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Biology PhD Day 2025

Our science stories

Wednesday, March 12, 2025

Ecole Polytechnique, Amphi Arago

Program and Book of abstracts

From lab benches to bright lights
IP Paris Biology PhD students talk science

Organizers and co-organizers
Maria Imezar, Elise Muller, Laurianne Li En Tay
Bhumika Garkhal, Tom Garnier, Elsa Balduzzi, Valentina Varriale
Aichata Sidi Abdel Jelil, Robin Kuhner, Yara Hassanein

PROGRAM

8h30 **Registration and Welcome Reception**

9h15 **Opening Remarks**

Session 1. Molecular Regulation : A focus on RNA

9h25 **Characterization of two RNA modification enzymes involved in intellectual disorders**
Laurianne Li En TAY
Laboratory of Structural Biology of the Cell (BIOC) , Laboratory of Molecular Chemistry (LCM)

9h40 **Exploring RNA circularization by Pab1020 in *Pyrococcus abyssi***
Maria IMEZAR
Laboratory for Optics and Biosciences (LOB)

Session 2. Molecular Regulation : A focus on Proteins

10h **Human PYURF, a mitochondrial TRMT112-like protein**
Wanwan HU
Laboratory of Structural Biology of the Cell (BIOC)

10h15 **Unraveling the function of a new protein modification enzyme**
Bhumika GARKHAL
Laboratory of Structural Biology of the Cell (BIOC)

10h30 **Coffee Break**

Session 3. Invited Speaker

10h55 **AlphaReps artificial repeat proteins as modular bioengineering tools**
Marielle VALERIO-LEPINIEC
Institute for Integrative Biology of the Cell (I2BC), Université Paris-Saclay

Session 4. A look to the future

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Morgane CORRE and Jordan FERRIA
Laboratory of Structural Biology of the Cell (BIOC) , Laboratory of Hydrodynamics (LadHyx)

12h10 **Lunch, Poster Session**

Session 5. Optics for molecular processes

14h30 **Mapping DNA replication in a polyploid archaea by superresolution microscopy**
Dorian NOURY
Laboratory for Optics and Biosciences (LOB)

14h45 **Elucidating the intracellular organisation of archaeal translation machinery by microscopy**
Tom GARNIER
Laboratory of Structural Biology of the Cell (BIOC) , Laboratory for Optics and Biosciences (LOB)

15h **Ultrasensitive *in vitro* diagnostics using luminescent lanthanide-based nanoparticles**
Robin KUHNER
Laboratory for Optics and Biosciences (LOB)

15h15 **Coffee Break, Poster Session**

Session 6. Cells and migration

15h50 **Role of the NHS protein in cell migration**
Ekaterina TSYDENZHAPOVA
Laboratory of Structural Biology of the Cell (BIOC)

16h05 **Impact of cold atmospheric plasma on cell migration**
Benjamin LABERIE
Laboratory of Structural Biology of the Cell (BIOC), Laboratory of Plasma Physics (LPP)

Session 7. Chip and Tissue

16h25 **Mechanical regulation of growth by FERONIA in Marchantia gemmae**
Elise MULLER
Laboratory of Hydrodynamics (LadHyx)

16h40 **Monitoring renal pathophysiological processes using quantitative imaging in Glomerulus-on-a-chip models**
Aichata SIDI ABDEL JELIL
Laboratory for Optics and Biosciences (LOB)

17h **Concluding Remarks and awards**

ORAL PRESENTATIONS

Session 1.

Molecular Regulation:
A focus on RNA

Characterization of two RNA modification enzymes involved in human pathologies

Speaker : Laurianne Li En TAY
Laboratory of structural biology of the cell (BIOC), Laboratory of Molecular Chemistry (LCM)

RNA modification enzymes targeting factors involved in mRNA translation have become a topic of interest due to their roles in maintaining the efficiency, fidelity and regulation of protein synthesis by chemically modifying key factors (eg. tRNA, mRNA, rRNA). This thesis focuses on two related proteins: ALKBH8 and TRMT9B. ALKBH8 is considered to be crucial for mRNA decoding fidelity as it is involved in the formation of a complex modification at position 34 of the anticodon loop of some tRNAs. Furthermore, ALKBH8 mutations have been identified in patients suffering from intellectual disorder (ID), emphasizing the importance of this protein for brain development and function. TRMT9B acts as a tumor growth repressor in certain cancers but its biochemical function is yet unclear. Based on its strong homology with the catalytic domain of ALKBH8, it is supposed to modify tRNAs. The aims of this thesis are thus: 1) to characterize *in vitro* and *in vivo* the effect of pathogenic mutations on ALKBH8 function; and 2) to identify the TRMT9B substrate and the chemical modification it is catalyzing. To date, we have shown that the small methyltransferase activator protein TRMT112 increases the solubility of TRMT9B and co-purifies with TRMT9B. Validation of the results of an RNA-crosslinking experiment will soon follow. Efforts are also ongoing to study *in vitro* the enzymatic activity of WT ALKBH8 and pathogenic mutants found in patients diagnosed with ID. In parallel, we have validated the use of LC-MS for nucleoside quantification and MALDI-FT-ICR for modification mapping via a partial digest approach. We are now applying chemical derivatization to improve the ionization of uridines and modified uridines in ESI-MS, to further improve detection and quantification of these nucleosides in biological samples.

