibition cooperates with gemcitabine inducing *in vitro* and *in vivo* pancreatic cancer regression by enhancing DNA damage

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Pancreatic cancer (PC) is the seventh most common cause of death due to oncological diseases. Nearly 80% of patients are diagnosed at advanced stages and chemotherapy, based on gemcitabine (GEM) remains the main treatment. However, PC develops chemoresistance to GEM so an alternative therapeutic regimen should be investigated combining GEM with other drugs. PC shows metabolic alterations based on the increased glucose consumption to fuel glycolysis. This leads to an upregulation of the hexosamine biosynthetic pathway (HBP) to produce, UDP-N-acetylglucosamine necessary for protein glycosylation, a nutrient- and stress-responsive post-translational modification. Previous results establish that FR054, a small inhibitor of PGM3, a HBP enzyme, leads to growth arrest in PC cells *in vitro*, highlighting the role of HBP in promoting PC survival. *In vivo*, GEM and FR054 administration is well tolerated and suppresses almost completely tumor growth in xenograft and PDX mice. The activity of some metabolic pathways can influence the DNA damage repair (DDR) by regulating substrates' availability required for the repair process and the function of its players. In this work we demonstrate that FR054 enhances GEM's efficacy. Indeed, their combination promotes apoptosis through augmentation of the DNA damage and a significant change in the phosphorylation status of several proteins involved in DDR response. We demonstrate that homologous recombination is reduced. This is an important achievement since this DDR mechanism is correlated to chemoresistance. In addition, some proteins directly involved in DNA damage response are O-glycosylated, as shown by proteomics analysis. In conclusion, the study sheds light on the correlation between proteins glycosylation and DDR, suggesting a potential role of HBP in regulating cancer progression. Supported by grants from MUR (# 2022ZBZFX3 PRIN-2022 Unione Europea - NextGenerationEU) to FC and DS, Fondo di Ateneo-Quota competitiva 2021, FAR 2022 to FC.

Cancer Biochemistry

SpT-27-1
Tissue metabolite composition driving metastatic organotropism in prostate cancer: the role of asparagine

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Prostate cancer (PC) is the second-leading cause of cancer death in men, mainly caused by metastatic disease. PC preferentially metastasizes to bone and lung. Emerging evidence underlines the crucial role of the distant organs' microenvironment in influencing metastatic cell distribution and tumour-reforming ability. Here, we investigated the metabolic factors affecting metastatic organotropism of PC. The specific organ metabolite composition was analyzed by gas-mass spectrometry (GC-MS) on tissues collected from athymic healthy mice, identifying asparagine as one of the most common enriched metabolites in bone and lung. Asparagine is critical in tumour progression, supporting cell proliferation under metabolic stresses. The relevance of asparagine in metastatic colonization of PC cells was investigated *in vitro* by comparing normally adherent cells (2D cultures) and cells grown in non-adherent conditions (3D cultures) as a model mimicking loss of extracellular support occurring during early stages of metastasis. We observed that asparagine exogenous supplementation increases 3D cell growth, while it does not enhance 2D cell proliferation nor increase cell migratory potential, indicating a specific role for asparagine in metastatic niche colonization. 3D cells display decreased mitochondrial oxidative metabolism and reactive oxygen species (ROS) accumulation. Mitochondrial ROS scavenging enhances 3D cell clustering, indicating the importance of limiting ROS during cell clustering. In addition, providing exogenous asparagine is sufficient to raise the oxygen consumption rate of 3D cells under metabolic stressful conditions. Asparagine supplementation in 3D cultures also increases the mammalian target of rapamycin complex 1 (mTORC1)-pathway activation and enhances protein synthesis. Together, these data advise that lowering asparagine availability in the bone and lung districts represents a promising strategy to target metastatic dissemination of PC cells. * The authors marked with an asterisk equally contributed to the work.

Cancer and Metabolism

SpT-26-4
A mass spectrometry imaging-based multiomics approach to map the tumour-immune landscape of clear cell renal cell carcinoma and gain insights into resistance to immunotherapy

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The advent of immune checkpoint inhibitors (ICIs) has revolutionised cancer treatment, but despite this significant advancement, the efficacy of ICIs is confined to inherently immunogenic cancers, and effectiveness of response varies from patient to patient. Many hypotheses regarding the primary or acquired resistance mechanisms to ICIs revolve around the tumour microenvironment (TME), which involves the presence on tumor infiltrating lymphocytes (TILs), as well as further heterogeneous sets of cell populations, whose presence is known to correlate with tumour response to treatment. In this context, the opportunity to investigate which immune cells characterise this environment, along with the ability to delve deeper into the molecular and spatial interactions which occur in their native spatial context, could be crucial in comprehending the mechanisms that influence the response to ICI treatment. To achieve this goal, a MALDI-HiPLEX-IHC based approach was employed to explore the TME of clear cell renal cell carcinoma (ccRCC) and leverage this multi-omics workflow for the for the subsequent mapping of the lipidome, multiplexed imaging of targeted immune cells and lastly proteome on a single formalin fixed paraffin embedded tissue section of patient-derived ccRCC. This exploration allowed the tissue distribution of TILs and further immune cells to be mapped and to determine their interface of interaction. Moreover, by correlating MALDI data with LC-MS identifications, it underlined which aberrant molecular mechanisms may be involved in immune-tumour cell communication. Concurrently, advanced co-culture cellular models were established and treated with ICIs to confirm the hypothesis formulated based on *ex-vivo* umour resections, and provide deeper insights into the factors that play a role in therapy resistance. Advancements in this realm can provide insights into the metabolic pathways steering the immunogenic environment and influencing resistance to immunotherapy. *The authors marked with an asterisk equally contributed to the work.

