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TALKS

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Abstracts submitted to the 47th FEBS Congress from 8th to 12 th July 2023 and accepted by the Congress Management Board are published in this Supplement of *FEBS Open Bio*. Late-breaking abstracts are not included in this supplement. The abstracts are available as two PDF files: Talks (Plenary Lectures, Symposia and Speed Talks) and Posters.

About these abstracts

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

TALKS

Plenary Lectures

Saturday 8 July
17:00–18:00, Auditorium François 1er

FEBS Sir Hans Krebs Lecture – Opening Plenary Lecture

PL-01-1
Diet, metabolism and cancer progression

K. Vousden
The Francis Crick Institute, London, UK

"Deregulating cellular energetics" is a hallmark of cancer that reflects the importance of metabolic changes that support tumor development and progression. Multiple factors that can be tumor type and stage dependent affect the metabolic requirements of cancer cells, and metabolic rewiring contributes to all steps of tumor progression. One important role of metabolism in cancers is to drive enhanced antioxidant defense and buffer cells from the increased oxidative stress that accompanies the oncogenic process. While ROS limitation is important to maintain cancer cell viability throughout tumor development and metastatic progression, we have found that ROS can also promote various stages of tumorigenesis through various cell intrinsic and extrinsic mechanisms. The altered metabolic demands of cancers can drive selective vulnerabilities that provide targets for therapeutic intervention. Our work has explored the concept that cancer cells become dependent on an exogenous supply of specific nutrients, such as the non-essential amino acid serine, that can be limited by dietary depletion. These studies suggest that targeted diet modulation will provide an effective addition to current treatment options, and we are currently testing the clinical efficacy of serine limited diets.

Saturday 8 July
18:00–19:00, Auditorium François 1er

FEBS Datta Lecture

PL-02-1
tRNA modifications and fidelity of decoding

E. Westhof
IBMC-CNRS, Université de Strasbourg, Strasbourg, France

An integrated view of decoding based on the pairing energy between the first two codon base pairs and the anticodon triplets was described. This information-rich representation of the 64 codons is circular with an uneven distribution of codons leading to a segregation between GC-rich 4-codon boxes and AU-rich 2:2 and 3:1 boxes. This representation is structurally supported by molecular recognition in the A decoding site: A-minor contacts of the first two Watson-Crick base pairs with asymmetric recognition for the third pair. The multiplicity and complexity of nucleotide modifications, particularly at positions 32, 37, and 38 of the anticodon loop, varies and corresponds well with the need to stabilize AU-rich codon-anticodon pairs. To avoid coding inefficiency or errors at the third base pair, which is recognized primarily at position 3 of the mRNA codon, modifications at position 34 of the tRNA are also crucial. The standard GoU pair is not isosteric upon inversion (GoU is not superimposable on UoG). While the standard wobble pair is only observed with U3 on the message and G34 on the tRNA (U3oG34), modifications of U34 (U34*) are required to stabilize in the decoding site an anionic pair U34*oG3 that is isosteric to U3oG34. Molecular recognition in the decoding site of the codon-anticodon complex is thus conditioned by the geometry of the pairs. As experimentally shown by the Rodnina and Uhlenbeck teams, the current structure-based network of tRNA interactions and modifications results in uniform dynamic decoding of all native tRNAs. The evolution and expansion of decoding can then be seen as initially based on GC-rich pairs with the gradual introduction of A/U pairs stabilized by tRNA modifications through the presence of modifier enzymes, and later the recognition of the third pair and the use of wobbling. Indeed, the triplet coding frame is imposed by the folding of the anticodon loop and the helicity of the complex between the anticodon and the codon.

Sunday 9 July
11:30–12:30, Auditorium François 1er

FEBS/EMBO Women in Science Award Lecture

PL-04-1
Mapping the human body: one cell at a time

S. Teichmann
Wellcome Sanger Institute, Cambridge, UK

The 37 trillion cells of the human body have a remarkable array of specialized functions, and must cooperate and collaborate in time and space to construct a functioning human. Harnessing cutting-edge single cell genomics, my lab has been attempting to understand this cellular diversity, how it is generated during development and how it goes wrong in disease. My talk will illustrate the relevance of cell atlasing to understand the cellular composition of the body, with a particular focus on how spatial transcriptomics technologies can define the diverse tissue microenvironments that regulate cell fate and function.

Sunday 9 July
15:00–16:00, Auditorium François 1er

Molecular Oncology Lecture

PL-03-1
Targeting KRAS: light at the end of the tunnel

M. Barbacid
Spanish National Cancer Research Center (CNIO), Madrid, Spain

KRAS oncogenes are responsible for at least one fourth of all human cancers. Although this oncogene was identified in human tumors four decades ago, the first KRAS selective inhibitor was not approved until 2021. Despite this long-awaited breakthrough, approved KRAS inhibitors only block one of the multiple oncogenic isoforms, KRASG12C, primarily present in lung adenocarcinomas. Moreover, tumor resistance is becoming a significant issue in the clinic (Awad *et al.*, *N Engl J Med*, 2021). To provide experimental information that might help to improve current KRAS targeting strategies, we have compared the therapeutic consequences of eliminating, instead of inhibiting, KRAS oncoproteins in two experimental mouse tumor models. In addition, we have interrogated those mechanisms responsible for the induction of resistance in KrasG12C-driven tumors. Therapeutic strategies to target KRAS mutant tumors also include those that block KRAS signaling pathways. Previous studies in our laboratory have shown that ablation of RAF1 in Kras/p53 driven-lung adenocarcinomas induce effective tumor regression without significant toxicities (Sanclemente *et al. Cancer Cell*, 2018; Esteban-Burgos *et al.*, *PNAS*, 2020). Likewise, concomitant ablation of RAF1 and EGFR in Kras/p53 driven-pancreatic ductal adenocarcinomas (PDAC) resulted in the complete regression of a limited fraction of tumors (Blasco *et al.*, *Cancer Cell*, 2019). We have now identified STAT3 activation as the main mechanism for the resistance of PDAC tumors to RAF1/EGFR ablation. Indeed, combined ablation of RAF1/EGFR/STAT3 completely blocked proliferation of mouse tumor cell lines and organoids. Moreover, tumors induced in syngeneic orthotopic models regressed completely with no evidence of tumor progression. These results open the door to the development of pharmacological strategies, that in combination with forthcoming KRAS inhibitors, should have a significant impact on the treatment of KRAS mutant tumors in a clinical scenario.

Monday 10 July
11:30–12:30, Auditorium François 1er

EMBO Lecture

PL-05-1
How do enhancers function to regulate embryonic development?

E. Furlong
EMBL, Heidelberg, Germany

Precise regulation of gene expression is essential for embryonic development, and when it goes awry it can lead to a wide range of human diseases. Gene expression is initiated by enhancers, which are non-coding elements that recruit transcription factors to regulate the initiation of gene expression at their target gene's promoter. As many enhancers are located upstream, downstream or in the introns of other genes, they need to come into physical proximity to their cognate target gene. What controls the specificity, timing and regulation of these enhancer-promoter loops are current key questions. I will discuss how we are combining genetic, genomics and microscopy to dissect this during embryonic development, to uncover general properties of genome regulation. Enhancers act as the regulatory nodes within gene regulatory networks, which drive cell fate decisions and tissue differentiation during embryonic development. We are also exploring the extent to which combining single cell regulatory genomics with both natural sequence variation (perturbations in *cis*) and transcription factor (TF) loss-of-function mutants (*trans*) can functionally dissect enhancer activity and developmental phenotypes, at both a cellular and molecular level. By sampling regulatory changes over a dense time-course of both mesoderm development and the entire embryogenesis of *Drosophila*, a very well-characterized test case, we could reconstruct developmental trajectories, uncover the TFs involved and the enhancers they regulate. We developed a nuclear genotyping strategy to systematically assess recessive lethal mutations from embryo pools with mixed genotypes. We have shown how deep learning on a dense time-course can predict developmental time, allowing the formation of continuous trajectories and to zoom into the scale of minutes. I will discuss how we are using these approaches as a framework to reassess mutant phenotypes, and identify new phenotypes at both a cellular and molecular level.

Monday 10 July
14:30–15:30, Auditorium François 1er

The FEBS Journal Richard Perham Prize Lecture

PL-07-1
Molecular mechanism allowing mycobacteria to evade natural antibiotics targeting the essential ClpCP degradation pathway

G. Taylor[1], J. Leodolter[1,2], H. Cui[1], M. Ziemski[1,3], **E. Weber-Ban**[1]
[1]*ETH Zurich, Institute of Molecular Biology and Biophysics, Zurich, Switzerland,* [2]*Research Institute of Molecular Pathology, Vienna, Austria,* [3]*ETH Zurich, Institute of Food Nutrition and Health, Zurich, Switzerland*

Mycobacterium tuberculosis (Mtb) remains one of the deadliest human pathogens, killing 1.5 million people every year. Multidrug-resistant strains have increased in incidence, creating an urgent need for novel drugs and drug targets and a thorough understanding of evasion mechanisms the bacterium employs. The essential protease complex ClpCP has recently emerged as a promising drug target. Employing a bacterial adenylate cyclase two-hybrid approach to screen the proteome of an Mtb ORF library, we revealed toxin-antitoxin (TA) systems as a major group of Mtb ClpCP interactors. Mtb encodes an unusually high number of TA modules (> 80 compared to only a few in the

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non-pathogenic *M. smegmatis*), and they appear to contribute to Mtb survival in the host [1]. To date, several antitubercular compounds have been shown to target the ClpCP chaperone-protease, including cyclic heptapeptide cyclomarin A (CymA) [2]. CymA exerts its toxicity by binding to AAA^+ chaperone ClpC1 that associates with the proteolytic core cylinder ClpP to form the ClpCP protease [3]. We show that a partial ClpC1 homolog, annotated as ClpC2, also binds CymA [4]. A ClpC2-dependent evasion mechanism allows Mycobacteria to evade antibiotics targeting the ClpC1 component of the essential ClpCP degradation pathway.

References: [1] Ziemski, Leodolter, Taylor, Kerschenmeyer & Weber-Ban (2020) Genome-wide interaction screen for Mtb ClpCP protease reveals toxin–antitoxin systems as a major substrate class. *FEBS Journal* 288, 99–114. [2] Schmitt et al. (2011) The natural product CymA kills Mtb by targeting the ClpC1 subunit of the caseinolytic protease. Angew Chem 50, 5889–91. [3] Taylor, Frommherz, Katikaridis, Layer, Sinning, Carroni, Weber-Ban & Mogk (2022) Antibacterial peptide CymA creates toxicity by deregulating the Mtb ClpCP protease. JBC 298, 102202. [4] Taylor, Cui, Leodolter, Giese & Weber-Ban (2023) ClpC2 protects mycobacteria against a natural antibiotic targeting ClpC1-dependent protein degradation. Comm Biol, in press.

FEBS Letters Award Lecture

PL-06-1
Plant lipid engineering to cut agricultural greenhouse gas emissions

R.K. Bhunia, G. Menard, H. van Erp, F. Bryant, J. Martin-Moreno, L. Michaelson, **P. Eastmond**
Rothamsted Research, Harpenden, UK

Global food production methods must change to minimize our impact on the environment, while continuing to feed the world's population. It's estimated that agriculture contributes 13–21% of total global greenhouse gas emissions. Most emissions from this sector are attributable to livestock production, with the predominant source being enteric methane generated by ruminants. Worldwide, livestock production occupies ~ 70% of agricultural land, or ~ 30% of the Earth's land surface. In addition to pasture, more than 60% of arable crop production is also committed to animal feed. Improving the efficiency and sustainability of livestock production systems and adopting a more plant-based diet are both interventions that can help our environment. In my lab we study the regulation of plant lipid metabolism, and I will describe two projects where we are attempting to apply our knowledge to help reduce agricultural greenhouse gas emissions. The goal of the first project is to produce high lipid forage crops, using mutant breeding and gene editing techniques. Modest increases in the lipid content of ruminant livestock diets can both increase productivity and reduce enteric methane emissions. As part of this project, we developed a simple gene editing strategy to achieve transgene-free transcriptional gain-of-function, which we published in *FEBS Letters*. The goal of the second project is to produce oilseed crops that make better animal fat substitutes. Animal fats, such as those found in milk, possess distinct types and arrangements of fatty acyl groups that are not found in plants and these help to define the physical, sensory and health properties of foods that they contain.

Tuesday 11 July
11:30–12:30, Auditorium François 1er

FEBS Special Lecture

PL-08-1
The ribosome and the protein folding code of translation

M. Rodnina
Max Planck Institute for Multidisciplinary Sciences, Goettingen, Germany

The ribosome is a macromolecular machine that synthesizes proteins and guides their folding towards the correct, functional structures. Defects in protein folding cause many human diseases; thus, understanding co-translational folding is of eminent importance. Cotranslational protein folding starts as soon as the N-terminal parts of nascent proteins become available, but is confined by the narrow space of the polypeptide exit tunnel of the ribosome. Compared to protein folding in solution, the ribosome alters the folding pathway, destabilizes native folds and stabilizes non-native intermediates of nascent proteins. Translation is a non-uniform process with periods of rapid translation interspersed with pauses. The stop-and-go rhythm of translation not only defines the rate with which a protein is synthesized, but also modulates folding and misfolding of nascent proteins. The sequence of the protein, the codon-specific translation rates and the interactions between the nascent peptide and the ribosome define the folding code of translation, which ultimately determine the quality of the cellular proteome.

Tuesday 11 July
14:30–15:30, Auditorium François 1er

FEBS Theodor Bücher Lecture

PL-09-1
Systematic cell biology – using high-throughput screens to reveal the unknown unknowns

M. Schuldiner
Weizmann Institute of Science, Rehovot, Israel

During the last decades, biology has gone through a technology-driven revolution with the emergence of high-throughput methods. Yeast has been an excellent model organism for these efforts, allowing the collection of large amounts of systematic data. Despite all of this data, we still do not have any idea of the function of over 25% of yeast proteins and a large fraction of the proteome remains poorly characterized. Hence, there is a huge gap between the amount of data generated and its ability to put forward high-quality predictions that can be followed up to obtain true mechanistic understanding of protein functions. I will share our efforts to bridge the gap by using high throughput methods in combination with detailed follow up to uncover functions for uncharacterized proteins and provide mechanistic understanding of fundamental unresolved questions in cell biology.

Wednesday 12 July
11:30–12:30, Auditorium François 1er

FEBS Open Bio Lecture

PL-10-1
Molecular mechanisms underlying neurotransmitter release and its regulation

J. Rizo
UT Southwestern Medical Center, Dallas, TX, USA

The release of neurotransmitters by calcium-triggered synaptic vesicle exocytosis is crucial for communication between neurons. Previous work by many groups and recent results that I will present have outlined the molecular mechanisms that lead to a primed state in which synaptic vesicles are ready for fast fusion with the plasma membrane upon calcium influx (in the microsecond time scale). Central roles are played by synaptotagmin-1, which acts as the calcium sensor, and by the SNARE proteins syntaxin-1, SNAP-25 and synaptobrevin, which form a tight complex that brings the synaptic vesicle and plasma membranes together and is critical for membrane fusion. This complex is disassembled by NSF and SNAPs to recycle the SNAREs. Munc18-1 and Munc13 orchestrate SNARE complex assembly in an NSF/SNAP-dependent manner through a mechanism that starts with Munc18-1 bound to syntaxin-1 folded in a self-inhibited closed conformation. Munc18-1 later binds to synaptobrevin, forming a template to assemble the SNARE complex while Munc13 bridges the two membranes and stimulates opening of syntaxin-1. The assembled SNARE complex binds to complexin and to synaptotagmin-1, which also interacts with the plasma membrane, resulting in the primed state that is ready for fast fusion. Elucidating the steps that lead from this primed state to fast membrane fusion has been highly challenging, but we have obtained crucial insights into these steps using all-atom molecular dynamics simulations together with NMR studies in solution and FRET assays on membranes. Our results suggest that: (i) the primed state includes a spring-loaded macromolecular assembly in which synaptotagmin-1 and complexin keep the SNARE complex almost fully assembled but inhibit progress towards fusion; (ii) upon calcium binding, synaptotagmin-1 acts as a lever that re-orients on the membrane and pulls the SNARE complex, which rapidly initiates membrane fusion.

Wednesday 12 July
12:30–13:30, Auditorium François 1er

IUBMB Lecture – Closing Plenary Lecture

PL-11-1
Turbo-charging synaptic vesicles for explosive release of neurotransmitters

J.E. Rothman
Department of Cell Biology, Yale University, New Haven, CT, USA

How neurotransmitter release occurs thousands of times faster than other forms of SNARE-dependent membrane fusion has been a vexing problem. We have now discovered a simple principle that can explain this involving two layers of SNAREpins. A ring of 6 "central" SNAREpins is released by Ca^{2+} to open the fusion pore. Unlike fusion reactions, we suggest that synaptic vesicles are "turbo-charged" by an outer set of 6 "peripheral SNAREpins" that provides additional force. This model has its origin in cryo-EM tomography that revealed a protein structure with 6-fold symmetry at the interface of each ready-release synaptic vesicle with the active zone plasma membrane. Our current studies involve single molecule counting of SNAREpins in single synthetic ready-release vesicles (using fully-defined reconstitutions of vesicles with suspended bilayers) and novel high resolution cryo-EM structures of the membrane-bound SNARE-assembling chaperone Munc13. Together, the new results suggest that the observed 6-fold symmetry results from the *de novo* assembly of a series of sequential, symmetrical supramolecular machines. In this model, the vesicle is initially captured by a hexagonal "basket" of 18 copies of the chaperone Munc13 in their upright conformation. Munc13 then transits through a lateral hexagon arrangement, moving the vesicle closer to the plasma membrane. The six peripheral SNAREpins are coordinately assembled during this transition. The six central SNAREpins are assembled later, each by one subunit of the Munc13 hexagon as it flattens onto the plasma membrane to form an outer ring. The central SNAREpins are bound to the inner Ca^{2+}-sensitive ring of Synaptotagmin, each paired with a peripheral SNAREpin by a bridging molecule of Complexin in the previously observed trans-clamping arrangement. Binding of Ca^{2+} then releases all 12 SNAREpins simultaneously. This model makes many novel and testable predictions, and is consistent with available structural and genetic evidence.

Symposia

Sunday 9 July
9:00–11:00, Auditorium François 1er

Cancer and Aging

S-01.1-2
Understanding the role of aging in cancer

P. Adams
Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA

Age is the biggest single risk factor for most adult human cancers. The reasons for this are not well understood. Aging is associated with multiple so-called "hallmarks of aging", including accumulation of genetic mutations, epigenetic and metabolic changes, telomere shortening, protein misfolding, accumulation of senescent cells, stem cell exhaustion, altered cell–cell communication and changes to the immune system. Many of these hallmarks can be potential drivers of cancer. Our lab is working to dissect the contribution of these hallmarks to the age-dependence of cancer. This can reveal targets for cancer diagnosis, risk assessment and prevention.

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S-01.1-1
Mitochondrial DNA mutations in aging and cancer – what's the connection?

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[1]*Newcastle University, Newcastle Upon Tyne, UK,* [2]*Cancer Research UK Beatson Institute, Glasgow, UK*

Alterations in mitochondrial metabolism are major hallmarks of both aging cells and cancer. Age is the biggest risk factor for the development of a significant number of cancer types and this therefore raises the question of whether there is a link between age-related mitochondrial dysfunction and the advantageous changes in mitochondrial metabolism prevalent in cancer cells. A common underlying feature of both aging and cancer cells is the presence of somatic mutations of the mitochondrial genome (mtDNA). MtDNA mutations are particularly enriched in colorectal cancers (Gorelick *et al. Nat Metab* 3: 558–570, 2021), and we have previously shown that individual normal human colonic crypt stem cells also accumulate somatic mtDNA point mutations with age (Greaves *et al. Exp Gerontol* 45: 573–579, 2010). This shows that somatic mtDNA mutations and altered metabolic pathways are present in colonic crypts prior to malignant transformation, suggesting that mtDNA mutations may either increase the risk of malignant transformation, promote tumor progression, or are selectively propagated during tumor development. To investigate this, we generated a mouse model in which we induced tumors specifically in intestinal stem cells with and without mtDNA mutation-induced mitochondrial dysfunction. We found that the mice with mitochondrial dysfunction had similar numbers of tumors to controls but they were growing significantly faster, resulting in a shortened lifespan (Smith *et al. Nature Cancer* 1: 976–989, 2020). Multi-omics analysis revealed the underlying mechanism to be an upregulation of the *de novo* serine synthesis pathway and mitochondrial one-carbon metabolism in response to mitochondrial dysfunction. These anabolic pathways are important regulators of cellular biomass production and, excitingly, may represent metabolic vulnerabilities for therapeutic exploitation in human colorectal cancer. *The authors marked with an asterisk equally contributed to the work.

S-01.1-3
Early life influences on aging trajectories of stem cells and metabolism

L. Rudolph
Leibniz Institute on Aging – Fritz Lipmann Institute (FLI), Jena, Germany

Organism aging leads to impairments in tissue maintenance and increases in disease development. Genetic studies from our lab indicate that growth and metabolism activity at early life influences aging trajectories of stem cells in later life. We propose that early life stress factors install a memory in stem cells and tissues that in turn influences aging by impairing metabolic functions, adaptive stress responses, and cell differentiation. Our data indicate that it is possible to reprogram metabolism and metabolic stress responses to a more youthful state by late life dietary interventions. Such interventions may have the capacity to ameliorate increases in cancer incidence during aging.

ShT-01.1-1
DNA mismatch repair protects the genome from oxygen-induced replicative mutagenesis

D. Szüts[1], R. Lózsa[1], E. Németh[1], J.Z. Gervai[1], B.G. Márkus[2], S. Kollarics[2], F. Simon[2]
[1]*Research Centre for Natural Sciences, Institute of Enzymology, Budapest, Hungary,* [2]*Department of Physics, Institute of Physics, Budapest University of Technology and Economics, Budapest, Hungary*

DNA mismatch repair (MMR) corrects mismatched DNA bases arising from multiple sources including polymerase errors and base damage. By detecting spontaneous mutagenesis using whole genome sequencing of cultured MMR deficient cells, we show that a primary role of MMR is the repair of oxygen-induced mismatches. We found an approximately twofold higher mutation rate in two different MMR deficient human cell lines exposed to normoxic conditions as opposed to mild hypoxia, which correlated with oxidant levels measured using electron paramagnetic resonance spectroscopy. The oxygen-induced mutations were dominated by T to C base substitutions found primarily on the lagging strand. A broad sequence context preference, dependence on replication timing and a lack of transcriptional strand bias further suggested that oxygen-induced mutations arise from polymerase errors rather than oxidative base damage. We defined separate low and high oxygen–specific MMR deficiency mutation signatures and showed that the effect of oxygen is observable in MMR deficient cancer genomes, where it best correlates with the contribution of mutation signature SBS21. A separate analysis of clonal and subclonal mutations shed light on the evolution of MMR deficient tumors, revealing a preferential increase of the hypoxia-specific signature among subclonal mutations signaling hypoxic conditions in later tumors, especially those that lack vascularization.

ShT-01.1-2
Mutant p53 (mutp53)-driven HMGA1 secretion promotes pancreatic ductal adenocarcinoma (PDAC) proliferation and chemoresistance

F. Danzi[1], G. Butera[1], A. Fiore[1], C. Mortali[1], R. Pacchiana[1], J. Brandi[2], D. Cecconi[2], M. Donadelli[1]
[1]*Department of Neurosciences, Biomedicine and Movement Science, Biological Chemistry Section, University of Verona, Verona, Italy,* [2]*Department of Biotechnology, University of Verona, Verona, Italy*

Pancreatic ductal adenocarcinoma (PDAC) is among the most lethal cancers. Recent evidence demonstrated a key role of mutant p53 (mutp53) in altering the secretion of signaling molecules, thus manipulating the tumor microenvironment (TME) to drive invasion. Since a deeper characterization of the cancer secretome may lead to the identification of druggable targets for tumor treatments, we focused our study on the roles of the mutp53-dependent secretome in the TME of PDAC cells. We demonstrated the oncogenic role of the mutp53-driven secretome on PDAC cells. Then, 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 whose high intracellular expression levels are correlated with poor prognosis of PDAC patients. Our data show that mutp53-induced secretion of

HMGA1 promotes PDAC cell hyperproliferation and resistance to gemcitabine (GEM) treatment, suggesting a critical role of this protein in tumor aggressiveness. Furthermore, we proved that GEM increases HMGA1 secretion in mutp53-PDAC cells. Since we previously published that GEM aberrantly stimulates mutp53 activity in PDAC, we assumed that mutp53-driven HMGA1 secretion may constitute a mechanism of chemoresistance to GEM. Therefore, HMGA1 can be a therapeutic target in mutp53-PDAC cells. Our preliminary data suggest that mutp53-driven secretion of HMGA1 may act in an autocrine/paracrine manner stimulating crucial anabolic and oncogenic pathways. In conclusion, we demonstrated that hypersecretion of HMGA1 by mutp53 induces the hyperproliferation and invasiveness of PDAC cells. This represents a promising secreted target in aggressive PDAC with mutations in the TP53 gene, thus confirming that the alteration of TME might provide new therapeutic opportunities counteracting chemoresistance in mutp53-PDAC patients.

Sunday 9 July
9:00–11:00, Auditorium Ronsard

Protein Life Cycle I: Localization, Dynamics, Functioning

S-06.1-1
Mitochondrial control of nuclear condensates

I. Díaz-Moreno, L. Guerra-Castellano, A. Guerra-Castellano, G. Pérez-Mejías, A. Velázquez-Cruz, M.A. Casado-Combreras, R. Giner-Arroyo, B. Baños-Jaime, J. Tamargo-Azpilizcueta, A. Fernández-Veloso, J.I. Pérez López, A.V. Cabello Aguilera, P. Rivero García, M.A. De la Rosa Acosta
Institute for Chemistry Research, cicCartuja – University of Seville – CSIC, Seville, Spain

The regular functioning of the nucleolus and nucleus-mitochondria crosstalk are considered unrelated processes, yet cytochrome *c* (C*c*) migrates to the nucleus and even the nucleolus under stress conditions. Nucleolar liquid–liquid phase separation usually serves the cell as a fast, smart mechanism to control the spatial localization and trafficking of nuclear proteins. Actually, the alternative reading frame (ARF), a tumor suppressor protein sequestered by nucleophosmin (NPM) in the nucleoli, is shifted out from NPM upon DNA damage. DNA damage also triggers early translocation of respiratory C*c* to the nucleus before cytoplasmic caspase activation. Here, we show that C*c* can bind to nucleolar NPM by triggering an extended-to-compact conformational change, driving ARF release. Such an NPM–C*c* nucleolar interaction can be extended to a general mechanism for DNA damage in which the lysine-rich regions of Cc—rather than the canonical, arginine-rich stretches of membrane-less organelle components—control the trafficking and availability of nucleolar proteins.

S-06.1-3
The role of highly flexible regions in orchestrating the properties of multi-domain proteins: the example of N from SARS-CoV-2

I. Felli
University of Florence, Florence, Italy

Highly flexible regions of complex multi-domain proteins introduce an additional dimension in protein function, still exploiting simple modules (globular domains and highly flexible regions themselves). This modular protein architecture is shared by many proteins involved in recognition, signaling and regulation, all processes in which structural and dynamic heterogeneity play a fundamental role. Protein malfunction, linked to the onset of incurable diseases, is often related to highly flexible regions. NMR represents a unique tool for their investigation at the atomic level. However, when globular and disordered domains are simultaneously present in a protein, the NMR spectra can become quite complex. ^{13}C detection offers an elegant approach to study them not only in isolation but also when part of complex multi-domain proteins, such as the Nucleocapsid protein from SARS-CoV-2.

S-06.1-2
Protein N-terminal acetylation: machinery and impact

T. Arnesen[1,2,3]
[1]*Department of Surgery, Haukeland University Hospital, Bergen, Norway,* [2]*Department of Biomedicine, University of Bergen, Bergen, Norway,* [3]*Department of Biological Sciences, University of Bergen, Bergen, Norway*

Most human proteins are N-terminally acetylated. In recent years, we have identified and defined the responsible machinery in human cells, the N-terminal acetyltransferases (NATs). There are seven human NATs known to date, and these may act as ribosome-associated co-translational modifiers of large substrate groups, or post-translationally on specific protein substrates. In the latter group, the Golgi-associated NAA60 acts on specific membrane proteins while the cytosolic NAA80 acetylates the N-termini of actins. At the protein level, the impact of N-terminal acetylation is very diverse and ranges from subcellular targeting to protein degradation or stabilization. The physiological importance of N-terminal acetylation is stressed by the pathologies caused by dysfunctional NAT enzymes. Here, the basic molecular principles and physiological impact of N-terminal acetylation will be outlined.

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ShT-06.1-2
Complex study of the toxic effect of SARS-CoV-2 ORF6 protein

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The genome of the SARS-CoV-2 virus, the causative agent of the COVID-19 pandemic, encodes 29 proteins instrumental in the transcription and replication of the virus and the inhibition of host cell defense. It was shown that accessory protein ORF6 limits the immune response to infection by antagonizing STAT1 nuclear translocation and preventing mRNA transport via interaction with the nucleopore components Nup98 and Rae1. However, the detailed mechanism of the pathogenicity of ORF6 on target cells is not yet understood. In this study, we focus on the effects of SARS-CoV-2 ORF6 on the DNA replication of the host cells. By *in silico* modeling and immunofluorescence labeling, we confirmed the interaction and co-localization of ORF6 and Rae1 in cells overexpressing ORF6. The cell cycle profiles of these cells exhibit a significant decrease in the percentage of cells in S- and G2-phases. This indicates that ORF6 impedes S-phase entry, explained by the observed tenfold decrease in the cyclin E mRNA levels due to the sequestration of Rae1 by ORF6. In cells that do enter S-phase, DNA fiber labeling showed that ORF6 reduces replication fork rate as a consequence of co-transcriptional RNA–DNA hybrids (R-loops). The increase of R-loops detected by FRAP analysis of an R-loop binding fluorescent sensor is likely due to defects in mRNA export. Our findings show that the toxic effects of ORF6 on DNA replication in infected cells are mediated through ORF6 interaction with Rae1 (with respect to proliferation) and are manifested in at least two ways: on the one hand, ORF6 compromises cell progression into S-phase, and on the other, it promotes the accumulation of R-loops, which are a major source of genome instability. Acknowledgements: This work was supported in part by the Bulgarian Science Fund under Grant KP-06-DK1/5/2021. Computational resources were provided at the BioSim HPC cluster at the Faculty of Physics of Sofia University "St. Kl. Ohridski".

ShT-06.1-1
Structural insights into the plant PIN-FORMED auxin transporter

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Auxin is a major class of plant growth hormone that orchestrates almost all developmental steps and growth in plants. Auxin response is mediated by an auxin gradient across the plant tissues. The secondary transporter PIN-FORMED (PIN) proteins are at the heart of this process as they govern auxin export from the cytosol to the extracellular space [1]. For decades, the lack of structural and biochemical data has impeded detailed comprehension of the molecular mechanism behind PIN-mediated auxin transport. In our recent discoveries [2], we present three cryo-EM structures of the *Arabidopsis thaliana* PIN8 at two main conformations: outward-facing conformation with and without auxin bound, and inward-facing conformation in the presence of the commonly used herbicide naphthylphthalamic acid (NPA). A detailed structural and biophysical analysis highlighted key residues involved in auxin recognition and transport by PIN as well as NPA binding. We showed that PIN8, in the homodimer form, is constitutively active and uses an elevator mechanism to transport auxin. This transport is independent of ion and proton gradients and our data support a transport mechanism driven by the negative charge of the auxin. In line with the findings, we recently caught a new PIN configuration during auxin transport. These discoveries will bring important new knowledge enabling an understanding of the fundamental mechanism for auxin interaction and transport and paving the way for developing new generations of eco-friendly and plant-specific herbicides. References: [1] Ulrich Z. Hammes, Angus S. Murphy, Claus Schwechheimer (2022) Auxin Transporters-A biochemical view. Cold Spring Harb. Perspect. Biol. DOI: 10.1101/cshperspect.a039875. [2] Kien Lam Ung*, Mikael Winkler*, Lukas Schulz, Martina Kolb, Dorina P. Janacek, Emil Dedic, David L. Stokes, Ulrich Z. Hammes & Bjørn Panyella Pedersen (2022) Structures and mechanism of the plant PIN-FORMED auxin-transporter. Nature. DOI: 10.1038/s41586-022-04883-y. *The authors marked with an asterisk equally contributed to the work.

Sunday 9 July
9:00–11:00, Auditorium Descartes

Climate Change: Biochemical CO_2 Fixation

S-03.3-1
Mechanism of multi-protein phase separation in beta-carboxysome biogenesis

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Photosynthesis is a fundamental process in biology as it converts solar energy into chemical energy and thus, directly or indirectly, fuels all life on earth. The chemical energy is used to fix atmospheric CO_2 and produce reduced carbon compounds in the Calvin-Benson-Bassham cycle. The key enzyme for this process in all photosynthetic organisms is ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), which is responsible for the conversion of an estimated amount of $\sim 10^{11}$ tons of CO_2 per annum into organic material. Rubisco is the most abundant enzyme in nature, owing in part to its low catalytic turnover rate and limited specificity for CO_2 versus O_2. To avoid the reaction with O_2, cyanobacteria have evolved proteinaceous microcompartments called carboxysomes, in which the enzymes Rubisco and carbonic anhydrase (CA) are enclosed. Dissolved CO_2 in the form of HCO_3^- diffuses through the proteinaceous carboxysome shell and is converted to CO_2 by CA, generating a high concentration of CO_2 for carbon fixation by Rubisco – the so-called CO_2-

concentrating mechanism. The shell also prevents access to reducing agents, generating an oxidizing environment inside the carboxysome. In beta-cyanobacteria, the assembly of the beta-carboxysome first involves the aggregation of Rubisco and CA, followed by shell formation. Recent advances have shown that early in the process of pro-carboxysome assembly, a specialized scaffolding protein called CcmM initiates phase-separation of both Rubisco and CA into biomolecular condensates. The talk will describe our present understanding of the complex multivalent interactions that result in the sequestration of four proteins into a pro-carboxysome. Understanding carboxysome biogenesis will be important for efforts to engineer a CO_2-concentrating mechanism into C3 crop plants.