Functional and structural analysis of the RNA ligase Pab1020 responsible of RNA circularization in *Pyrococcus abyssi*

Speaker : Maria IMEZAR
Laboratory for optics and biosciences (LOB)

During the last years, the interest in circular RNAs has dramatically increased, since different reports have convincingly demonstrated that circular RNAs are abundant and found in all kingdoms of life, including Archaea. Circular RNAs result from the covalent ligation of the 5' and 3' free ends of linear RNA, which makes them more resistant to exonucleases and therefore more stable. Our team has previously identified Pab1020 as the enzyme responsible for RNA circularization in hyperthermophilic archaeaon *Pyrococcus abyssi*, establishing it as the founding member of the RNA ligase family 3 (Rnl3). Using an RNAseq approach, we have also identified a large number of circular RNAs in the hyperthermophilic archaea *Pyrococcus abyssi* cells (Brooks and al. 2008, Becker et al., 2019).

As the functional importance of RNA circularization remains poorly documented we are currently investigating the physiological significance of this unexpected RNA circularization activity. To provide insights into the cellular processes and pathways in which RNA circularization is involved, we identified the protein interactants of Pab1020, by *in cellulo* immunoprecipitation experiments in *P. abyssi* cells and mass spectrometry analyses. Currently, we aim to validate these potent interactions by *in vitro* immunoprecipitation assays complemented by intracellular co-localization through immunofluorescence microscopy.

We are also exploring the link between Pab1020's atypical homodimeric structure and its catalytic mechanism. Our biochemical and cryo-EM studies have revealed significant conformational rearrangements upon RNA binding. The analysis of new RNA-Pab1020 structures is ongoing to decipher further the mechanism of RNA circularization.

Session 2.

Molecular Regulation: A focus on proteins

Human PYURF regulates coenzyme Q biosynthesis and complex I assembly in mitochondria

Speaker : Wanwan HU
Laboratory of structural biology of the cell (BIOC)

Post-transcriptional modifications play a pivotal role in governing the translation of messenger RNA (mRNA) for optimal cellular function. Methylation, among these modifications, stands out as one of the most prevalent. In mitochondria, PYURF emerges as a regulator for several S-adenosylmethionine-dependent methyltransferases (SAM-MTs). It interacts with and stabilizes SAM-MTs within the complex network of NADH: ubiquinone oxidoreductase (complex I, MT-ND1) and coenzyme Q (CoQ) pathways1. This stabilization likely extends to essential components of these pathways, including COQ5 and NDUFAF5, both critical for respiratory processes1. PYURF's structural resemblance to the zinc knuckle domain of TRMT112, another SAM-MT chaperone, further underscores its functional significance2. The impact of PYURF dysregulation is evident in individuals harboring homozygous PYURF mutations, characterized by a spectrum of debilitating symptoms such as muscle hypotonia, failure to thrive, developmental delay, optic atrophy, and elevated lactate levels in blood and cerebrospinal fluid1. Magnetic resonance imaging reveals abnormalities in cerebellar white matter and cerebellar atrophy, highlighting the vital role of PYURF in mitochondrial function. Unraveling the intricacies of PYURF regulation is thus imperative for understanding mitochondrial physiology and addressing associated pathologies.

Unravelling the function of a new protein modification enzyme

Speaker : Bhumika GARKHAL
Laboratory of structural biology of the cell (BIOC)

The 5'- end caps protecting eukaryotic mRNAs from uncontrolled degradation is removed by the decapping holoenzyme composed of the Dcp2 catalytic subunit and its intrinsic partner Dcp1. Additional factors such as Pat1 or Edc3 interact with Dcp2 in Saccharomyces cerevisiae to stimulate the decapping activity of Dcp2. Recently, my host lab has characterized Pby1 as a new Dcp2 partner. A former PhD student from the lab has determined the crystal structure of the C-terminal Pby1 domain either alone or as a complex with the Dcp1-Dcp2-Edc3 complex [1]. Based on its crystal structure, Pby1 belongs to the ATP-grasp protein family, which is responsible for the ATP-dependent ligation of a nucleophilic group to an acid group. Yet, its function in mRNA decapping is still unknown.