Tuesday 2 July
13:10–13:30, Silver Room

Marine Biochemistry

SpT-11-1
Evolution of marine contagious cancers in cockles characterized by genomic instability

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Transmissible cancers are malignant cell clones that spread among individuals through transfer of living cancer cells. Several such cancers, collectively known as bivalve transmissible neoplasia (BTN), are known to cause leukaemia-like disease in marine molluscs such as the common cockle which inhabits the Atlantic coasts of Europe and Africa. To investigate the origin and evolution of contagious cancers in common cockles, we collected 6854 specimens and diagnosed 390 cases of BTN. We then generated a reference genome and assessed genomic variation in the genomes of 61 BTN tumours. Tumour-specific variants confirmed the existence of 2 cockle BTN lineages with independent clonal origins, and gene expression patterns supported their status as haemocyte-derived blood cancers. Mitogenomes revealed several mitochondrial capture events in BTN, as well as co-infection of cockles by different tumour lineages. Cytogenetic and copy number analyses uncovered genomes marked by pervasive instability and karyotypic plasticity. Whole-genome duplication, amplification of oncogenes *CCND3, MDM2* and *MYC*, and deletion gene *MGMT*, are likely drivers of BTN evolution. Characterization of satellite DNA identified elements that are absent from tumours despite vast expansions in the cockle germ line, suggesting ancient BTN clonal origins. Our study illuminates the evolution of transmissible cancers under the sea and reveals indefinite tolerance of extreme instability in neoplastic genomes.

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SpT-11-2
Cubosome drug nanocarriers loaded with algae extract for photodynamic therapy in pancreatic cancer treatment

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Pancreatic cancer, an extremely deadly malignancy, presents significant challenges in treatment, mainly because of its complex tumor microenvironment, difficult to bypass for drug molecules. On the other hand, the marine environment is full of biomass rich in compounds that may act as such anticancer drugs. *Ulva rigida* is an example of an alga containing photoactive chlorophylls, which, when combined with photodynamic therapy (PDT) and nanotechnology, constitute a potentially effective drug for pancreatic cancer. The use of novel liquid-crystalline drug nanocarriers (cubosomes) allows the protection of encapsulated molecules against degradation in tumor microenvironment, as well as against aggregation related to low solubility of the extracted pigments due to their hydrophobic nature. In order to evaluate this idea, the pigments were extracted from *U. rigida* with green microwave-assisted extraction method, then encapsulated in cubosomes and studied in series of biological experiments for the assessment of their biocompatibility, and biological activity. The comparative physicochemical characteristics of two types of cubosome-based nanoformulations along in tests for the generation of reactive oxygen species (ROS), MTT cytotoxicity, flow cytometry and bioimaging on pancreatic cancer cell lines (BxPC-3), will be presented in this work. Moreover, the presented results will demonstrate the effectiveness in PDT for pancreatic cancer of the obtained novel more biocompatible cubosome nanoformulations loaded with chlorophylls extracted in a green way from algae biomass. Bioimaging results show a particular advantage of the encapsulated drug compared to its pure form. We believe that our work will contribute to the development of science in the field of drug nanocarriers, treatment of pancreatic cancer, and the management of marine biomass. The research project was supported by the Department of Chemical and Geological Sciences, University of Cagliari.

Cutting edge approaches for sustainable and environmental biotechnology

SpT-07-1
A multi-omic study of nicotine catabolism in *Paenarthrobacter nicotinovorans*

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Paenarthrobacter nicotinovorans is a soil actinobacterium that degrades nicotine using the pyridine pathway encoded by the pAO1 catabolic megaplasmid. Only half of the 40 putative *nic*-genes have experimentally proven functions. The strain is a potential key biological agent for the degradation of nicotine from tobacco waste and contaminated natural resources. Since the bacterium can convert toxic nicotine into non-toxic derivatives, it could be used as a green chemicals factory to produce valuable compounds such as 6-hydroxy-L-nicotine, γ-aminobutyric acid, methylamine, succinic acid, and α-ketoglutaric acid. The interplay between the general metabolism of the bacterial cell and the nicotine degradation pathway encoded by pAO1 is still unexplored. Further knowledge of the mechanisms which regulate nicotine catabolism would facilitate the biotechnological applications of *P. nicotinovorans*. This study aims to perform the first transcriptomic analysis of nicotine catabolism in this strain and to integrate it with the already available proteomic data (ProteomeXchange: PXD008756). The bacterium was grown in the absence and presence of nicotine. Cultures were sampled at three key time points of nicotine catabolism: start of exponential phase, late exponential phase, and late stationary phase. Direct-RNA long-read sequencing was performed using the MinION Mk1B device coupled to a Flongle adapter. Raw data was base-called using Guppy_6.3.2 with high accuracy. The generated transcriptomic data (NCBI GEO: GSE240220) was analysed using nf-core/nanoseq v3.1.0, and DE genes at the three key time points were evaluated. We identified 8 pAO1 genes and 10 chromosomal genes (padj<0.1) that were previously unknown as having nicotine-related expression. The transcriptomic data is currently being integrated with the proteomic dataset using Pathview and SBGNview for pathway enrichment analysis. Finally, this study will provide the first multi-omic study of nicotine catabolism in bacteria. *The authors marked with an asterisk equally contributed to the work.

SpT-07-4
Bifidobacterium animalis subsp. *lactis* HN019 live probiotics and postbiotics: Production strategies and bioactivity evaluation for potential therapeutic properties

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B. animalis subsp. *lactis* HN019 is a commercially available well-characterized probiotic with documented effects on human health, such as the ability to enhance the immune function and to balance the intestinal microbiome. Therefore, optimizing the manufacturing process to increase biomass yields and viability is currently of interest. Moreover, the growing demand of vegan grade food supplements further addresses scientific research towards the design of alternative growth media for lactic acid bacteria (probiotics). Besides the established use of live probiotic cells, alternative supplements like non-viable cells and/or probiotic derived bioactive molecules, indicated as postbiotics, might be considered as potential next generation biotherapeutics. In fact, the latter may present lower variability during storage, as well as easier production processes and scale-up. In this work, to better characterize the physiology of *B. lactis* HN019, medium design together with different fermentation strategies (batch, fed-batch and *in situ* product removal) on lab-scale bioreactors were

combined to increase the titer of viable cells up to $2.9 \pm 0.1 \times 10^{10}$. On the other hand, exopolysaccharides (EPS) were isolated from the fermentation broth, characterized, and tested, in comparison to live cells and whole heat inactivated broth for the first time up to date, in *in vitro* biological assays, using differentiated CaCo-2 cells challenged by LPS of *S. minnesota*. Interestingly, all samples demonstrated immune-modulating properties by downregulating the expression of TLR-4 and NF-kB in LPS challenged cells. In addition, the proposed treatments, and EPS in particular, showed the ability to restore the integrity of the tight junctions by up-regulating the expression of zonulin.