S-03.3-3
Harnessing nature's solutions for more efficient CO_2 fixation

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The CO_2-fixing enzyme Ribulose-1,5-carboxylase/oxygenase (Rubisco) represents the major point of carbon entry into the biosphere. Rubisco has a slow catalytic rate and exhibits poor substrate specificity such that Rubisco catalysis often limits the growth rate of photosynthetic organisms, including crop species. But what about the wealth of Rubisco functional diversity that has evolved in different lineages under different selection pressures? What lessons can we learn from these organisms? For example, the catalysis of certain "red" Rubisco isoforms from non-green algae, such as *Griffithsia monilis*, far surpass "green" Rubiscos found in higher plants and green-algae. However, efforts to transplant these Rubiscos into green plants have been thwarted by their folding and assembly requirements not being met by plants (or *Escherichia coli*). The sequence-structure basis for the superior kinetic prowess of *G. monilis* Rubisco is, at best, poorly understood. Dr. Gunn will present prior successes, and ongoing efforts, to express red type Rubiscos in green-type plants for enhanced CO_2 fixation efficiency using biochemistry, structural biology, synthetic biology and protein engineering approaches. Harnessing the superior carbon fixing efficiency of *G. monilis* Rubisco in plastids will have major implications for the efficiency of crop species.

S-03.3-2
How pyrenoid-based CO_2-concentrating mechanisms can boost plant performance

A. McCormick
University of Edinburgh, Edinburgh, UK

Many photosynthetic species have evolved CO_2-concentrating mechanisms (CCMs) to improve the efficiency of CO_2 assimilation by Rubisco and reduce the negative impacts of photorespiration. As the majority of plants (i.e., C3 plants) lack an active CCM, introducing a functional heterologous CCM into crops is a key engineering ambition to enhance yield potential. Most algae, including the green alga *Chlamydomonas reinhardtii*, possess a pyrenoid-based CCM that enhances CO_2 concentrations in the chloroplast and aggregates Rubisco into a liquid-like phase separated condensate resulting in faster catalysis and decreased photorespiration. Through advances in our understanding of the Chlamydomonas CCM, the complex task of building a functional pyrenoid-based CCM in crops has moved several steps closer to reality, particularly now with a model-based roadmap to guide future engineering efforts. I will outline how pyrenoid-based CCMs can boost plant performance and resilience to climate change and our recent progress in transferring key components and features into plant chloroplasts.

ShT-03.3-4
The evolution of the Rubiscosome

G. Hochberg
Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

Rubisco is the central CO_2-fixing enzyme of the Calvin cycle and probably the most abundant protein on earth. Changes in Rubsico's biochemistry have had profound effects on the trajectory of life on earth, because they are very directly tied to the efficiency of photosynthetic CO_2 fixation. In the course of its history, Rubisco recruited a new subunit, improved its ability to discriminate between CO_2 and its undesired side substrate O_2, and recruited a set of dedicated assembly chaperones that became completely essential to assemble the enzyme in plants. In this talk, I will summarize our work in recapitulating these events using ancestral sequence reconstruction and the experimental resurrection of ancient forms of this enzyme.

ShT-03.3-1
Rubisco regulation in crops

E. Carmo-Silva
Lancaster University, Lancaster Environment Centre, Lancaster, UK

Rubisco catalyzes the first step in the conversion of carbon from CO_2 into plant biomass: the carboxylation of ribulose-1,5-bisphosphate (RuBP). This reaction is essential for life on earth. Rubisco is frequently regarded as an inefficient enzyme because it also catalyzes the oxygenation of RuBP and is prone to making mistakes during catalysis. However, the more we know about Rubisco, the more we appreciate how good it is for its job! Many chloroplast components, including proteins, sugar-phosphates, ions, etc, interact with Rubisco. These components respond to fluctuations in the prevailing environment of the leaf, and dynamically modulate the activity of Rubisco, adjusting the rate of CO_2 assimilation as conditions change. Incident light, for example, is constantly changing for leaves within crop canopies in field settings. With increasing frequency of heat waves, leaf temperature changes also impact Rubisco activity. While Rubisco has been evolving and adapting to the specific chloroplast environment, there is scope to fine tune the regulation and thereby improve the efficiency of crop Rubiscos.

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Sunday 9 July
16:30–18:30, Auditorium François 1er

Supramolecular Assemblies I: Signal Transduction

S-07.1-3
Elucidating energy coupling of tripartite transport machineries in biomimetic environments

M. Picard
IBPC, Paris, France

The need to overcome antibiotic resistance in common Gram-negative pathogens remains unmet and represents a challenging aspect of the research in the field of antimicrobials. Among the various resistance mechanisms developed by bacteria, efflux pumps are on the front line. They consist of membrane proteins that expel noxious compounds outside of the bacteria, across its two-membrane environment. Efflux pumps constitute promising therapeutic targets, as their blockage could restore the actual arsenal utility. We have developed an original and unique methodology that allows us to specifically monitor the activity of *in vitro* reconstituted efflux pumps from Gram-negative bacteria. This test relies on the reconstitution of the efflux pumps in proteoliposomes mimicking the two-membrane architecture of the envelope of Gram-negative bacteria, and on the monitoring of the transfer of substrates through the reconstituted pump using fluorescent reporters. Our *in vitro* approach allows us to study the molecular determinants behind efflux pump assembly and transport and to tackle their structure–function analysis by crystallography, cryo-EM and NMR. In addition, the methodology was miniaturized and automatized on a 96-well microplate reader, hence making it possible to automatically test large-scale chemical libraries. Our project is to identify efflux pump inhibitors (EPI) that consist of adjuvant molecules without intrinsic antibiotic activity that work by blocking efflux pump and therefore restore the activity of antibiotics.

S-07.1-1
Misregulation of Wnt/β-catenin signaling at the plasma membrane and the regulatory role of membrane lipids in hepatocellular carcinoma

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Wnt/β-catenin signaling plays fundamental roles in numerous physiological activities such as cell fate determination, differentiation, and migration during development and homeostasis. Since Wnt pathway misregulation has been widely linked to pathological conditions including malignant tumors, a thorough understanding of pathway regulation is essential for the development of effective therapeutic approaches. A prominent feature of cancer cells is that they significantly differ from healthy cells with respect to their plasma membrane composition and lipid organization. Hepatocellular carcinoma (HCC) is largely associated with aberrant activation of Wnt/β-catenin signaling. Nevertheless, how membrane lipid composition is altered in HCC cells with abnormal Wnt signaling remains elusive. By exploiting comprehensive lipidome profiling, we unravel the membrane lipid composition of different HCC cell lines with mutations in components of Wnt/β-catenin signaling, leading to differences in their endogenous signaling activity. Among the numerous differentially regulated lipids, diacylglycerol (DAG) and ceramide were downregulated at the membrane of all HCC cells after Wnt3a stimulation. DAG and ceramide enhanced Wnt/β-catenin signaling in two different HCC cell types, one type with a relatively lower level of endogenous Wnt/β-catenin activity that can be stimulated with Wnt3a, and another type with a high level of endogenous Wnt/β-catenin activity that cannot be stimulated with Wnt3a. In contrast, depletion of DAG and ceramide suppressed Wnt/β-catenin signaling and significantly impeded the proliferation, tumor growth, and *in vivo* migration capacity of SNU475 and HepG2 cells. This study, by pioneering plasma membrane lipidome profiling in HCC cells, exhibits the remarkable potential of lipids to correct dysregulated signaling pathways in cancer and stop abnormal tumor growth.

S-07.1-2
The pleiotropic actions of arrestin proteins

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Research Centre for Natural Sciences, Budapest, Hungary

β-arrestins are key regulators of G protein-coupled receptors (GPCRs). The binding of β-arrestins to GPCRs induces receptor desensitization, internalization, and activation of a set of signaling pathways, which complement G protein-dependent ones. Generally, β-arrestins interact with the agonist-activated GPCRs through the receptor core and the phosphorylated C terminus. We found that β-arrestins are able to bind even the inactive state of the receptor, if the "stability lock" is formed. The stability lock represents a strong interaction between phosphate groups attached to Ser/Thr amino acids in the receptor C terminus and positively charged residues in β-arrestins. The phosphorylation of receptors can be triggered by both G protein-coupled receptor kinases or second messenger-activated kinases, such as protein kinase C, thus enabling β-arrestins to take part in heterologous regulatory mechanisms. The formation of the stability lock is sufficient for the activation of β-arrestins and assembly of β-arrestin-dependent signaling complexes. The stable binding is also responsible for the co-trafficking of β-arrestins and the receptors to endosomes. Interestingly, in a systematic analysis of β-arrestin activation by various AT1 angiotensin receptor agonists, we observed the largest kinetic differences in endosomal receptor-β-arrestin complex formation. In contrast, we detected much less variance in β-arrestin translocation to the plasma membrane. Therefore, the stability lock not only permits the β-arrestin-mediated regulation of inactive receptors but provides an important link for the spatiotemporal modulation of signaling efficacy of agonists. We also discovered that the serine/threonine motifs involved in the stability lock may have a broader impact on β-arrestin-dependent signaling than previously thought. *The authors marked with an asterisk equally contributed to the work.

ShT-07.1-1
Holdup Multiplex assay for high-throughput measurement of protein-ligand affinity constants using a mass-spectrometry readout

F. Delalande[1], G. Gogl[2], C. Carapito[1], G. Travé[2], **E. Monsellier**[2]
[1]*Laboratoire de Spectrométrie de Masse BioOrganique (LSMBO), IPHC-DSA, Université de Strasbourg, CNRS UMR 7178, Strasbourg, France,* [2]*IGBMC, Illkirch-Graffenstaden, France*

The accurate description and subsequent modeling of protein interactomes requires quantification of their affinities at proteome-wide scale. Here we develop and validate the Holdup Multiplex, a versatile assay for high-throughput measurement of protein-ligand affinity constants that uses mass-spectrometry as readout. The method can quantify thousands of affinities in one single run, with high precision and over several orders of magnitude. We applied this strategy to the seven human 14-3-3 isoforms, quantifying in a few sample-runs their interaction with 1000 different phosphopeptides. We were able to identify hundreds of new 14-3-3 binding sites. We showed that the seven human 14-3-3 display similar specificities but staggered affinities, 14-3-3γ being always the best binder and 14-3-3ε and σ, the weakest. Finally, we identified dozens of 14-3-3 bindings sites, some intervening in key signaling pathways, that were either stabilized or destabilized by the phytotoxin Fusicoccin-A. Our approach, for which throughput can be pushed up to the sensitivity limit of the mass-spectrometry set-up, is applicable to any category of protein-ligand interactions and thus has wide potential for both high-throughput interactomics and chemoproteomics.

ShT-07.1-2
Comparative analysis of PDZ-binding motifs in the diacylglycerol kinase family

B. Zambo, G. Gogl, H. Moine, G. Travé
I.G.B.M.C. – Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch-Graffenstaden, France

Diacylglycerol kinases (DGKs) are evolutionarily conserved lipid kinases whose enzymatic function is to convert DAG to PA by phosphorylation. DGKs are highly expressed in the brain and their impaired function leads to neurological disorders such as epilepsy, obsessive compulsive disorder, schizophrenia, bipolar disorder or fragile X syndrome. Some of these enzymes possess C-terminal sequences that encode potential PDZ-binding motifs (PBMs) that may be involved in their recruitment into supramolecular signaling complexes. In this study, we used the ProfAff database containing PDZ-PBM affinities to compare the binding profiles of the different types of DGK enzymes across the whole PDZome. We combined the obtained data with our novel native holdup (nHU), affinity purification coupled to mass spectrometry (AP-MS) and evolutionary conservation analyses to investigate the functionality of the PBMs in the family. We concluded that potential PBM sequences of type II enzymes are likely to be non-functional, while type IV enzymes possess a highly promiscuous PBM with very similar binding profiles and we identified several new partners for these enzymes. This study demonstrates that combining quantitative interactomics and evolutionary analyses is a useful strategy to identify and characterize functional domains and motifs within different enzyme families.

Sunday 9 July
16:30–18:30, Auditorium Ronsard

Biotech Solutions to Current Problems

S-03.1-3
PET recycling: from enzyme and process optimization to an industrial plant

A. Marty[1], V. Tournier[1], N. Chabot[1], I. André[2], G. Lippens[2]
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Plastics are found everywhere in our daily life due to exceptional properties. The worldwide market reaches 400 million tons. However, they represent a major environmental issue with 125 million tons of generated plastic waste annually. Only 10% of collected plastics are recycled, and, at best, plastic wastes are incinerated but an unacceptable quantity are lost in nature, with 9 million tons ending each year in the oceans. Carbios (http://www.carbios.com), a young innovative green chemistry company, in collaboration with the laboratory TBI (Toulouse Biotechnology Institute; INSA/CNRS/INRAE; http://www.toulouse-biotechnology-institute.fr), developed an enzymatic process to recycle one of the main plastics, PET (~ 100 million tons per year). A first breakthrough was reached with the optimization of an extraordinary PETase used to break down PET returning to monomers (Nature; Vol. 580 Issue 7802, 9 April 2020). Since then, we continue to optimize this enzyme, to improve kinetics and yields and the performances of our best enzymes will be presented. The scale-up of the process in an industrial demonstrator will be presented with a 20 m^3 reactor and all the downstream processing to purify both terephthalic acid and ethylene glycol. Carbios is building a first industrial unit in France, operational in 2025, which will recycle 50 000 tonnes of PET waste per year. Key Words: PET recycling, enzyme, PETase, industrial plant.

S-03.1-2
Designing live catalysts for large-scale environmental interventions

V. de Lorenzo
National Centre for Biotechnology, Madrid, Spain

Metabolic engineering for whole-cell catalysis has evolved in the last few years in the context of a major climate crisis characterized by unacceptably high atmospheric levels of greenhouse gases, the worrying pollution of the oceans with very recalcitrant plastics and microplastics and the noxious effects of micropollutants on many ecosystems. Global problems ask for global solutions and the environmental microbiome—because of its dimension and amazing activities—may end up being out best instrument to both counter the impact of industrial development and enable a new, sustainable interplay with the natural world. While the whole planet is afflicted at a global scale by chemical pollution and anthropogenic emissions, the ongoing development of systems and synthetic biology, modern chemistry and some key concepts from ecological theory allow us to tackle this phenomenal challenge and propose large-scale interventions aimed at reversing and even improving this state of affairs. This involves (i) identification of key reactions or processes that need to be

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re-established (or altogether created) for ecosystem reinstallation, (ii) implementation of such reactions in natural or designer hosts able to self-replicate and deliver the corresponding activities when/where needed in a fashion guided by sound ecological modeling, (iii) dispersal of niche-creating agents at a global scale—what has been called *Environmental Galenics*—and (iv) containment, monitoring and risk assessment of the whole process. The pillar of this new scenario includes a deep engineering of microorganisms as live chassis for delivering beneficial activities and multi-scale environmental interventions for pollution prevention/remediation (including climatic change). Current advances in the use of environmental bacteria (e.g. *Pseudomonas putida*) as a SynBio chassis of choice for meeting some of these environmental objectives and some genetic tools developed to this end will be addressed.

S-03.1-1
Enzymes as game changers to address the sustainability challenge in synthesis

S. Gandomkar, S. Roth, M. Aleotti, F. Rossi, **M. Hall**
University of Graz, Graz, Austria

Enzymes are gaining increased attention in the context of sustainable synthesis, as they operate under environmentally friendly reaction conditions and can contribute to the 'greening' of chemical manufacturing. Two selected examples will illustrate how biocatalysis combined with biotechnology can offer attractive approaches to address the sustainability challenge in modern synthesis. (i) γ- and δ-lactams are – in contrast to smaller ß-lactams – molecules with remarkable stability. The chemical hydrolysis of their amide bond requires harsh reaction conditions (reflux and strong acid) and up to now, no enzyme active on monocyclic γ- and δ-lactams has been reported. We aim at establishing a broad-spectrum biocatalytic platform for the ring opening of γ- and δ-lactams, thereby contributing to the understanding of the underlying biochemistry of these highly stable molecules. Our approach relies on microbial strains exposed to metabolic pressure through growth on lactams as sole source of C/N, and on heterologously expressed proteins, such as ATP-dependent enzymes. (ii) The nitro functionality is one of the major functional groups used in organic synthesis. Its use as an enabling tool in synthesis for the production of dyes, polymers, and pesticides, is complemented by its occurrence in a range of compounds (e.g., chemotherapeutic agents and prodrugs). However, access to nitro compounds relies on poorly selective and environmentally unfriendly methods (e.g., use of halogenated compounds and strong acids and oxidants). Inspired by the occurrence of nitro compounds in nature, we are currently designing innovative enzymatic strategies for nitration reactions toward the next generation of bio-based chemical building blocks in an environmentally acceptable manner, while reducing both waste and toxicity of the process. Acknowledgement is made to the Austrian Science Fund (FWF P32815-N) and the ACS GCI Pharmaceutical Roundtable Research Grant for partial support of this research.

ShT-03.1-1
Structural analysis of antigens on virus-like particles in vaccine formulations

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Structural analysis of adjuvant-coupled antigens is important for rational vaccine development, but has been impeded by the lack of appropriate techniques. In principle, depending on the mobility of the antigen site, solution NMR or magic-angle spinning (MAS) solid-state NMR is pertinent to the study of such dynamic and heterogeneous systems. However, their application has been hampered by the relatively low antigen content in vaccine formulations. We show that isotope labelling together with sensitivity enhancement techniques allow us to overcome the penalty associated with antigen dilution. This makes it possible to assess the structure of antigens, both in their free, unformulated form and once chemically coupled or genetically fused to the surface of large virus-like particles (VLPs). Comparison of the NMR fingerprints between the free and VLP-coupled forms of the antigen provides site-specific information of the structural change or conservation occurring upon bioconjugation. Thereby, this work demonstrates that NMR can play a major role in vaccine design, formulation studies, and manufacturing process development. Previously published in: Jaudzems K et al. (2021) Angew Chem Int Ed 60, 12847–12851.

ShT-03.1-2
Revealing the mechanisms of TnpB: biochemical characterization of DNA target recognition

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Mobile genetic elements, such as transposons, plasmids, phages, and insertion sequences (IS), are responsible for high genomic plasticity in bacteria, allowing microorganisms to evolve rapidly towards changing environmental conditions. IS200/IS605 family insertion sequences are one of the most widely distributed groups of mobile genetic elements among prokaryotes, encoding TnpA, a transposase, responsible for the IS mobility, and TnpB, which is unnecessary for transposition. It was suggested that TnpB proteins might be evolutionary predecessors of CRISPR-Cas12 nucleases, which are widely used as genome editing tools. Previously, we demonstrated that TnpB acts as a miniature reRNA-guided nuclease, capable of cleaving targets *in vitro* and in cells in a TAM-dependent manner, similarly to much larger Cas12 proteins. In this study, we aimed to expand the knowledge of the target cleavage mechanism by TnpB nuclease from *Deinococcus radiodurans*. Here, we present the mechanistic insights into target requirements for efficient substrate cleavage. Moreover, we

determine the impact of single nucleotide mismatches in TAM or reRNA complementary target sequences on the binding and cleavage of dsDNA substrates. Altogether, the obtained results reveal novel mechanistic details of DNA target recognition and cleavage by TnpB, and provide the framework for further development of TnpB-based genome editing tools. *The authors marked with an asterisk equally contributed to the work.

Sunday 9 July
16:30–18:30, Auditorium Descartes

Autophagy

S-02.1-1
Nonselective autophagosome biogenesis balances vesicle maturation with cargo inclusion

Z. Elazar, O. Shatz, M. Fraiberg
Weizmann Institute, Rehovot, Israel

Autophagy sequesters cytoplasmic content into newly built vesicles targeted for lysosomal degradation. In selective autophagy, the isolation membrane (IM) elongates while tightly engulfing specific cargo. In contrast, the manner by which expansion of the nonselective IM is coordinated with sequestration of bulk cytoplasm is yet unknown. Here we combine yeast genetics with live imaging to establish expansion of the nonselective IM in a spherical shape while maintaining a narrow rim. We further show that artificial augmentation of the rim delays maturation of the autophagic vesicle, while excessive constriction of the rim excludes internalization of large cargo such as ribosomes. We therefore propose that the narrow opening of the nonselective autophagic vesicle is tightly regulated in space and time to balance maturation with inclusion of cargo.

S-02.1-3
WIPI beta-propeller proteins in the control of autophagy and longevity

T. Proikas-Cezanne
Eberhard-Karls-University, Tübingen, Germany

Human WIPI beta-propeller proteins act as key PI3P effectors in (macro)autophagy, with WIPI4 and WIPI3 being able to link autophagy control to autophagosome formation via the major regulators of autophagy, AMPK and TORC1. At the nascent autophagosome, WIPI1 is thought to assist WIPI2 in the efficient recruitment of the ATG16L complex, which in turn promotes LC3/GABARAP lipidation and autophagosome maturation. Here, we will discuss our current understanding of the role of human WIPIs in autophagy, lifespan control, and neurodegeneration.

S-02.1-4
Initiation of autophagy and the role of the ATG8 conjugation machinery

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[1]*The Francis Crick Institute, London, UK,* [2]*University of Tokyo, Tokyo, Japan*

Autophagy is a highly conserved intracellular pathway which is essential for survival in all eukaryotes. In healthy eukaryotes, autophagy is used to remove damaged intracellular components, which can be as simple as an unfolded proteins or as complex as whole mitochondria. Once the damaged component is captured, the autophagosome engulfs it and closes, isolating the content from the cytoplasm. The capture and engulfment process can be either non-selective (bulk or macroautophagy, here called autophagy) or selective (ERphagy, mitophagy etc.). The autophagosome then fuses with the late endosome and/or lysosome to deliver its content to the lysosome for degradation. Formation of the autophagosome requires dedicated machinery, the ATG proteins, which act in a hierarchy of complementary activities. Initiation of autophagy begins with the formation of a phagophore, a double membrane, curved cisterna, which expands, grows, captures content (cargo) and closes, becoming an autophagosome. The phagophore and autophagosome are well described in morphological studies and there are approximately 15 core ATG genes in mammalian cells whose functions are under intense investigation. Visualization and monitoring autophagy relies on the detection of lipidated-ATG8s both *in vitro* and *in vivo* experiments. Recent data reveals the importance of small membrane-associated domains in the ATG proteins, in particular, alpha-helical structures in the ATG8 conjugation machinery and ATG8s. These studies illustrate how unique membrane association modules can control the formation of autophagosomes. *The authors marked with an asterisk equally contributed to the work.

ShT-02.1-2
Regulation of BNIP3L/NIX receptor dimerization in development-induced mitophagy

I. Novak Nakir
School of Medicine, University of Split, Split, Croatia

Mitophagy has been the most studied selective autophagy pathway. Its involvement in the elimination of impaired mitochondria is essential for cellular homeostasis and survival. Moreover, programmed mitophagy of healthy mitochondria, mostly governed by mitophagy receptors, plays an important role in the differentiation of many cell types, including erythrocytes, retinal ganglion cells, or oligodendrocytes. More than a decade ago, the BNIP3L/NIX mitophagy receptor was shown to be essential for the programmed removal of mitochondria during terminal erythropoiesis. Recent discoveries have proven that BNIP3L/NIX-mediated mitophagy is regulated by receptor dimerization as well as phosphorylation of the LC3-interacting region (LIR), which is essential for the recruitment of autophagy machinery. Here, we further investigate which phosphatases and kinases are responsible for receptor dimerization that provides the formation of stronger interactions between the receptor and autophagosomal LC3 proteins, and thus increased recruitment of mitochondria to autophagosomes. Our particular interest is to unveil signaling pathways that trigger and regulate mitophagy in erythroid lineage cells.

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ShT-02.1-1
ULK1/Atg1-mediated phosphorylation regulates the interaction between GAPR-1 and Beclin 1

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Utrecht University, Utrecht, The Netherlands

GAPR-1 is a negative regulator of autophagy through retaining Beclin 1 at the Golgi apparatus in mammalian cells. To study the molecular interactions between GAPR-1 and Beclin 1, we recently developed an *in vivo* protein–protein interaction assay in *Saccharomyces cerevisiae* that is based on mutual interference of condensate formation upon co-expression of the amyloidogenic proteins GAPR-1 and Beclin 1. Here, we used the same *Saccharomyces cerevisiae* model system to study the effect of phosphorylation on the oligomeric state of GAPR-1 and Beclin 1 and to determine the role of phosphorylation in the GAPR-1/Beclin 1 interaction. We found that ULK1/Atg1 is capable of phosphorylating both GAPR-1 and Beclin 1, thereby affecting their amyloidogenic properties and enhancing their interaction. Our study provides new insights into the regulation of autophagy, and has important implications for the development of therapeutic strategies for various diseases.

Monday 10 July
9:00–11:00, Auditorium François 1er

Gene Expression/Epigenetics

S-04.1-1
How transcription factors find their binding sites in the large genome?

N. Barkai
Weizmann Institute of Science, Rehovot, Israel

Transcription factors (TFs) contain long regions that are predicted to lack stable 3D folds. Those regions are located outside the DNA binding domains (DBDs), and remain mostly uncharacterized. I will discuss the roles of IDRs in directing TF binding specificity along the genome. After a short review of our initial findings, showing such a role in two TFs, I will describe new results addressing the mechanism, molecular grammar and generality of these IDR-based promoter preferences.

S-04.1-4
Understanding gene regulation using single molecule genomics

A. Krebs
Genome Biology Unit, EMBL, Heidelberg, Germany

Cis-regulatory elements (CREs) control the expression of genes involved in the acquisition of cellular identity during development, and its maintenance in healthy tissues. CRE activation typically requires the binding of multiple transcription factors (TFs) in the context of chromatin. The precise mechanisms underlying their cooperative action and the impact of epigenetic signals on their function is largely unknown. Current assays used to map TF occupancy average binding events arising from millions of cells, without informing on the potential cooperativity and the antagonisms that organize their binding at CRE. To move beyond this boundary, my lab developed Single Molecule Footprinting (SMF) that allows us to simultaneously measure the occurrence of multiple TFs, nucleosomes and DNA methylation on individual molecules genome-wide. I will illustrate how we leveraged this new layer of information to understand mechanisms of TF cooperativity and the epigenetic regulation of TF binding at enhancers. Detecting multiple TF binding events on single DNA molecules has enabled us to determine TF co-binding frequencies *in vivo*. Systematic analysis of the co-occupancy patterns of thousands of TF pairs reveals widespread evidence of cooperative binding. It elucidates the binding cooperativity mechanism used by TFs in absence of strict organization of their binding motifs, a characteristic feature of most enhancers. Additionally, we simultaneously quantified DNA methylation and TF binding on individual DNA molecules and tested if TF binding can occur at enhancers when their target DNA molecules are methylated *in vivo*. We identified a subset of cell-type specific enhancers that showed reduced accessibility on methylated molecules, suggesting that these enhancers are epigenetically regulated. Genetic perturbation experiments suggest a direct epigenetic control of TF binding at these loci. Sönmezer C, et al. *Mol Cell*. 2021; Kreibich E, et al. *Mol Cell*. 2023; Krebs, AR, TIGs. 2021.

S-04.1-1
The double-edged sword of transcription factor clustering

J. Meeussen[1,2], W. Pomp[1,2], I. Brouwer[1], W. de Jonge[1], H. Patel[1], **T. Lenstra**[1,2]
[1]*Netherlands Cancer Institute, Amsterdam, The Netherlands,* [2]*Oncode Institute, Amsterdam, The Netherlands*

Many transcription factors (TFs) localize in nuclear clusters of locally increased concentrations, but how TF clustering is regulated and how it influences gene expression is not well understood. Here, we use quantitative microscopy in living cells to study the regulation and function of clustering of the budding yeast TF Gal4 in its endogenous context. Our results show that Gal4 cluster formation is facilitated by, but does not completely depend on DNA binding and intrinsically disordered regions. Gal4 cluster properties are regulated by the Gal4-inhibitor Gal80 and Gal4 concentration. Moreover, we discover that clustering acts as a double-edged sword: self-interactions aid TF recruitment to target genes, but recruited Gal4 molecules that are not DNA-bound do not contribute to, and may even inhibit, transcription activation. We propose that cells need to balance the different effects of TF clustering on target search and transcription activation to facilitate proper gene expression.

ShT-04.1–2
Cell type-specific contribution of CTCF to transcriptional regulation

B. Dehingia*[1], M. Milewska-Puchała*[1], M. Janowski[1], M. Rafiee[2,3], P. Błaut[1], A. Piotrowska[1], J. Senge[4], J. Severino[2,5], D. Walsh[6], D. Chaudhury[1], E. Duński[1], R. Casellas[7,8,9,10], W. Huber[2], P. Dłotko[4], T. Zimmermann[6], J. Krijgsveld[2,11], **A. Pękowska**[9,12]

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DNA regulatory elements, including promoters, enhancers, and insulators, control the level of gene activity, thereby contributing to the establishment of cell identity during development. Insulators bind CTCF, an eleven-zinc finger chromatin structural protein. CTCF binding may impinge on the spatial arrangement of the genome and impact interactions between enhancers and promoters. Recent data, including ours, revealed a gradual increase in the strengths of insulators during the earliest stages of mammalian development, indicating a unique connection between the restriction of the developmental potential of the cell and chromatin topology. However, the role and the molecular bases of this phenomenon remain unknown. We found substantial differences between the transcriptional consequences of the acute removal of CTCF from embryonic stem (ES) cells, and from neural stem (NS) that were differentiated from the ES cells. When integrated with epigenomic data, our results are consistent with a more pronounced insulatory role of CTCF in NS than in the ES cells. To define the mechanisms that contribute to the gain of insulatory functions of CTCF in development, we took advantage of Chromatin IP (ChIP)-Selective Isolation of Chromatin Associated Proteins (SICAP) to identify CTCF partners in ES and NS cells. We detected established CTCF interactors (e.g., cohesins, topoisomerases), testifying the high quality of the ChIP-SICAP data. Our data revealed a pervasive alteration of the protein interactome of CTCF upon differentiation. We focused on two proteins that feature an enhanced interaction with CTCF in the NS cells. We reasoned that this type of CTCF partner might help to understand mechanisms driving the maturation of chromatin topology. We removed the candidate factors and uncovered a differentiation stage-specific effect of these proteins on the biophysical and biochemical properties of CTCF. Our data shed new light on how chromatin structure changes during development. *The authors marked with an asterisk equally contributed to the work.

ShT-04.1–3
Transcriptional co-activator PGC-1α cooperates with nuclear cap-binding proteins to activate gene expression

X. Rambout[1,2], H. Cho[1,2], N. Rina[1,2], R. Blanc[3], J.M. Miano[4], G.M. Nelson[5], H.K. Yalamanchili[6], K. Adelman[5], L.E. Maquat[1,2]

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PGC-1α is well-established as a transcriptional coactivator of the cellular adaptation to environmental changes. However, how PGC-1α activates gene expression is incompletely understood. We report that PGC-1α induces distinct sets of genes via its binding to either the CBP20–CBP80 nuclear cap-binding protein complex (CBC) or to an elusive CBC accessory protein that has recently raised interest among RNA biologists. In a first mechanism, which is an early pre-mRNP quality-control pathway, PGC-1α senses CBP80 at the cap of nascent pre-mRNAs deriving from stress-activated genes during promoter-proximal pausing of RNAPII to allow for transcription elongation [1]. To elucidate the underlying mechanism, we performed protein immunoprecipitations (IPs), precision run-on sequencing (PRO-seq), RNA-seq and RNA-IP (RIP)-seq using C2C12 myoblasts in which PGC-1α had been knocked down, with or without re-expression of PGC-1α variants. We found that direct binding of the C-terminal region of PGC-1α to CBP80 and the MED1 subunit of Mediator (i) prevents the recruitment of the premature transcription termination complex Integrator, and (ii) facilitates the recruitment of the positive transcription elongation factor b (P-TEFb). We used an in-house mouse model to show that this mechanism ensures efficient differentiation of primary myoblasts to myofibers and timely skeletal-muscle regeneration after injury. Additional analyses of our PRO-seq and RNA-seq data showed that, in a very distinct and seemingly independent pathway, PGC-1α also activates target genes post-transcriptionally (unpublished). In particular, our results show that stabilization of transcripts deriving from energy-metabolism genes requires a poorly characterized nuclear protein, which we found strongly associates with PGC-1α in a transcription-independent manner and competes away the RNA decay machinery from PGC-1α-induced transcripts. [1] Previously published in: Rambout X et al. (2023) Mol Cell 83(2), 186–202.

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Monday 10 July
9:00–11:00, Auditorium Ronsard

Immunometabolism in Cancer Development and Therapy

S-01.2-2
Immunosuppression by the tumor nutrient microenvironment

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Bellvitge Biomedical Research Institute, Barcelona, Spain

Tumor cells require nutrients and oxygen at amounts that exceed their supply. This leads to the secretion of pro-angiogenic molecules in order to restore balance. Additionally, nutrient avidity of tumor cells leads to an immunosuppressed environment thought to be due to nutrient competition between tumor and immune cells. We subjected a variety of tumor cell lines to nutrient restriction and studied their secretome in order to explore the tissue responses that this would promote. Cytokine arrays unveiled that starved tumor cells secrete molecules that modulate immune functions. Acute starvation triggered the secretion of the neutrophil chemoattractant IL-8 and the inflammatory mediator IL-6, both of which participate in tumor growth and correlate with poor prognosis. I will present these and other results that indicate that the hypoglycemic conditions of the nutrient microenvironment promote paracrine responses in cells of the immune system and endothelial cells, which together can lead to persistent inflammation, angiogenesis and cancer immunosuppression. *The authors marked with an asterisk equally contributed to the work.

S-01.2-3
Targeting immunometabolism in patient-derived breast cancer explant cultures

J. Klefstrom[1,2], P. Munne[1], R. Turpin[1]
[1]*University of Helsinki, Helsinki, Finland,* [2]*UCSF, San Francisco, CA, USA*

Combining explorative or standard anticancer therapies with immunotherapies holds great promise in the treatment of advanced cancers, with variable response due to influence from the tumor immune microenvironment (TIME). Therefore, there is a clear need for pharmacologically tractable models of the TIME to dissect its influence on individualized treatment response. We have established a Patient Derived Explant Culture (PDEC) model of breast cancer, which retains the immune contexture of the original tumor, recapitulating cytokine profiles and CD8+ T cell cytotoxic activity. We explored the therapeutic action of standard of care chemo paclitaxel and a MYC synthetic lethal BCL2 inhibitor venetoclax + metformin drug combination *ex vivo*, discovering metformin cannot overcome the specific lymphodepleting action of venetoclax. Instead, metformin exerts mitochondrial complex I dependent immunomodulatory effects on populations of antigen presenting cells, increasing their capacity to co-stimulate CD4+ T cells and thus facilitating anti-tumor immunity. Our results highlight PDECs as a feasible model to identify immuno-metabolic functions of anticancer drugs in the context of patient-specific TIME.