Using a combination of enzymology and mass spectrometry analysed, our collaborators have identified a Pby1 substrate, revealing that Pby1 catalyzes the formation of a novel protein post-translational modification on a factor distantly related to mRNA decay. My PhD project aims at combining various biochemical (pull-down, enzymology, ITC, fluorescence…) and structural biology (X-ray crystallography, cryo-EM…) approaches to obtain molecular details about the recognition of its substrate by Pby1 and about Pby1 catalytic mechanism. This information is essential to help unravelling the function of this protein in mRNA metabolism.

[1]. Charenton et al (2020); *Nucleic Acids Research,* Volume 48, Issue 11, 19 June 2020, Pages 6353–6366

Session 3.

Invited Speaker

AlphaReps artificial repeat proteins as modular bioengineering tools

Marielle VALERIO-LEPINIEC, Agathe URVOAS, Philippe MINARD
Protein modeling and Engineering (MIP) team, Institute for Integrative Biology of the Cell (I2BC), Université Paris-Saclay CNRS CEA, 91198, Gif-sur-Yvette

Our overall objective is to develop efficient methods for creating new proteins and binding sites using combinatorial biology and directed evolution. In this context, we have designed αReps, a family of artificial repeat proteins engineered for specific binding interactions[1]. The αReps are constructed from a 31-residue consensus helical motif with six hypervariable positions, resulting in a highly diverse library of around 2 billion variants. Each αRep shares a common fold but has a unique hypervariable surface, enabling precise interactions with various targets. Selected using phage display, αReps exhibit high-affinity binding with dissociation constants (Kd) ranging from nM to µM.

This versatility in binding specificity has allowed αReps to be used across a large range of fields. In structural and cellular biology, they have operated as crystallization helpers[2,3] and intracellular tracers in living cells[4] or antiviral Nanoligands[5]. In chemistry and physics, αReps have enabled the development of generic biosensors[6,7], nanobiomaterials[8], supramolecular assemblies[9,10], and artificial metalloenzymes with stereoselective or inducible activity[11]. Their stability, ease of production, and versatility make αReps a powerful platform for a wide range of scientific and biotechnological applications.

References

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Session 4.

Everything you need to know about postdocs

A look to the future : Everything you need to know about postdocs

Morgane CORRE[(1)] and Jordan FERRIA[(2)]

[(1)]Laboratory of structural biology of the cell (BIOC), [(2)]Laboratory of Hydrodynamics (LadHyx)

As PhD graduates transition into the next phase of their careers, they are often faced with critical decisions regarding their professional future. This presentation will provide tips and tricks to navigating post-PhD life for those considering an academic career. It covers key steps to secure a postdoc, from identifying motivations to preparing strong applications. The goal is to equip PhD students with some tools and knowledge necessary to transition successfully into their next professional step.

Session 5.

Optics for Molecular processes

Mapping DNA replication in a polyploid archaea by super-resolution microscopy

Speaker : Dorian NOURY
Laboratory for optics and biosciences (LOB)

DNA replication is essential for the proliferation of all living beings. In most organisms it starts at specific sequences called replication origins where the replisome is formed. The two replication forks progress along the chromosome in opposite direction, synthetizing a single replicon each. Little is known about it in archaea, the prokaryotic domain of life from which eukaryotes emerged. As such, some archaea species provide a unique pattern: a main circular chromosome, a bacterial-like feature, with multiple replication origins, a eukaryotic-like feature. Moreover the vast majority of the components of the archaeal replisome are homologous to their eukaryotic counterpart. Finally, the genome's ploidy varies among archaea species, up to 25 copies in some. This makes archaea an interesting model to deepen our understanding of replication dynamics, raising important questions: is each genome copy replicated once per cell cycle, and are all replication origins activated when a chromosome is replicated?

To answer those question, we use the halophilic euryarchaea Haloferax volcanii for which an extensive repertoire of genetic and biochemistry tools is available. Its chromosome has 4 replication origins, and growing cells have an average of 12 chromosomal copies, ranging from 5 to 30. This heterogeneity makes it crucial to implement methods allowing single-cell study. We aim to characterize the replication sites: their number and size, the protein actors and the potential chromosomal organization around them. The replisome being at a subdiffraction level, we established a protocol for STORM, allowing super-resolved imaging of replication proteins in Haloferax volcanii. Thanks to this increase in precision and sensitivity we are able to study quantitatively RPA2 localizations in proliferating and stressed cells, giving us insights on a replication regime contradictory with an usual cell cycle.