Enzyme Engineering and Biotechnology

SpT-20-1
Discovery and engineering of improved biocatalysts for the synthesis of biodegradable plastic copolymers (BioCat4BioPol)

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Inadequate waste management practices have rendered petroleum-based plastics a global problem. Their accumulation in the environment and their gradual breakdown into micro- and nano-plastics represents a global threat. For this reason, research aims at the production of new polymers derived from renewable sources and new polymerization strategies. Bioplastics, like polylactic acid (PLA) and poly-hydroxy-alkanoates (PHAs), seem to be a viable solution to mitigate the impact of plastic pollution worldwide. However, both polymers present disadvantages that reduce their widespread commercial adoption: PLA has excellent transparency but has demonstrated inefficient degradation in the environment; on the other hand, PHAs are highly biodegradable but present less resistance and stiffness. For these reasons, the ability to copolymerize different monomers may be the key to obtaining bioplastic with desirable characteristics. [P(LA-co-3HB)] copolymers have been designed to exhibit enhanced properties, both in terms of mechanical strength and biodegradability. To reach this target, recombinant *Escherichia coli* that expresses lactyl-CoA (LA-CoA)-polymerizing enzymes (LPE) have been employed to prepare [P(LA-co-3HB)] copolymers. This goal has never been achieved enzymatically (*in vitro*) or with microbial cell factories (*in vivo*). The recently launched BioCat4BioPol project has the objective of designing hybrid P3HB-PLA polymerases that can perform the elongation of alternative copolymers in a one-pot/two-enzyme system. For this, we will exploit advanced artificial intelligence (AI) methods to identify target enzymes and predict the mutation that can improve the catalytic features of these biocatalysts, thus demonstrating that enzymatic synthesis of bioplastic copolymers using a rational approach can become attainable and implementable.

SpT-20-2
Unlocking the potential of cohesin-dockerin interactions: mini-cellulosomal complexes from *Clostridium thermocellum* and their impact on enzymatic biomass degradation

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Anaerobic bacteria use ordered protein–protein interactions to anchor cellulose-degrading protein nano-machines (cellulosomes) to the cell surface, to facilitate uptake of sugars released through biomass degradation. Cellulases and hemi-cellulases are assembled onto a non-catalytic protein subunit (scaffoldin) through interactions between enzyme-bearing dockerins and cohesin domains upon the scaffoldin in *Clostridium thermocellum*. We produced a mini-scaffoldin, individual cohesins and several (natural) enzyme-dockerin fusions to create multi-enzyme complexes through cohesin–dockerin interactions. Interactions of selected biomass-degrading (dockerin-bearing) enzymes with individual cohesin domains were analysed through SEC, native PAGE, thermophoresis and mass spectrometry, and structural studies of all protein components were performed through DSC, CD and fluorescence, to assess protein thermal and chemical stabilities. Relative cohesin–dockerin affinities and partner preferences emerged from these studies, with insights for cellulose function. In other studies, since different linkers needed to be used for producing protein fusions, we examined various linkers in respect of proteolytic stability by producing engineered constructs joining a cohesin (Coh2) and an enzyme (the BSX Xylanase). We found that short flexible linkers resist proteolysis by facilitating rapid and independent protein domain motions that, in turn, facilitate steric protection of linkers from proteolysis, by acting like nunchucks. We are using engineered cellulosomal proteins for PET degradation, as an immobilizing platform for esterases. By fusing dockerins with PET-degrading enzymes and utilizing mini-scaffoldin, we aim to enhance the efficiency of PET degradation. Our research contributes to enzyme-based solutions for key environmental and sustainability challenges, showcasing the potential of protein engineering and bioinspired design to create enzymatic systems for industrial applications.

Tuesday 2 July
13:10–13:30, Red Room

Long ncRNA and microRNA Networks

SpT-04-1
Type 2 transglutaminase inhibition deregulates MIR210HG lncRNA expression impacting hypoxia-related genes in triple-negative breast cancer cell line

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Type 2 transglutaminase (TG2) is a ubiquitously expressed protein involved in several physiological and pathological processes,

strongly associated with cancer. Among these several roles attributed to TG2, its involvement as a gene expression modulator has also been described, through serotonylation of histones [Previously published in Farrelly LA et al. (2019) Nature 567, 535-539], as well as the cross-linking of transcription factors [Previously published in Farrelly LA et al. (2019) Nature 567, 535-539]. This study aimed to inhibit TG2 using the AA9 inhibitor, followed by a bulk RNA-sequencing, to highlight alterations in lncRNA and canonical gene expression of MDA-MB-436 triple-negative breast cancer (TNBC) cells. MIR210HG ($log2FC = -1.56$) has been identified as significantly down-regulated upon treatment of 24 h. MIR210HG is an antisense long non-coding RNA (lncRNA) that has been associated with several types of cancers, particularly with TNBC, in which it enhances the Warburg's effect [Previously published in Du Y et al. (2020) Front Oncol 10:580176]. The down-regulation of MIR210HG, which acts as a miRNA sponge to the miR-1226-3p, allows the latter to target HIF-1α, leading to the down-regulation of genes encoding for glycolytic enzyme in MDA-MB-436 cells. We assessed their expression along with other key hypoxia-related markers, under the control of HIF-1α such as the metabolic enzymes ALDOA, ENO, LDHA, ACOT, PGAM1, and TPI1, angiogenesis-related genes like VEGF and ADM, the cyclin-dependent kinase inhibitor CDKN2A, or structural protein such as P4HA1 and TUBB. All the mentioned markers resulted down-regulated by AA9. These data agree with the effects on cancer metabolism already demonstrated, correlating with decreasing cell motility and reduced oncogenicity of cancer cells. In this context, we underline the relevance of our results, highlighting the role of TG2 in the regulation of ncRNAs expression, which has never been investigated so far.