S-01.2-1
Harnessing tumor metabolism to overcome immunosuppression

M. Mazzone
VIB KU Leuven Center for Cancer Biology, Leuven, Belgium

Anti-cancer immunotherapy has provided patients with a promising treatment. Yet, it has also unveiled that the immunosuppressive tumor microenvironment (TME) hampers the efficiency of this therapeutic option and limits its success. The concept that metabolism is able to shape the immune response has gained general acceptance. Nonetheless, little is known on how the metabolic crosstalk between different tumor compartments contributes to the harsh TME and ultimately impairs T cell fitness within the tumor. This lecture will decipher some of the metabolic changes in the TME impeding proper anti-tumor immunity. Starting from a meta-analysis of public human datasets, corroborated by metabolomics and transcriptomics data from several mouse tumors, we ranked clinically relevant and altered metabolic pathways that correlate with resistance to immunotherapy. Using a CRISPR/Cas9 platform for their functional *in vivo* selection, we have identified cancer cell intrinsic metabolic mediators and, indirectly, distinguished those belonging specifically to the stroma. By means of genetic tools and small molecules, we have targeted promising metabolic pathways in cancer cells and stromal cells (particularly in tumor-associated macrophages) to harness tumor immunosuppression. Finally, we went back to patient samples to assess the relevance of these metabolic networks in humans. By analyzing the metabolic crosstalk within the TME, this lecture would like to shed some light on how metabolism contributes to the immunosuppressive TME and T cell maladaptation.

ShT-01.2-1
Decrease of phosphocholine metabolism in 2-hydroxyflutamide-resistant prostate cancer cells

A. Boert*[1], B. Sánchez*[2], J.M. Mora-Rodríguez[2], A. Díaz-Yuste[2], A. Sebastián-Martín[2], **I. Diaz-Laviada**[2]
[1]*Yale University School of Health, New Haven, CT, USA,* [2]*University of Alcalá, Alcalá de Henares, Spain*

Metabolic reprogramming is a hallmark of cancer and allows tumor cells to meet the increased energy demands required for rapid cell growth, invasion, and metastasis. Moreover, metabolic reprogramming prevents cancer cells from chemotherapy-induced death contributing to cancer drug resistance and recurrence. This phenomenon is associated with the emergence of dormant cancer cells characterized by cell cycle arrest, which are largely insensitive to conventional anti-cancer therapies. Herein, we have used a chemoresistant prostate cancer cell line to study their metabolic adaptations compared to a sensitive cell line. Prostate cancer LNCaP cells were adapted to grow in the presence of the antiandrogen 2-hydroxyflutamide (FLU), by culturing in a stepwise increasing concentration of the compound. When cells were resistant to FLU they were renamed LN-FLU. Those cells exhibited features of cancer stem cells and were drug-resistant but duplicated at a very low rate. To understand the adaptive response linked to chemotherapy resistance, we previously used a metabolomic approach. Besides changes in fatty acid oxidation and methionine metabolism, we found a decrease in phosphocholine. In this work, we have determined the expression of the enzyme

CTP-choline cytidylyl transferase (CCT), which is involved in phosphocholine biosynthesis. Both phosphorylcholine and the enzyme CTT were decreased in the cancer stem-like LN-FLU cells compared to LNCaP cells, implying a diminution of the phosphatidylcholine biosynthesis pathway. To confirm this result, we determined the expression of CTT in prostate cancer biopsies by qPCR. We found an inverse correlation between CTT and the stem cell marker Oct4. Our results indicate that the resistant prostate cancer cells are metabolically dormant and that the decrease in phosphocholine metabolism could be linked to the acquisition of stem cell properties and therapy resistance. *The authors marked with an asterisk equally contributed to the work.

ShT-01.2-2
Metabolic targeting of neuroblastoma

L. Catalano[1], S. Aminzadeh-Gohari[2], J. Tevini[2], T. Maheshwor[3], R. Poupardin[4], S. Derdak[5], V. Stefan[2], R.F. Feichtinger[2], W.J. Smiles[2], D.D. Weber[2], **B. Kofler**[2]
[1]Research Program for Receptor Biochemistry and Tumor Metabolism, Department of Pediatrics, University Hospital of the Paracelsus Medical University, Salzburg, Austria, [2]Paracelsus Medical University, Salzburg, Austria, [3]Shuzhao Li Lab, The Jackson Laboratory for Genomic Medicine, Farmington, CT, USA, [4]Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCI-TReCS), Cell Therapy Institute, Paracelsus Medical University, Salzburg, Austria, [5]Core Facilities, Medical University of Vienna, Vienna, Austria

Neuroblastoma (NB) is a childhood cancer with a subgroup of high-risk patients, which harbor the amplification of the MYCN oncogene. MYCN-amplified NB rely on both glucose and oxidative metabolism. Several pre-clinical studies have revealed the anti-tumor effect of a low carb, high fat ketogenic diet (KD), especially when combined with conventional cytotoxic therapy. Since oxidative phosphorylation (OXPHOS) plays a critical role in cancer (immune)-metabolism, targeting mitochondrial energy metabolism has attracted growing interest. The antidiabetic drug metformin (MET) targets complex I of OXPHOS. Therefore, the aim was to elucidate whether MET can enhance the anti-tumorigenic effects of a KD in NB. MET reduced the basal and maximal respiration in MYCN-NB cells. NB xenografts were then established in CD-1 mice with MYCN-amplified SKNBE (2) and KELLY NB cells. MET enhanced the anti-proliferative effect of the KD (ketogenic ratio 8:1) when combined with a low dose of cyclophosphamide (CP), increasing overall survival in both MYCN-amplified NB models. From 20 different biomarkers potentially involved in tumor progression, fibroblast growth factor 21 (FGF-21) was higher in all KD groups compared to the control diet groups. RNA-seq analysis revealed that KD increased the expression of genes involved in β-oxidation, inter alia Carnitine palmitoyltransferase 1 (CPT1 and CPT2), whereby MET enhanced this effect. Moreover, MET + CP + KD decreased protein expression of acetyl-CoA carboxylase (ACC), a rate limiting enzyme of fatty acid synthesis. Pharmacological inhibition of ACC has been shown to reduce tumor burden in NB. We are currently analyzing metabolomics data from plasma and tumor samples as well as the tumor environment in an attempt to untangle the complexity behind the antitumor effect of a KD in combination with MET and CP in NB.

Monday 10 July
9:00–11:00, Auditorium Descartes

Chemical Biology

S-03.2-4
Expanding the genetic code – new chemistries for biology

K. Lang
ETH Zurich, Zurich, Switzerland

Nature uses a limited set of 20 amino acids to synthesize proteins. In recent years it has become possible to site-specifically incorporate designer amino acids with tailored chemical properties into proteins in living cells by reprogramming the genetic code. Together with developments in designing chemical reactions that are applicable to and selective within living systems, these strategies have begun to have a direct impact on studying biological processes. In this talk, I will present our lab's efforts to expand the genetic code and to endow proteins with novel chemical moieties within their physiological environment. By site-specifically incorporating artificial designer amino acids into proteins, we have developed tools to image and probe proteins [1,2] to study protein–protein interactions and stabilize low-affinity protein complexes [3–6] and to re-engineer and manipulate molecular networks and biological pathways such as ubiquitylation in living cells [7–9]. We envision that these approaches and technologies will enable the study of biological processes that are difficult or impossible to address by more classical methods. References: [1] K. Lang et al.; *Chem. Rev.* 2014, 114, 4764. [2] S.V. Mayer et al.; *Angew. Chem. Int. Ed.* 2019, 58, 15876. [3] M. Cigler et al.; *Angew. Chem. Int. Ed.* 2017, 56, 15737. [4] T.A. Nguyen et al.; *Angew. Chem. Int. Ed.* 2018, 57, 14350. [5] T.A. Nguyen et al.; *Angew. Chem. Int. Ed.* 2022, 61, e20211108. [6] J. Du et al., *Nat. Commun.* 2021, 12, 460, doi: 10.1038/s41467-020-20,702-2. [7] M. Fottner et al.; *Nat. Chem. Biol.* 2019, 15, 276. [8] M. Fottner et al.; *Nat. Commun.* 2021, 12, 6515. [9] M. Fottner et al.; *J. Am. Chem. Soc.* 2022, 144, 13118.

S-03.2-1
Chemogenetic tools for imaging, sensing and controlling cell biology

A. Gautier
Sorbonne University, Paris, France

Cells and organisms are complex machines driven by a set of dynamic biological events tightly orchestrated in space and time. Our understanding of their inner workings is intricately related to our ability to observe and control how their constituents organize and interact. Our lab recently developed chemogenetic tools for observing and controlling biomolecules and dynamic biochemical events in live cells and tissues. Made of organic synthetic molecules coupled to genetic tags, these systems combine the advantage of synthetic molecules with the targeting selectivity of genetically encoded tags, challenging the paradigm of fully genetically encoded systems. During this talk, I will present how these systems can be used for imaging, sensing and controlling cell biology with high spatial and temporal resolution.

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S-03.2-2
Microscopic evidence for malaria infection in viscera tissue of the Medici family

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The Medici were a powerful family from Florence (Italy) that rose to prominence in the early 15th century. After death, deceased Medici family members were subjected to an embalming process that included the removal of the viscera which were placed in large terra cotta embalming jars (orci). We subjected viscera tissue from selected orci to microscopic and molecular analyses. During our initial histological analysis, we could identify a possible blood vessel that still contained traces of red blood cells and a potential first indication of a parasite that apparently resided within the red blood cells. Additional experimental approaches indicated the presence of the malaria parasite *Plasmodium falciparum* inside the red blood cells. Our results provide the first potential microscopic evidence for the occurrence of the most fatal form of malaria in the Medici family. *frank.maixner@eurac.edu, nicole.kilian@med.uni-heidelberg.de/ nicole.kilian@aya.yale.edu, albert.zink@eurac.edu. *The authors marked with an asterisk equally contributed to the work.

ShT-03.2-3
Ways for enhancement of anticancer action of novel synthetic and natural heterocyclic compounds

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Synthetic derivatives of thiazoles and 4-thiazolidinones are attractive scaffolds for design of novel drug-like compounds. Here, we addressed the anticancer potential of these compounds. Two methodological approaches were applied: (1) chemical modification of the structure of heterocyclic rings and attached functional groups that enhanced their bio-activity [1,2]; (2) immobilization of created heterocyclic compounds on a polymeric nanoplatform that improved their water solubility and biotolerance *in vivo* [3]. 5-ene-4-thiazolidinone hybrid molecules were found to be promising pro-apoptotic antitumor agents targeting PPAR gamma, topoisomerase II, Bcl-2, and tubulin [1]. *In silico* modeling was applied for searching these biological targets. Recently, we detected that bioisosteric replacement of 1H-1,2,3-triazole with a 1H-tetrazole ring significantly enhanced antileukemic activity of (5-benzylthiazol-2-yl) benzamides [2]. We also described that condensation of thiopyrano[2,3-d]thiazoles with Juglone (5-hydroxy-1,4-naphthoquinone that is found in Juglandaceae plants) via hetero-Diels-Alder reaction, considerably elevated anticancer activity of created hybrid molecules *in vitro*, while its toxic action towards normal human blood lymphocytes and adverse effects in laboratory mice were much less expressed, compared to such effects of doxorubicin (paper under submission). We did not succeed in creating water-soluble forms of bioactive 4-thiazolidinones via structural modifications of their molecules. However, their complexes with amphiphilic nano-scale poly(VEP-co-GMA)-graft-PEG carrier were found to be soluble in water, be more potent inducers of apoptosis in tumor cells, and demonstrated much higher bio-tolerance in treated mice, compared to free form of those derivatives [3]. References: [1] Finiuk N et al. (2022) Eur J Med Chem. Aug 5. 238. 114422. [2] Pokhodylo N et al. (2023) Eur J Med Chem. Online. Jan 25, 115126. [3] Kobylinska L et al. (2018) Cancer Nanotechnol. 9, art. 11.

ShT-03.2-1
An enhanced peptide–peptide ligation system facilitates highly efficient modular coupling for robust decoration of the cell surface

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[1]*Department of Biochemistry, University of Oxford, Oxford, UK,* [2]*Department of Pharmacology, Cambridge, UK*

Peptide–peptide ligation enables the assembly of constructs with novel architecture and functionality, with potential application across a broad range of fields from therapeutics to materials science. The SnoopLigase system spontaneously forms an isopeptide bond between SnoopTag and DogTag peptide tags, allowing precise ligation of proteins at the termini or internal sites. Here the SnoopLigase2 system was validated, showing 1000-fold improvement in reaction speed and high activity across diverse conditions. SnoopLigase2 was then applied to produce a modular tool for robust decoration of the cell surface with growth factors. The efficacy of exogenous growth factors and cytokines is often limited by rapid clearance, necessitating high dosing which can lead to severe side effects. We engineered Transglutaminase 2 (TG2) to create a modular scaffold capable of high affinity decoration of the cell surface. However, this TG2 module is limited to expression in *Escherichia coli*. We demonstrated that SnoopLigase2 coupling enables high-yield TG2 functionalization with transforming growth factor alpha (TGFα), which is impossible through genetic fusion. This TG2:TGFα construct was applied to DU145 cells, resulting in high stability retention of TGFα at the cell surface and enhancing TGFα induction of cell differentiation. The broad compatibility of SnoopLigase2 presents new possibilities for molecular assembly, introducing modularity into other biotechnologies to significantly enhance their utility. *Acknowledgements: QinetiQ, Farnborough for EPSRC iCASE Award.

Monday 10 July
16:00–18:00, Auditorium François 1er

Cell Death, and Inflammation

S-02.2-3
Cell stress, cell death and inflammation

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

Inflammation is initiated in response to infection, tissue injury or cell stress, that can all elicit the production of a battery of cytokines and chemokines that recruit and activate cells of the immune system and initiate wound repair. It is well established that conserved components of infectious agents, called PAMPs (pathogen-associated molecular patterns), and molecules released by necrotic cells, called DAMPs (damage-associated molecular patterns), promote inflammatory responses. However, it is less well appreciated that cell stress (e.g. protein misfolding-induced ER stress, mitochondrial depolarization, heat shock, DNA damage) can also instigate inflammatory responses in a manner that is very poorly understood at present. Here, I will discuss stress-induced inflammation and argue that inflammation in this context is regulated by "stress-associated molecular patterns" (SAMPs) that are upregulated/activated in response to divergent forms of cell stress. ER Stress-induced upregulation and activation of Death Receptor 5/TRAIL-R2 will be discussed as a paradigm example of a SAMP that is induced by diverse forms of ER stress to drive inflammation, as well as cell death.

S-02.2-1
Deconstructing inflammasomes: from mechanisms to applications

I. Hafner Bratkovic
National Institute of Chemistry, Ljubljana, Slovenia

Inflammasomes are central components of the early immune response. Upon sensing infection or disrupted tissue homeostasis, inflammasome sensors through oligomerization lead to the assembly of inflammasomes and activation of inflammatory caspases. Caspase-1 subsequently proteolytically activates proinflammatory cytokines IL-1b and IL-18. Gasdermin D is another substrate of inflammatory caspases. In a dormant state, gasdermin D molecules are present in autoinhibited form, while cleavage and recognition of negatively charged phospholipids enable the N-terminal domain of Gasdermin D to form pores in the membranes and facilitate pyroptotic cell death. Despite enormous progress that has been achieved in the past 20 years since the discovery of inflammasomes, there are still major gaps in our understanding of how inflammasomes are activated and the regulatory processes involved. We are investigating the regulation of the initial steps of NLRP3 inflammasome assembly and downstream responses that are common to all inflammasomes. Using inflammasome reconstruction in physiologically relevant cells (macrophages), we previously defined the shortest NLRP3 molecule that is able to sense inflammasome triggers and induce inflammasome assembly, and we are currently investigating how NLRP3 localization affects trigger sensing and inflammasome assembly. While the current emphasis of NLRP3 inflammasome research is focused more on its detrimental involvement as a major driver of inflammation, the canonical role of inflammasomes is nevertheless to protect the organism against threats. Cancer is an example of a disease where the immune system fails to detect the threat and several types of tumor have been shown to downregulate inflammasome components. Using the synthetic biology approach combined with the mechanistic insight into inflammasome assembly, we are currently deconstructing inflammasomes to provide efficient priming of immune responses in cancer immunotherapy.

S-02.2-2
Gasdermin-executed pyroptosis in antitumor immunity

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Pyroptosis is a proinflammatory cell death executed by the gasdermin-family pore-forming proteins. Among the family, gasdermin D (GSDMD) is cleaved by inflammasome-activated caspase-1 and LPS-activated caspase-11/4/5. The cleavage unmasks the pore-forming domain in GSDMD that perforates the plasma membrane. Using a bioorthogonal chemical biology approach allowing controlled delivery of active gasdermin into tumors in mice, we found that pyroptosis of <15% tumor cells could clear the entire 4T1 mammary tumor graft, which was absent in immune-deficient mice or upon T-cell depletion. Thus, pyroptosis stimulates potent and effective antitumor immunity. In antitumor immunity, cytotoxic lymphocyte relies on granzymes to kill target cells. We found that natural killer cells and cytotoxic T lymphocytes kill GSDMB-positive cells through pyroptosis, mediated by granzyme A (GZMA) cleavage of GSDMB. IFN-γ upregulates GSDMB expression and promotes pyroptosis of cancer cells including that by CAR-T/TCR-T cells. Thus, gasdermin-executed pyroptosis serves as a cytotoxic lymphocyte killing mechanism, playing an important role in cancer immunotherapy.

ShT-02.2-1
Mitochondrial nicotinic acetylcholine receptors: the role in cell survival and apoptosis

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Nicotinic acetylcholine receptors are ligand-gated ion channels mediating fast synaptic transmission in neuro-muscular junctions and autonomic ganglia. They are also expressed in many non-excitable cells to control survival, proliferation and neurotransmitter or cytokine release. The neuronal-type nicotinic receptors are found in the outer mitochondria membrane to regulate the release of pro-apoptotic substances like cytochrome c or reactive oxygen species. In the intracellular environment, nicotinic acetylcholine receptor signaling is ion-independent and involves intramitochondrial kinases. In my presentation, I will describe the data obtained during the last 5 years, including post-translational glycosylation as a targeting signal to mitochondria, mechanisms of mitochondrial nicotinic acetylcholine receptor signaling studied with subtype-specific agonists, antagonists, positive allosteric modulators and knockout mice lacking certain nicotinic acetylcholine receptor subunits, interaction of mitochondrial nicotinic acetylcholine receptors with Bcl-2 family proteins and their involvement in important pathologies like neuroinflammation, liver damage and SARS-CoV-2 infection.

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ShT-02.2-2
NatB-dependent acetylation protects procaspase-8 from UBR4-mediated degradation and is required for full induction of extrinsic apoptosis

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N-terminal acetyltransferase B (NatB) is a major contributor to the N-terminal acetylome, which is suggested to influence several key cellular processes including apoptosis and proteostasis. However, the molecular mechanisms linking NatB-mediated N-terminal acetylation to apoptosis and its relationship with protein homeostasis remain uncharacterized to date. Mouse embryonic fibroblasts (MEFs) containing an inactivated catalytic subunit of NatB ($Naa20^{-/-}$) were engineered. We combined quantitative N-terminomics with label-free quantification, and targeted proteomics in this background. The results obtained reveal that NatB is not involved in the proteostasis of all its substrates. Rather, by focusing on putative NatB-dependent apoptotic factors, we show that NatB acetylation of these factors acts as a protective shield from UBR4 and UBR1 Arg/N-recognin-mediated degradation. Importantly, $Naa20^{-/-}$ MEFs are less responsive to extrinsic pro-apoptotic stimuli, but this is partially reversible when UBR4 Arg/N-recognin is silenced and procaspase-8 degradation is blocked. Collectively, our results shed light on the role of NatB-mediated acetylation into the interdependence of N-degron pathways on regulation of apoptosis, providing new insights that open up new perspectives in the field, including therapeutic interventions.

Monday 10 July
16:00–18:00, Auditorium Ronsard

Supramolecular Assemblies II: RNA–Protein Complexes, Molecular Machines

S-07.2-3
TnpB—a bridge between transposition and CRISPR-Cas antiviral defense

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Abstract not available.

S-07.2-1
mRNA scanning alteration in colorectal cancer

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Protein synthesis is an essential cellular process relying on translation of the messenger RNA (mRNA) into polypeptide chains of amino acids that fold into functional proteins. The key player of translation is the ribosome that is composed of a core of ribosomal RNA (rRNA) embedded by dozens of ribosomal proteins of different structures and functions. Recently, it has been shown that the loss of the 3-a-amino-a-carboxypropyl (acp3) rRNA modification at position U1248 is a major feature of colorectal cancer and it can be used as a fingerprint for cancer. Our objective is to characterize functionally and structurally how the lack of the acp3 rRNA modification in the ribosomal P-site influences the translation initiation, where the P-tRNA interacts and scans the incoming codons during the initiation stage. We investigate functionally and structurally by cryo-electron microscopy this specialized distinct class of ribosomal complexes in cancer cells. Our preliminary results indicate the alteration of the position of the initiator tRNA when the modification is absent from U1248. Thus, we hypothesize that this slightly different tRNA accommodation induces an altered reading of the incoming mRNA, which in turn could impact the efficiency of synthesis of different proteins, yielding abnormal translation rates and altering the proteostasis of the cell.

S-07.2-2
Virus traps and other molecular machines of the future

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Our research is focused on creating molecular devices and machines that can perform specific tasks as directed by users. We draw inspiration from natural macromolecular assemblies such as viruses and molecular motors and seek to implement similar principles in synthetic molecular machinery. DNA origami is a particularly promising technique that we use for programmable molecular self-assembly. We employ computational design and cryo-electron microscopy to enhance the accuracy and complexity of our synthetic molecular creations, which lead to several interesting accomplishments:

- By studying viruses, we have successfully programmed DNA blocks to self-assemble into icosahedral shells, which have potential applications as programmable antiviral drugs. These virus traps can be designed to neutralize targeted viruses.
- We have developed DNA origami structures capable of carrying genetic instructions that can be read by mammalian cells. This opens up possibilities for a wide range of applications, including gene therapy and targeted drug delivery.
- We have constructed nanoscale assemblies with controllable movements, such as autonomous, power-generating rotary DNA motors, and turbines driven by ion flux across membranes. These machines offer potential for executing energy-consuming tasks in synthetic cells.

ShT-07.2-1
How does a kinase and small GTPases regulate the function of scaffolding proteins?

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LRRK2 (*Leucine Rich Repeat Protein Kinase-2*), an autosomal dominant gene associated with familial as well as the sporadic

forms of Parkinson's disease (PD), encodes a multidomain protein which phosphorylates a group of Rab GTPases including Rab8A and Rab10 at conserved Ser/Thr residues located at the center of the effector binding switch II motif. Previous studies have revealed that LRRK2-phosphorylated Rab8A and Rab10 interact with a group of scaffolding proteins containing an RH2 domain including RILPL1 (RILP-like protein 1), RILPL2 (RILP-like protein 2), JIP3 (JNK-Interacting Protein 3), and JIP4 (JNK-Interacting Protein 4). Our affinity-enrich mass spectrometry approach identified a lysosomal transmembrane protein named TMEM55B that binds specifically to the phosphorylated Rab8A: RILPL1 complex. We have identified a Cysteine-rich region (TMEM55B Conserved Domain-TCD) within TMEM55B that recognizes a conserved motif (TMEM55B Binding Motif-TBM) in the C-terminus of RILPL1. By combining alpha-fold analysis and docking, we were able to model the structure of TCD and TBM and determine the interface of this interaction, which was validated biochemically. We further confirmed this interaction by organelle immunoprecipitation and confocal microscopy. This study reveals how post-translation modification of Rab-GTPases by a kinase can change the conformation of its effector protein and facilitate the binding of a new set of interactors.

ShT-07.2-2
The Rab5 effector complex Rabenosyn5/Vps45 coordinates transition from membrane tethering to fusion

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Tethering of a vesicle to the membrane of its target compartment is a key event in intracellular trafficking. By recruiting tethering factors, Rab GTPases regulate this process. Tethering is required to ensure specificity and efficiency of membrane fusion. Fusion itself is mediated by trans-pairing of v-SNAREs on the tethered vesicle and t-SNAREs on the target compartment. How are incoming vesicles tethered exactly at the location where the appropriate fusion machinery is located? How are vesicles brought close to the target compartment, within a distance which allow recognition and trans-pairing of v and t-snares? We reconstituted *in vitro* the minimal early endosomal (EE) fusion machinery. We developed a microscopy fusion assay to visualize single tethering and fusion events, and study the process kinetics. We were able to observe fusion events between Rab5/v-SNAREs proteoliposomes and Rab5/t-SNAREs proteoliposomes only when the EE Rab5 effector complex Rabenosyn5/Vps45 was added to the reaction. Structural data showed that Rabenosyn5 has a globular domain binding the EE membrane and Vps45, and a flexible tail ending with a Rab5 binding site. Moreover, we also showed *in vitro* that Rabenosyn5/Vps45 is sufficient to tether EE-like endosomes to membrane-coated beads harboring Rab5. We believe that with its conformation, Rabenosyn5 can capture vesicles harboring Rab5 and bring them closer to the target compartment to a fusion compatible distance. Then, the SNARE chaperone Vps45 would stimulate fusion by clasping together v and t-SNAREs. Finally, we sought to uncouple Rabenosyn5 tethering function from Vps45 chaperone function by designing a Rabenosyn5 mutant missing its flexible tail and Rab5-binding domain. We expect that the mutated complex would fail to induce tethering and fusion events. That would suggest that coupling Rabenosyn5 tethering function with Vps45 chaperone activity is critical to efficiently and specifically coordinate membrane tethering and fusion.

Monday 10 July
16:00–18:00, Auditorium Descartes

Protein Life Cycle II: Degradation

S-06.2-3
Cellular roles of proteasomal degradation in mycobacterial stress responses

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Bacterial proteasomes are exclusive to mycobacteria and other actinobacteria, and they were acquired by horizontal gene transfer to support survival under adverse conditions, for example during persistence of the human pathogen *Mycobacterium tuberculosis* inside macrophages or during the mycobacterial DNA damage response [1,2]. Proteasomes are encoded in a gene cluster together with a post-translational protein modification system that attaches the small, prokaryotic ubiquitin-like protein (Pup) to lysine side chains of target proteins [3]. Pupylation recruits these proteins to the mycobacterial proteasomal ATPase Mpa, a hexameric ring complex associated with the 20S proteasome for the energy-dependent degradation of pupylated proteins [3,4]. In addition, an alternative proteasome interactor called Bpa is involved in pupylation-independent protein degradation. I will discuss molecular mechanisms by which the Pup-proteasome system (PPS) gene locus contributes to survival of mycobacteria under stress. References: [1] von Rosen T, Keller LML & Weber-Ban E (2021) Survival in Hostile Conditions: Pupylation and the Proteasome in Actinobacterial Stress Response Pathways. Front Mol Biosci 8, 685757. [2] Müller AU, Kummer E, Schilling CM, Ban N & Weber-Ban E (2021) Transcriptional control of mycobacterial DNA damage response by sigma adaptation. Science Advances 7, eabl4064. [3] Müller AU & Weber-Ban E (2019). The bacterial proteasome at the core of diverse degradation pathways. Mini Review. Front Mol Biosci, 6:23. 10.3389/fmolb.2019.00023. [4] Kavalchuk M, Jomaa A, Müller A & Weber-Ban E (2021) Structural basis of prokaryotic ubiquitin-like protein engagement and translocation by the mycobacterial Mpa-proteasome complex. Nature Communications, 13(1):276.

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S-06.2-1
EDEM selective functions in physiological and acute ER stress

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Misfolded secretory proteins are retained within the endoplasmic reticulum and targeted for proteasomal degradation. Accumulation of misfolded proteins increases ER stress and EDEM are transcriptionally activated. The EDEM family includes three proteins, EDEM-1, EDEM-2 and EDEM-3, that signal and target ER misfolded polypeptides for degradation, as found in cultured cells. To understand the function of EDEM at an organism level, we investigated their role in *C. elegans*, a model organism that, similar to mammals, has three homologous EDEM proteins. The most drastic phenotypic alterations were displayed by edem-2 mutants, as in its absence, the clearance of misfolded proteins from the ER is impaired. In contrast, EDEM-1 and EDEM-3 roles become prominent under ER stress. The alterations induced by edem-2 silencing are not phenocopied by edem-1 and edem-3 and all three EDEM act in a common degradation pathway. EDEM-2 appears to have a major role in ERAD under physiological conditions. Thus, there is a striking difference between EDEM-2, involved in the clearance of misfolded proteins under physiological conditions and EDEM-1 and EDEM-3 that act mostly under acute ER stress. The physiological role of EDEM-2 was validated in a mammalian cell system, by discovering the first endogenous glycoproteins whose turnover was regulated by EDEM2, indicating an active role for EDEM-2 in gpERAD. Using *C. elegans* and mammalian cells, this work proposes a novel role for EDEM in adjusting the ER stress responsiveness that affects ER homeostasis and survival.

S-06.2-2
ER-phagy and ER-to-lysosome-associated degradation

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Institute for Research in Biomedicine, Bellinzona, Switzerland

The endoplasmic reticulum (ER) is an organelle of nucleated cells that produces proteins, lipids and oligosaccharides. ER volume and activity are increased upon induction of unfolded protein responses (UPR) and are reduced upon activation of so-called ER-phagy programs. ER-phagy relies on activation of so-called ER-phagy receptors, i.e., of ER membrane proteins that engage cytosolic autophagy gene products and control fragmentation of the ER and clearance of ER subdomains by lysosomal compartments. ER-phagy operates constitutively to maintain ER homeostasis. It can be induced on nutrient deprivation to gain nutrients, during recovery from acute ER stresses to restore physiological size and activity (recov-ER-phagy) and to remove ER subdomains containing misfolded polypeptides (ER-to-Lysosome-Associated Degradation, ERLAD). *The authors marked with an asterisk equally contributed to the work.

ShT-06.2-1
COP I and II-dependent trafficking directs ER-associated degradation in mammalian cells

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Tel Aviv University, Tel Aviv, Israel

Secretory protein folding and quality control mechanisms are localized in the endoplasmic reticulum (ER). Terminally misfolded molecules are targeted to ER-associated degradation (ERAD) by cytosolic proteasomes. We have observed in previous studies that machinery components involved in quality control and ERAD concentrate together with misfolded proteins in the ER-derived Quality Control compartment (ERQC), adjacent to the centrosome, suggesting it as a staging ground for ERAD. By tracking the chaperone calreticulin and an ERAD substrate we have now determined that the trafficking to the ERQC is reversible and recycling back to the ER is slower than the movement in the ER periphery. The dynamics, studied by live cell imaging and fluorescence loss in photobleaching (FLIP) experiments, suggest vesicular trafficking rather than diffusion. Indeed, by interfering with the activities of COPI or COPII, using dominant negative mutants of ARF1 and Sar1 or the drugs Brefeldin A and H89, we observed that COPI inhibition causes accumulation in the ERQC and increased retrotranslocation and ERAD, whereas COPII inhibition has the opposite effect. Our results with established membrane-bound (asialoglycoprotein receptor H2a) and soluble luminal (α1-antitrypsin variant null Hong Kong (NHK)) ERAD substrates suggest that targeting of misfolded proteins to ERAD involves COPII-dependent transport to the ERQC and that they can be retrieved to the peripheral ER in a COPI-dependent manner. Surprisingly, vesicular trafficking controls misfolded protein targeting to ERAD. *The authors marked with an asterisk equally contributed to the work.

ShT-06.2-2
Selective degradation of ARF monomers controls auxin response in Marchantia

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The plant signaling molecule auxin controls a variety of growth and developmental processes in land plants. Auxin regulates gene expression through a nuclear auxin signaling pathway (NAP) consisting of a ubiquitin ligase auxin receptor TIR1/AFB, its Aux/IAA degradation substrate, and the DNA-binding ARF transcription factors. While extensive qualitative understanding of the pathway and its interactions has been obtained by studying the flowering plant *Arabidopsis thaliana*, it is so far unknown how these translate to quantitative system behavior *in vivo*, a problem that is confounded by large NAP gene families in this species. Here, we used the minimal NAP of the liverwort *Marchantia polymorpha* to quantitatively map NAP protein accumulation and dynamics *in vivo* through the use of knock-in fluorescent fusion proteins. Beyond revealing the native accumulation profile of the entire NAP protein network, we discovered that the two central ARFs MpARF1 and MpARF2 are proteasomally degraded. This degradation serves two functions: it tunes the stoichiometry of auxin-responsive, positively acting MpARF1 and auxin-independent, negatively acting MpARF2, thereby permitting auxin response. Secondly, through mapping a

minimal degradation motif, we found that degradation is likely selective for MpARF2 monomers and favors accumulation of dimers. Interfering with MpARF1:MpARF2 stoichiometry or preventing degradation of MpARF2 monomers caused strong growth defects associated with auxin response defects. Thus, quantitative analysis of the entire Marchantia NAP, allowed to identify a novel regulatory mechanism in the auxin response, built on regulated ARF degradation.

Tuesday 11 July
9:00–11:00, Auditorium François 1er

Protein Life Cycle III: Ribosomes, Folding, Chaperones

S-06.3-3
Folding of ABC-transporter CFTR: a simple story

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Proteins must fold into their native conformation to function properly, which is a challenge because of the many different conformations a protein theoretically may acquire. Mutations in the CFTR gene may cause misfolding of the CFTR protein and lead to CFTR dysfunction and the disease cystic fibrosis (CF). CFTR is a 1480-amino-acid-large multispanning membrane protein, but its folding is nonetheless quite robust, as we concluded from kinetic studies with incorporated radioactive amino acids and limited proteolysis as conformational assay. Characterization of ~60 CF-causing and numerous designed mutations showed that CFTR folds its domains during synthesis, followed by mainly post-translational assembly of the domains. Disease-causing CFTR mutants often have defects in domain assembly due to misfolding of the mutant domain. Rescue of misfolded CFTR requires correction of domain assembly rather than domain folding. Using our assays, we analyze mode of action of molecular chaperones and (pre)clinical modulator compounds.

S-06.3-2
Chaperoning protein self-assembly and phase transitions

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Liquid–liquid phase separation (LLPS) of biomacromolecules into membrane-less condensates is implicated in physiological functions as well as pathological roles in the cellular context. Many disease-associated protein aggregates are thought to nucleate initially in biomolecular condensates, but the molecular level determinants that promote pathological liquid-to-solid transitions are poorly understood. The microtubule-associated protein Tau, the aberrant aggregation of which is associated with a number of neurodegenerative diseases, has been found to undergo reversible LLPS. We are using single-molecule fluorescence techniques, including FRET and fluorescence correlation spectroscopy, to monitor the intra- and intermolecular changes of Tau during liquid–liquid phase separation leading to the formation of condensates. Our data demonstrate that the N- and C-terminal regions of Tau become extended and the microtubule-binding region is exposed. These changes facilitate intermolecular interactions to form nanoscale clusters of Tau which nucleate microscale condensate formation and promote fibrillization of Tau which can be dramatically accelerated by disease-related mutations. Molecular chaperones Hsp70 and Hsp40 can co-phase separate with Tau *in vitro* and *in vivo*, which maintains the proteins in a functional condensed phase and prevents their maturation into pathological aggregates. Our findings provide new insights into the mechanism of protein phase separation, the conversion from functional liquid droplets to pathological aggregates, and the ways these processes may be regulated by chaperones in the cell.

S-06.3-1
Functional and aberrant membrane interactions of α-synuclein

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University of Naples, Naples, Italy

The aggregation of α-synuclein (αS), a neuronal protein that is abundant at the presynaptic terminals, is associated with a range of highly debilitating neurodegenerative conditions, including Parkinson's Disease. Fibrillar aggregates of αS are the major constituents of proteinacious inclusions known as Lewy bodies that form in dopaminergic neurons of patients suffering from these conditions. The function of αS, however, is currently unknown, with evidence suggesting a role in the regulation of the trafficking of synaptic vesicles (SV). To elucidate the nature of the normal and pathological forms of αS, we have established a research program based on structural biology and cellular biophysics to reveal the properties of its transient interactions with biological membranes. A major focus of our studies is the binding to SV and to the plasma membrane, a key step in the regulation of the SV homeostasis during neurotransmitter release [1–3]. In the context of αS aggregation, we focus on the pathological membrane interactions of oligomers and fibrils of αS [4–5] that form on pathway during the self-assembly into mature amyloids as found in post-mortem analyses of patients affected by synucleinopathies. References: [1] Man et al, Nat Commun, 2021, 12:927 [2] Fusco et al, Nat Commun, 2016, 7:15623 [3] Fusco et al, Nat Commun, 2014, 5:3827 [4] Fusco et al, Science, 2017, 358:1440–3 [5] Cascella et al, ACS Chem Biol, 2019, 14:1352–1362.

ShT-06.3-1
GSSG at the center of the protein oxidative folding scene

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Oxidized glutathione (GSSG) was considered for many years the main actor for protein oxidative folding, but 20 years ago, ER oxidoreductin 1 and the protein disulphide isomerase were indicated as being mainly responsible for this process. However, recent findings are bringing GSSG back to center stage. In fact, we discovered that in five proteins (albumin, lysozyme,

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ribonuclease, trypsinogen and chimotrypsinogen), a few structural cysteines, only devoted to form disulfides, display astonishing hyper-reactivity toward GSSG when the polypeptide is present in a reduced molten globe conformation. This is noteworthy because GSSG is present at high concentrations in the endoplasmic reticulum. We report here that a similar phenomenon is present in Lactoferrin. This protein displays 16 disulfides and, in a reduced molten globule conformation, a single cysteine displays reactivity toward GSSG more than 3000 times higher than that of an "unperturbed" protein cysteine. This hyper-reactivity is specific for GSSG and is probably due to a productive transient protein-GSSG complex. Fluorescence spectroscopy indicated the existence of this complex with a dissociation constant of 0.55 mM. The crucial role of GSSG was also suggested by the replication of milestones experiments on the oxidative folding of ribonuclease (RNase) as previously published: Gambardella G et al. Int J Mol Sci. (2022), 14, 7759. Contrary to what found by Anfinsen, the spontaneous re-oxidation of reduced RNase does not give a complete recovery of activity, also showing structures significantly different from that of the native enzyme. Only in the presence of the reshuffling mixture of GSH/GSSG were we able to completely restore RNase activity and native conformation. These results confirm the incontestable role of GSSG in protein oxidative folding. *The authors marked with an asterisk equally contributed to the work.

ShT-06.3-2
Contribution of heat shock protein A2 to the maintenance of human epidermal homeostasis

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HSPA2, a heat shock-non-inducible chaperone of the HSPA (HSP70) protein family, was over the years considered as a testis-specific and male fertility related factor. Now it is clear that HSPA2 is expressed also in human somatic tissues, including various multilayered epithelia. The contribution of HSPA2 to somatic cells phenotype is poorly understood. Therefore, in this study we aimed at finding which biological processes rely on HSPA2 in the epidermis, a type of stratified epithelium composed mostly of keratinocytes. The levels of HSPA2 expression were modified in immortalized epidermal keratinocytes. HSPA2-null and HSPA2-overproducing cells were established by CRISPR/Cas9 gene editing system or lentiviral gene transfer, respectively. Cells were cultured *in vitro* either as a standard 2D cell culture or as a 3D reconstructed human epidermis (RHE) model. RNA-seq-based transcriptomic profiling of cells grown in 2D and 3D models showed HSPA2-dependent changes in gene expression; the effect was stronger in the 3D model. Unsupervised principal component analysis revealed a set of 138 genes, the expression of which was affected due to HSPA2 loss. The HSPA2-sensitive signaling pathways were those related to immunological reaction and keratinocyte differentiation. Functional analysis confirmed that HSPA2 is required for keratinocyte growth in 2D and 3D models. Histological and ultrastructural analysis of RHE revealed that HSPA2-null, but not HSPA2-overexpressing cells, formed RHE with granular layer under-development and decreased expression of late keratinocyte differentiation markers. HSPA2-null RHE also produced higher levels of pro-inflammatory proteins. In summary, we have found, for the first time, that HSPA2 regulates late stages of keratinocyte differentiation and it may impact on immunomodulatory processes in the human epidermis.

This work was supported by the National Science Centre, Poland grant 2017/25/B/NZ4/01550.

Tuesday 11 July
9:00–11:00, Auditorium Ronsard

Innate Immune Pathogen Sensing

S-05.2-3
Why do we survive or die from infections?

M. Soares
Fundação Calouste Gulbenkian – Instituto Gulbenkian de Ciência, Oeiras, Portugal

Why do some individuals develop severe disease and succumb to infection while others develop only mild forms of the disease and survive the same type of infection? The answer is, in part, because individuals that manage to clear invading pathogens develop milder forms of disease while those that develop severe and often lethal forms of the disease fail to clear those same pathogens. It is under this conceptual framework that medical interventions against infectious diseases, such as vaccination or antimicrobial drugs, are used with overwhelming success. Yet, there are "exceptions to the rule", with major consequences to global human morbidity and mortality. For example, life-threatening organ dysfunction caused by a dysregulated host response to infection, a condition known as sepsis, can develop in individuals carrying pathogen burdens that are similar to those that do not develop sepsis. The same is true for severe and often-lethal outcomes of malaria, caused by Plasmodium infection. More recently we became aware of the same phenomenon occurring in individuals that develop or not severe COVID-19, caused by SARS-CoV-2 infection. These apparent discrepancies can be explained by variations in the establishment of disease tolerance to infection, an evolutionary conserved defense strategy that does not rely on the elimination of pathogens. In this seminar I will discuss experimental evidence supporting the notion that disease tolerance to infection by different classes of pathogens relies on evolutionary conserved stress- and damage-transcriptional programs. These are essential to reprogram energy metabolism and to maintain the function of vital organs, at levels compatible with survival to infection.

S-05.2-1
Regulation and function of CARD-CC/MALT1 signaling in immunity and inflammation

R. Beyaert, A. Aidarova, M. Carels, I.S. Afonina
VIB-UGent Center for Inflammation Research, Ghent, Belgium

The caspase-recruitment domain – coiled coil (CARD-CC) adaptor proteins CARD9, −10, −11 and −14 play an important role in innate and adaptive immune responses. They all form a

signaling complex with the paracaspase MALT1, which mediates NF-κB signaling and modulates the threshold for activation of several immune and nonimmune cells in response to a rapidly growing number of stimuli. Our original discovery that MALT1 not only functions as a scaffold protein for other signaling proteins but also holds a unique proteolytic activity has led to a conceptual breakthrough and initiated a strong interest in the therapeutic targeting of MALT1 in the context of autoimmunity and cancer. On the other hand, genetic defects within CARD-CC and MALT1 are associated with human immunodeficiency syndromes, autoinflammation as well as cancer. The formation of distinct CARD-CC/MALT1 signaling complexes is largely determined by the cell type-specific expression of specific CARD-CC family proteins. CARD11 is mainly expressed in lymphocytes and mediates antigen-induced B and T cell responses. CARD9 is highly expressed in antigen-presenting cells including dendritic cells and macrophages, and CARD9 signaling initiated by C-type lectin receptors has been recognized as an important protective mechanism against infections of selected fungi. CARD14 is specifically expressed in epithelial cells but specific receptors activating CARD14 signaling remain enigmatic. Knowledge of the role of CARD14 signaling in innate immunity mainly originates from the activity of gain-of-function CARD14 variants in humans. To further study the role of CARD14 in innate immunity, we have developed mice specifically expressing a gain-of-function CARD14 variant in either keratinocytes or intestinal epithelial cells. I will present published and unpublished data showing the important role of CARD14 in skin and gut innate immune responses.

S-05.2-2
Cell-death and innate immunity in the gut

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Several regulated cell death pathways are now accepted as drivers of chronic inflammatory conditions, such as inflammatory bowel disease (IBD). The evolving literature suggests that innate immune myeloid cells appear to be especially susceptible to undergoing inflammatory cell death. These cells play critical roles in maintaining tissue homeostasis in the intestinal tract, where they are central for not only sensing and responding to commensal microbiota-derived ligands and metabolites, but also for coordinating the adaptive immune responses to both commensal and pathogenic microbes. We therefore postulate that loss of these cells, through increased cell death, plays a role in pathogenic processes. Using a model for early-onset IBD, caused by the genetic loss of expression of the X-linked inhibitor of apoptosis protein (XIAP), we show that specific subsets of innate immune cells are more susceptible to undergoing inflammatory cell death, in the murine small intestine. We link the function of these cells, namely microbial sensing, to their sensitivity to inflammatory cell-death. We propose that this process also drive the changes in the composition of the commensal microbiome. Together with changes in epithelial cell functions, also linked to increased cell death and microbial sensing, we propose a model in which the increased cell death of innate immune cells underlies the chronic inflammation observed in IBD.

ShT-05.2-1
Abstract Withdrawn

ShT-05.2-2
Lactobacillus crispatus S-layer proteins modulate innate immune inflammation in the lower female reproductive tract

I. Krasias, L.A. Roberts, P.R. Bennett, D.A. MacIntyre, J.R. Marchesi, **A. Decout**
Imperial College London, London, UK

Preterm birth is the leading cause of death among children under the age of 5 years. In 30% of cases, it is preceded by preterm prelabor rupture of the fetal membrane (PPROM). A risk indicator for PPROM is vaginal dysbiosis. Term pregnancy is associated with an optimal vaginal microbiota characterized by low diversity, with lactobacilli species constituting the vast majority of the bacteria therein. Conversely, a switch towards a more diverse microbiota containing opportunistic pathogenic species and a reduced proportion of lactobacilli is associated with increased risk of preterm birth. Inflammatory pathway activation associated with PPROM can be triggered by pro inflammatory stimuli such as TLR4 ligands. Yet, how the vaginal microbiota modulate the fetal immunological environment and thus PPROM risk is not fully understood. Using clinical isolates of vaginal pathogens and commensals we identified that lactobacilli associated with term birth selectively interacted with anti-inflammatory innate immune receptors. Conversely, bacteria associated with adverse pregnancy outcomes, *Lactobacillus iners* and *Gardnerella vaginalis*, interacted with both pro- and anti-inflammatory receptors. Specific pathogenic bacteria commonly associated with bacterial vaginosis, including *Fannyhesse vaginae*, were shown to selectively activate pro-inflammatory receptors TLR2 and TLR4. A unique feature of lactobacilli surfaces is the presence of surface layer proteins (SLPs) forming a crystallin array. Removal of SLPs from the surface of *L. crispatus* restored a strong TLR2 activation, indicating that TLR2 ligands are present on *L. crispatus*, but are shielded by the S-layer proteins. Furthermore, these S-layer proteins interacted selectively with anti-inflammatory receptors such as DC-SIGN, possibly promoting an anti-inflammatory environment. These data provide new insights into immunological mechanisms of microbial associated preterm birth and may offer new targets for novel preventive strategies.

Tuesday 11 July
9:00–11:00, Auditorium Descartes

The Exposome and Cancer

S-01.3-1
Longitudinal profiling of the molecular features of mesothelioma – a fatal malignancy causally linked to asbestos exposure

M. MacFarlane
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Mesothelioma is a uniformly fatal malignancy of the lining of the chest cavity and lungs that is causally linked to inhalation of

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asbestos fibers. The UK currently endures the highest incidence of mesothelioma worldwide: 45 fatalities/million population; 1/212 male lifetime risk, making asbestos exposure the leading cause of occupation-related mortality in the UK. Worldwide, mining and use of asbestos continues in many counties with inadequate labor protection and mesothelioma rates are rising rapidly. The disease does not respond to conventional therapy and lacks obviously druggable driver mutations. Due to a prolonged latency period and difficulty in distinguishing premalignant from benign lesions, the molecular features of early stage disease are presently uncharacterized. Chronic inflammation, arising from frustrated fiber phagocytosis, is an established risk factor in disease initiation and predictor of poor outcome, but its role in progression and maintenance remains unclear. A much deeper understanding of the basic biology underpinning disease progression *in vivo* is needed in order to develop new strategies for prophylaxis and treatment. The late onset of clinically symptomatic pleural disease that precedes mesothelioma (typically several decades after asbestos exposure), combined with very short survival from time of diagnosis, has limited studies in human subjects to the analysis of very advanced disease. Besides the established link to inhalation of asbestos fibers, we know very little about how this cancer develops prior to the late emergence of clinical symptoms. We have pioneered longitudinal *in vivo* analysis of asbestos-exposed wildtype mouse models and uncovered asbestos-driven molecular changes that occur prior to mesothelioma development. Furthermore, we have shown that biopersistent synthetic fibers (Carbon Nanotubes) that physically resemble asbestos can drive mesothelioma in mice via a near-identical sequence of molecular events, potentially heralding future outbreaks of synthetic fiber-driven MPM. Our future work focusses on longitudinal analysis of genetically-defined mouse models with predictable outcomes to provide mechanistic insight into premalignant cancer emergence & progression, linking disease-relevant mutations to evolving tumor phenotypes and their bi-directional interactions with the immune microenvironment. Our goal is to use these platforms (i) to determine mechanisms of progression to mesothelioma and (ii) to identify strategies to improve responses to immunotherapy.

S-01.3-3
Formaldehyde-induced endogenous DNA damage disrupts blood regeneration, nutritional homeostasis and promotes aging

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Endogenous DNA damage is an important and dominant cause for the accumulation of mutations in all organisms. This can be seen in the mutation signatures associated with cells obtained from aged animals and in most cancers. The established factors and processes that cause endogenous DNA damage are oxygen, water, structured DNA and DNA replication and transcription. However, a fundamental question is whether there are other factors that are prevalent drivers for endogenous DNA damage and whether DNA repair pathways mitigate against such damage. Our research has identified that simple aldehydes such as formaldehyde and acetaldehyde are produced in our cells that can cause DNA damage possibly through the formation of DNA crosslinks. We uncovered that a two-tier protection mechanism (aldehyde detoxification and DNA repair) ensure that these metabolites do not cause DNA damage and mutations. I will discuss how this two-tier protection mechanism ensures this protection and how its dysfunction damages blood regeneration, alters nutritional homeostasis, causes kidney failure and neurodegeneration. This research therefore defines endogenous aldehyde, particularly formaldehyde, as an important prevalent source of endogenous DNA damage, and thus manipulating the original source of these reactive chemicals might provide a means to preserve blood, renal and brain function over time.

S-01.3-4
Cell fate decisions upon exposure to ionizing radiation in tumor and normal tissue: Implications for the treatment outcome of cancer radiotherapy

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Radiotherapy is a central component of multi-modal cancer therapy. The induction of tumor cell death and the abrogation of clonogenic tumor cell survival are considered to be central determinants of its therapeutic success. At the same time, radiotherapy damages cells of the normal tissue, thus giving rise to acute and chronic radiation injury. On the subcellular level ionizing radiation induces DNA damage leading to the activation of the highly sophisticated and finely tuned signaling cascade of the DNA damage response and – depending on the extent of damage – to transient or permanent cell cycle arrest, and/or other cell fate decisions, respectively. However, these cell fate decisions reveal a wide range of heterogeneity and dynamics depending on physical (radiation quality, dose, etc.), cell biological (origin of the cell, genetic repertoire, cell cycle phase, cell state, etc.), microenvironmental (oxygenation, nutrient supply, pH, etc.), and systemic (overall condition, age, sex, time-of-day, etc.) parameters and have distinct cell autonomous and non-cell autonomous consequences with large impact on the treatment success and adverse side effects. The long-term goal of our work is to understand the mechanistic drivers of this heterogeneity and to develop and evaluate combined modality radiotherapy approaches personalized towards cell fate-related vulnerabilities.

S-01.3-2
Chemical exposome, microenvironment & breast cancer aggressiveness

X. Coumoul
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Breast cancer (BC) is a major public health concern, and its prognosis is very poor once metastasis occurs. The tumor microenvironment and chemical pollution have been recently suggested to contribute, independently, to the development of metastatic cells. The BC microenvironment consists, in part, of adipocytes and preadipocytes in which persistent organic

pollutants (POPs) can be stored. We conducted an exploratory case–control study in which the concentrations of 49 persistent organic pollutants (POPs) were measured in both adipose tissue (AT) and serum samples from BC patients, with or without lymph node metastasis. The concentrations of several POPs in AT were positively associated with the risk of lymph node metastasis and the tumor size. We then developed a co-culture model using BC MCF-7 cells or MDA-MB-231 cells together with hMADS preadipocytes to investigate the contribution of the microenvironment and 2,3,7,8-tetrachlorodibenzo-p-dioxin TCDD, one of the identified POPs. Global differences were characterized using a high-throughput proteomic assay. Subsequently, we measured the BC stem cell-like activity, analyzed the cell morphology, and used a zebrafish larvae model to study the metastatic potential of the BC cells. We found that co-exposure to TCDD and preadipocytes modified BC cell properties; moreover, it induced the expression of ALDH1A3, a cancer stem cell marker, and the appearance of giant cancer cells with cell-in-cell structures (CICs), which are associated with malignant metastatic progression, that we demonstrated *in vivo*. The results of our study using BC cell lines co-cultured with preadipocytes and a POP and an *in vivo* zebrafish model of metastasis suggest that the interactions between BC cells and their microenvironment could affect their invasive or metastatic potential.

Tuesday 11 July
16:00–18:00, Auditorium François 1er

Emerging Technologies for the Future

S-03.4-1
A field of dreams: synthetic biology towards engineering microbial transformation of lignin

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Developing a sustainable bio-based process to convert low-value substrates of natural origin to high-value products is an increasingly attractive strategy due to the lower ecological footprint as compared to chemical synthesis. Lignin is the second most abundant polymer in nature, which is also widely generated during biomass fractionation in lignocellulose biorefineries. At present, most technical lignin is simply burnt although it represents the richest natural source of aromatics, and thus a promising feedstock for generation of value-added compounds. Lignin is heterogeneous in composition and recalcitrant to degradation, which substantially hampers its use. Notably, microbes have evolved enzymes and specialized metabolic pathways to degrade this polymer and metabolize its numerous aromatic components. In order to make lignin biotransformation a sustainable process, we used different strategies: (1) identification of interesting lignin degrading/modifying enzymes from natural sources; (2) generation of evolved enzyme variants by protein engineering; (3) set up of *in vitro* cascade multi-enzymatic pathways; (4) engineering microbial strains; and (5) metabolic engineering of synthetic pathways. Here, we report a case study based on the production of cis, cis-muconic acid (ccMA), a petroleum-derived dicarboxylic acid, the global market of which is >$22 billion (for its relevance in the production of biodegradable plastic materials), from both lignin and wheat bran. The engineered *E. coli* strain produced ccMA with a > 95% conversion yield starting from ferulic acid, corresponding to 4.2 mg of ccMA/g of Kraft lignin, and at approximately 5% of the commercial cost of ccMA, see Molinari F. et al. ACS Sust Chem Eng (2023) 11(6), 2476–2485. The upgrade of this global waste stream promises a sustainable product portfolio, which will become an industrial reality in the future, especially with the broader use of synthetic biology approaches.

S-03.4-3
Life beyond the pixels: single-cell analysis using deep learning and image analysis methods

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In this talk, I will give an overview of the computational steps in the analysis of single cell-based large-scale microscopy experiments. First, I will present a novel microscopic image correction method designed to eliminate illumination and uneven background effects which, left uncorrected, corrupt intensity-based measurements. New single-cell image segmentation methods will be presented using differential geometry, energy minimization and deep learning methods (www.nucleAIzer.org) (*Hollandi et al. 2022, Trends Cell Biol 32, 4, 295–310*). I will discuss the Advanced Cell Classifier (ACC) (www.cellclassifier.org), a machine learning software tool capable of identifying cellular phenotypes based on features extracted from the image. It provides an interface for a user to efficiently train machine learning methods to predict various phenotypes. For cases where discrete cell-based decisions are not suitable, we propose a method to use multi-parametric regression to analyze continuous biological phenomena. To improve the learning speed and accuracy, we propose an active learning scheme that selects the most informative cell samples. Our recently developed single-cell isolation methods, based on laser-microcapturing and patch clamping, utilize the selection and extraction of specific cell(s) using the above machine learning models (*Brasko et al. 2018, Nat Commun 9, 226*). I will show that we successfully performed DNA and RNA sequencing, proteomics, lipidomics and targeted electrophysiology measurements on the selected cells (*Mund et al. 2022, Nat Biotechnol 40, 1231–1240*).

S-03.4-2
Use of hyperspectral imaging in biomedical applications

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Hyperspectral imaging (HSI) is a powerful optical modality that collects a specific part of the spectrum at each point in the imaging plane producing a so-called hypercube. Using mathematical algorithms, spectra from each pixel are then used to reveal major differences (called "principal components") or compared to previously acquired spectral libraries. All pixels whose spectra match the target spectra to a specified level of confidence are then marked as potential targets, forming so-called HSI component

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images. Owing to the richness of collected spectral information, HSI enables us to greatly enhance the contrast between spectrally different components and to reveal features otherwise invisible to the naked eye. The massive amount of collected data can be analyzed by a variety of advanced image processing and machine learning algorithms. HSI has been used in a variety of fields ranging from remote sensing to art history. The use of HSI for biomedical applications is just beginning. For now, it has been focused mainly on body surfaces to which there is direct access (e.g., skin cancer detection or monitoring external wounds). Yet, the development of ever more sensitive cameras, advances in tunable filters, optical fiber bundles, and other optical and computational components makes it now possible to envision flexible catheters capable of acquiring HSI hypercubes in real-time via percutaneous access. This opens the door for the widespread use of HSI methodology for high-resolution in-surgery imaging of internal tissues. Another promising direction includes the use of HSI for the automated analysis of both stained and unstained histological specimens. HSI can also be used to separate different components of complex microscopy samples that include fluorescent proteins, quantum dots, and various endogenous fluorophores and chromophores.

ShT-03.4-1
Mapping LDL receptor hot spot pathogenic residues through integration of predictive software

A. Larrea[1], U. Galicia[1], S. Jebari[1], A. Benito[1], K. Belloso[1], A. San Jose[1], H. Gonzalez[2], S. Arrasate[2], C. Martin[1]
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Familial hypercholesterolemia (FH) is an inherited metabolic disease causing the malfunction of cholesterol metabolism. In 90% of cases, FH is caused by mutations in the LDL receptor (LDLr) gene, with missense mutations being the most common. Predictive software has arisen as a powerful tool to predict the pathogenicity of LDLr variants. However, each predictive software uses different criteria to infer substitution's pathogenicity and therefore, the results often show discrepancies. The aim of this work is integrating the most used software to predict the pathogenicity of LDLr mutations in a new predictive model and to map LDL receptor hot spot pathogenic residues. Four predictive software were selected: Polyphen-2, SIFT and MutationTaster (not specifically devoted to LDLr) and MLb-LDLr (specific for LDLr). Software accuracy was tested with the characterized variants annotated in ClinVar so far and, by bioinformatic and machine learning techniques we integrated all models into a more accurate one. The resulting optimized model presents a specificity of 96.67% and a sensitivity of 95.73%, surpassing the accuracy of the analyzed predictive software. The optimized model was then used to obtain a hot spot map of LDLr, where each residue was colored depending on the average pathogenicity of all possible mutations that could occur on a residue. The results of this work provide a powerful tool to classify LDLr pathogenic variants and also contribute to decipher pathogenic hot spots within the receptor. This study clearly shows that combination of several predictive software results in a more accurate prediction to help clinicians in FH diagnosis.

ShT-03.4-2
Profiling affinities across the interactome

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IGBMC, Illkirch-Graffenstaden, France

Human protein networks have been widely explored, but most binding affinities remain unknown because nearly all medium/high-throughput methods used to detect protein interactions are qualitative, hindering quantitative interactome-function studies. We developed several quantitative high-throughput interactomic assays that can not only screen for protein–protein interactions but also quantify their intrinsic equilibrium binding properties at the same time with high precision. These methods are capable of measuring the biophysical properties of either isolated minimal interacting fragments, such as domains and motifs, or full-length proteins taken from total cell extracts. We propose rational strategies exploiting advances in quantitative interactomics to study either molecular mechanisms behind macromolecular interactions or the impact of interaction networks on cellular processes. We show how the "native holdup" approach can be used to measure affinities of any detectable endogenous full-length protein, or protein complex, directly from cellular extracts. Then, putative minimal interaction motifs can be synthesized as peptides to be used in the "fragmentomic holdup" assay to measure site-specific intrinsic affinities at similarly high-throughput. We demonstrate these strategies through a case study of BIN1, an SH3 domain-containing membrane remodeling protein involved in centronuclear myopathy. We identify hundreds of interaction partners for its SH3 domain and decipher the binding mechanism of most. Then, we use the same strategies to quantify the interactomic impacts of missense mutations of this SH3 domain. These strategies are simple, can be implemented in most laboratories, and can be powerful complementary approaches to conventional qualitative interactomics by providing affinities and completeness of coverage, putting a full human interactome affinity survey within realistic reach.

Tuesday 11 July
16:00–18:00, Auditorium Ronsard

Cell Metabolism and Stress

S-02.3-3
Active transcriptional repression by BACH1 as a safeguard mechanism for the functional specificity and diversity of tissue macrophages

L. Nagy
Johns Hopkins University School of Medicine, St. Petersburg, FL, USA

Transcriptional master regulators of the myeloid lineage (PU.1, Cebpa/b, etc.) are believed to be the drivers of chromatin reprogramming during differentiation and polarization acting by their pioneer ability to open chromatin. However, recent studies challenged this mode suggesting that signal-dependent TFs, particularly repressors, can also have a pioneer role. Such an expanded model predicts that active transcriptional repression and chromatin bookmarking by signal-dependent master regulators are key mechanisms for safeguarding the enhancer repertoire and

proper priming and resolution of an inflammatory response. Here, we provide evidence that BACH1, a heme-sensitive transcriptional repressor, acts as such a pervasive epigenomic safeguard, which we termed pioneer repressor, to regulate tissue macrophage identity. Using epigenomic and transcriptomic approaches combined with mouse loss of function genetic models in experimental tissue injury models and competitive bone-marrow transplant assays, we show that BACH1 is part of the core hardwired transcriptional program of ground-state macrophages shaping chromatin accessibility. Furthermore, the BACH1 cistrome is extensive, dynamically expanding upon inflammatory stimuli and correlates with pre-formed and *de novo* formed enhancer-promoter networks. We further show that myeloid BACH1 is indispensable *in vivo* for tissue regeneration upon injury. Finally, BACH1 was found to be required *in vivo* for competitive fitness and tissue adaptation of several tissue-resident macrophage populations (e.g., lung, peritoneal macrophages), further supporting its pervasive role in acquiring and maintaining the functional specificity and diversity of tissue macrophages. Taken together, our data suggest that BACH1 acts as a pioneer repressor, a signal-dependent master regulator that directly controls key transcriptional circuits during tissue macrophage subtype function, specification, heterogeneity, and inflammatory responses *in vivo*.

S-02.3-2
Riboregulation of serine hydroxymethyltransferase, a novel mechanism controlling serine metabolism across cellular compartments

F. Cutruzzolà
Sapienza University of Rome, Rome, Italy

Serine catabolism through one-carbon metabolism (OCM) is important for organismal development, cancer cell growth, and immune function [1]. Serine consumption and serine synthesis from glycine is mainly controlled by Serine hydroxymethyltransferase (SHMT), the enzyme interconverting serine and glycine in the presence of folates. The two major isoforms of the enzyme are in the cytosol (SHMT1) and in the mitochondria (SHMT2). Serine to glycine interconversion is highly dynamic, and yield serine or glycine according to the cell needs. In many tissues, 1C units are produced from serine via the mitochondrial pathway, while liver relies on SHMT1 activity to produce serine (and NADPH); reversal of the flux can occur, depending on the cell's requirements, both in physiological and pathological conditions. We discovered another regulatory mechanism of serine metabolism. We showed that SHMT's catalytic activity can be controlled by RNA [2], in a process called riboregulation, and showed *in silico* and in cell lines that RNA can dynamically control the levels of serine/glycine across cellular compartments [3]. Here we present a complete structural, functional, and phylogenetic analysis of the mechanism of riboregulation of SHMT1. We show that the RNA modulator competes with polyglutamylated folates and acts as an allosteric switch, selectively altering the enzyme's reactivity vs. serine. We also present data suggesting that riboregulation may have played a role in the evolution of eukaryotic SHMT1 and the need to compartmentalize one-carbon metabolism. We will also provide the proof-of-concept that RNA molecules acting as molecular switches of this metabolic pathway can be successfully employed *in vitro* and *in vivo* to control serine metabolism in cancer. References: [1] Amelio I, Cutruzzolá F, Antonov A, Agostini M, Melino G. Trends Biochem Sci. 2014;39(4):191–8. [2] Guiducci, et al., Nucleic Acids Research, 2019, 47:4240–4254. [3] Monti M, et al. Comput Struct Biotec 2021; 19: 30343041.

S-02.3-1
A multisite phosphorylation system controlling the decision between cell differentiation and proliferation enables multi-input logic gate switching

M. Loog
University of Tartu, Tartu, Estonia

At the cell fate decision points, different signals are processed into alternative outputs. We present a mechanism regulating the decision between differentiation and proliferation in *S. cerevisiae*. The differentiation via the mating is triggered in G1 by the pheromone pathway leading to phosphorylation of Far1 by MAP kinase Fus3. Phosphorylated Far1 inhibits the cyclin-dependent kinase (CDK) to prevent the cell cycle. Alternatively, the CDK switches off the inhibition until the next G1 by also phosphorylating Far1, leading to its degradation. The CDK and MAPK signals with similar specificity for S/TP motifs are differentially processed via competing diversionary pathways on Far1 by phospho-adaptor Cks1 and short linear docking motifs (SLiMs) to prevent cross-circuiting. We also demonstrate synthetic logic gates built based on multisite phosphorylation modules of Far1.

ShT-02.3-1
Non-genomic integrin-mediated action of thyroid hormone on PC-12 cells during hypoxia

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[1]*Ilia State University, Tbilisi, Georgia,* [2]*Ivane Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia*

Triiodothyronine (T3) has a central role in normal brain functions, such as development, growth, neural differentiation and metabolic regulation. Brain damage is associated with reduced activation of T3, altered metabolism and neuronal death. Hypoxia is one of the major factors in the pathogenesis of many neurological disorders. Our previous studies have shown, that T3 may modulate the G/F actin ratio via the Rac1 GTPase/NADPH oxidase/cofilin1 signaling pathway and αvβ3 integrin-dependent suppression of Fyn kinase phosphorylation. The precise mechanisms of Fyn kinase regulation by T3 through αvβ3 integrin during hypoxia are currently unclear. The fate of signaling molecules in the cell response is regulated through post-translational modifications, such as S-palmitoylation, that is regulated by S-palmytoiltransferases-1-23. We assumed that T3 changes the expression level of genes encoding enzymes involved in the S-palmitoylation process via nongenomic action through αvβ3 integrin during short-term hypoxia. We analyzed the palmitoyltransferase genes according to their role in brain metabolism: ZDHHC2, ZDHHC3, ZDHHC8, ZDHHC9, and ZDHHC16. Differentiated PC-12 cells were incubated with T3 and αvβ3 integrin blocking antibody under hypoxic conditions for 1 hour, after which we extracted RNA from the samples with TRIZOL reagent, synthesized cDNA using the RevertAid RT kit and detected transcription levels of the palmitoyltransferase genes by real-time PCR.

Our experiments revealed that T3 significantly reduced palmitoyltransferase-2 (ZDHHC2) gene expression and this effect was relieved by αvβ3 integrin blocking. T3, also modulated the expression level of ZDHHC9 and ZDHHC16, but these effects were not mediated by αvβ3 integrin. In conclusion, palmitoyltransferase-2 may have a central role in the regulation of the response to hypoxia and in the prevention/protection of brain hypoxia-induced pathological processes. Key words: PC-12, S-palmitoyltransferase, Hypoxia, T3, αvβ3-integrin. *The authors marked with an asterisk equally contributed to the work.