SpT-04-2
RNA regulates transcription via hybrid-triplex formation

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Triplexes are triple-helical geometries comprising all-DNA, all-RNA, or mixed molecules. They are formed within the same strand or comprise two or three individual strands with intermolecular hydrogen bonds across the nucleobases. Such bonds expand the common Watson-Crick base-pairing, including Hoogsteen interactions between a homopurinic strand and a third strand containing CT-only (or CU-only, in case of RNA) or AG-only bases. Recently, hybrid RNA-DNA triplexes were proposed as mediators of long non-coding RNA (lncRNA) function in chromatin architecture and gene expression. Two studies demonstrated that triplex formation at the promoter site of designed artificial transcription units can inhibit or enhance the observed transcription rate of the fluorogenic aptamer Broccoli, providing clues that support triplex-mediated lncRNA function. In fact, the up- or down-regulation was dictated by triplex motif, triplex position, and homopurinic location (i.e., template or sense strand). These new rule set was used to develop logic gates and a threshold gate, using triplex forming RNAs as inputs, and the transcription rate as output. To further study triplex function in cells, triplex targets were identified in *E. coli* genome and transferred into artificial transcription units. Quite remarkably, the different configurations previously tested with designed sequences, confirmed the effect of hybrid triplexes on transcription. The proposed system to study triplexes is expected to shed some light on these elusive structures, especially in the context of non-coding RNAs.

Biochemistry for Drug Repurposing

SpT-34-2
Repositioning a novel precursor of the HIV-1 protease inhibitor darunavir: antiproliferative effect, autophagy activation and proteasome inhibition by RDD-142 in hepatocarcinoma cell line HepG2

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In spite of cancer diagnostic methods and therapeutics making great strides in the last decades, there is a continual demand for improved therapeutic options for managing oncology patients. Repurposing approved and market-available drugs is an encouraging way to reduce cost and the timeframe for developing new cancer therapies. Noteworthy, certain protease inhibitors, authorized in the treatment of AIDS, demonstrated a pleiotropic antitumor activity. In previous works we investigated the pro-apoptotic activity of some HIV-protease-inhibitor precursors on different hepatoma lines with encouraging results [Previously published in Rinaldi, R. et al. (2021) Cells 10, 3052; Caddeo C et al. (2023) Int J Mol Sci 24, 4552]. However, a major drawback for the adoption of HIV-protease-inhibitors in cancer therapy relies on their relatively high dose requirement as pro-apoptotic agents and the important side effects. Consequently, we studied the hydroxyethylamine derivative RDD-142, a precursor of HIV-1 protease inhibitor Darunavir, under its IC50 concentration in order to evaluate a possible anti-cancer activity beyond apoptosis cascade activation. The anti-proliferative effect of RDD-142 on HepG2 cells was observed at lower concentrations of its IC50 performing the Agilent xCELLigence real-time proliferation assay. The flow cytometry cell cycle analysis, at the same concentrations, highlighted an arrest in G2/M of RDD-142-treated HepG2 cells, confirmed by decrease of CDK1 and cyclin B protein levels detected by western blotting. Furthermore, RDD-142 treated cells showed autophagy activation, through the initiation of autophagosome formation by ULK-kinase complex, and inhibition of proteasome catalytic activity by fluorogenic assay. Molecular docking of RDD-142 into the proteasome structure also predicted its ability to bind the proteolytically active β2 subunit in a similar way to other well-known proteasome inhibitors.
*The authors marked with an asterisk equally contributed to the work.

SpT-34-1
Structural characterization of thioredoxin reductase from *Cryptosporidium parvum* and its interaction with auranofin

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Cryptosporidium parvum, an apicomplexan protozoan parasite, is the causative agent of cryptosporidiosis, one of the most common causes of diarrheal disease worldwide. Cryptosporidiosis is strongly related to early childhood mortality and is a potential life-threatening complication in individuals with poor health or weakened immune system (HIV/AIDS patients, cancer, and transplant patients). No fully effective treatments or vaccines are available as the only FDA-approved drug, nitazoxanide, is effective in immunocompetent patients while showing reduced efficacy in immunocompromised ones. By means of drug repurposing, Auranofin (AF), a gold-containing compound and FDA-orphan drug, has been identified as an antiparasitic drug for the treatment of many human parasitic diseases. AF shows efficacy *in vitro* against *C. parvum* in the micromolar range, comparable to nitazoxanide [Previously published in Debnath A et al. (2013) Gut Microbes)]. It is well known that AF can target thioredoxin reductase (TrxR), a crucial parasite enzyme involved in the detoxification of reactive oxygen species. Using X-ray crystallography, we solved the crystal structure of *C. parvum* TrxR in the apo form (1.95 Å) and in complex with AF (3.3 Å). The 3D structure classifies CpTrxR as a type II high-molecular-weight TrxR, sharing a characteristic spacer of four residues between the two redox active Cys residues at the C terminus. This distinctive redox motif characterizes TrxRs from apicomplexan protozoa, including *Plasmodium falciparum*, a malaria parasite, and, to the best of our knowledge, it has been observed for the first time in our crystallographic structure. This result will allow a thorough investigation of the catalytic mechanism of these enzymes. The second structure results from co-crystallization with AF in reducing conditions and shows AF bound to the protein, providing insights into the molecular mechanism of inhibition against these parasites.

G protein Coupled Receptors

SpT-29-1
Exploring interaction sites of arrestin and Y1R/Y2R utilizing genetic code expansion

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Arrestins, next to G proteins, are the most prominent interaction partners of G protein- coupled receptors (GPCRs), known for their essential role in terminating G protein signaling, and driving GPCR internalization. Most GPCRs interact with non-visual arrestins (Arr2 and Arr3), but show significant differences in interaction mode and binding strength. Even receptors of the same ligand/receptor-system, like the neuropeptide Y(NPY)-system, have been reported to bind arrestins with different strength and dependence on the arrestin finger loop.[1] To investigate GPCR/arrestin interaction on a structural level within the NPY-system, genetic code expansion is used to express arrestin carrying non-canonical amino acids in living cells. This allows for applying different crosslinking strategies. Photo-crosslinking is used to map the GPCR/arrestin interface in regard to the proximity of relevant regions of arrestin.[2] Based on previous observations[1], the finger loop was the first region mapped, yielding distinct patterns for the NPY-receptors Y_1R and Y_2R. The screening identified a strong, unstructured crosslinking of the finger loop for Y_1R in general, while crosslinking with Y_2R only occurred at the middle of the finger loop. Based on these results, arrestin mutants have been tested for arrestin recruitment, which further highlighted the importance of the middle of the finger loop (Arr3 67-72) for Y_2R. Replacing these six amino acids with alanine reduced the maximal BRET-response to 33%, an effect comparable to the deletion of the whole finger loop (Arr3 64–78). Mapping of other selected areas of arrestin (*e.g.,* 160-loop and C-loop) revealed further receptor-specific contacts with arrestin. In conclusion, this approach has showed a strong and global association of arrestin and Y_1R, in contrast to only few contacts with Y_2R, thus, agreeing with the different reported behavior [1]. References: [1] Wanka L et al. (2018) Cell Signal 50, 58–71 [2] Böttke T et al. (2020) EMBO 21: e50437.

Molecular Basis of Diseases - Part B

SpT-36-1
VDAC1-based gene therapy recovers mitochondrial respiration by enhancing the complex I–sirtuins axis in a mice model of ALS

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On the outer mitochondrial membrane, the voltage-dependent anion-selective channel 1 (VDAC1) stands out as the most abundant pore-forming protein and the main permeability pathway for ions and small metabolites (ATP/ADP, NAD^+/NADH, Krebs's cycle intermediates)[1]. However, in neurodegeneration, VDAC1 behaves as a hub for the selective recruitment of misfolded proteins on the cytosolic surface of mitochondria. Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease, and ~20% of inherited cases depends on mutations in the gene encoding the antioxidant enzyme Cu/Zn Superoxide Dismutase (SOD1). The mitochondrial accumulation of SOD1 mutants affects VDAC1 conductance, ADP/ATP exchanges and oxygen consumption, triggering the organelle dysfunction[2]. As a matter of fact, VDAC1 downregulation in SOD1 transgenic rats accelerates the pathology onset and decreases the animals' lifespan. In the attempt to counteract the mitochondrial malfunctioning in ALS, we enhanced the expression of VDAC1 in the spinal cord of SOD1 transgenic mice by a neonatal intraspinal injection of an adeno-associated virus. For the first time, here we demonstrate that a stable VDAC1 upregulation rescues the whole respiratory profile of pre-symptomatic mice. Mechanistically, VDAC1 activates a mitochondrial quality control pathway

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involving the TOM complex, the main gateway for the import of proteins within the organelle, leading to the selective increase of expression and function of the electron transport chain (ETC) complex I and the NAD^+-dependent deacetylases sirtuin 3 (Sirt3). Overall, by stabilizing the complex I-Sirt3 axis, VDAC1 emerges as a highly promising for advancing ALS therapeutics, establishing a 'virtuous cycle' in affected mitochondria: it increases the NADH availability and reoxidation by complex I, and the derived NAD^+ strongly stimulates Sirt3 activity, a pivotal regulator of the ETC. References: 1 Magrì et al, Front Chem, 2018, 6: 10. 2 Magrì et al, Cell Death Dis, 2023, 14: 122.

Tuesday 2 July
13:10–13:30, Blue Room

Molecular Basis of Diseases – Part B

SpT-32-1
MCJ: a mitochondrial target for cardiac intervention in pulmonary hypertension

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Pulmonary hypertension can affect both the pulmonary arterial tree and cardiac function, often leading to right heart failure and death. Despite the urgency, the lack of understanding about the molecular mechanisms happening at the cardiac level has limited the development of effective cardiac therapeutic strategies. Our research reveals that methylation-controlled J protein (MCJ; encoded by the nuclear gene *DNAJC15*) modulates the mitochondrial response to chronic hypoxia. MCJ levels elevate under hypoxic conditions, as in the lungs of patients affected by chronic obstructive pulmonary disease (COPD), mice exposed to hypoxia, and myocardium from pigs subjected to right ventricular overload. Remarkably, the absence of MCJ preserves the right ventricular function, safeguarding against both cardiac and lung remodelling induced by chronic hypoxia. Importantly, cardiac-specific silencing is enough to protect against cardiac dysfunction despite the adverse pulmonary remodeling. Mechanistically, the absence of MCJ triggers increased cardiac levels of ROS that resulted in activation of the mTOR pathway and elevated HIF-1α levels in basal conditions. We demonstrated that MCJ^{KO} mice preserved the right ventricular function following hypoxia exposure due to the blunted ROS increase following hypoxia exposure and the preconditioning state generated by HIF-1α. These discoveries provide a potential avenue to alleviate chronic hypoxia-induced PH, highlighting MCJ as a promising target against this condition.

SpT-32-2
Investigation of the role of Wnt/β-catenin signaling in development of Alzheimer's disease in a zebrafish model of amyloid-β toxicity