ShT-02.3-2
Regulation of the DNA-damage response by phosphorylation clusters in the p53 signaling network

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The transcription factor p53 coordinates the cellular response to DNA damage. P53 protein level and activity are highly regulated by a signaling network comprising the DNA damage response kinases ATM/ ATR/ DNA-PK (PIKKs), the kinase Chk2, the E3-ubiquitin ligase Mdm2 and the phosphatase PPM1D/Wip1. The activity of the kinases/phosphatases and reciprocal feedbacks generate repeated p53 accumulation pulses, whose duration and number determine p53-mediated transcriptional responses and cell fate. Interestingly, Chk2 and Mdm2 contain clustered phosphorylation sites of PIKKs (SQ/TQ motifs). Since multisite phosphorylation can intricately regulate protein function, we hypothesized that these additional phosphosites act as a buffer to set thresholds and molecular timers for modification of key regulatory residues upon DNA damage. Therefore, multisite phosphorylation could fine-tune Mdm2 and Chk2 activity and thereby regulate the p53 network and its long-term dynamics. By using Cas9-mediated genomic engineering, we established breast epithelial cell lines with mutations in several SQ/TQ motifs in the N-terminal domain of Chk2. Time-lapse live-cell imaging indicates that these phosphosite mutations affect Chk2 activation, leading to higher basal p53 levels and altered timing of p53 pulses upon DNA damage. To gain further insights into this regulatory mechanism, we will elucidate how mutations in SQ/TQ motifs affect phosphorylation kinetics of the remaining phosphosites in Chk2 by using NMR spectroscopy. Furthermore, we will monitor the consequences of these mutations on p53 dynamics, target gene expression and cellular outcome. Our aim is to examine the functional role of individual phosphosites in the p53 network and to evaluate how the balance between PIKK, Chk2, and Wip1 activities shapes p53 dynamics.

Tuesday 11 July
16:00–18:00, Auditorium Descartes

Host–Microbial Interactions

S-05.1-2
Organelle and metabolic dynamics of the host-pathogen interaction

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Max Planck Institute for Biology of Ageing, Cologne, Germany

The rewiring of mitochondrial metabolism serves as an innate immune-type defense mechanism that restricts the growth of the human parasite *Toxoplasma gondii*. We recently reported a previously undescribed structure we term SPOT (structure positive for outer mitochondrial membrane (OMM)) that emerges from the OMM during infection. We propose that Toxoplasma hijacks the formation of SPOTs, a cellular response to OMM stress, to counteract mitochondria and promote its growth. Here, I discuss the fate and function of these peculiar structures.

S-05.1-1
Commensal genotoxin tilimycin generates colonic stem cell mutations and drives *de novo* emergence of antibiotic resistance in mice

L. Pöltl[1], M. Kitsera[1], S. Raffl[1], S. Schild[1], A. Cosic[1], S. Kienesberger[1], K. Unterhauser[1], C. Lembacher-Fadum[2], R. Breinbauer[2], G. Gorkiewicz[3], C. Sebastian[4], G. Hoefler[3], **E. Zechner**[1]
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Members of the *Klebsiella oxytoca* species complex (KoSC) colonize the human gut within days after birth. As early arrivers, they have the potential to impact the composition and function of the community that assembles afterwards. KoSC bacteria carry *til* genes for biosynthesis of non-ribosomal peptide enterotoxins tilimycin (TM) and its derivative tilivalline (TV). TM is a DNA-alkylating pyrrolobenzodiazepine that is secreted in a chemically stable bioactive form. We colonized mice with *til* + *Klebsiella* or genotoxin-deficient mutants to show that TM inhibited competitors from multiple phyla in the gut ecosystem and increased rates of mutagenesis in co-resident opportunistic pathogens, as shown by *de novo* emergence of antibiotic resistance [1]. In patients, overgrowth of this population as an off-target effect of antibiotic use can result in apoptotic erosion of the epithelium, necrotizing enterocolitis in infants and antibiotic-associated hemorrhagic colitis in children and adults. Renewal of the intestinal lining and response to injury requires the activities of stem cells located at the base of intestinal crypts. Since TM is a diffusible small molecule, we charted the spatial distribution and luminal quantities of *til* metabolites in *Klebsiella*-colonized mice in the context of a complex microbial community. We then interrogated the consequences of TM-induced DNA damage to cycling stem cells in mice after a brief period of antibiotic-induced overgrowth. Loss of marker gene *G6pd* function revealed

genetic aberrations in colorectal stem cells that became stabilized in monoclonal mutant crypts. Mice colonized with TM-producing *Klebsiella* displayed both higher frequencies of somatic mutation and more mutations per affected individual than animals carrying a non-producing mutant. Our findings imply that genotoxic *til* + *Klebsiella* may drive somatic genetic change in the colon and increase disease susceptibility in human hosts.
References: [1] Kienesberger et al 2022 Nat Microbiol 7:1834–8.

ShT-05.1-1
Role of uracil in the antigenic variation and genetic diversity of the malaria parasite *Plasmodium falciparum*

R. Izrael[1,2], R. Babai[1,3], M. Hegedüs[1,3], G. Papp[1], A. Békési[3], K. Wengelnik[4], B.G. Vértessy[1,3]
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Malarial infections threaten hundreds of millions of people annually despite the immense effort to eradicate the disease in recent decades. The main underlying reason is the unparalleled genetic diversity of the malaria parasite *Plasmodium falciparum*, the main causative agent of lethal malarial infections. The quick emergence of resistance against different antimalarial drugs and the antigenic variation to evade the immune system makes it a challenging pathogen to eliminate. Here, we focus on understanding the role of the non-canonical base uracil in the genomic integrity of *P. falciparum*. Previously, we have demonstrated elevated levels of genomic uracil, which begs the question of how and why it is present in the genome in spite of working DNA repair machinery. To investigate the genomic distribution of uracil, we utilized a DIP-Seq-like method that has been developed by our laboratory. The method noted as U-DNA-Seq exploits a catalytically inactive uracil DNA glycosylase (UNG), the primary protein in base excision repair that recognizes uracil in the genome. Using this sensor to pull down uracilated DNA fragments, we generated next-generation sequencing data to determine uracil-enrichment and to explore its genomic distribution. Furthermore, we have established a CRISPR-Cas9-based conditional knock-out system to examine the role of *Pf*UNG and base excision repair on the genetic variability. Our results indicate an interesting role of uracil in antigenic variation. The genomic data showed an unusually high and structured enrichment in the main variable antigenic families of *var*, *rif* and *stevor* genes alike. We also characterized the impact of *Pf*UNG knock-out on the cell physiology of *P. falciparum* parasites using CRISPR-Cas9. In this study, we dissected the different aspects of how and why uracil is incorporated in a distinct manner, during which we identified a putative cytidine deaminase that can have a prominent role in the observed uracil enrichment.

ShT-05.1-2
Quantifying differential modes of cytosolic access of *Mycobacterium tuberculosis* to the host cell cytosol

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Mycobacterium tuberculosis (Mtb), the etiologic agent of tuberculosis, is an intracellular bacterium that was for a long time thought to be only an intraphagosomal pathogen. Indeed, the bacteria can subvert many host pathways to prevent phagosome acidification and maturation through effectors secreted by five distinct secretion systems (ESX-1 to ESX-5), which turn the environment of the phagosome into a more "friendly" one for replication and survival of the bacteria. However, the dogma of an exclusive intraphagosomal Mtb lifestyle has been challenged during the past decade with an increasing number of studies showing that the bacilli are able to access the cytosol of the host cell. Importantly, it has remained debated when damage of the phagolysosomal membrane occurs and what are the consequences of this. To address this in a quantitative way, we created macrophage cell lines stably expressing fluorescent galectin 3 (Gal-3), a host galactoside-binding protein, to use it as a reporter for vacuolar rupture. This reporter does not only allow us to count vacuolar damage at the single phagolysosome level, it also provides information on whether membrane rupture occurs rather discretely, in an eruptive manner, or whether bacilli remain within damaged vacuoles. We infected our reporter-containing macrophages with DsRed Mtb (H37Rv strain) and selected mutant strains, and we recorded the cells by time-lapse microscopy for a total of 5 days. This enabled us to discern different vacuolar rupture events. In addition to the previously reported late cytosolic escape, we identified frequent early damage events within the first 24 h of bacterial infection. Also, distinct subpopulations of bacteria remaining in contact with vacuolar membrane remnants and fully cytosolic bacteria could be differentiated in a dynamic manner. Currently, we aim to decipher whether the Mtb secretion systems are directly involved in initiating the first vacuolar damage events.

Wednesday 12 July
9:00–11:00, Auditorium François 1er

Mitochondria in Health and Disease

S-05.3-2
Mitochondria as regulators of cellular protein homeostasis

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IMol Polish Academy of Sciences, Warsaw, Poland

Mitochondria are multifunctional organelles, primarily involved in the fundamental biological process of respiration, but also many other biochemical reactions. Their dysfunction causes, or is observed in, many pathologies. The efficient functioning of mitochondria depends on the proper transport, sorting and assembly of mitochondrial proteins that originate either from nuclear or mitochondrial genomes. The nuclear-encoded proteins make up the large majority of proteins involved in the formation of

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mitochondria, including the respiratory chain complexes. These proteins are transient residents in the cytosol. Stress frequently leads to mitochondrial defects that in turn negatively impact mitochondrial protein uptake by the organelles. This seems to be a large challenge of cellular protein homeostasis. To preserve physiological state of the cellular proteomes, the cells activate several stress response mechanisms which are in principle focused on an increase in specific chaperone activities, improving the degradation capacity of the ubiquitin-proteasome system and modulation of cytosolic protein synthesis. This interplay between defective mitochondria and cytosolic protein homeostasis mechanisms may have a direct impact on understanding the molecular consequences of mitochondrial diseases, as well as age-related degeneration. Slow protein import and mislocalization of mitochondrial proteins increases their chances to be degraded by the proteasome in the cytosol. Thus, UPS inhibition can provide a benefit to malfunctioning mitochondria and cells as a potential therapeutic strategy for mitochondrial diseases.

S-05.3-3
Mitochondrial proteostasis

P. Rehling
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Mitochondrial proteins are predominantly encoded in the nucleus and post-translationally imported into the organelle. The translocase of the outer mitochondrial membrane (TOM complex) mediates protein transport across the outer membrane. Transport across the inner membrane requires one of two translocases in the inner membrane (TIM complexes). A subset of the mitochondrial proteome however is encoded on mitochondrial DNA. These proteins are co-translationally exported across the inner membrane by Oxa1 and assemble with newly imported proteins into membrane protein complexes of the respiratory chain. In order to maintain mitochondrial function, the assembly of respiratory chain complexes from imported and mitochondria-encoded subunits has to be tightly regulated to be adapted to cellular requirements. However, malfunction of these regulatory processes is linked to human disorders. To understand such regulatory processes, we have focused on the cytochrome c oxidase assembly process. Here, translational regulation is coupled to the assembly state of the enzyme complex. Our analyses provide new insights into the mechanism of inner mitochondrial membrane complex assembly.

S-05.3-1
Mitochondrial biogenesis and turnover in neurodegeneration and aging

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Aging is driven by the inexorable and stochastic accumulation of damage in biomolecules vital for proper cellular function. Although this process is fundamentally haphazard and uncontrollable, senescent decline and aging is broadly influenced by genetic and extrinsic factors. It is becoming increasingly apparent that longevity is intimately related to the ability of the organism to effectively cope with both intrinsic and extrinsic stress. Key determinants of this capacity are the molecular mechanisms that link aging to main stress response pathways, and mediate age-related changes in the effectiveness of the response to stress. Mitochondria, the energy-generating organelles in all eukaryotic cells, play essential roles in fundamental cellular processes. Neuronal cells depend on proper mitochondrial function. Mitochondrial impairment is a major hallmark of several age-related neurodegenerative pathologies. Interestingly, accumulation of damaged mitochondria has been observed in post-mortem brain of Alzheimer's disease patients. Mitophagy is a selective type of autophagy mediating elimination of damaged mitochondria, and the major degradation pathway by which cells regulate mitochondrial number in response to their metabolic state. Although disease-associated tau and amyloid β are known to deregulate mitochondrial function, it remains elusive whether they also directly influence the efficiency of mitophagy. We developed an *in vivo* imaging system to monitor mitophagy in neurons. We demonstrated that neuronal mitophagy is impaired in *C. elegans* models of Alzheimer's disease. Urolithin A- and nicotinamide mononucleotide-induced mitophagy ameliorates several pathological features of Alzheimer's disease, including cognitive defects. Mitophagy stimulation restores memory impairment. Our findings suggest that impaired removal of damaged mitochondria is a pivotal event in Alzheimer's disease pathogenesis, highlighting mitophagy as a potential therapeutic intervention.

ShT-05.3-2
Role of mitochondrial DNA depletion in pathogenesis of mitochondrial diseases

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Mitochondrial DNA (mtDNA) depletion syndromes (MDS) are a heterogeneous group of autosomal recessive disorders that are characterized by a severe reduction in mtDNA content caused by mutations in nuclear genes that function in either mitochondrial nucleotide synthesis or mtDNA replication, leading to impaired energy production in affected tissues and organs. MDS are phenotypically heterogeneous and may affect either a specific organ or a combination of organs, including muscle, liver, brain, and kidney. We report the molecular investigation by next-generation sequencing (NGS) of two consanguineous families including patients with clinical features suggestive of MDS. In addition, bioinformatic analyses were carried out as well as mtDNA deletion screening and copy number quantification in the blood of the patients. For the first family, NGS revealed a homozygous c.2391G > T POLG mutation (p.M797I) and analysis of the mitochondrial genome in the two patients disclosed mtDNA depletion in blood, but no deletion. Bioinformatics investigations supported the pathogenicity of the p.M797I mutation that is located in the C-terminal subdomain and might change POLG 3D structure, stability and function. In the second family, NGS revealed a mutation c.1205T > A (p.L402Q) within exon 9 of the TYMP gene. In addition, mtDNA analysis revealed a decrease of the copy number in the blood of the two patients. The p. Leu402Gln mutation was located in a conserved amino acid within the α/β domain of the TP protein. In addition, molecular docking and dynamic simulation analyses revealed that L402Q caused a conformational change in TP mutated structure and could therefore alter its flexibility and stability as well as the formation of stable homodimer leading to partial or complete loss of its catalytic activity. Overall, bioinformatic investigations

could be valuable to support experimental functional studies for a better understanding of physiopathological mechanisms leading to MDS.

ShT-05.3-1
Insights to mitochondria-regulated metabolism and mechanotransduction of hepatic cancer cells utilizing liver tumor-derived 3D cell culture system

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Advanced cell culture techniques, such as 3D cell culture methods, hold promise in drug discovery by pointing towards *in vivo*-like conditions of original/natural cellular microenvironment. Traditional 2D monolayer cell culture on a stiff glass-like material is a well-established system for the analysis of various cellular functions. However, 3D cell cultures based on derivatives of the extracellular matrix (ECM) support cells with a microenvironment that generates qualitatively and quantitatively distinct mechanical cues compared to conventional 2D cultures. Of note, functionally specialized liver cells such as hepatocytes are surrounded by functional and specific ECM, that contains cross-linked proteins and carbohydrates, including collagen. An increase in stiffness (10–75 kPa) of the liver is directly linked to various pathological conditions, including cancer progression. Moreover, cancerous tissue displays mechanical heterogeneity: edge tissue is considerably stiffer than the core of the tumor. Needless to say, low ECM stiffness (1–5 kPa) is associated with cancer stemness promotion. To prepare a soft (<1 kPa) liver-like 3D environment for cell culture, we designed and synthesized soft collagen scaffold (CS) with Young's Modulus ~0.1 kPa, utilizing bovine collagen type I. We utilized these CSs as a 3D platform for hepatocellular carcinoma (PLC/PRF/5) and hepatoblastoma (HepG2) cancer cell culturing. We revealed that physical constraints of 3D CS culture caused decreased proliferation, down-regulated YAP mechanotransducer and modulated mTOR metabolic activity by promoting autophagy. Moreover, we detected glycolysis upregulation, mitochondrial depolarization, and downregulation of mitochondria-encoded cytochrome c oxidase I. To sum up, we exposed how mechanical cues of CS affect hepatic cell behavior. We conclude that 3D cell culture systems are of great usefulness to study fundamentals of mechanics-regulated cellular fate and metabolic rewiring of liver tumor cells.

Wednesday 12 July
9:00–11:00, Auditorium Ronsard

RNA Biology

S-04.2-1
Poly(a) tail metabolism as a regulator of cell growth and homeostasis

A. Kondrashov[1], K. Willliams[1], S. Lawrence[1], R. Singhania[1], J. Waldron[2], J. Lin[1], M. Bushell[2], **C.H. de Moor**[1]
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The polyadenosine tail of mRNAs has long been linked to many aspects of the mRNA life cycle. Cleavage and mRNA polyadenylation are linked to transcription termination, in the cytoplasm the poly(A) tail is involved in translation initiation and removal of the poly(A) tail generally precedes mRNA decay. In recent years, the CNOT deadenylation complex has also been linked to translational control and RNA buffering. RNA buffering is the phenomenon where changes in RNA production or decay are compensated, leading to a lack of change in RNA concentration. A similar phenomenon is observed in changes in cell size, where the concentration of many mRNAs is kept constant. We have shown that mRNA polyadenylation is less uniform than previously thought, with mRNAs exiting the nucleus with a variety of poly(A) tail sizes. Some of this regulation is mediated by the CNOT complex, and CNOT subunit knockdown also affects mRNA synthesis rates as well as their decay. Immunoprecipitation of chromatin associated RNA identified the genes associated with the nuclear CNOT complex. Moreover, inhibition of polyadenylation and knockdown of polyadenylation factors affects a similar group of genes and causes downregulation of cell growth-associated signal transduction pathways. The affected genes are associated with inflammatory diseases, cancer and aging. Indeed, the polyadenylation inhibitor cordycepin and its derivatives are already showing promise in these areas. We propose a key role of poly(A) metabolism in cell growth, stress responses and homeostasis.

S-04.2-4
The opposing roles of the eIF4F and CCR4-NOT complexes in delivering gene expression programs

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The Ccr4-Not complex and the eIF4F complex are two crucial regulatory complexes involved in controlling translation and mRNA decay, which are fundamental processes in gene expression. While the CCR4-Not complex primarily influences mRNA decay, the eIF4F complex is mainly responsible for translation initiation. The Ccr4-Not complex is a multi-subunit complex found in eukaryotic cells. It plays a pivotal role in mRNA turnover and degradation. The complex is composed of several subunits, including the enzymes responsible for mRNA deadenylation, to promote mRNA decay and CNOT3 that monitors ribosome elongation. On the contrary, the eIF4F complex is involved in translation initiation, the process of synthesizing proteins from mRNA templates. The complex consists of three subunits: eIF4E, eIF4G, and eIF4A. eIF4E binds to the 5' cap structure of mRNA, while eIF4G acts as

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a scaffold protein, bringing together other translation initiation factors. eIF4A, an RNA helicase, unwinds the secondary structures present in the mRNA, facilitating the binding of the ribosome and initiation of protein synthesis. We are exploring how these two major complexes are responsible for controlling mRNA levels and protein production, and orchestrate delivery of correct gene expression programs. Critically, we find that these controls are not only modulating protein production but also delivering these proteins to the correct cellular compartment and helping co-translation assembly of protein complexes.

S-04.2-3
A nuclear function for an oncogenic microRNA as a modulator of splicing

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miRNAs are regulatory transcripts established as repressors of mRNA stability and translation that have been functionally implicated in carcinogenesis. miR-10b is one of the key onco-miRs associated with multiple forms of cancer. Malignant gliomas exhibit particularly striking dependence on miR-10b. However, despite the therapeutic potential of miR-10b targeting, this miRNA's poorly investigated and largely unconventional properties hamper clinical translation. We utilized Covalent Ligation of Endogenous Argonaute-bound RNAs and their high-throughput RNA sequencing to identify the miR-10b interactome and a combination of biochemical and imaging approaches for target validation. We demonstrate that miR-10b binds to U6 snRNA, a core component of the spliceosomal machinery. We provide evidence of direct binding between miR-10b and U6, *in situ* imaging of miR-10b and U6 co-localization in glioma cells and tumors, and biochemical co-isolation of miR-10b with the components of the spliceosome. We further demonstrate that miR-10b modulates U6 N-6-adenosine methylation and pseudouridylation, U6 binding to splicing factors SART3 and PRPF8, and regulates U6 stability, conformation, and levels. These effects on U6 result in global splicing alterations, exemplified by the altered ratio of the isoforms of a small GTPase CDC42, reduced overall CDC42 levels, and downstream CDC42-mediated effects on cell viability. In conclusion, we identified U6 snRNA, the key RNA component of the spliceosome, as the top miR-10b target in glioblastoma. We, therefore, present an unexpected intersection of the miRNA and splicing machineries and a new nuclear function for a major cancer-associated miRNA.

ShT-04.2-2
Noncoding RNAs, the next generation of biomarkers and therapeutics for cardiovascular diseases

A. Jusic
HAYA Therapuetics, Lausanne, Switzerland

Despite advances in treatments and therapies, cardiovascular diseases (CVD) are the leading cause of death worldwide. An outstanding improvement of the high-throughput next generation sequencing (NGS) technologies have revealed a broad spectrum of the non-coding RNA (ncRNAs) associated with cardiovascular disease. NcRNAs come in a variety of forms such as circular RNAs (circRNA), long non-coding RNAs (lncRNA), microRNAs (miRNA) and piwi-associated RNAs (piRNAs). Trends in cardiovascular research in the last decade highlighted ncRNAs as emerging, fundamentally novel diagnostic and therapeutic targets. Several clinical trials involving ncRNAs, particularly miRNAs, and heart failure are in progress, with the aim of their validation in the clinical arena. In addition, lncRNAs playing a role in heart fibrosis were successfully targeted with an antisense oligonucleotide (ASO) in mice and porcine post myocardial infarction model. Finally, the different expression profiles of ncRNAs in various CVD phenotypes as well as their response to therapy, highlighted them as attractive biomarkers in clinical drug discovery and development. Key words: ncRNAs, cardiovascular diseases, biomarkers, therapeutics targets.

ShT-04.2-1
The SARS-CoV-2 protein NSP2 regulates host gene expression by enhancing microRNA-induced translational silencing

P. Naeli[1], X. Zhang[2], P. Harris Snell[1], S. Chatterjee[1], M. Kamran[1], T. Duchaine[2], N. Sonenberg[2], S.M. Jafarnejad[1]
[1]*Patrick G. Johnston Centre for Cancer Research, Queen's University Belfast, Belfast, UK,* [2]*Department of Biochemistry and Goodman Cancer Research Centre, McGill University, Montreal, Canada*

Maintaining cell homeostasis is reliant on regulation of mRNA stability and translation by microRNAs (miRNAs). Therefore, viruses commonly co-opt this pivotal cellular mechanism to impair the host antiviral immune system and facilitate viral infection. We recently discovered that the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) encoded non-structural protein 2 (NSP2) interacts with the GIGYF2/4EHP translation repressor complex. This interaction hijacks the GIGYF2/4EHP complex to block translation of the Ifn1-b mRNA that encodes the key cytokine Interferon-ß (IFN-ß), thereby impairing the host antiviral immune response (Previously published in: Zhang Xu et al. (2022) PNAS 119(32):e2204539119). Translation of Ifn1-b mRNA was previously shown to be regulated by miR-34a in a 4EHP-dependent manner (Previously published in: Zhang Xu et al. (2021) Mol Cell 18;81(6):1187-1199.e5). However, the mechanism by which NSP2 enhances the repression of Ifn1-b mRNA by the GIGYF2/4EHP complex is not understood. We hypothesized that NSP2 could enhance the recruitment of the GIGYF2/4EHP complex by miRNAs to repress the translation of their target mRNAs. We first demonstrated that NSP2 interacts with components of the miRNA-Induced Silencing Complex (miRISC). By using sensitive luciferase reporter assays, we investigated the effect of NSP2 on miRNA-induced silencing and found that NSP2 enhances the translational repression of cellular mRNAs by miRNAs in a pervasive manner. Our data revealed a novel mechanism by which SARS-CoV-2 manipulates the host gene expression program through manipulating the miRNA-induced silencing of the host mRNAs.

Wednesday 12 July
9:00–11:00, Auditorium Descartes

Supramolecular Assemblies III: Metabolons, Multienzyme Complexes

S-07.3-2
Exploring metabolon formation in purine biosynthesis

A.M. Pedley
The Pennsylvania State University, State College, PA, USA

A longstanding question in cellular metabolism is how multi-step metabolic processes are regulated to maximize the efficiency of biomass generation to meet cellular demand. An emerging hypothesis is that enzymes within a given metabolic pathway assemble into dynamic multi-enzyme complexes called metabolons to facilitate substrate channeling between sequential pathway enzymes. Examples of this phenomenon are growing within a variety of metabolic pathways and across many different organisms. Here, I will present our work on the characterization and regulation of a human metabolon within *de novo* purine biosynthesis called purinosomes. Purinosomes have been shown to assemble through a liquid–liquid phase transition driven by the collective transient interactions between pathway enzymes under conditions of high purine demand such as during the G1 phase of the cell cycle and in many disease states. The properties of these assemblies are hypothesized to be regulated through a variety of different mechanisms such as post-translational modifications and through the assistance of molecular chaperones. Advancements made in understanding how these regulatory mechanisms influence metabolon behavior will be presented. Together, this discovery represents a newfound level of regulation within cellular metabolism and has propelled investigations into whether analogous metabolons exist in other metabolic pathways.

S-07.3-1
Genome-scale metabolic models reveal targetable vulnerabilities of metastatic colorectal cancer

M. Cascante[1], S. Marin[1], M. Tarrado-Castellarnau[1], J. Tarrago-Celada[2], C. Foguet[3], C. Hernandez-Carro[1], D. Lopez[1], R. Perez[1], B. Bori[1], N. Aleixandre[1], J.J. Centelles[1], F. Mas[1], S. Madurga[1], P. de Atauri[1]
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Metastatic colorectal cancer (mCRC) is the second leading cancer in mortality. Few genetic changes are identified during the metastatic process and epigenetic or metabolic changes have emerged as hallmarks of metastasis. Current standard-of-care for mCRC patients includes chemotherapy and anti-angiogenic or anti-EGFR in MSS tumors. The efficacy of chemotherapy in CRC is limited by the emergence of chemoresistance, which marks the need for new tools for patient selection for specific therapies and for the design of new combined therapies to overcome chemoresistance. Since metabolic reprogramming is known to be an important hallmark of cancer, tumor metabolism is considered a sensitive target in the design of new combined therapies. Here, our recent results characterizing metabolic adaptations of mCRC and on tools towards a new metabolic classification of mCRC tumors will be presented. Thus, the workflow developed to integrate multiple layers of -omics data and to characterize putative drug targets to impair metabolic adaptations that sustain CRC metastatic potential will be shown. Using this workflow, genome-scale metabolic flux models (GSMMs) were built and were used to predict and validate cystine uptake and folate metabolism as potential targets against mCRC. Next, our work in progress towards the integration of enzyme abundance and kinetic data to better identify supramolecular assembly, metabolons and multienzyme complexes to improve predictive power of GSMMs in the design of patient-tailored combined therapies will be presented. Finally, the possibilities that offer GSMMs to complement integrated physiology toward the identification of metabolic key players in metabolic diseases and the design of new combined therapies will be discussed. Acknowledgments: AGAUR (2021SGR00350) from Catalan Government, ICREA Academia, MCIN/AEI/10.13039/501100011033 (PID2020-115051RB-I00) and Instituto de Salud Carlos III (CIBEREHD, CB17/04/00023).

S-07.3-3
Biological energy transduction in action – deciphering the molecular mechanism of the remarkable Complex I superfamily

V.R.I. Kaila
Department of Biochemistry and Biophysics, Center for Biomembrane Research, Stockholm University, Stockholm, Sweden

Complex I is a gigantic ($\sim$1 MDa) membrane bound enzyme that transduces redox energy into a proton motive force across a biological membrane, powering cellular respiration. Despite major advances in recent years, its energy transduction principles remain unsolved, and a major challenge for biochemistry. In this talk, I describe our integrative biophysical, computational, and structural approach to derive a molecular understanding of the long-range (>200 Å) proton-pumping principles within the Complex I superfamily. Our findings reveal how substrate reduction triggers conformational and hydration changes, how the protonation signal propagates across the membrane domain of the complex, and how various modular adaptations have enabled both H_2 gas production and CO_2 concentration in different enzyme isoforms. We suggest that the Complex I superfamily operates by electric field effects that control both catalysis and ion-transport, with striking physical similarities to other enzymes.

ShT-07.3-1
Actinobacteria do it differently: surprising features of a mixed PDH/ODH metabolic supercomplex

L. Yang*, E.M. Bruch*, T. Wagner, P.M. Alzari, **M. Bellinzoni**
Institut Pasteur, Paris, France

The pyruvate dehydrogenase (PDH) and the 2-oxoglutarate dehydrogenase (ODH) complexes are among the most conserved, multienzymatic machineries in central metabolism. Their distinctive architecture, structured around a highly symmetric and hollow core made by the acyltransferase component, is a milestone in biochemistry textbooks. Here, I will illustrate how Actinobacteria, one of the largest and most relevant prokaryotic phyla,

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show a surprisingly different picture where the PDH and ODH complexes converge into a mixed "supercomplex" with unique structural and regulatory features. I will illustrate how reshuffling and fusion of domains led to an 'all-in-one', ribosome-sized 2-oxoglutarate dehydrogenase enzyme with a unique homohexameric architecture and allosteric regulation. At the same time, a subtle insertion makes the complex core, borrowed from the PDH complex, lose its canonical highly oligomeric, symmetrical arrangement. These findings challenge the field by raising new questions that concern not only the subunit composition and three-dimensional architecture of 2-oxoacid dehydrogenase complexes, but also their evolution, their regulation, and their intracellular localization. *The authors marked with an asterisk equally contributed to the work.

ShT-07.3-2
Engineering the yeast model *Saccharomyces cerevisiae* as a biofactory platform for optimizing monoterpene indole alkaloid production

T. Perrot*[1], G. Guirimand*[2], S. Besseau[1], V. Courdavault[1]
[1]*University of Tours, Plant Biomolecules and Biotechnology, Tours, France,* [2]*Molecular Biotech and Genomics Biotechnology Research Center, Abu Dhabi, UAE*

Plants produce a diversity of highly valuable compounds which have many interests for the industrial sector, particularly for pharmaceutic and cosmetic applications. Among these beneficial plant natural products, monoterpene indole alkaloids represent a broad family composed of more than 3000 molecules identified so far. The biosynthetic pathway of monoterpene indole alkaloids has been widely studied and a key precursor named strictosidine has been identified as the crucial starting point for producing a diversity of alkaloid derivatives. To explore the complex metabolic routes leading to the desired alkaloid products, synthetic biology approaches have been considered and microbial engineering strategies have been applied (Guirimand et al., 2021; Kulagina et al., 2021). Amidst these strategies, colocalization of biosynthetic enzymes has remained critical for avoiding metabolic bottlenecks and maximizing the metabolic flux. In this work, we optimized strictosidine synthesis and its deglucosylation in the yeast *Saccharomyces cerevisiae* in order to produce alkaloids on demand. To do that, three subcellular localizations of the strictosidine synthase and strictosidine glucosidase have been tested and strictosidine titers were measured by liquid chromatographic-mass spectrometry. Afterwards, the best engineered yeast strains were then selected for producing the tetrahydroalstonine alkaloid. Taken together, this work contributed to design of a yeast chassis which can be used for producing tailor-made alkaloids. Ultimately, the next step will be the scale-up of the optimized chassis strain in bioreactors for producing higher levels of desired plant alkaloid metabolites. Guirimand G et al. (2021) Trends in Biotechnology. 39, 488–504. Kulagina N et al. (2021) Frontiers in Bioengineering and Biotechnology. 9, 1–6. *The authors marked with an asterisk equally contributed to the work.

Speed Talks

Sunday 9 July
12:35–13:00, Auditorium François 1er

Cancer and Aging

SpT-01.1-1
Mitochondrial transplantation enhanced the effect of cisplatin in prostate and ovarian carcinoma

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Prostate and ovarian cancers are among the most frequently diagnosed cancers worldwide. Traditional approaches to combat prostate and ovarian cancers involve chemotherapy and surgery. However, since drug resistance is developing against the used first-line drugs in both types of cancer, new treatment approaches are being researched. Aerobic glycolysis is a hallmark of cancer and is present in nearly all invasive human cancers and persists even under normoxic conditions and is correlated with tumor aggressiveness. These data suggest that mitochondrial dysfunction may confer a significant proliferative advantage during the evolution of cancer and that this glycolytic phenotype may be a crucial component of malignancy. In this study, we investigated the effect of mitochondrial transplantation on the therapeutic efficacy of cisplatin in slightly resistant DU145 and PC3 prostate cancer cell lines and resistant SKOV3 ovarian cancer cell line using *in vitro* and *in vivo* xenograft tumor models. The results demonstrated that mitochondrial transplantation has no effect on cancer cell proliferation; however, it decreased migration ($P < 0.001$) and altered the cell cycle significantly ($P < 0.0001$). Also, mitochondrial transplantation significantly increases chemotherapeutic sensitivity by increasing Caspase-3 and Caspase-9 levels ($P < 0.01$) and providing similar apoptotic levels with low-dose chemotherapy as that achieved with high-dose chemotherapy ($P < 0.0001$). Additionally, cisplatin when combined with mitochondria transplantation significantly decreased DU145 and SKOV3 tumor size as compared to cisplatin alone as determined by both BLI and by caliper measurement in *in vivo* studies ($P < 0.01$, $P < 0.001$, respectively). Histological evaluation of tumors was illustrated by H&E, Masson's trichome, and TUNEL staining. These results suggest that mitochondrial transplantation could be an alternative approach for the early treatment of focal cancers prior to metastasis.

SpT-01.1-2
Influence of the tumor microenvironment on the formation of brain metastases by breast cancer cells

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The tumor microenvironment (TME) plays a critical role in regulating the formation of brain metastases, which are the most severe clinical manifestation of malignancies. Breast cancer (BC) is a common cause of brain metastases. To understand the molecular processes and metabolic susceptibilities that cancer cells develop during brain metastasis formation, we are using a specific model that mimics the brain microenvironment. We have developed a complex model mimicking the brain microenvironment, including brain extracellular fluid (from mouse), BC cells able to metastasize to the brain, and BBB derived endothelial cells. The brain microenvironment is characterized by the presence of many metabolites, as well as cytokines released by immune and cancer cells. Cancer cells rewire their metabolism to cross the BBB and enter the brain parenchyma in response to those metabolites and inflammatory molecules present in the TME. We are investigating the role of glutamate, a significant metabolite on the brain side of the BBB, and cytokines in facilitating the migration of metastatic BC cells across the BBB. Our results suggest that glutamate enhances the migratory ability of metastatic BC cells when they attempt to cross the BBB. We have observed that metastatic BC cells detect and import glutamate via the mGLUR1 receptor and EAAT1 transporter, respectively. We are also investigating the presence of cytokines to understand if there is a specific cytokine signature related to BC cells that can cross the BBB. Based on our findings, we propose that inhibiting the molecular pathways utilized by metastatic BC cells to exploit extracellular glutamate and cytokines could be an effective approach to inhibit the formation of brain metastases.

SpT-01.1-3
A probe-based 3D co-culture screening platform to repolarize tumor-associated macrophages and cancer-associated fibroblasts

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Immunotherapy has revolutionized the treatment of cancer, yet many tumor types fail to respond due to the tumor microenvironment (TME). Overcoming this TME can dramatically improve cancer immunotherapies. Two of the main contributors are tumor-associated macrophages (TAMs) and cancer-associated fibroblasts (CAFs). Hypothesizing that most of the suppression is mediated by TAMs and CAFs, re-education of these cells could profoundly improve the efficacy of cancer therapies. Therefore, we aim to develop an improved *in vitro* model better representing the complexity of the TME and to subsequently develop strategies to overcome the TME-induced suppression by converting CAFs and TAMs. To re-create the TME, we cultured patient-derived tumor organoids with human fibroblasts and macrophages in a collagen matrix to resemble the 3D environment. To assess the polarization status of TAMs and CAFs, we used non-invasive fluorescent probes with TAM/CAF-specific features. The TAM probe targets cathepsins, which are highly expressed by M2-like TAMs (pro-tumorigenic) but not by M1-like TAMs (anti-tumorigenic). The CAF probe measures the activity of the serine protease FAPα located on the surface of CAFs, but not normal fibroblasts. We were able to setup a co-culture model in 3D, in which the presence of macrophages and fibroblasts induces an immense boost in tumor growth. Further, we confirmed that the TAM probe specifically visualizes macrophages in the co-culture system and showed higher activity in M2-like macrophages, indicating the sensitivity of the probe to visualize an immunosuppressive phenotype. We also show that the CAF probe is activated by cell-bound FAPα in FAPα-overexpressing fibroblasts in a time-dependent manner. These results indicate the suitability of both probes for fluorescence-based readouts. Pending further validation, we will screen compound libraries for blockade of polarization to an immunosuppressive phenotype and/or repolarization of the cells.

SpT-01.1-4
MCPIP1 protein, gender and hepatocellular carcinoma – what is the relationship between them?

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The MCPIP1 protein is one of the regulators of inflammation. As a result of RNase activity, it regulates levels of many transcripts, including pro-inflammatory cytokines, for example, IL-1β and IL-6. Recent studies showed that the MCPIP1 level decreases during the progression of breast cancer and clear cell renal cell carcinoma (ccRCC), leading to faster tumor growth and metastatic progression. However, the role of MCPIP1 protein in the progression of hepatocellular carcinoma (HCC) has not yet been investigated. HCC is one of the most prevalent cancers worldwide, and the incidence of HCC is almost 2–7 times higher in men compared to women. Our research model is based on the chemical induction of HCC in mice, but it is effective only in males, as in females the effectiveness ranges from 10 to 30%. We observed that the effectiveness of HCC induction is dependent on the MCPIP1 protein in our model and its lack in female mice livers activates tumor development. There are several possible alternatives to this disparity: our results indicate that one of them is the dependence of HCC development on the MCPIP1 protein that somehow regulates the signaling pathway for sex hormones. Therefore, the results obtained so far indicate the initiation and progression of cancer according to different pathways depending on gender, for example β-catenin, STAT and NFκB. In addition, in mice lacking the MCPIP1 protein, we observed chronic inflammation and increased infiltration of certain groups of immune cells. Obtained results indicate that the MCPIP1 protein may have a protective role during HCC progression, and its absence leads to disturbance of the hormone signaling pathway, persistent inflammation and, consequently, the development of cancer.
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SpT-01.1-5
Diverse tumor microenvironment composition influences the effectiveness of therapy with the use of a STING agonist and an anti-vascular compound

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Vascular disrupting agents (VDAs) are effective at tumor growth inhibition. However, after initial tumor reduction, it rapidly revascularizes and regrows. Therefore, combining VDAs with additional immunostimulation seems to be a rationale to awaken a potent antitumor immune response to eradicate the remaining cancer cells. The aim of the study was to examine whether a combination of immunotherapy and anti-vascular treatment will succeed in poorly immunogenic melanoma and triple negative breast tumor models. Experiments were performed on B16-F10 melanoma and 4T1 breast tumor murine models, characterized by high and low STING protein expression, respectively. Combretastatin A4 phosphate (CA4P) was used as VDA. STING agonist (cGAMP) was used as immunostimulating agent. We performed analyzes of immune cell infiltration and their state of activation (macrophages, NK cells, CD8 T cells). We have shown that a combination of CA4P with cGAMP exhibits antitumor effects only against 4T1 breast cancer. We have demonstrated that the combination boosts innate immunity. Macrophages were polarized into anti-tumorigenic M1 phenotype, NK cells massively infiltrated tumor and exhibited activated phenotype. The combination therapy was unable to activate CD8 T cells but reduced the number of exhausted $CD8^+PD\text{-}1^+$ T cells. Therefore, combination with anti-PD-1 inhibitor failed to improve overall benefit. In B16-F10 melanoma, cGAMP was sufficient to induce a potent antitumor response. No additional therapeutic effect was observed following CA4P treatment. In this model, tumor growth inhibition was due to the infiltration of TME with activated NK cells. cGAMP stimulated the infiltration of $CD8^+PD\text{-}1^+$ T cells, hence additional benefits of anti-PD-1 inhibitor were observed. The study provides preclinical evidence of effectiveness of antivascular therapy in combination with immunotherapy only in certain tumor microenvironment composition. The work was financed by National Science Center Poland, UMO-2018/31/B/NZ5/01825.

SpT-01.1-6
CK2 plays a critical role in tyrosine kinase inhibitor resistance driven by the Bcr-Abl T315I mutation in chronic myeloid leukemia

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Chronic myeloid leukemia (CML) is a myeloproliferative neoplasia characterized by the expression of the Bcr-Abl fusion kinase, and the use of tyrosine kinase inhibitors (TKIs), such as imatinib, is the standard of care. Mutations in Bcr-Abl, particularly the T315I mutation, lead to resistance to TKIs. Protein kinase CK2, a tetrameric ubiquitous Ser/Thr kinase, was previously found to be highly expressed in imatinib-resistant CML cells without Bcr-Abl mutations. Here, we investigated the roles of CK2 in TKI-resistance mediated by the Bcr-Abl T315I mutation. We found that the CML cell line KBM5 harboring the Bcr-Abl T315I mutation (KBM5-T315I) displays significantly higher CK2 protein level and activity compared to the parental KBM5 cells. KBM5-T315I cells are highly sensitive to various CK2 inhibitors (CX-4945, SGC-CK2-1 and GO289), with apoptotic cell death observed in the low μM range, suggesting survival dependency of these cells on CK2. Mechanistically, CK2 inhibition reduced Bcr-Abl autophosphorylation, phosphorylation of its downstream targets, and signaling by STAT-3 and LYN, both reported as key players in CML TKI resistance. Results were further confirmed in cells with transient down-regulation of CK2 catalytic subunits by siRNA, or in cells with one of the CK2 catalytic isoforms knock-out (KO). The KO cells exhibited improved sensitivity to TKIs. The CK2 inhibitor CX-4945 effectively induced cell death even when KBM5-T315I cells were cocultured with MS-5 stromal cells, which, resembling the tumor microenvironment, could exert a protective effect on tumor cells. Moreover, CX-4945 was effective in reducing colony formation, with only partial protection by the stromal cells. Collectively, our results demonstrate that CK2 plays a critical role in survival of Bcr-Abl-T315I mutant CML cells and its targeting may overcome resistance to and improve the efficacy of TKIs in patients with this mutation, thus paving the way for further investigation.

Autophagy

SpT-02.1-1
Tumor-derived CTF1 is a critical mediator of stroma-assisted and autophagy-dependent breast cancer cell migration, invasion and metastasis

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Autophagy is an evolutionarily conserved cellular stress response and degradative mechanism whose activation fuels cellular metabolism. It is rapidly upregulated during cellular stress, hence allowing them to survive unfavorable conditions. Autophagy dysregulations play critical roles in the pathogenesis and progress of several human health problems, including neurodegenerative disorders, degenerative syndromes, lysosomal storage disorders, inflammation and cancer. In recent years, attention to autophagy in tumor stroma which is referred as "autophagic tumor stroma" has created a new paradigm to understand the role of autophagy in cancer. However, communication with surrounding cells and the mechanism of autophagy stimulation in the tumor microenvironment are not fully documented. We identified a cytokine CTF1 (cardiotrophin 1) as an activator of autophagy in fibroblasts and breast cancer-derived carcinoma-associated fibroblasts (CAFs). We showed that CTF1 stimulated phosphorylation and nuclear translocation of STAT3, initiating transcriptional activation of key autophagy proteins, and leading to AMPK and ULK1. Moreover, promotion of breast cancer cell migration and invasion by activated fibroblasts depended on CTF1 and autophagy. Analysis of the expression levels of CTF1 in patient-derived breast cancer samples led us to establish a correlation between CTF1 expression and autophagy in the tumor stroma [1] CTF1-associated changes in signaling may also be dependent on the autophagic capacity of fibroblasts. Hence, the molecular basis of autophagy and fibroblast activation was further dissected. Our results suggested that CTF1 is crucial for tumor-stroma interactions, stromal fibroblast activation and cancer metastasis, and autophagy resides at the nexus of these cancer-related events. Previously published in: [1] Akkoc et al., (2023) Autophagy 19 (1):306–323.

Sunday 9 July
12:35–13:00, Auditorium Ronsard

Protein Life Cycle I: Localization, Dynamics, Functioning

SpT-06.1-1
Structural basis of Nedd4-2 regulation by 14-3-3 protein

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Nedd4-2, a human HECT E3 ubiquitin ligase is the last component of the ubiquitination cascade reaction that transfers the ubiquitin molecule to its specific target. Since it has many substrates, most of them membrane transporters and ion channels, its dysregulation contributes to the development of a whole spectrum of disorders, but it is mostly known as one of the causes of Liddle syndrome. Different adaptor proteins regulate the function of this enzyme by interacting with it, but in my research, the focus was the family of 14-3-3 proteins. They are known negative regulators of Nedd4-2 because their binding prevents the accessibility of the Nedd4-2 WW domains to the PY motifs of target molecules. Though this has been studied in the past, the specific molecular mechanism remains unknown. In our previous study, by using fluorescence spectroscopy, it was demonstrated that binding of the 14-3-3 dimer to the ubiquitin ligase causes the steric hindrance of its WW3 and WW4 domains, which keeps them secluded, but has an opposite effect on its catalytic HECT domain since it exposes it to the environment [1,2]. The goal of the present study is to structurally characterize the interaction between full-length human Nedd4-2 and 14-3-3 protein in the presence and absence of $Ca2+$ ions, known to be partly responsible for the activation of this enzyme. By using integrative methods of structural biology (chemical crosslinking and hydrogen-deuterium exchange coupled to MS, SAXS and modeling), we shed light on the architecture of the Nedd4-2:14-3-3 complex. References: [1] Pohl P et al. (2021) Commun Biol., 4, 899. [2] Joshi R et al. (2022), Biophys. J. 121, 1299–1311 This study was supported by the Czech Science Foundation (Projects 20-00058S), the Czech Academy of Sciences (Research Projects RVO: 67985823 of the Institute of Physiology) and by Grant Agency of Charles University (Project No. 348421).

SpT-06.1-2
LIM Kinases, crucial actors of Actin cytoskeleton dynamics: a new intriguing mechanism of regulation highlighted

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LIM kinases, LIMK1 and LIMK2, play a crucial role in cytoskeletal dynamics by preventing the turnover of actin filaments through the phosphorylation and inhibition of cofilin.

They are involved in many physiological processes including cell division, migration and neurite plasticity, but also in several pathological mechanisms such as cancer, neurological diseases and neurofibromatosis. Many LIMK inhibitors have been developed these last years but none have reached clinical trial phase III. Therefore, it is crucial to better understand the molecular mechanisms regulating LIMK activity on cofilin, in order to develop new therapeutic strategies targeting LIMKs. Here, we showed that the kinase domain of LIMK2 is not sufficient for its activity on cofilin, as its C-terminal part is also essential for this process. Moreover, this C-terminal part is phosphorylated, suggesting the existence of a phosphorylation site necessary for LIMK2 activity on cofilin. By site-directed mutagenesis, we identified an amino acid located in the C-terminal end of LIMK2, tyrosine 630, which is necessary for LIMK2 activity on cofilin. When Y630 is mutated in alanine, the protein is still able to interact with cofilin and with ROCK, one of its upstream activating kinases, but is not phosphorylated on the LIM kinase canonical phosphorylation site of activation by ROCK, threonine 505. Moreover, a constitutively active mutation of LIMK2, T505EE, combined with Y630A mutation only partially restores LIMK2 activity on cofilin, suggesting that phosphorylation of T505 is not sufficient for optimal activity on cofilin. Indeed, Y630 seems also to play a crucial role in LIMK2 phosphorylation and activity. We are currently further characterizing the role of tyrosine 630 in these processes. Taken together, our results suggest a new unsuspected regulatory mechanism of LIMK2 activity on its main substrate cofilin and question previously established exclusive activation of LIMK2 by T505 phosphorylation.

SpT-06.1-3
The role of correlated motions of conserved polar motifs and the binding modes of agonists in the activation mechanism of G protein-coupled receptors

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G protein-coupled receptors (GPCRs) are transmembrane proteins of high pharmacological relevance. It has been proposed that their activity is linked to dynamically interconverting structural and functional states and the process of activation is controlled by an interconnecting network of conformational switches in the transmembrane domain. However, it is yet to be uncovered how ligands with different extent of functional effect exert their actions. According to our recent hypothesis, the transmission of the external stimulus is accompanied by the shift of macroscopic polarization in the transmembrane domain, furnished by concerted movements of conserved polar amino acids and the rearrangement of polar species [1]. We have examined the μ-opioid (MOP), β_2-adrenergic (β_2AR) and type 1 cannabinoid (CB1) receptors using molecular dynamics simulations. The results revealed that the orthosteric binding pocket and the intracellular G protein-binding surface are connected through a polar signaling channel in all three receptors. The interplay of this polar signaling channel in the activation mechanism was evidenced by systematic mutation of the channel residues of the MOP receptor, followed by characterization of the mutant receptors by performing *in vitro* receptor binding and G protein stimulation assays. Apart from one exception, all mutants failed to bind the endogenous agonist endomorphin-2 (EM2) and to stimulate the G_i protein complex. Furthermore, mutation results indicated strong allosteric coupling between the binding pocket and the intracellular surface. The correct orientation of the bound agonist was found to be crucial for the initiation of correlated motions and consequent signaling in all three studied receptors. The epitopes of EM2 that form contacts with the MOP receptor during activation were unveiled by saturation transfer triple difference (STTD) NMR spectroscopic measurements in live cell samples. References: [1] Mitra et al. (2021) Biomolecules 11, 670.

SpT-06.1-4
Unraveling protein and DNA dynamics with video-rate atomic force microscopy

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Studying molecular dynamics and structural conformations is important for understanding the function and biological significance of samples ranging from single membrane proteins to complex macromolecular systems, and further developing appropriate therapeutic applications. Recent atomic force microscopy (AFM) developments have led to unprecedented imaging rates in fluid, enabling temporal resolution on the sub-20-ms scale. We will give three examples in which high-speed AFM was applied for studying of structural transitions and biomolecular dynamics in samples, containing bacteriorhodopsin (BR), annexin V (A5), and thermodynamic DNA rehybridization. BR is a light-driven protein pump in some purple membrane-containing Halobacterium species, where it assembles in a characteristic trimeric bundle. We have studied the structural transition of the described trimers, assembled in a 2D protein crystal. We will demonstrate how photon absorption can lead to a conformational change in the BR trimers, as shown by the reversible transition between a photolyzed and non-photolyzed states. A5 serves as an important regulator of membrane repair in eukaryotic cells, where it shows a strong Ca^{2+} binding affinity to phosphatidylserine. We have used high-speed AFM to study the 2D crystal formation in a model system containing supported lipid bilayers and A5 molecules. We demonstrate the lateral dynamics and preferred structural orientations of the mobile A5 trimers. We previously demonstrated that pUC19 plasmids bind to poly-L-ornithine substrate in supercoiled states that are very high in torsional energy, thereby driving dehybridization of the double-helical DNA strands. Here we have quantified the process kinetics with a temporal resolution of 25 ms per frame and identified stages that include formation of metastable dehybridization bubbles, thermodynamic single strand fluctuations, and ultimately rehybridization to an intact double-stranded state. *The authors marked with an asterisk equally contributed to the work.

SpT-06.1-5
A tissue specific post-translational modification (PTM) map of the human proteome

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Protein functions are closely linked to their spatial distribution in cells and tissues. Post-translational modifications are the key regulators of protein functions and their spatial distribution. Phosphorylation plays a crucial role in many cellular processes and in disease states. Several studies have demonstrated the tissue specific expression of proteins and in particular kinases, clearly indicating differential phosphorylation across tissues. We performed a proteome wide identification and functional analysis of tissue specific as well as global phosphoproteins and phosphosites (P-sites) in human using large scale re-processed proteomics experiments from PRIDE. We compared the identified tissue specificity of proteins and P-sites to the functional domains, structural, biophysical and disorder annotations. Our preliminary results shows that the protein instances of the same domain family have different tissue specificity. P-sites were most frequently found in the inter-domain region of proteins compared to domain regions. Domains involved in more generic functions like structural support (actin, tubulin) and involved in multiple functions were associated with more non-tissue specific P-sites. Most or all of the P-sites identified in domains that are involved in very specific functions, such as trypsin, tyrosine and Serine/Threonine kinase, and heat shock proteins were tissue specific sites. Based on our preliminary results, we hypothesize that, though these proteins have similar biological functions, there are some inherent properties of these protein instances in their disordered and functional domain regions that differentiate the phosphorylation patterns and tissue specificity. In summary, our work on identifying tissue specific phosphoproteins and P-sites show our initial steps towards understanding differential phosphorylation across different tissues which can be easily extended to other common PTM types such as acetylation, methylation and ubiquitination. *The authors marked with an asterisk equally contributed to the work.

Molecular Basis of Disease (Excluding Cancer)

SpT-08.5-3
Iron overload impairs autophagy and decreases lifespan in the yeast model of Niemann-Pick type C1

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Niemann Pick Type C1 (NPC1) is an endolysosomal transmembrane protein involved in the export of cholesterol and sphingolipids to other cellular compartments such as the endoplasmic reticulum and plasma membrane. NPC1 loss of function is the major cause of NPC disease, a rare lysosomal storage disorder characterized by an abnormal accumulation of lipids in the late endosomal/lysosomal network, mitochondrial dysfunction, and impaired autophagy. NPC phenotypes are conserved in yeast lacking Ncr1, an orthologue of human NPC1, leading to premature aging. Herein, we performed a phosphoproteomic analysis to investigate the effect of Ncr1 loss on cellular functions mediated by the yeast lysosome-like vacuoles. Our results revealed changes in vacuolar membrane proteins that are associated mostly with vesicle biology (fusion, transport, organization), autophagy and ion homeostasis, including iron, manganese, and calcium. Consistently, the Cytoplasm to vacuole targeting (Cvt) pathway was increased in *ncr1*Δ cells and autophagy was compromised despite TORC1 inhibition. Moreover, *ncr1*Δ cells exhibited iron overload mediated by the low-iron sensing transcription factor Aft1. Iron deprivation restored the autophagic flux of *ncr1*Δ cells and increased its chronological lifespan and oxidative stress resistance. These results implicate iron overload in autophagy impairment, oxidative stress sensitivity and cell death in the yeast model of NPC1. This work was funded by national funds through FCT – Fundação para a Ciência e a Tecnologia, I.P., under the project UIDB/04293/2020. T.S.M. (SFRH/BD/136996/2018), V.T. (CEECIND/00724/2017 and CEECIND/00724/2017/CP1386/CT0006) and C.P. (IF/00889/2015) are supported by FCT.

SpT-08.5-2
Studies of three missense mutations involved in 3-phosphoglycerate dehydrogenase deficiency—a syndrome associated with reduced L-serine synthesis

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Human 3-phosphoglycerate dehydrogenase (hPHGDH, EC1.1.1.95) catalyzes the first step of the "phosphorylated pathway" (PP), a glial-exclusive pathway that represents the primary source of L-serine (L-Ser) in the central nervous system. Here, L-Ser participates in excitatory neurotransmission, being the precursor of D-serine (D-Ser) and glycine (Gly), the two co-agonists

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of the N-methyl-D-aspartate receptors (NMDAR). Considering the importance of L-Ser in brain physiology, impairments at the level of its biosynthesis may lead to severe pathologies. In this work, the effect of three missense mutations in the human PHGDH gene, discovered in the context of 3-PHGDH deficiency (a rare neurometabolic disorder characterized by reduced levels of L-Ser in plasma and cerebral spinal fluid) were investigated. In particular, the proposed inactivating amino acid substitutions V261M, V425M and V490M (Tabatabaie L, et al. (2009) Hum Mutat. 30, 749–56) were characterized. *In vitro* biochemical analyses showed reduced activity in the physiological direction and an altered protein conformation and oligomeric state of the recombinant hPHGDH variants compared to the wild-type enzyme. Cellular studies were performed by ectopically expressing the different PHGDH variants in the U251 human astroglioma cell line. A consistent upregulation of endogenous PSP expression, coupled to an increase in L-Ser content in cells overexpressing wild-type hPHGDH, was observed. On the other hand, the formation of large protein aggregates were apparent in cells expressing the V425M and V490M PHGDH variants, with altered cellular L-Ser levels. These studies, aimed to shed light on the alteration in structure–function relationships in pathological PHGDH variants, will allow us to propose new approaches to treat neurological disorders by acting on the modulation of brain L-Ser levels. This project was founded by "PRIN-2017 – Dissecting serine metabolism in the brain". *The authors marked with an asterisk equally contributed to the work.

SpT-08.5-1
The circadian modulation of melanin biosynthesis by binding to the ROR regulatory motif in the retinal pigment epithelium

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Albinism is a pigment disorder characterized by a reduction or complete lack of melanin pigment in the eyes, skin and/or hair. The circadian clock is a molecular pacemaker comprised of transcriptional and translation feedback loops involving transcription factors such as *BMAL1*, *CLOCK*, *PER1-2*, and *CRY1-2*. Little is known about the role that the circadian clock plays in melanogenesis. Herein, we investigated this link by bioinformatics, RT-PCR and bright-field microscopy. We found that serum-shock synchronization induces rhythmic mRNA expression of clock genes *BMAL1*, *CLOCK*, *CRY2*, *PER1*, *PER2*, and *REV-ERBα* and melanogenesis-related genes *TYR*, *CDH3*, *DCT* and *PMEL* in 8–12-week-old embryonic stem cell-derived retinal pigment epithelium (hESC-RPE). By bioinformatics, we found that melanogenesis-related pathways are enriched in up-regulated genes at night compared to late-afternoon time points in mouse RPE. Using UCSC database with 21 currently known human albinism disease genes, we selected mouse/human/zebrafish conserved promoter regions including peaks of histone marks and analyzed motif enrichment by TF Motif View. We found over-representation of binding sites for negative regulators of transcription and the known clock gene binding site for ROR. Results of bright-field microscopy showed that supplementing culturing medium with the ROR clock gene agonist Nobiletin decreased pigment/field of view area compared to vehicle control in hESC-RPE. Conversely, supplementing medium with the REV-ERBα clock gene agonist SR9009 significantly increased pigmentation in hESC-RPE cells compared to vehicle treated controls. RT-PCR revealed that Nobiletin decreased mRNA expression of melanogenesis related genes: CDH3, PMEL and RAB1A. Conversely, SR9009-treated cells had increased PMEL mRNA compared to Nobiletin-treated ones. Overall, these results suggest that melanogenesis regulation is affected by the circadian clock. Modulation of the clock could be a potential target for treating albinism.

Sunday 9 July
12:35–13:00, Auditorium Descartes

Biotech Solutions to Current Problems

SpT-03.1-1
One step closer to the 3D bioprinted meniscus

F. Porzucek, M. Mańkowska, A. Mieloch, A. Mleczko, T. Szymański, P. Cywoniuk, A. Augustyniak, J. Semba, **J. Rybka**
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In the knee, the meniscus is essential for articular surface protection, shock absorption. Its injuries are extremely common, but the regenerative capability of the meniscus is relatively low. Therefore, new therapeutic alternatives, which will allow for restoring meniscus physiological properties, are needed. Using 3D bioprinting offers an unmatched chance to develop and fabricate meniscal constructions that accurately resemble the intricate nature of their natural architecture. Efficient meniscal implant bioprinting requires optimized conditions which will allow achieving proper accuracy, integrity, and durability of printed objects while simultaneously providing an optimal microenvironment for cell growth and proliferation. The aim of this work was to develop a protocol for porcine meniscal ECM extraction, allowing for retention of its native properties, while offering good printability, and providing a stimulatory environment for cell proliferation and differentiation towards meniscus-like phenotype. In line with these goals, a procedure that incorporates homogenization, a number of extractions (including supercritical CO_2 extraction), hydrolysis, and lyophilization was designed. The rheological properties of bioinks were evaluated, and a printing accuracy measurement and SEM imaging were performed. Subsequently, scaffolds were bioprinted either with suspended human adipose derived mesenchymal stem cells or 3D spheroids to assess the biocompatibility and chondrogenic capability of the dECM-based bioinks. The LIVE/DEAD assay was used to determine the viability of the cells, and RT-qPCR analysis of a subset

of genes related to chondrogenesis and the synthesis of ECM components were carried out. In summary, this study provides an in-depth description of dECM production and bioink formulation to be used in tissue engineering for meniscus implant 3D bioprinting. This work was supported by the National Center for Research and Development TECHMATSTRATEGIII/0027/2019-00 grant.

SpT-03.1-2
Development of immuno-PCR for sensitive quantification of SARS-CoV-2 nucleocapsid protein

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Accurately diagnosing people with suspected SARS-CoV-2 infection is essential to help manage COVID-19. Currently available SARS-CoV-2 diagnostics detect either RNA of the virus by RT-PCR or the presence of viral antigens in biological fluids by ELISA or similar techniques. Low sensitivity of antigen tests could lead to the risk of false negative results. Therefore, this study aimed to develop a highly sensitive immuno-PCR method for quantifying SARS-CoV-2 nucleocapsid (N) protein that combines the specificity of sandwich ELISA with the sensitivity of PCR. Recombinant N protein fragment was produced in *E. coli* as an expression system and purified using immobilized metal ion affinity chromatography. The antibodies against the N protein were raised in rabbits and mice. High-affinity polyclonal mice and rabbit N protein-specific antisera were purified using ammonium sulfate precipitation and used to develop sandwich ELISA for the quantification of N protein. Mice polyclonal serum was used as a capture for N protein. N protein bound to mice antibodies was detected with rabbit polyclonal sera. A double-stranded amino-DNA molecule of 77 base pairs was PCR-synthesized, covalently conjugated to a secondary goat anti-rabbit antibody and subsequently amplified and quantified by real-time PCR. The results were compared to analogous sandwich ELISA consisting of alkaline phosphatase-labeled goat anti-rabbit antibody. The sensitivity of immuno-PCR for quantification of N protein was increased by up to 7-fold compared to analogous ELISA, having a limit of detection of 92 pg/mL and a limit of quantification of 840 pg/mL. The developed immuno-PCR method thus has the potential to be used as a new antigen test for COVID-19 and beyond.

SpT-03.1-3
Bioactive natural peptides: from fighting antimicrobial resistance to anticancer opportunities

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Marine organisms, normally exposed to pathogens, are a promising source for bioactive peptides of biotechnological interest, whose composition, sequence and structures are different from those found in terrestrial species. Among them, antimicrobial peptides (AMPs) from fishes are highly studied due to their key role in the innate immune system of these vertebrates and their action against bacteria, viruses, yeasts and protozoa. As they show a broad spectrum of activity against multidrug resistant bacteria, AMPs could replace conventional antibiotics and slow antimicrobial resistance, that represents a global health crisis. Beyond this application, there is also a great interest towards their use as anticancer agents. Hence, we focused on a new natural AMP (chionodracine) from an Antarctic fish (*Chionodraco hamatus*) that has adapted to live in an extreme environment. Based on its scaffold, we successfully designed peptide mutants effective against ESKAPE/fungal pathogens. Previously published in: Olivieri C et al. (2018) RSC Adv 8, 41,331–41,346; Bugli F et al. (2022) Int J Mol Sci 23, 2164. As for the best active peptide mutant, we investigated its effect on bacterial virulence factors and then, the anti-biofilm activity against bacterial clinical isolates, which are the leading cause of nosocomial infections. Previously published in: Artini M et al. (2022) Int J Mol Sci 23, 13,494. Our results highlight the effect of this AMP on protease secretion. We also demonstrated that the peptide impaired biofilm development in the tested clinical strains. Moreover, a cytotoxic effect of the mutant was observed, only at the highest tested peptide concentrations, on human lung adenocarcinoma cells. The identification of AMPs that are active against human pathogens or that induce cancer cell death, but spare normal cells, may be beneficial for developing strategies to increase peptide selectivity toward specific cell targets and for understanding their mode of action.

SpT-03.1-4
Genomic and functional analysis of *Bacillus velezensis* P3.3S – a putative biocontrol agent of plant pathogens

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Plant diseases cause considerable losses in fruit and vegetable production during cultivation, handling, transport and storage. Therefore, increased interest was shown in recent years for the

use of bacteria as a solution to manage diseases caused by plant pathogens. In particular, inoculants prepared from *Bacillus* strains were considered as effective and environmentally friendly alternatives to chemical pesticides. In this line, the aim of this study was to identify the antimicrobial potential of *Bacillus velezensis* P3.3S strain isolated from saline soil cultivated with maize. The draft genome of the strain was sequenced using the Illumina NovaSeq 6000 platform and assembled using SPAdes 3.15. The antiSMASH 7 webserver was used for the identification of secondary metabolite biosynthetic gene clusters and MALDI-TOF mass spectrometry was employed to confirm the production of lipopeptides. Four phytopathogenic fungal strains (*Rhizoctonia solani, Fusarium oxysporum, Sclerotinia sclerotiorum, Verticillium dahliae*) and one bacterial strain, *Agrobacterium tumefaciens* GV220, were used for direct antagonism tests. Our results showed that 11 secondary metabolite gene clusters encoding NRPS, PKS, transAT-PKS, T3PKS and terpene were predicted in the whole genome of the P3.3S strain. Among these, six clusters showed 100% similarity and were linked to the synthesis of bacillaene, fengycin, difficidin, bacilysin, macrolactin H and bacillibactin. One cluster had 91% similarity (responsible for surfactin production) and four clusters showed no similarity with the database. *B. velezensis* P3.3S exhibited antagonistic activity against all five tested plant pathogens. Using MALDI – TOF mass spectrometry, three classes of lipopeptides were detected: surfactin, iturin, and fengycin. In conclusion, *B. velezensis* P3.3S showed important antifungal and antibacterial potential, probably related to its ability to produce lipopeptides and polyketides.

SpT-03.1-5
A system biology approach integrating cyanide assimilation into central metabolism

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Among cyanotrophic organisms, *Pseudomonas pseudoalcaligenes* CECT 5344 is a prominent candidate for the treatment of cyanide-containing wastes because it tolerates a high concentration of free cyanide, using it as the sole nitrogen source for growth at alkaline pH, which prevents it volatilizing as HCN. Despite the role of cyanide as a critical precursor of some building blocks of life, the metabolic contextualization of cyanide assimilation in biological systems has remained surprisingly elusive so far. Addressing this topic by means of metabolic analysis at genome scale using a high-quality metabolic reconstruction of *P. pseudoalcaligenes* CECT 5344 allows the integration of cyanide assimilation into central metabolism. Remarkably, cyanide assimilation is directly interconnected with respiration, the Krebs cycle, and nitrogen, phosphorous and sulfur metabolism. These connections may add new clues both in the context of the role of cyanide as a (pre)biotic component of life and in the context of the biodegradation of cyanide-containing wastes.

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Food and Nutrition in Biochemistry

SpT-08.3-1
Wood-based lignans and polyphenols improve intestinal health

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Lignans and polyphenols derived from wood are known to exhibit a broad spectrum of biological activities, indicating promising potential as relevant constituents of food and feed supplements. We aimed to investigate two extracts derived from wood lignan-based feed additives ('*ROI*' and '*Protect*') regarding their impact on selected parameters of intestinal functionality. The antioxidant and anti-inflammatory properties of the extracts were determined by measuring the effects on reactive oxygen species (ROS) and pro-inflammatory cytokine production *in vitro*. The impact on intestinal barrier integrity was evaluated in Caco-2 cells and in *Drosophila melanogaster* by documenting leaky gut formations. Furthermore, feeding trials with *ROI* and *Protect* using chickens and infected piglets, respectively, were conducted. Intestinal samples of the chickens were taken to study gene expression of pro-inflammatory target genes by qPCR. Oxidative lesions recovered from the intestine of infected piglets were analyzed for the levels of superoxide dismutase, glutathione and lipid peroxidation. *Protect* lowered ROS production in Caco-2 cells and reversed the stress-induced weakening of barrier integrity. *ROI* inhibited the expression or secretion of interleukin-8 (IL-8), interleukin-6 (IL-6), interleukin-1β (IL-1β) and tumor necrosis factor α (TNFα) in THP-1 macrophages. Moreover, *ROI* decreased leaky gut formation and mortality rates in *Drosophila melanogaster*. *ROI*-fed chickens showed decreased gene expression levels for IL-1β and interleukin-8-like 2 (IL-8 L2). Dietary supplementation with *Protect* improved the antioxidant status and barrier integrity of the intestines of infected piglets. In conclusion, wood lignan-enriched feed supplements are valuable tools to support intestinal health by exerting antioxidant, anti-inflammatory and barrier-strengthening effects. Previously published in: Heckmann et al. (2022) Molecules 27, 6327.