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The Wnt/β-catenin signaling pathway, an evolutionarily conserved and pivotal pathway associated with synapse formation in adulthood, plays a crucial role in Alzheimer's disease (AD). AD, marked by various pathologies, is primarily linked to the accumulation of extracellular beta-amyloid plaques. The interplay between this accumulation and disruptions in the Wnt/β-catenin signaling pathway triggers synaptic degeneration, resulting in synaptic dysfunction and AD progression. In this study, we modeled AD induced by the Aβ42 peptide using adult transgenic (6XTCF) zebrafish. To establish the zebrafish AD model, we employed cerebroventricular microinjection (CVMI) with the Aβ42 peptide. Fish, anesthetized prior to CVMI, were positioned on a stable platform, and the Aβ42 peptide was injected into the telencephalon region of the brain by a capillary needle. Brain samples were collected on 1, 3, 4, 7, and 14 days post-CVMI (dpi) to analyze changes in Aβ42 peptide accumulation, the immune system response, synaptic degeneration, apoptosis, and the expression of genes related to proliferation using qPCR and immunofluorescent staining. To examine the role of the Wnt/β-catenin signaling pathway in the molecular mechanism of AD development, fish exhibiting high levels of regeneration on days 7 and 14 were treated with the IWR-1 drug, which inhibits the Wnt/β-catenin signaling by stabilizing the Axin2 protein, thereby suppressing the regenerative response. Our results revealed that the AD model manifested on 3dpi, with the regenerative response reaching its peak on 7dpi and 14dpi. Treatment with IWR-1 resulted in increased Aβ42 accumulation, accelerated synaptic degeneration, and elevated cell deaths in fish where the Wnt signaling pathway was inhibited. In conclusion, our adult zebrafish AD model is poised to elucidate the molecular mechanisms connecting the Wnt signaling pathway and AD, thereby contributing to the development of alternative therapeutic approaches for AD patients.

SpT-32-3
Alternative low-populated conformations prompt phase transitions in polyalanine repeat expansions

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Abnormal trinucleotide repeat expansions alter protein conformation producing malfunction and contribute to a significant number of incurable human diseases. Due to the repetitive, polymorphic and dynamic nature of expanded homorepeats, their structural study is highly challenging and only limited structural information is available. PHOX2B is a transcription factor with a 20 polyalanine tract whose elongation is frequently observed in patients suffering from congenital central hypoventilation syndrome (CCHS), a rare disease affecting mainly children. The length of the polyalanine expansion is correlated with the development and severity of symptoms related to CCHS [Matera I. (2004) J. Med. Genet, 41, 373-80]. However, the pathogenic mechanisms of expanded PHOX2B remain unknown. The main hypothesis proposed as the fundamental mechanism of PHOX2B dysfunction is its aggregation. Nevertheless, aggregation in polyalanine-expanded proteins in not always apparent, and additional pathogenic mechanisms could determine cell toxicity. In particular, aberrant phase transitions and the overpower of the proteostasis network could determine cell degeneration in CCHS. To help understand the pathogenic nature of polyalanine expansions in PHOX2B, we have determined by NMR spectroscopy the structure and dynamic properties of PHOX2B C-terminal fragment in addition to a subpathogenic construct with 23 alanines and a pathogenic construct containing 26 alanines in the polyalanine tract. Interestingly, while there are no significant structural differences between mutants and the wild-type protein, at least in its major conformation, mutants promote nascent conformations that prompt length-dependent liquid-to-solid transitions into biomolecular condensates that capture wild-type. The unprecedented direct observation of the nascent polymorphs in expanded PHOX2B leads us to propose unbalanced phase separation as a novel pathophysiological mechanism in homorepeat expansion diseases.

SpT-32-4
Analysis of the molecular basis of senataxin-associated amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is the most common neurodegenerative disease affecting upper and lower motor neurons (MNs). A juvenile form of this disease (ALS4) is caused by dominant mutations in the gene encoding the putative RNA helicase senataxin (SETX). Former studies involved SETX in transcription termination and the metabolism of R-loops, which are three-stranded nucleic acid structures that form during transcription when the nascent RNA invades and hybridizes with the template DNA. Accumulation of R-loops can lead to increased DNA damage and genome instability. However, it is unclear how ALS4-associated mutations perturb SETX function and result in MN degeneration. To assess the effects of ALS4-associated mutations on MN biology, we generated a human induced pluripotent stem cell line carrying an ALS4 mutation in one of SETX alleles and we characterized MNs obtained by differentiation of these cells. Importantly, we found that the ALS4 mutation provokes a reduction in axon length suggestive of axonal degeneration, a typical feature of the early stages of this disease. We also observed a partial relocation of mutant SETX from the nucleus to the cytoplasm, spreading along neurites. This mislocalization can lead not only to a gain of neurotoxical function but also to a loss of function at natural sites of action. However, we found that the ALS4 mutation does not result in DNA damage accumulation, which is a typical consequence of SETX loss of function. Furthermore, transcriptomic analyses revealed more than 1000 genes that appear deregulated in ALS4 MNs, among which are genes of the TGF-b superfamily signalling pathway. These features have been observed in MNs from ALS4 patients and in patients of other ALS forms. We propose that deregulated SETX activity in transcription termination and R-loop resolution in the nucleus leads to alterations in the expression of genes important for MN function and drive MN impairment in ALS4.

Bioinformatics and AI for Precision Medicine

SpT-28-1
The cancer genome explorer (TCGEx): a powerful visual interface for sophisticated analyses of high throughput cancer data

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Analyzing high-throughput genomics data requires programming expertise, and it remains challenging for many experimental researchers. While visual interfaces have eased access to this data, their limited flexibility in accommodating complex custom analyses remains a significant hurdle. To address these shortcomings, we have developed The Cancer Genome Explorer (TCGEx), a web-based R/Shiny application that can work with preprocessed

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The Cancer Genome Atlas (TCGA) transcriptomics data and user-provided external datasets. TCGEx serves as a centralized hub, offering a diverse array of analytical tools including survival modeling, exploratory graphing, gene set enrichment, gene-to-gene correlation, dimensionality reduction, and machine learning. Our utilization of TCGEx in investigating gene expression profiles within human primary and metastatic melanoma revealed distinctive tumor subsets characterized by unique immune signatures and survival outcomes. Delving deeper, we explored miRNA networks associated with intratumoral immunity, harnessing TCGEx's machine learning algorithms. Aligning with existing literature, our study highlighted miR-155 as among the prominently upregulated miRNAs in immune-enriched melanoma biopsies. Intriguingly, heightened miR-155 levels correlated with transcriptomic enrichment in lipid catabolism pathways and depletion in ribonucleoside catabolism pathways. Expanding our inquiry to previously published datasets from melanoma immunotherapy trials, we discerned transcriptomic patterns linked to therapeutic benefits. While our study predominantly focused on immune-associated noncoding RNAs within the melanoma tumor microenvironment, TCGEx extends its capabilities to investigate 32 other TCGA cancer projects as well as user-uploaded external datasets. In essence, TCGEx emerges as a powerful and adaptable platform facilitating the analysis of high-throughput cancer data.

SpT-28-2
Dissecting the role of glutamine metabolism in anaplerosis and redox homeostasis in K-ras-transformed mouse fibroblasts

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The enhanced growth and survival of K-ras-transformed cells rely on deep changes in metabolism, including glutamine addiction and increased oxidative stress. We study glutamine roles in metabolism, signal transduction, and redox homeostasis in K-ras-transformed NIH3T3 mouse fibroblasts (NIH-RAS), by complementing glutamine deprivation with dimethyl-α-ketoglutarate (AKG) and nonessential amino acids (NEAA). The combination AKG + NEAA only partially rescues glutamine deprivation, likely due to low glutamine synthetase (GS) activity in NIH-RAS cells. This substitution results in low levels of nucleotides and the non-use of reductive carboxylation of AKG – predicted by ENGRO model – to synthesize lipids, whose content is lower due to downregulated expression of genes involved in lipogenesis that correlates with lower NADPH levels. Thus, in NIH-RAS cells glutamine is essential as a carbon and nitrogen source for biosynthesis (amino acids, nucleotides, and glutathione) and as a signalling molecule. We successfully exploit an integrated, systems biology approach to study nutritionally perturbed transformed cells, pushing forward a system-level understanding of complex diseases like cancer.

Tuesday 2 July
13:10–13:30, Yellow Room

D-amino acids and Pathological States

SpT-22-2
A multi-omic analysis reveals gender-specific D-serine signatures in Alzheimer's disease

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Alzheimer's disease (AD), affecting millions worldwide, remains a significant barrier to healthy aging and is the predominant cause of dementia. Despite AD neuropathology has been well defined, the underlying causes of the disease remain debatable. Recently, sex and gender are emerging as crucial drivers of development and progression of AD dementia. So, using an integrated omics approach, this study investigated gender-related alterations in the molecular composition of postmortem hippocampus samples of healthy individuals (CTR) and AD patients. Comparative analyses spotlighted the gender-dependent omic changes, revealing profound differences in energetic metabolism, cytoskeleton organization, and oxidative stress response [previously published in Maffioli et al. (2022) Cell Rep 40(10):111271]. A marked decrease in insulin response is evident in AD females compared to males, indicating a potential vulnerability for targeted therapeutic strategies. Moreover, serine metabolism, closely tied to the glycolytic pathway and the production of the N-methyl-D-aspartate (NMDA) receptor co-agonists D-serine (D-Ser) and Glycine (Gly), is modulated across genders. The D-Ser/total serine ratio emerged as a critical factor to counteract age-related cognitive decline: in females it is mainly due to Ser metabolism upregulation, while in males it is linked to Pro/Arg metabolism. Our data support the evidence that altered L-Ser level may contribute to damaged neurotransmission and synaptic plasticity in aging and in AD patients (triggering an increase in brain D-Ser availability) and highlight how different pathophysiological mechanisms are active across genders. This project was founded by "PRIN-2017 - Dissecting serine metabolism in the brain".

SpT-22-1
Investigating the impact of the pathological human phosphoserine phosphatase N133S variant on serine synthesis

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L-serine plays crucial roles in various metabolic pathways, especially in the brain since it can be converted into D-serine and glycine, which are the main co-agonists of NMDA receptors. It is mainly synthesized starting from a glycolytic intermediate via the phosphorylated pathway (PP). This cytosolic pathway involves three enzymes, which have recently been proposed to form a transient assembly named "serinosome" [previously published in: Rabattoni V et al. (2023) FEBS J 29015, 3877–3895]. Phosphoserine phosphatase (PSP) catalyzes the last and irreversible step of the pathway, which is essential to push the overall pathway towards L-serine synthesis. Genetic alterations in PP enzymes are related to serine deficiency disorders that lead to severe neurological phenotypes. A homozygous missense variant (c.398A > G, p.N133S) in the PSP encoding gene was identified in two siblings exhibiting a neurodevelopmental syndrome and myelopathy. Despite no significant alterations in protein conformation, dimeric oligomerization, enzymatic activity, and PP functionality, the recombinant N133S PSP shows reduced stability compared to wild-type PSP, a characteristic evident at the cellular level as well. Patients' fibroblasts present decreased levels of PP enzymes, along with partial nuclear and perinuclear localization of the PSP variant and increased perinuclear aggregates formation. These findings suggest that these alterations contribute to the formation of a dysfunctional serinosome, leading to reduced levels of L-serine, glycine, and D-serine, observed both in fibroblasts and serum of patients, which may explain their neurological traits. This study on patients presenting the N133S PSP substitution contributes to our understanding of the molecular mechanisms underlying alterations in serine levels. It also offers valuable insights into potential therapeutic strategies aimed at restoring the balance and mitigating the associated neurological manifestations.

SpT-22-3
Human serine racemase: structural flexibility and interaction with protein partners

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Human serine racemase (hSR) is a pyridoxal-5′-phosphate (PLP)-dependent enzyme involved in the production of D-serine, a co-agonist of N-methyl-D-aspartate receptors (NMDARs). Given its role in the regulation of excitatory glutamatergic neurotransmission, hSR is involved in the pathophysiology of different brain disorders, such as Alzheimer's and Parkinson's disease or schizophrenia [previously reviewed in Raboni S et al. (2019) Front Mol Biosci 5, 112]. hSR is a dimer able to catalyze the formation of D-serine from L-serine, as well as the dehydration of both L- and D-serine to form pyruvate and ammonia. The activity of hSR is regulated by Mg^{2+}, small molecules such as ATP, glycine or malonate and by the interaction with protein partners. To evaluate the structural flexibility of hSR upon ligand binding and expand the knowledge on its interactome, we exploited PLP ^{31}P-NMR and limited proteolysis to study the binding of small-molecule interactors and assessed the binding of the third PDZ domain of PSD-95, a protein involved in trafficking and localization of glutamate receptors. Although some preliminary measurements suggested the presence of an interaction between hSR and PSD-95 PDZ3, NMR and ITC indicated that the two proteins bind only weakly [previously published in Giaccari R et al. (2022) Int J Mol Sci 23(9):4959]. The dehydratase activity of hSR was not significantly affected by PSD-95 PDZ3 in the presence of ATP, while a moderate increase of the activity was observed in the absence of ATP. Moreover, our results show that the presence of ATP and malonate shifts hSR towards a closed conformation, in agreement with crystallographic data reported in the literature.