SpT-08.3-2
Peptides obtained after *in vitro* digestion of intracellular proteins of *S. thermophilus* display anti-inflammatory activity in THP-1 cells

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Streptococcus thermophilus is a dairy starter granted "Generally Recognized as Safe" by the FDA and "Qualified Presumption of Safety" by EFSA. A significant part of the world's population ingests this bacterium when consuming fermented products. Some strains of *S. thermophilus*, either in the live or heat-inactivated state, and peptides released after shaving and hydrolysis of the surface proteins of some strains of this bacterium displayed anti-inflammatory activity *in vitro* (Allouche et al., 2022). *S. thermophilus* cells could undergo lysis during their passage through the digestive tract. Consequently, its intracellular proteins could be hydrolysed by endogenous proteases leading to the release of peptides. We hypothesized that peptides generated from digestion of intracellular protein of *S. thermophilus* might also contribute to its overall anti-inflammatory effect. Therefore, intracellular proteins from *S. thermophilus* CNRZ-21 N strain were recovered after sonication. After fractionation by size exclusion chromatography, the resulting 3–10 kDa protein fraction was hydrolysed by Corolase PP, a mixture of pancreatic proteases. MS–MS analysis showed that most of the identified peptides belonged to the ribosomal proteins. The hydrolyzed fraction showed anti-inflammatory activity on macrophage-like THP-1 cells inflamed by LPS since their secretion of IL-8 and IL-1β cytokines and expression level of Pro-IL-1β were reduced. The results suggest that the peptides released from a fraction of intracellular proteins of *S. thermophilus* after digestion by Corolase PP may contribute to the anti-inflammatory activity of this bacterium and could be used as a functional ingredient to prevent low-grade inflammation.

Monday 10 July
12:35–13:00, Auditorium François 1er

Gene Expression/Epigenetics

SpT-04.1-1
The role of Rab27 in melanoma cell invasion and exosomal secretion

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Rab27A and Rab27B are isoforms of the Rab27 subfamily of small GTPases that have 71% amino acid similarity. Rab proteins participate in intracellular vesicular transport, which is essential to maintain physiological homeostasis. Rab27 promotes exosome secretion in particular, which is crucial for cell-to-cell communication. Rab27A and Rab27B have been reported to promote melanoma cell proliferation, invasion, and metastasis, which are the hallmarks of this malignant tumor progression. This phenomenon appears to be particularly related to exosome secretion modulation, which enables the interaction between melanoma cell-derived exosomes and non-tumor cells i.e., immune cells, endothelial cells, and fibroblasts. Rab27A/B overexpression has been associated with poor prognosis in melanoma cancer patients. We created Rab27A and Rab27B knockouts (KO) in selected melanoma cell lines using CRISPR/Cas9 technology to examine the role of Rab27 in cell growth and invasion. The wound healing and matrigel assays were used to evaluate cell migration and invasion. Using a proteome profiler, we investigated the expression of many cancer-related proteins in KO and wild type cells. Furthermore, we utilized Western blotting and flow cytometry to analyze the exosomes released by KO cells. Rab27A/B knockout significantly affected the expression of proteins involved in apoptosis, cell signaling, and epithelial-mesenchymal transition. Furthermore, mutant cells exhibited impaired migration. Interestingly, exosome secretion remained unaffected. These findings suggest that Rab27A/B control pro-invasive activity in melanoma cells which is related to mechanisms other than vehicle production, which require further investigation. This work was supported by the National Science Centre of Poland (NCN, Poland), grant number UMO-2019/35/B/NZ7/03256, and by statutory funds of the Centre of Molecular and Macromolecular Studies of the Polish Academy of Sciences, Lodz.

SpT-04.1-2
The role of the SWI/SNF chromatin remodeling complex in organ-specific transcription start site choice

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Chromatin structure control is essential to enable DNA accessibility for various regulatory proteins: transcription factors, activators or repressors controlling gene expression. Therefore, to secure proper gene expression, chromatin is subject to various modifications. One of them is chromatin remodeling executed by evolutionarily conserved among eukaryotes SWI/SNF (SWItch/Sucrose Non-Fermentable) multiprotein complexes utilizing energy from ATP hydrolysis. In Arabidopsis, mutations in genes encoding BRM (central ATPase) and SWI3C subunits of SWI/SNF complex cause pronounced leaf and flower defects. The presented research aims to unravel the role of the SWI/SNF complex in organ-specific alternative transcript formation through TSS (Transcription Start Site) choice in Arabidopsis. RNA-seq analysis of leaves and flowers of wild-type (WT), *brm*, and *swi3c* plants revealed alterations in both gene expression level and alternative transcript formation in the SWI/SNF mutants. Focusing on organ-specific TSS usage disruption, a few genes were chosen for further qPCR verification. Chromatin immunoprecipitation followed by qPCR screening of their promoter sequence was performed to evaluate direct BRM and SWI3C influence. Finally, chromatin structure and status were analyzed to reveal the potential mechanism of SWI/SNFs' action in organ-specific TSS choice. Our results prove the direct functional relationship

between chromatin remodeling executed by SWI/SNF chromatin remodeling complexes and organ-specific alternative transcript formation through TSS choice in Arabidopsis. Furthermore, our findings support the hypothesis that changes presented by *brm* and *swi3c* mutant lines may arise not only from altered transcript levels but also can be a consequence of alternative transcript formation. Project foundation: National Science Centre DEC-2018/29/B/NZ1/01935 and 2021/43/D/NZ2/02461 given to SK.

Cell Death, and Inflammation

SpT-02.2-1
Healthy cells accelerate death of oxidatively damaged cells by blocking their transcriptional response to oxidative stress

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Current research efforts to delay aging and age-related diseases mostly target cell autonomous mechanisms such as oxidative stress and DNA damage. In recent years, non-cell autonomous mechanisms regulating health and longevity are emerging as promising alternatives (Miller HA et al. 2020; Radman M, 2019). In this project we investigate the impact of healthy cells on the phenotype of adjacent damaged cells in a mammalian cell culture model. Specifically, we evaluated the survival of oxidatively damaged cells grown in co-culture with healthy cells and compared it with the survival of the damaged cells in monoculture. Surprisingly, we observed that the survival of cells treated with low doses of UVC/UVB significantly decreased when they were co-cultured with healthy cells. This "assisted suicide" effect of the healthy cells was contact-dependent (Eroglu M & Derry WB, 2016). To gain deeper understanding of the mechanism, we performed transcriptional analysis of the damaged cells in co-culture and monoculture. While the UVC/UVB treatment induced major transcriptional reprogramming in the cells grown in monoculture, cells in co-culture retained the transcriptional signature of the healthy state. In conclusion, we propose a model that healthy cells accelerate death of aberrant cells via a contact-dependent mechanism that disables transcriptional response to oxidative damage. References: Miller HA et al. (2020) eLife 9, e62659. Radman M (2019) Open Biol. 9, 180,250. Eroglu M & Derry WB (2016) Cell Death Diff 1, 1110–1118.

SpT-02.2-2
The distinct role of keratinocyte and myeloid Mcpip1 in skin inflammation

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Monocyte chemotactic protein-1-induced protein 1 (MCPIP1) is a RNase that negatively regulates transcript stability, thereby acting as a fundamental modulator of inflammatory processes. Importantly, its expression is elevated in human psoriatic epidermis. Our previous study found that keratinocyte-specific Mcpip1-deficient ($Mcpip1^{EKO}$) mice exhibited impaired skin integrity and accelerated imiquimod-induced psoriasis-like inflammation through the IL-23/Th17 and Stat3 pathways. In contrast, myeloid-specific knockout ($Mcpip1^{MKO}$) mice showed predominant pro-allergic phenotype in the skin. Thus, the aim of this study was to investigate the role of Mcpip1 in the crosstalk of keratinocytes and myeloid cells in skin function. To this end, we generated double knockout mice with Mcpip1 depletion in both keratinocyte and myeloid cells ($Mcpip1^{EMKO}$) and performed RNA-seq analysis from 12-week-old mouse skin (Control, $Mcpip1^{EKO}$, $Mcpip1^{MKO}$, $Mcpip1^{EMKO}$). We found many differentially expressed genes between double and single knockout mice related mostly to processes such as cytokine-mediated inflammation and leukocyte infiltration (such as *Marco*, *Ebi3*, *Il36g*), skin development (such as *Cldn3*, *Mc1r*), lipid metabolic processes and regulation of calcium ion transport. Interestingly, we found an increase in the thickness of subcutaneous layer in $Mcpip1^{EKO}$ mouse skin, compared to a strong decrease in $Mcpip1^{MKO}$. Imiquimod-induced psoriasis-like inflammation showed a similar enhanced sensitivity in all knockout mice compared to the control, based on PASI score. On the histological level, we found a significant increase in the epidermis thickness of $Mcpip1^{EMKO}$ mice skin after 24 h of treatment. However, 2 days later this increase was already similar in all knockout mice. In conclusion, our work demonstrated that keratinocyte and myeloid Mcpip1 are equally important, but modulate different processes in skin biology. This study was supported by the National Science Centre grant no 2020/37/N/NZ5/00575.

SpT-02.2-3
Molecular insights into an ancient mechanism of rapid cell death involving endoplasmic reticulum and nuclear remodeling

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Imagine being able to deliver anti-cancer drugs into tumor cells at a super fast-speed and induce non-toxic apoptosis like cell death. This vision was realized by a new group of membrane-active lipid-peptide/protein-complexes (LPCs) derived from components of human breast milk in cancer models and clinical studies [1,2]. Blocking classical cell death (apoptosis, necroptosis and autophagy) and endocytosis pathways did not alter the uptake of LPC and the cell death response. Here, we identify the mechanistic details of how the novel LPC achieves this outcome by

rapidly engaging with the largest membranous organelle in the cell, the endoplasmic reticulum (ER). We identify a previously unknown mechanism of tumor cell death, involving ER and nuclear remodeling and the creation of a joint ER and nuclear compartment for the scavenging of cellular contents. Induced by the membrane-active alpha1-oleate complex, this response also affected molecular mechanisms of cancer and cell adhesion gene networks. Cell death was sensitive to ion channel inhibitors but refractory to inhibitors of apoptosis, necrosis or autophagy. Massive detachment of dying cells with increasingly degraded nuclei containing the complex was also observed in patients with bladder cancer after intra-vesical alpha1-oleate instillations, in a placebo-controlled trial. The results identify a new, ER-driven mechanism for capturing constituents of dying cells for final processing and shedding from treated tumors, to avoid tissue toxicity. References: [1] A. Brisuda et al., Bladder cancer therapy using a conformationally fluid tumoricidal peptide complex. Nat Commun 12, 3427 (2021). [2] T. T. Hien et al., Bladder cancer therapy without toxicity-A dose-escalation study of alpha1-oleate. Int J Cancer 147, 2479–2492 (2020).

SpT-02.2-4
Modulation of NF-kB-mediated inflammation via inhibition of p65 translocation

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Cells of the innate immunity protect the human body by inducing inflammation, primarily as a protective response. NF-kB is one of the most important signaling pathways that is activated in the inflammation process. In many diseases however, like allergies, auto-immune diseases and cancer, there is an over-activation of NF-kB which can lead to cell and tissue damage and thus needs to be controlled. In our study, we aimed to select bioactive small molecules which can modulate the inflammatory response by attenuating NF-kB activation, mainly via the inhibition of nuclear translocation of the p65 component in cells of the innate immune system (macrophages and epithelial cells). Cells were treated with different proinflammatory stimuli (LPS and food allergen Act d 1), with or without pre-treatment with the small molecules. Gene expression of proinflammatory cytokines was analyzed using PCR and fluorescent microscopy and flow cytometry were employed to confirm the activation of NF-kB after transfection with reporter NF-kB-GFP plasmid. Nuclear translocation of components p65 and p50 was analyzed in nuclear extracts using ELISA with coupled NF-kB-specific nucleotide sequences. Western blot was used to analyze the expression levels of p65 in the cell lysates. Cells that were pre-treated with the small molecules showed decreased expression of proinflammatory cytokines (IL-1b, IL-6, IL-25, TNFα). The transcription factor was activated upon Act d 1 treatment and subsequently attenuated by the small molecules. Regarding the specific components, p65 was down-regulated in both macrophages and epithelial cells after pre-treatment with the selected molecules, while the change in p50 did not show equal statistical significance. These results highlight the immunomodulatory effect of small molecule NF-kB inhibitors and emphasize their therapeutic potential in inflammatory diseases.

Monday 10 July
12:35–13:00, Auditorium Ronsard

Supramolecular assemblies II: RNA–protein complexes, molecular machines

SpT-07.2-1
Structural and functional analysis of OrfG, a central component of the type IV secretion system of ICESt3

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Conjugation is one of the main mechanisms of horizontal gene transfer (HTG). It allows the exchange of mobile genetic elements (i.e., conjugative plasmids and Integrative Conjugative Elements (ICEs)) between bacteria in contact. The transfer of conjugative plasmids and ICEs is mediated by a multiprotein complex localized in the cell envelope of the donor cell and is called the type IV secretion system (T4SS). Little is known about the architecture and the function of T4SSs in Gram-positive bacteria compared to their Gram-negative homologs. The main goal of my PhD project is to identify transfer proteins involved in Gram-positive T4SS assembly and to determine their function. Our model is ICE*St3*, a mobile genetic element found in *Streptococcus thermophilus*, shown to propagate efficiently by conjugation to various Gram-positive bacteria. ICE*St3* contains a conjugation module which encodes a set of proteins involved in ICE*St3* T4SS assembly. Here, we describe the structure–function analysis of OrfG, a core component of ICE*St3* T4SS. By mating experiments, we demonstrate that OrfG is essential for ICE*St3* conjugative transfer. Using bacterial two-hybrid assays and SEC-MALS analysis, we showed that OrfG multimerizes and that both OrfG subdomains are involved in its multimerization. The structure of the soluble domain of OrfG solved by X-ray crystallography reveals a trimeric quaternary structure in the crystal and a NFT2-like fold, a signature of VirB8 proteins recovered in Gram-negative T4SSs. Cysteine crosslinking approach combined with X-ray crystallography confirmed that OrfG forms trimers in solution. Further analysis using Cryo-EM are conducted to determine the structure of full-length OrfG and to analyze the impact of cysteine crosslinking on its assembly and dynamics. This study provides insights into the quaternary structure of a central component of T4SS and thus contributes to a better understanding of Gram-positive T4SS assembly and function.

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SpT-07.2-2
Assembly factor of *E. coli* ribosomal small subunit biogenesis: structural insights

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The late steps of small ribosomal subunit (SSU) assembly are sprinkled with kinetic traps, that are avoided thanks to redundant mechanisms involving multiple assembly factors. Defects of those factors lead to misassembly of the ribosome, often causing cell death, that makes them high potential therapeutic targets for anticancer and antimicrobial therapies. Among these assembly factors, the prokaryotic functional counterpart of human hCINAP, is required for SSU rRNA maturation and for the positioning of essential ribosomal proteins in prokaryotes. However, the mechanism involved during this maturation step is not yet fully understood. In order to characterize protein–protein interactions involving our assembly factor of interest (AFI), we have cloned a polycistronic construct of our His6 tagged AFI and untagged proteins from its interactome. The proteins are expressed in *E. coli* and then co-purified thanks to their interaction. For structural insights, protein complexes are concentrated up to 40 g/L to identify crystallization conditions. Using AFI depleted cells (ΔAFI), we observed by agarose gel electrophoresis that its deletion is responsible for accumulation of 3 different unmatured SSU rRNA. Density gradient purification and analysis of ribosomes from ΔAFI cells showed accumulation of 30S and 50S ribosomal subunits compared to WT cells. Using cryogenic electron microscopy, we are solving at this moment the structure of unmatured SSU purified from the depleted cells. First analysis clearly shows that the deletion of the AFI is responsible for the lack of critical ribosomal proteins in the SSU. This functional and structural study will highlight the active sites involved in this maturation step, and we believe will help the understanding of the late steps of ribosomal subunit assembly.

Protein Life Cycle II: Degradation

SpT-06.2-1
Insulin degrading enzyme affects proteasome assembly and activity by regulating the dynamics of 20S maturation

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The proteasome is a multi-subunit proteolytic complex that degrades most intracellular proteins. The canonical holocomplex is made up of the 19S regulatory particle and the 20S core particle. The latter is a barrel-shaped assembly made up by two outer α and two inner β rings, each composed of seven subunits. Given the central role of proteasome activity in cell metabolism, the regulation of proteasome assembly and activity has gained considerable interest. During 20S biogenesis, the α-ring is assembled from the free α-subunits through the assistance of PAC 1-4 chaperones. Thereafter, the α-ring serves as a scaffold for subsequent insertion of β-subunits through the contribution of the POMP chaperone to constitute the half-20S. Two half-20S then assemble into a mature 20S. The activity of proteasome particles can be regulated through decoration with Proteasome Interacting Proteins, which modulate their composition and proteolytic specificities. In this regard, Insulin Degrading Enzyme (IDE), a ubiquitous metalloenzyme, has been documented to bind to the 20S, affecting its interaction with the 19S *in vitro*. It has been previously reported that downregulation of IDE expression in SHSY5Y cells increases the bulk proteasome activity, envisaging that IDE modulation has a global effect on targeted proteolysis. In the present study, we have deepened the molecular features of IDE-proteasome interaction using both siRNA delivery strategies and catalytic inhibition of the enzyme (dispensing the IDE-specific inhibitor 6bk) in SHSY5Y cells. By applying multi-disciplinary approaches, we have documented that modulation of IDE expression or activity brings about relevant effects on the abundance of main intracellular assemblies and of the main 20S precursor (16S), likely through a not yet fully characterized interaction with POMP. Taken together, these data suggest that IDE affects proteasome assembly and activity by regulating the dynamics of 20S maturation.

RNA Biology

SpT-04.2-2
From mapping to mechanism: ribosome rRNA methylation in plants

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Plant development involves two major transitions: seedling establishment and flowering. Both require rapid changes: in translation efficiency of specific mRNA to express development-specific proteins and in ribosome assembly and activity, which are affected by differential rRNA modifications. This project focuses on 2'-*O*-ribose methylation (2'-*O*-Me), a major nucleotide modification in eukaryotic rRNA, which is guided by small nucleolar RNAs of C/D-box type (C/D snoRNA) and performed by the methyltransferase Fibrillarin (FIB) associated with the C/D snoRNA ribonucleoprotein complex (C/D snoRNP). RiboMeth-Seq in *Arabidopsis thaliana* mapped 117 rRNA 2'-*O*-Me sites and we have identified most of their corresponding C/D snoRNAs by immunoprecipitation (IP) of FIB2, previously published in: Azevedo-Favory J *et al.* (2021) RNA Biology 18:11, 1–18. Recently, RiboMethSeq data revealed hypomethylation of sites critical for proper ribosome activity, like (a) in the central pseudoknot, (b) the A- and (c) P-site, in 4- compared to 15-day old seedlings.

This correlates with the expression of 45S rRNA from the Nucleolar Organizer Regions 2 and may be caused by altered expression or activity of corresponding C/D snoRNP complexes. We address this issue by small RNA-seq analysis and using snoRNA knockouts plants. Alternatively, mislocation of snoRNAs could also lead to hypomethylation, which could be verified by fluorescence *in situ* hybridization. In parallel to the developmental 2'-*O*-hypomethylation, epitranscriptomic analyses of rRNA from IP ribosomes (Flag-tagged_RPL18) and from agarose gel showed consistently altered levels for 2 of 19 tested base modifications, namely: 1-methylguanosine and 2-methyladenosine. Now, we intend to identify the respective rRNA methyltransferases and methylation sites. Overall, we aim to address the contribution of chemical modifications of rRNAs to the production of heterogeneous ribosomes and how differential ribosome methylation affects plant functions.

SpT-04.2-1
Application of an innovative nanopore sequencing-based approach to identify novel circRNAs of the human PRMT1 gene in a panel of breast cancer cell lines

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Circular RNAs (circRNAs) constitute a class of RNA molecules formed via back-splicing. CircRNAs have been shown to be involved in important cellular functions and influence the onset and progression of breast cancer. Although it has been proven that PRMT1-mediated methylation affects the characteristics of breast cancer cells, the impact of circular transcripts deriving from this gene remains unknown. In this study, a plethora of novel PRMT1 circRNAs were discovered in breast cancer cell lines, and distinct alternative splicing events were detected. In brief, total RNA was extracted from 11 breast cancer cell lines of distinct molecular subtypes and from a normal human breast epithelial cell line, followed by reverse transcription with random hexamer primers. Next, nested PCRs were performed using exon-specific divergent primers, in order to selectively amplify PRMT1 cDNAs derived from circRNAs. Then, targeted long-read (3rd generation) sequencing based on nanopore technology was performed in the MinION Mk1C platform with the Flongle adapter. Extensive bioinformatics analysis was conducted, using publicly available tools such as minimap2 and SAMtools, as well as in-house developed algorithms. More than 120 unique PRMT1 circRNAs consisting of the gene's exonic and intronic regions were discovered in this way. Additionally, many alternative splicing events and three novel PRMT1 exons were revealed. Numerous cryptic splice sites of each exon were discovered, while most circRNAs had truncated exons that form the back-splice junction. Extended exons were frequent; moreover, genomic regions beyond the annotated PRMT1 gene boundaries were used in a few novel circRNAs. Interestingly, poly(A) stretches were also present in several circRNAs. In conclusion, the complete sequences of novel circRNAs of the PRMT1 gene were determined in this study; the remarkable diversity in these sequences implies distinct molecular features and regulatory functions for these molecules.

Immunometabolism in Cancer Development and Therapy

SpT-01.2-1
Modulation of NLRP3 inflammasome assembly, cytokine production and pyroptosis cell death: Implications for a potential therapy of breast cancer

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All stages of carcinogenesis are associated with oxidative stress, inflammatory processes and inflammasome complex mechanism. Once assembled and activated, NLRP3 inflammasome determines the cleavage of caspase-1, and secretion and maturation of pro-interleukin (IL)-1β and pro-IL-18, leading to pyroptosis cell death. Specific molecules target inflammasome components, conditioning its activation. Adenosine triphosphate (ATP) acts as a danger signal and triggers the activation of the NLRP3 inflammasome and IL-18 production. MCC950 is a small-molecule inhibitor of the NLRP3 assembly mechanism, which interferes with ATP hydrolysis and ASC adaptor protein oligomerization. Also, reactive oxygen species (ROS) are one of the main mediators of NLRP3 inflammasome activation. The aim of this study was to investigate the potential of inflammasome regulation, using two main modulators of NLRP3 inflammasome to prevent breast cancer progression and aggressiveness. To accomplish this study, MDA-MB-231 breast cancer cells were exposed to ATP inducer and different concentrations of MCC950 inhibitor. Afterwards, cell viability, caspase-1 activity and ROS production were quantitatively determined from culture media. The differential expression of the major components of the NLRP3 inflammasome mechanism (NLRP3, caspase-1, IL-1β or IL-18, etc.) were determined using Real-Time PCR and immunofluorescence techniques. The exposure of MDA-MB-231 cells to ATP induced NLRP3 oligomerization, caspase-1 activation and inflammatory interleukin secretion and maturation. On the other hand, the presence of MCC950 inhibited in a dose-dependent manner caspase-1, IL-1β and IL-18 activity, thereby blocking NLRP3 inflammasome and pyroptosis mechanisms. Moreover, MCC950 acted as an antioxidant and decreased ROS production. To conclude, regulation of the NLRP3 inflammasome, using both activators and inhibitors, represents a promising strategy for the development of new efficient treatment for breast cancer.

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Monday 10 July
12:35–13:00, Auditorium Descartes

Chemical Biology

SpT-03.2-1
Deciphering retinoid storage and metabolic pathways using bio-orthogonal chemistry-based strategies

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Vitamin A and its metabolites, also known as retinoids, are small lipophilic molecules of nutritional origin, essential for normal development and physiology in vertebrates. Despite years of research, their storage, metabolism and functions in specific cell types throughout the body remain still not completely understood. Indeed, some retinoids display low abundance and are weakly ionizable (e.g., retinaldehyde) making them difficult to detect with standard techniques like HPLC-MS2. The aim of our project is to generate and test modified retinoids suitable to undergo click reactions with functionalized probes for their visualization and quantification at cellular and subcellular levels. Copper-free Strain-Promoted Azide-Alkyne Click Chemistry (SPAAC) was chosen to study azide-retinaldehyde (N3-RAL). Its activity was validated biologically via assays of enzymatic conversion (Raldh3), transcriptional activity (luciferase reporter) and differentiation induction of P19 embryonal carcinoma cells into GABAergic neurons (immunocytochemistry). Optical imaging of N3-RAL was tested in P19 cells by reaction with strained alkyne DBCO, BCN, or TMTHSI probes functionalized with fluorophores. Such reactions carried out in living cells revealed an internalization of probes in lysosomes. Moreover, using these probes for reactions on fixed cells, led to non-specific signal. On the other hand, reacting N3-RAL with probes carrying mass-tags (TMPP or TMTI) allowed retinoid detection in biological matrix (plasma) with high sensitivity. In conclusion, the use of classical and new mass-tag functionalized SPAAC probes is a promising method to study metabolism of small molecules with high sensitivity. Instead, the use of fluorescent strained alkyne probes for SPAAC in living cells is limited by their endocytosis, whereas their use in fixed tissue was inefficient and points to a need to identify new probes to visualize azide-modified small molecules in biological material.

SpT-03.2-2
Engineering of chemogenetic rigid probes for polarization microscopy

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Fluorescent polarization microscopy (FPM) enables monitoring of the orientation of molecules within living cells in real time. Efficient FPM imaging requires rigid linking of proteins of interest to fluorescent probes to conserve orientation information. Here, we explore the use of pFAST (Benaissa et al. (2021) Nat Commun 12, 6989) and HaloTag (Los et al. (2008) ACS Chemical Biology 3 (6), 373–382), two chemogenetic fluorescent protein tags enabling the monitoring of protein localization in live cells and organisms. As a model for molecular engineering of the best candidate probe, we used the actin – LifeAct complex. LifeAct is a small peptide able to bind filamentous actin (F-actin) with high affinity. When F-actin is labeled with LifeAct fused to fluorescent reporter in a rotationally constrained manner, the orientation of the averaged alignment of actin filaments can be determined as an ensemble orientation of fluorescence polarization observed with FPM. We developed several protein engineering approaches to rigidly link LifeAct to variants of pFAST or HaloTag. The binding of the probes to actin was first validated by confocal microscopy. Analysis of the promising functional candidates by FPM allowed us to identify new rigid probes. Here we show how different approaches in protein engineering can be applied for making rigid probes for FPM. *The authors marked with an asterisk equally contributed to the work.

Medicinal Biochemistry

SpT-08.2-1
Could GroEL1 be a potential anti-tuberculosis drug target?

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Tuberculosis (TB) remains a major global health issue, especially for patients infected with *M. tuberculosis* multidrug-resistant strains. My Ph.D. research goal is to investigate the role of GroEL1 as a potential new anti-TB drug target. GroEL1 is important to mycobacteria to escape drug treatment and various environmental stresses. A structure–function analysis was performed on purified recombinant GroEL1, which eventually mutated according to the structure prediction model. The protein structures were verified by FTIR and their enzymatic activities were compared to wild-type GroEL1. Interactions between potential new compounds and GroEL1 were also performed by thermal shift assay. Furthermore, using a proteomic approach, we identified that GroEL1 is required for phthiocerol

dimycocerosate biosynthesis, lipids involved in cell wall impermeability, but also that GroEL1 is involved in metabolic adaptation when facing stressful environments. Susceptibility drug assays performed on *M. bovis* BCG allowed not only the identification of potential anti-TB drug candidates but also to highlight new potential anti-TB drug regimens constituted of potential synergistic combinations.

SpT-08.2-2
Fighting resistance to aminoglycosides – design of bacterial enzyme inhibitors

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Multidrug resistance is a major health problem, and the WHO highlighted the need to hurry to develop new treatments against Priority-1 pathogens (*P. aeruginosa*, *A. baumannii* and Enterobacteriaceae). Aminoglycoside antibiotics are used for severe infections, but the development of resistance increases therapeutic difficulties. Aminoglycoside PHosphotransferases (APH) are bacterial enzymes responsible for resistance (*Ramirez and Tolmasky, 2010*) and the aim of this project is to design specific inhibitors of these proteins in order to restore sensitivity of bacteria to aminoglycosides (*Stogios et al., 2013*). Small molecule screening has been performed by *in vitro* activity measurements and thermal shift assay experiments on an APH model; this led us to the identification of interesting fragments. The selected fragments have been characterized in more detail by IC50 and Kd measurements. Structures of APH-fragment complexes have been solved and allowed us to characterize at an atomic scale the interactions involved. Moreover, MIC measurements have been performed on either laboratory or clinical strains to characterize the capability of these inhibitors to restore sensitivity to aminoglycosides. These experiments have highlighted a chemical consensus motif and after optimisation, we identified an interesting inhibitor capable of inhibiting the activity of 5 different APH. This compound shows a sub-micromolar IC50 for two of these enzymes and a good affinity shown by micromolar Kd values. Structures of two APH in complex with this inhibitor have been solved at a high resolution. Finally, MIC measurements show a capability of this molecule to restore sensitivity of bacterial strains to aminoglycosides. To conclude, in this work we have identified an interesting molecule with a high therapeutic potential to be further investigated. These results also guided the chemical synthesis of new derivatives of this inhibitor. This work was supported by an ANR contract (SIAM, ANR-19-AMRB-0001-01).

Undergraduate Teaching/Learning

SpT-09.1-1
Selected problems/cases to stimulate collaborative learning regarding the dynamic flexibility of metabolism

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Metabolic reprogramming has been identified as one of the hallmarks of cancer. Furthermore, it is becoming more and more frequent to establish connection between other diseases and metabolic rewiring. One of us has recently argued that metabolic reprogramming is not driven by disease but instead is the main hallmark of metabolism, based on its dynamic behavior that allows it to continuously adapt to changes in the internal and external conditions [1]. Metabolism is considered by many students a remarkably difficult subject, due to its broad contents and the need to integrate them in a biologically meaningful manner. Collaborative learning strategies could contribute to make it easier for students to study metabolism. Since 2015, we have designed and used problem-based learning (PBL)-like exercises to help our students to study metabolism and its regulation. In the present communication, the experience with a PBL-like exercise devoted to show that reprogramming is a main hallmark of metabolism will be analyzed and discussed. This collaborative learning approach was monitored through comparisons between scores in pre- and post-tests of knowledge, perception and satisfaction questionnaires, evaluation of students' final reports and final exam scores. Results show an overall high level of satisfaction and higher final exam scores by enrolled students, as compared to those of students that did not take part in these tasks.
This work is supported by an Educative Innovation Project (PIE22-118, funded by University of Málaga). [Grants: PID2022-138181OB-I00, PID2019-105010RB-I00 and RTI2018-098560-BC22 (Spanish Government), UMA18-FEDERJA-220, and PY20_00257 (Andalusian Government and FEDER). Funds from BIO 267 (Andalusian Government)]. References: [1] Medina MA. Metabolic reprogramming is a hallmark of metabolism itself. BioEssays 42, 2000058, 2020.

SpT-09.1-2
Entrance tests: about devils or angels

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Entrance test (ET) is a well-established education tool for assessing students' knowledge prior to a new course with a goal to synchronize the education process with the level of student's knowledge. Yet, there is a conflict between the teacher's needs for ETs and the student's experience as well as their ability to cope with the ET results. In this pilot study, we performed a single run survey in a real time before the Immunology course, on

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143 students of the University of Zagreb Faculty of Pharmacy and Biochemistry in March 2023. Kahoot quiz was used to ensure active students' attitude and engagement. In addition to the questions on students' knowledge, their attitude towards ETs was tested before and after the test. It was found that students perceived their knowledge in physiology and pathophysiology significantly lower than grades obtained in those courses. Still, most of the students did not take any action in improving their knowledge to prepare themselves for the Immunology course. Although most of them promote students' engagement in shaping education process, they have negative attitude towards ETs. Even if they perform well on ETs, they still do not perceive it as a positive education tool. In addition, < 1% perceive a bad performance on ET as motivating. Only 38% of correct answers related to knowledge were collected in this test. Most of the students collected 10–20% of correct answers, with a gauss distribution plotted between 0 and < 40%. 83% of students perceived the test as hard and 63% perceived the experience as awful. No change in students' attitude towards ET related to their achievements and only 5% decrease in negative attitude towards ETs was observed after ET. In conclusion, although ET showed low level of students' ability to follow the course properly, they do not perceive ETs as a useful education tool but rather as a highly stressful experience. *The authors marked with an asterisk equally contributed to the work.

Postgraduate Teaching/Learning

SpT-09.2-1
A workshop that integrates master's degree content develops soft skills for the professional future

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In Higher Education, besides being concerned with achieving significant learning in relation to the contents reviewed, we must train different soft skills and, thus, prepare our students for the job market. Here we report the assessment of soft skills developed in the workshop *Design and selection of active biological molecules*, within the *Master in Therapeutic Targets in Cell Signaling: Research and Development* of our university. In this workshop, the design of a research project serves as an excuse to integrate the knowledge acquired in the different modules of the master's degree: (i) molecules and pathways involved in signaling, (ii) experimental methods in signaling, (iii) cellular and pathological processes and (iv) pharmacogenomics. The students (n = 18) were divided into four teams and were guided by the instructors in all phases of the project: (i) definition of the team name and logo, (ii) choice of the pathology, (iii) search for bibliographic information, (iv) choice of both the signaling pathway involved and therapeutic target, (v) selection of techniques and methodology, (vi) deduction of possible results, (vii) preparation of the presentation and (viii) oral presentation of the project. These phases served to develop several soft skills: collaborative work, creativity, critical thinking, problem solving, time management, initiative, collaborative work, adaptability and communication. A rubric was designed for the evaluation of these soft skills, which was completed according to the observations of the team members at the end of the workshop. This motivating experience resulted in the design of research projects of high scientific quality, as well as the training of fundamental soft skills in the development of competences essential for the professional insertion of our postgraduates. *InDoBio 5.0* Biochemistry Teaching Innovation Group (UAHGI21171).

Tuesday 11 July
12:35–13:00, Auditorium François 1er

Emerging Technologies for the Future

SpT-03.4-1
Rewiring innate immune pathways by synthetic biology tools for the development of novel biosensors of microbial activity

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Inflammasomes are intracellular multiprotein complexes that form in response to various pathogenic and endogenous danger signals and execute pyroptosis, an inflammatory form of programmed cell death. CARD8 is a human sensor that upon activation with HIV protease or DPP8/9 inhibitors assembles an inflammasome through direct recruitment and activation of procaspase-1. Active caspase-1 activates proinflammatory cytokines and gasdermin D, which assembles pores in cellular membranes, leading to pyroptosis. CARD8 is composed of a disordered N-terminal tail, ZU5-UPA, and CARD domains. Upon cleavage of the N-terminus by HIV protease, the N-terminus becomes destabilized and undergoes proteasome degradation, while the UPA-CARD fragment remains intact and forms filaments that facilitate the recruitment and activation of pro-caspase-1. We aimed to develop a viral detection biosensor by reformatting CARD8 to act as a biosensor of microbial activity independently of its function as a component of the inflammasome. We directly fused the CARD8 molecule with an enzymatic component (i.e., split luciferase system) that becomes functional only upon UPA-CARD filament formation, which leads to a restoration of enzyme activity that can be used as a readout. We first demonstrated the functionality of the system using HIV protease and through the incorporation of other viral protease cleavage sites illustrated the high adaptability of the CARD8-based biosensor for the detection of other viral proteases. Unlike methods that rely on detecting a specific nucleic acid sequence and can thus fail to detect mutated viruses, our biosensor detects the activity of pathogen proteases, which are crucial for virus propagation. Such biosensors have the potential to be used for diagnostics and also for the development of antiviral drugs targeting viral proteases.

SpT-03.4-2
Effect of presowing seed treatment with cold plasma on secondary metabolite biosynthesis in plant: an overview of *Stevia rebaudiana* Bertoni case

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Seed treatment with cold plasma (CP) stimulates seed germination, plant morphometric parameters, biomass production, and disease resistance in different plant species by inducing changes in plant biochemical phenotype; however, the underlying mechanisms of CP-induced changes in secondary metabolite biosynthesis are only partially elucidated. *Stevia rebaudiana* Bertoni is an economically valuable plant due to its secondary metabolites steviol glycosides (SGs) which are responsible for the sweetness of stevia and are widely used as natural sweeteners. Our research group reported for the first time that presowing seed treatment with CP can be a powerful technique for stimulating SG biosynthesis and/or accumulation [1]. The aim of this study is to overview the effect (changes in SGs and other secondary metabolites concentrations) dependence on CP type, treatment duration/dosage, seed origin, cultivar type, vegetation stage and the possibility to transfer the CP-induced stimulating effect to the vegetatively propagated plants. We have demonstrated that a short time (2–7 min) seed treatment with two types of CP (dielectric barrier discharge (DBD) and capacitively coupled (CC) CP) can increase Stev and RebA concentrations several times (from 14% up to 11-fold) depending on treatment duration and CP type. The stimulating effect persisted for 14 weeks; however, the CP-induced stimulating effect was lost in vegetatively propagated plants. The concentrations of other bioactive compounds such as phenolics and flavonoids were decreased or unchanged by both types of CP treatment leading to the lower or unchanged antioxidant activity of stevia leaf extracts rich in SGs. These trends were reproducible to varying extents across three cultivars. It can be concluded that a short-time presowing treatment of seeds with CP can be a powerful tool for the enhancement of biosynthesis/accumulation of SGs in stevia plants. References: [1] Judickaite A. et al., Plants, 2022, 11, 611.

Neurobiochemistry (Including Neurodegenerative Diseases)

SpT-08.1-1
Pretreatment with a plant-derived phenolic compound confers neuroprotection in a mouse model of neonatal hypoxia-ischemia

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Neonatal hypoxia-ischemia (HI) is a cerebrovascular injury caused by oxygen deprivation to the brain that affects newborns. It is one of the most common causes of neonatal mortality and morbidity worldwide and it often leads to lifelong sequelae such as cerebral palsy, seizures, or mental retardation. Therapeutic hypothermia is the current standard of care, but it can only be applied to a subset of newborns that meet strict inclusion criteria and does not provide complete neuroprotection. Since HI physiopathology involves oxidative stress and inflammation, there is a growing interest in studying natural compounds with antioxidant and anti-inflammatory properties, such as polyphenols or omega-3 fatty acids, as a novel therapeutic approach to prevent and reduce brain damage. In our lab, we are investigating the neuroprotective activity of a plant-derived phenolic compound (PDPC) in a mouse model of neonatal HI. For that, we induced HI in mouse pups at postnatal day 7 using the Rice-Vannucci method, with unilateral ligation of the common carotid artery followed by hypoxia. The PDPC was administered intraperitoneally to pups prior to HI induction at 20 or 100 mg/kg. Our results showed that pre-treatment with PDPC significantly reduced the severity of histological damage as well as the brain infarct area in a concentration-dependent manner. Besides, Iba1 immunohistochemistry revealed that PDPC pretreatment inhibited the rise in the number of microglia observed in HI samples in several sites of injury: the cortex, corpus collosum, and striatum. This suggests that PDPC may reduce the activation of the immune response following the HI insult. We are currently investigating the mechanisms by which PDPC may exert its neuroprotection, by analyzing the activity of antioxidant enzymes, the preservation of myelin sheaths, and the activation of astroglia. Overall, our results show that pre-treatment with PDPC confers neuroprotection and can prevent HI-induced brain damage.

SpT-08.1-2
Molecular organization of the Usher2 complex related to deafness and blindness

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Usher2 syndrome is a rare disease and the most common genetic cause of combined deafness and progressive blindness, affecting the sensory cells of the inner ear and the retina. Associated with Usher disease, two transmembrane proteins, usherin and the G protein-coupled (GPCR) ADGRV1, and the cytoplasmic proteins whirlin and PDZD7, form a dynamic quaternary complex in hair cells. Usher 2 proteins are transiently located at the base of the stereocilia during hair bundle development, contributing to the formation of links that connected stereocilia. The underlying molecular mechanisms being unknown, we have proposed to elucidate the composition, structure, regulation and downstream signaling of the Usher2 complex. To this end, we have implemented an integrative approach to study Usher proteins *in vitro*, *in cell* and in dissected cochlea using notably state-of-the-art biochemistry (production of soluble, multidomain membrane proteins), biophysics, NMR, SAXS, X-ray diffraction as well as single particle cryo-electron microscopy analysis (*Structure* 2017; *PNAS & J. Mol. Biol.* 2020; *BMC Bioinformatics* 2021; *Front. Mol. Science* 2022). We are also developing soft X-ray and cryo-electron tomographies as well as high-resolution STED fluorescence approaches on dissected tissues, in order to characterize complex assemblies *in situ*, and unveil the intricacy of complexes in their relevant cellular environment. Altogether, the obtained results aim at understanding the physiopathology of mutations

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associated with Usher2 syndrome. *The authors marked with an asterisk equally contributed to the work.

SpT-08.1-3
Nanoscale outer membrane vesicles from *Helicobacter pylori* alter astrocyte functionality inducing neuronal degeneration

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We study "gliosis," a process whereby glial astrocytes exposed to inflammation undergo hypertrophy, increase cell motility, and express reactivity markers, such as GFAP, αVβ3 integrin, and Connexin 43. Such features make astrocytes crucial to study outcomes associated with neurodegeneration. Considering the high prevalence of *Helicobacter pylori* (Hp) infection in the world population (> 50%) and its correlation with the appearance of several extra-gastric pathologies, including neurodegenerative conditions, we wondered if gliosis could be triggered directly by Hp components and particularly by its secreted outer membrane vesicles (OMVs), which have emerged as plausible links between the gut microbiota and distant organs like brain. Purified and characterized Hp 60,190 OMVs were added to astrocytes to test their effects *in vitro* by immunoblotting and immunofluorescence analysis. Dir-labeled OMVs were also administered orally or in tail-vein injected to monitor their biodistribution and impact in the mouse brain. The effects on neuronal function were evaluated following neurite retraction by microscopy *in vitro* and neuronal damage *in vivo*. Hp OMVs induced gliosis, which promoted neurite retraction *in vitro*. Ingested and injected Hp OMVs were detected in the brain of mice, indicating that they accessed the CNS. Furthermore, their presence in the brain coincided with astrocyte reactivity and neuronal damage. Hp OMVs altered astrocyte and neuronal function *in vivo* and *in vitro*. Hence, Hp could trigger systemic effects through Hp-generated nanovesicles in the gut that access the CNS and alter brain homeostasis. Given the observed deleterious effects of Hp infection in the brain, treating this highly prevalent worldwide bacterial infection would appear imperative. Acknowledgments, funded by ANID grants: FONDECYT 1171615 (MVV), 1200836 (LL), 1210644 (AFGQ), 1211223 (LLG) FONDAP 15130011 (AFGQ), FONDEF ID21I10210 (LLG), NIH R01HL149685/ P01HL151328 (JWH), UCEN CIP2019015 (MVV). *The authors marked with an asterisk equally contributed to the work.

SpT-08.1-4
Structural dynamics of human AADC: new perspectives for severity prediction in AADC deficiency

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Aromatic amino acid decarboxylase (AADC) is the enzyme responsible for the synthesis of the neurotransmitters dopamine and serotonin and its mutations lead to AADC deficiency (AADCd), a rare and severe neurodegenerative disorder. We used a combined approach of crystallography, molecular dynamic simulations (MD), limited proteolysis and SAXS to obtain new structural and dynamic data on AADC for the comprehension of the effects of the pathogenic substitutions and the molecular basis of the disease. AADC is an obligate dimer with the two active sites located at the conserved interface and composed of elements of both subunits. Crystallographic data of native and ligand bound AADC together with aaMD show for the first time that the active sites are connected to each other by a network of interactions and a water tunnel surrounded by conserved residues. When the substrate is bound at the active site pocket the highly flexible AADC catalytic loop (CL) is found in a catalytically competent closed conformation. Once closed, the CL of one subunit is stabilized by intra-CL interactions and by interactions with regions of the facing subunit, such as C-terminal and N-terminal elements leading to an increased interface surface. SAXS-derived models show that these interactions drive changes in the AADC geometrical parameters: the ligand-bound protein is more compact, less elongated and less asymmetric than holo and apoAADC. Interestingly, SAXS combined with limited proteolysis and coarse-grained MD indicate that AADC is a conformational heterodimer, and the flexibility of the N- and C-terminal domains is crucial for the acquisition of the correct active site conformation. Finally, these results have been useful for the interpretation of more than 40 recombinant pathogenic variants. When altered, protein flexibility and interface integrity impact AADC kinetic parameters. This approach could constitute a powerful tool for predicting the mildness/severity of AADCd.

Tuesday 11 July
12:35–13:00, Auditorium Ronsard

Cell Metabolism and Stress

SpT-02.3-1
Peroxi-ome – a near-complete compendium of yeast peroxisomal proteins

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The peroxisome is an organelle that hosts a myriad of essential metabolic processes, such as metabolism of fatty acids and detoxification of Reactive Oxygen Species (ROS). Peroxisomal loss or even dysfunction leads to severe genetic diseases. Therefore, there is great significance in the characterization of the complete peroxisomal proteome – the peroxi-ome – as a basis for understanding the various functions and processes taking place in peroxisomes. Our lab has been developing and using imaging-based screens to identify additional peroxisomal proteins in the model organism *Saccharomyces cerevisiae*. We have already uncovered tens of peroxisomal proteins, yet it was clear that more proteins awaited discovery. In this work, we established and utilized a collection of strains, each expressing a C terminal fusion of one yeast protein and the fluorescent protein NeonGreen in the background of a genomically integrated peroxisomal marker (Pex3-mCherry). We imaged all strains to find cases of co-localization and uncovered 11 proteins that were not observed in peroxisomes before. By defining their sub-peroxisomal localization, we found that seven are peroxisomal membrane proteins, all of which are dually localized to peroxisomes and mitochondria. We were also able to characterize the unusual targeting mechanism by which Eci1 and Dci1, paralogous enzymes that take part in fat catabolism, are targeted to peroxisomes. Our findings suggest a new binding interface between the peroxisomal cargo factor Pex5 and these enzymes. This work, alongside our previous efforts, now enables us to define a near-complete peroxi-ome in yeast, together with uncovering a novel peroxisomal targeting mechanism.

SpT-02.3-2
Enhanced ER-mitochondria association and its implications in mitochondrial function, glycogen metabolism and insulin resistance

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Endoplasmic reticulum mitochondria contact sites (ERMCs) are highly dynamic contact regions implicated in several biological processes. Disrupting the endoplasmic reticulum (ER)-mitochondria communication has been linked to metabolic disorders including insulin resistance (IR). Nevertheless, because of contradicting findings, the precise role of ERMCs in IR remains elusive. Starch binding domain-containing protein 1 (Stbd1) is an ER-resident, glycogen-binding protein that was previously demonstrated to undergo co-translational *N*-myristoylation. A non-*N*-myristoylated form of the protein Stbd1(G2A) is preferentially targeted to the ERMCs and enhances ER-mitochondria association. Therefore, the Stbd1(G2A) variant can serve as a useful tool to address the effects of increased ER-mitochondria association in mitochondrial function, glycogen metabolism and IR *in vitro*. To this end, AML12 cell lines overexpressing either Stbd1(G2A) or Stbd1(WT) were generated. AML12 cells overexpressing the unrelated GFP protein served as controls. Overexpression of Stbd1(G2A) resulted in increased ER-mitochondria interactions, altered mitochondrial morphology and respiratory function. Cells overexpressing Stbd1(G2A) exhibited significantly reduced glycogen content compared to the Stbd1(WT) cells. ATP-stimulated calcium influx into mitochondria and calcium content within the ER, mitochondria and cytoplasm were determined. Cells overexpressing Stbd1(G2A) displayed significantly reduced mitochondrial calcium uptake and increased mitochondrial calcium content. Overexpression of Stbd1(WT) was found to significantly improve IR induced by insulin treatment in AML12 cells, more efficiently than Stbd1(G2A). Our findings indicate that enhancement of ER-mitochondria association induced by Stbd1(G2A) results in disturbed mitochondrial morphology and respiratory function. The above appears to impact on glycogen metabolism and the response to insulin and may indicate broader changes in cell metabolism.

Protein Life Cycle III: Ribosomes, Folding, Chaperones

SpT-06.3-1
Structural characterization of starting codon recognition

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Protein synthesis is a highly controlled process divided into three stages: initiation, elongation and termination. While elongation is conserved between prokaryotes and eukaryotes, initiation and termination have major differences. In bacteria, three initiation factors, the initiator tRNA (tRNAi) and the Shine-Dalgarno sequence on the 5′ end of mRNA can start translation. In contrast, in eukaryotes, the process is more arduous and they have developed different mechanisms, the most common being canonical initiation. In this, at least a dozen initiation factors (eIFs) associate with the 40S ribosomal subunit for localizing the starting codon of the mRNA in the ribosomal P site. Unlike prokaryotes, start codon recognition in eukaryotes is poorly understood because the Kozak sequence is not as crucial as the Shine-Dalgarno sequence. In an eukaryotic mRNA, some AUG can be skipped when an unfavorable sequence flanks them. At the same time, non-AUG triplets (differing in one base from AUG) can be selected if they are in good flanking regions (previously published in Hinnebusch AG et al. (2016) Science 352 (6292), 1413–1426). We aim to use cryoelectron microscopy to understand, at a structural level, how the mRNA sequence influences codon

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discrimination. For that, we use *Saccharomyces cerevisiae* as a model to reconstitute *in vitro* preinitiation complexes (PIC). Our objective is to generate mRNAs with variations on the start codon or Kozak sequence and use it to achieve structures of different PICs. We also hope these structures can help us identify each factor/ribosomal element's contribution to the stringency of codon discrimination. We have produced eIFs, 40S ribosomes, capped-mRNA and the tRNAi. Also, we have obtained a PIC with an unstructured AUC-mRNA in which we can observe how the third base is discriminated. Now, we are attempting to get a PIC with an UUG-mRNA to compare the effect of variations in the first and third position of the mRNA starting codon.

Mitochondria in Health and Disease

SpT-05.3-1
Mutant p53 stimulates mitochondrial fragmentation in pancreatic ductal adenocarcinoma (PDAC)

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Mutations in the p53 tumor suppressor gene represent the most common genetic lesions in cancer and occur in about 75% of cases of pancreatic ductal adenocarcinoma (PDAC). Most of these mutations are missense, that, in addition to the loss of tumor suppressive function of the wild-type p53 protein, acquire new oncogenic functions that promote cancer progression by enhancing the ability of cancer cells to invade and metastasize, their chemoresistance and genomic instability. Given the pivotal role of mitochondria in cancer cells not only to produce energy, but also for the neo synthesis of macromolecules, to improve their fitness and maintain redox balance, we investigated the mutant p53-dependent regulation of mitochondrial dynamics in PDAC. We deeply analyzed mitochondria morphology with the transmission electronic microscope (TEM) that revealed a higher aspect ratio, as an index of mitochondrial length, in PANC-1 cells after transient knock down of mutant p53 compared to the scramble control. Further investigation of mitochondria morphology by live cell imaging with confocal microscopy confirmed that when mutant p53 gene is silenced, the mitochondria appear more elongated and more branched compared to the short and small mitochondria present in the negative control. The analysis of mitochondrial length and network formation were performed using the MiNA (Mitochondrial Network Analysis) macro tool that allowed us to analyze the three-dimensional length of mitochondria and revealed longer mitochondria and a more branched network after knock down of mutant p53. Lastly, we analyzed the mitochondria proteome by proteomic analysis with Orbitrap mass spectrometer in mitochondria isolated by mutant p53 CRISPR/Cas9-mediated knock-out PANC-1 cells. These morphological and mechanistic data suggest a direct correlation between mutant p53 and mitochondrial dynamics further highlighting mitochondria as a promising therapeutic target in PDAC.

Cardiovascular Diseases

SpT-08.4-1
Pro-resolving lipid mediator-loaded biomimetic nanoparticles as promising carriers for inflammation resolution in atherosclerosis

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Atherosclerosis (AS) is accompanied by a chronic inflammatory process in the arteries strongly associated with failed resolution (Res). Cellular effectors, an endogenous anti-inflammatory protein network, and specialized pro-resolving lipid mediators (SPMs) regulate this process. SPMs decrease as AS advances, creating an imbalance between SPMs and pro-inflammatory molecules that promotes plaque instability. To reduce inflammation and promote its Res, we envisioned a biomimetic nanocarrier system comprised of SPM-loaded lipid nanoemulsions (LN/SPMs), namely Bio-LN/SPMs that could efficiently accumulate at the AS lesions through their coating with macrophage (MΦ) membranes. The organic phase with five SPMs was evaporated and reconstituted in an aqueous phase to obtain LN/SPMs. Cell membranes isolated from murine MΦ were used to cover LN/SPMs and obtain Bio-LN/SPMs. Western blotting was used to detect MΦ membrane proteins on Bio-LN/SPMs' surface. The XTT assay was used to determine the effect of Bio-LN/SPMs on the viability of endothelial cells (EC), smooth muscle cells (SMC), and MΦ. Fluorescence microscopy and flow cytometry were used to evaluate the binding and internalization of Bio-LN/SPMs by the cells. Monocyte adhesion and transmigration and phagocytosis assays were used to evaluate the therapeutic effect of Bio-LN/SPMs. Our results revealed that Bio-LN/SPMs (i) are biocompatible, (ii) have a high affinity for the surface of activated EC and SMC via MΦ membranes and SPMs receptors, and are effectively internalized by both cell types; (iii) impede monocyte adhesion and transmigration to/through activated EC; and (iv) enhance MΦ phagocytic activity. In conclusion, the developed Bio-LN/SPMs could be instrumental in developing novel strategies for inflammation Res in AS using a targeted nanomedicine approach. Acknowledgments: This work was funded by the Romanian MECTS, CNCS–UEFISCDI (PNCDI III), NANORES project no. PN-III-P4-ID-PCE-2020-2465 (PCE 68/2021).

SpT-08.4-2
Boosting cholesterol efflux from foam cells by sequential administration of rHDL to deliver microRNA and to remove cholesterol in a triple-cell two-dimensional atherosclerosis model

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Cardiovascular disease, the leading cause of mortality worldwide, is primarily caused by atherosclerosis, which is characterized by lipid and inflammatory cell accumulation in blood vessels and carotid intima thickening, among others. Although disease management has improved significantly, new therapeutic strategies focused on accelerating atherosclerosis regression must be developed. Atherosclerosis models mimicking *in vivo*-like conditions provide essential information for research and new advances towards clinical application. Here, a therapeutic strategy to improve cholesterol efflux has been developed based on two-step administration of rHDL consisting of a first dose of antagomiR-33a-loaded rHDLs to induce ABCA1 transporter overexpression, followed by a second dose of DPPC rHDLs, which efficiently remove cholesterol from foam cells. A triple-cell 2D atheroma plaque model reflecting the cellular complexity of atherosclerosis has been used to overcome the translational gap providing a suitable model to improve the efficiency of the nanoparticles in promoting cholesterol efflux. The results show that sequential administration of rHDL potentiates cholesterol efflux indicating that this approach might be used *in vivo* to more efficiently target atherosclerotic lesions and improve prognosis of the disease. Sequential targeting of foam cells with nanoparticles including a first antagomiR-33a delivery by DPPC:CE:LPC rHDL followed by a second infusion of DPPC rHDL induces a potent cholesterol efflux from foam cells, which may overcome current technique barriers to promote clinical applications.

Tuesday 11 July
12:35–13:00, Auditorium Descartes

Host–Microbial Interactions

SpT-05.1-1
The bacterial pathogen *Shigella flexneri* subverts the human exocyst complex to enhance intercellular spread

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The bacterium *Shigella flexneri* causes bloody diarrhea or dysentery. This pathogen uses an actin-based motility (ABM) process to spread between cells of the colonic epithelium. ABM brings bacteria to the periphery of host cells, facilitating the formation of plasma membrane protrusions that enwrap bacteria and mediate their spread to adjacent human cells. Our previous work revealed that efficient protrusion formation and cell-to-cell spread of Shigella requires bacterial stimulation of host polarized exocytosis through exploitation of a human protein complex called the exocyst [1]. However, the molecular mechanism by which Shigella manipulates the exocyst was not known. The ability of Shigella to infect human cells requires a bacterial type III secretion system (T3SS), which transports ~25 microbial effector proteins into the cytoplasm of the host cell. Using bacterial mutants defective in formation of the T3SS, we found that this secretion apparatus is needed for bacterial-induced recruitment of the exocyst and stimulation of exocytosis in protrusions. In order to identify Shigella effector proteins that contribute to stimulation of host exocytosis, we screened a collection of bacterial mutant strains defective in each of the 25 effector genes. Our results show that the effectors IcsB and IpgD promote exocytosis, protrusion formation, and cell-to-cell spread of Shigella. Collectively, these findings reveal that Shigella uses its T3SS to co-opt the exocyst, thereby enhancing dissemination in host cell monolayers. Future work will focus on elucidating the molecular mechanisms by which IcsB and IpgD control exocyst function. Reference: [1] Herath, T.U.B., Roy, A., Gianfelice, A., and Ireton, K. 2021. Mol. Microbiol. 116: 1328–1346.

SpT-05.1-2
Pathogenic virus control of host viability at the level of the ribosome exit tunnel

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All nascent prokaryotic chains must be co-translationally deformylated. This process is ensured by peptide deformylase (PDF). Metagenomics analysis of oceanic samples revealed PDF genes as one of marine phage genomes' most abundant gene families. Why so many phages carry PDF genes in their genome remains unknown. Comparisons of sequence, structure and deformylase activity with known PDFs reveal that viral PDFs strongly resemble bacterial PDFs, despite the absence of the C-terminal extension, which is essential in bacterial PDF for ribosome binding and activity. Here, we show that the PDF occurring in the genome of Vibrio sp.16-specific bacteriophage Vp16, accumulates in the course of the infection, reaching a maximal expression at the beginning of cell lysis. Interestingly, preliminary results involving Vp16PDF KO from the phage genome indicate that absence of Vp16PDF inhibits phage release from the host, suggesting a key function of Vp16PDF in phage infectivity. Accordingly, the expression of Vp16PDF alone in Vibrio sp.16 induces a strong cold-sensitive growth inhibition. This Vp16PDF-dependent cold-sensitive inhibition was also detected when Vp16PDF was expressed in *E. coli*. We reveal that Vp16PDF expression causes cell lysis, affects outer membrane integrity, and induces the formation of aggregates, suggesting that Vp16PDF might specifically interfere with sec-dependent translocation. Unexpectedly, we observed that, despite the lack of the C-ter extension, Vp16PDF can still bind directly to *E. coli* ribosomes. Vp16PDF interacts with ribosomes next to the exit tunnel overlapping the specific binding sites for trigger factor (TF) and SecB. In agreement, overexpression of TF and SecB in both *E. coli* and Vibrio sp.16 overcomes the Vp16PDF inhibitory effect. In conclusion, our findings provide a basic understanding of a previously unrecognized mechanism based on viral control of host viability at the level of the ribosome exit.

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Supramolecular Assemblies III: Metabolons, Multienzyme Complexes

SpT-07.3-1
Evolution of a protein fractal

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Fractals are complicated yet fascinating patterns that are self-similar across multiple length scales. Divided into parts they result in a nearly identical reduced–size copy of the whole; one famous example is the Sierpiński triangle. Fractals are prominent in mathematics and science but also frequently employed in esthetics and art. Engineering fractal assemblies on a molecular level has become a popular while still challenging target e.g., using small molecules that assemble on chiral surfaces. In nature, molecular fractals have never been observed. Here, we report the discovery of a citrate synthase that self-assembles into complexes closely resembling the Sierpiński triangle. To understand how these complexes are possible, we have solved a cryo EM structure of the first fractal order which is built from 18 monomers: the structure relies on a symmetry violation within hexameric subcomplexes that make up the fundamental building blocks of the fractal pattern. Using ancestral sequence reconstruction, we show that this assembly evolved recently from simpler dihedral hexamers and further identified a single historical substitution that drove this process. The fractal assembly can be dissociated via a physiological shift in pH or the presence of high concentrations of the enzyme's substrates. We show that the resulting hexameric building blocks are more catalytically efficient compared to the fractal-like complexes, which indicates a form of allosteric regulation associated with the assembly state. Finally, we show that fractal assembly likely synchronizes citrate synthase activity to diurnal changes in *S. elongatus*' physiology. Our findings expand the space of known protein geometries to fractal-like complexes of different sizes and imply that these assemblies may be surprisingly easy to evolve.

SpT-07.3-2
Probing the mechanism of the peroxiredoxin decamer interaction with its reductase sulfiredoxin from the single molecule to the solution scale

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Peroxiredoxins from the Prx1 subfamily (Prx) are highly regulated multifunctional proteins involved in oxidative stress response, redox signaling and cell protection. Prx is a homodimer that associates into a decamer. The Prx monomer C-terminus plays intricate roles in Prx catalytic functions, decamer stability and interaction with its redox partner, the small reductase sulfiredoxin (Srx), which regulates the switching between Prx cellular functions as an antioxidant and a chaperone. As only static structures of covalent Prx-Srx complexes have been reported, we assessed the non-covalent interaction mechanism and dynamics in a solution of *Saccharomyces cerevisiae* Srx with the 10 subunits of Prx Tsa1 at the decameric level. A combination of multiscale biophysical approaches was used. Native mass spectrometry and atomic force microscopy imaging combined with solution scale techniques (anisotropy fluorescence and dynamic light scattering) showed that the 10 subunits of Tsa1 decamer can be saturated by 10 Srx molecules and that the interaction of Srx does not induce Tsa1 decamer dissociation. The single-molecule atomic force microscopy approach using a tip sized to the decamer dimensions, decorated with multiple Srx molecules, allowed resolving up to five native interactions with Tsa1 subunits within a decamer. Combining protein engineering and rapid kinetics allowed demonstration of a two-step mechanism of Srx binding to Tsa1 and the importance of Tsa1 C-terminus flexibility. This combined approach from the solution to the single-molecule level offers promising prospects for understanding oligomeric protein interactions with their partners. Previously published in: Beaussart A et al (2022) Nanoscale Horizons 7, 515–525. *The authors marked with an asterisk equally contributed to the work.

SpT-07.3-3
L-serine biosynthesis in human brain relies on a multienzyme metabolic assembly: the serinosome

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The non-essential amino acid L-serine (L-Ser) plays a major role in the development and function of the human CNS: various severe, infantile, neurological disorders have been linked to its deficiency. In the brain, L-Ser *de novo* biosynthesis relies on three astrocyte-specific enzymes, D-3-phosphoglycerate dehydrogenase (PHGDH), phosphoserine aminotransferase (PSAT) and phosphoserine phosphatase (PSP), starting from the glycolytic intermediate D-3-phosphoglycerate. The so called "phosphorylated pathway" (PP) is crucial in providing neurons with L-Ser, which is the precursor of the N-methyl-D-aspartate receptor (NMDAR) co-agonist D-serine. So far, very little is known about the control mechanisms allowing the PP to meet cellular needs. The generation of a stable complex using the recombinant human PHGDH, PSAT and PSP was not observed *in vitro*. However, kinetic studies of the reconstituted pathway (at physiological enzyme and substrate concentrations) support the formation of an enzymatic agglomerate and indicated that PHGDH catalyzes the rate-limiting step, while the PSP reaction is the driving force for the whole pathway. Cellular studies on differentiated human astrocytes, using different experimental approaches, showed that the PP enzymes are in close proximity and generate clusters of a size similar to the one reported for other metabolons (i.e. the purinosome). We propose that PHGDH, PSAT and PSP are capable of clustering in a transient metabolic assembly, the putative "serinosome", providing a channeling solution for the pathway

intermediates and delivering a relevant level of sophistication to the control of L-Ser biosynthesis. These results highlight and imply the need to shed light on the significance of PHGDH allosteric machinery for the integration of the metabolic signals that modulate the PP, likely controlling the agglomerate assembly and disassembly. This project was founded by" PRIN-2017 – Dissecting serine metabolism in the brain".

SpT-07.3-4
Multiple electron paths associated with *Flavobacterium johnsoniae* respiratory alternative complex III

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Bacterial Alternative Complex III (ACIII) is a membrane complex that catalyzes quinol oxidation, presumably fulfilling the role of cytochrome bc_1/b_6f complexes in organisms that lack these enzymes. Recently, the structure of *Flavobacterium johnsoniae* ACIII has been solved by cryo-EM, revealing no structural similarity to cytochrome bc_1/b_6f. Interestingly, ACIII is present in supercomplex with an aa_3 oxidase. However, it is still unknown how ACIII operates at the molecular level. In order to get experimental access to explore the function of individual ACIII subunits, we constructed a system for genetic manipulations within this complex in *F. johnsoniae*. We used this system to delete the heme-containing subunits, ActA and ActE, as the ones involved in the catalytic mechanism. The enzymatic activity of generated mutants was measured by tracking oxygen consumption in isolated native membranes. The experiment showed lack of cytochrome aa_3 activity only in ActA deletion mutant, but not in ActE mutant. This indicates that ActE is not required for electron transfer between ACIII and cytochrome aa_3 (Previously published in: Lorencik K et al. (2021) Microbiol Spectr 2: e00135-21). Another candidate that may act as a linker between ACIII and cytochrome aa_3 is a monoheme mobile domain of ActA (mdA). To verify the proposed role of mdA, we obtained a mutant that lacks mdActA (ΔmdA) and examined whether and how the absence of this domain alters the activity of the supercomplex. The results showed that in the ΔmdA mutant, the electron transfer from ACIII to the cytochrome aa_3 does not occur, and the consumption of oxygen by this strain is residual. This confirmed that mdA heme is the sole donor of electrons to aa_3 oxidase. The results of this work help us to define the electron transfer paths connecting ACIII with aa_3, and provide the first insight into the functional organization of the supercomplex. This work was supported by PRELUDIUM-20 grant from National Science Centre, Poland (to KL).

SpT-07.3-5
The cryo-EM structure of a fiber-forming bifunctional single-polypeptide enzyme explains the dual spectra of clinical presentations and of inheritance modes of its genetic disorders

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The bifunctional, single-chain enzyme Δ^1-pyrroline-5-carboxylate synthetase (P5CS) catalyzes through its glutamate-5-kinase (G5K) and glutamate-5-phosphate reductase (G5PR) domains the two initial steps of *de novo* synthesis of ornithine and proline. Our pioneering determination (Marco-Marin et al. J Mol Biol 2007) of the structure of bacterial G5K led us to become involved in the molecular study of the fifth family found with a congenital neurocutaneous syndrome (NC) and mutation in the P5CS gene. Unlike in the previous four families, which exhibited recessive inheritance, in our family a single, dominant, *de novo* pathogenic mutation was found (Martinelli e al. J Inher Metab Dis 2012). We were also pioneers in finding patients with spastic paraplegia (SPG) due to dominant (Panza et al. Brain 2016) or recessive (Magini et al. Ann Clin Transl Neurol. 2019) mutations in the gene for P5CS. Thus, four diseases were defined, NC-R, NC-D, SPG-R and SPG-D (R, recessive; D, dominant), which we have proven to be due to P5CS deficiency of decreasing severity in the order NC-R ≥ NC-D > SPG-R > SPG-D (Marco-Marin et al. J Inher Metab Dis 2020). We now report our cryo Electron Microscopy determination of the structure of human P5CS, which has enabled us to understand this continuum of severity and to clarify the mechanism of dual inheritance. P5CS is a self-aggregating homotetramer that can dissociate into dimers or stack into octamers, dodecamers of two types, and higher fibers. The stacked forms partially enclose the active centers of both enzyme components and favor the channeling of glutamate-5-phosphate between adjacent tetramers. Recessive mutations exclusively inactivate the mutant subunit without hindering stacking/channeling between tetramers, unlike dominant ones, whose severity is greater the more they favor dissociation to tetramers or dimers. Grants CIVP-20A6610 from the Ramón Areces Foundation and BFU2017-84264-P and PID2020-116880GB-100 from the Spanish Government.

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