Neurobiochemistry

SpT-30-3
Blood–brain barrier permeability increases with the differentiation of glioblastoma cells

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Glioblastoma multiforme (GBM) is a highly aggressive tumour, difficult to treat pharmacologically because of the blood–brain barrier (BBB), rich of ATP-binding cassette (ABC), and tight junction (TJ) proteins. BBB is disrupted in GBM bulk, but it is competent in brain-adjacent-to-tumour area, where GBM foci can trigger tumour relapse. How GBM cells influence the permeability of BBB is poorly investigated. To clarify this point, we co-cultured human BBB cells with 3 patient-derived GBM cells,

separating from each tumour the stem cell (SC) and the differentiated component (AC). The presence of GBM cells increased the permeability to doxorubicin and mitoxantrone, substrates of ABC transporters, and dextran-70, a parameter of TJ integrity; decreased TEER; down-regulated ABC transporters and TJ proteins at protein and transcriptional level. Interestingly the conditioned medium obtained from GBM SC and AC produced the same effects. The secretome analysis identified IL-6 as significantly higher produced by AC than by SC. Notably, AC-conditioned medium treated with an IL-6 neutralizing antibody reduced the BBB permeability to SC levels, while SC-conditioned medium enriched with IL-6 increased BBB permeability to AC levels. Mechanistically, the IL-6 released by AC GBM cells activated in BBB cells STAT3, which down-regulated ABC transporters and TJ expression, increased permeability and TEER. The opposite effects were obtained in BBB cells treated with STA-21, a pharmacological inhibitor of STAT3, or with a PROTAC targeting STAT3. Our work demonstrates for the first time that the degree of GBM differentiation influences BBB permeability. GBM cells via IL-6/STAT3 control the expression of ABC transporters and TJ proteins on BBB. These results may pave the way to novel therapeutic tools to tune BBB permeability and improve drug delivery to GBM. Funding: AIRC - IG21480; Fondazione Compagnia di San Paolo, Torino; PNRR - PNC – D3 4 HEALTH * The authors marked with an asterisk equally contributed to the work.

SpT-30-2
Boosting NAD in senescent microglia: a useful strategy to counteract neurodegeneration?

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In neurodegenerative disorders and senescence, microglia, the brain immune cells, acquire a disease-associated microglia signature that may favor tissue repair in early disease state but at late stages lose its capability to restore brain homeostasis and protect neurons and oligodendrocytes from cell death. Senescent microglia exhibit a secretory associated senescence phenotype, and impaired metabolism, with depletion of NAD, which plays a central role in genome integrity and cell metabolism. Emerging evidence highlighted lower levels of NAD in senescence and neurodegenerative diseases, with consequent impairment of sirtuins' activity. In this study we investigated changes that occur during senescence in microglia developing an *in vitro* model of chronically exposure (up to 30 days) to high iron concentration. Initially, iron treatment induces microglia to proliferate more, enhances phagocytosis, and increase NAD levels suggesting microglia activation. After 30 days of treatment microglia acquired a senescent-like phenotype characterized by proliferation arrest, decreased phagocytosis, upregulation of SASP markers with a significant increase in EVs production. Biochemical, transcriptome and metabolome analyses showed decreased levels in NAD and NADPH content in iron-treated microglia, concomitantly to an increased expression of CD38 (the major NAD consuming enzyme). Moreover, the levels and activity of Sirtuin 6, which is downregulated in aged/senescent cells, were strongly reduced compared to control microglia. Senescence in microglia could be prevented by boosting NAD synthesis through the administration of a NAD precursor: NMN. Senescent microglia co-cultured with healthy microglia induced senescent traits in healthy cells, as revealed by a significant increase in SA-ß-Gal and p21 positive cells and in reduced levels of NAD. In conclusion NAD boosting could represent a useful strategy to counteract senescence and senescence propagation to healthy microglia.

SpT-30-1
Bisphenol a, an endocrine disruptor that links obesity and neurodegeneration, a zebrafish study

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Bisphenol A (BPA) is an estrogenic endocrine disruptor used in food containers, adhesives, dye powders, and dental fillings. Of all the substances that cause obesity, environmental endocrine disruptors are the most noticeable. We have previously reported BPA-induced obesity in zebrafish and suggested impaired glucose tolerance, oxidant-antioxidant balance, and increased inflammatory response as the possible mechanisms [Previously published in: Beler et al. (2022) Toxicol Ind Health 38, 19-28]. Dopamine is one of the main actors in food reward and food intake management as shown by a variety of studies examining neural connections. Reward-deficit hypothesis of obesity suggests that a fundamental factor in overeating is the process of striatal dopaminergic hypofunctioning. Dopaminergic neuron degeneration in brain is considered as the major factor in the development of Parkinson's disease (PD). Considering the possible relationship between obesity and PD reported in recent years, we aimed to examine whether BPA has neurodegenerative effects in addition to its obesogenic effects. Zebrafish were divided into three groups, Control, Low-Dose (1 μM BPA), and High-Dose BPA (10 μM BPA), and at the end of 30 days locomotor activities were determined. Oxidant-antioxidant system parameters including lipid peroxidation, nitric oxide, superoxide dismutase, catalase and glutathione S-transferase were determined spectrophotometrically in brain. Acetylcholinesterase activity was also evaluated to determine cholinergic functions. Results of our study showed that, in addition to its obesogenic effects, BPA also had a neurodegenerative effect in zebrafish by changing locomotor and cholinergic activities and disrupting the oxidant-antioxidant system in the brain. Based on these results, we can suggest that the redox balance disrupted in the brain due to BPA exposure is one of the key mechanisms in the relationship between PD and obesity.