Elucidating the intracellular organization and biogenesis of Haloferax volcanii's translation machinery by microscopy

Speaker : Tom GARNIER
Laboratory of structural biology of the cell (BIOC), Laboratory for optics and biosciences (LOB)

Today, the strong phylogenetic proximity between eukaryotes and archaea supports the hypothesis of a potential eukaryotic emergence from an archaeal ancestor. In addition, many proteins, originally thought to be specific to eukaryotes, are now known to be shared between archaea and eukaryotes, including proteins involved in ribosomes and their biogenesis. Eukaryotic ribosome biogenesis is characterized by a high degree of functional compartmentalization, from the nucleolus to cytoplasm. Moreover, the translation process is separated from the stored genetic information. Accordingly, the early formation of intracellular organization in archaea may have facilitated the emergence of eukaryotes and their compartmentalization.
Therefore, to contribute to the resolution of this hypothesis, we aim to characterize the degree of intracellular organization of ribosome biogenesis in different model archaea, through the use of wide-field fluorescence microscopy and super-resolution techniques (dSTORM).

Ultrasensitive *in vitro* diagnostics using luminescent nanoparticles

Speaker : Robin KUHNER
Laboratory for optics and biosciences (LOB)

Swift identification of pathogens (viruses, bacteria, toxins, or parasites) and biomarkers in diverse environments (bioaerosols, water, food matrices, etc.) or biological samples (blood, serum, urine) is vital for preventative, diagnostic, and therapeutic actions. Current protein detection methods either boast ultrahigh sensitivity (~fM), with associated high costs and implementation challenges, or lack the sensitivity needed for early detection of specific biomarkers like p24 or botulinum toxin. Our research demonstrates that the application of luminescent YVO4:Eu nanoparticles effectively lowers the limit of detection (LOD) for multiple targets, employing an approach akin to conventional detection methods such as ELISA, leveraging the unique optical properties of these nanoparticles.

Rare-earth doped vanadate nanoparticles exhibit broad-band, high absorption coefficients around 280 nm, along with large Stokes shifts and narrow emission bands. These latter characteristics facilitate the efficient rejection of unwanted signals. These nanoparticles have enabled highly sensitive detection using a simple ELISA-like protocol and an economical, portable, homemade microplate reader. Specifically, the detection sensitivity for insulin (utilized as a proof-of-concept analyte), interferon-Î³, and HIV p24 was 10,000x, 100x, and 20x greater, respectively, compared to a commercial test employing the same antibodies. Moreover, at a low cost and high sensitivity, we've expanded this technology to nucleic acid detection, successfully identifying the n1 gene of SARS-CoV-2.

Session 6.

Cells and migration

Role of the NHS protein in cell migration

Speaker : Ekaterina TSYDENZHAPOVA
Laboratory of Structural Biology of the Cell (BIOC)

Cell migration relies on lamellipodia extension powered by the Arp2/3 complex upon activation by nucleation promoting factors (such as WASP/Scar/WAVE), where Rho family of small GTPases plays a crucial role. Although a lot is already well-established, the mechanism of cell motility remains rather complex. New regulators of cell migration are continually being discovered, but how these signals are integrated with biochemical pathways to control cell movement remains an active area of research. Here, my PhD project is built upon the NHS protein, founding member of the Nance-Horan Syndrome protein family. This protein is reported to have a so-called WAVE-homology domain, by which it might interact with the WAVE complex, a well-known regulator of migration persistence. According to our findings, the NHS affects migration persistence of the cell, yet the exact mechanism is to be figured out and described.

Impact of cold atmospheric plasma on wound healing

Speaker : Benjamin LABERIE
Laboratory of structural biology of the cell (BIOC), Laboratory of plasma physics (LPP)

Cold plasma is presented as the fourth state of matter, which follows the gaseous state. It is an ionized gas. In our case, it is weakly ionized. This low ionization allows the plasma to remain at ambient temperature. Plasma has multiple components; it emits light, UV, infrared radiation, and generates an electric field. Additionally, when plasma interacts with air or a liquid, it produces reactive oxygen and nitrogen species. It has been shown that a wound treated with plasma heals more quickly. The reasons for this effect are still unknown. To explain this phenomenon, two components of cold plasma are identified as the primary causes: reactive oxygen and nitrogen species (RONS) and the electric field. It has been shown that these two components of cold plasma influence cell migration. Since cell migration is an essential process in wound healing, more efficient cell migration could explain the beneficial effect of cold plasma on wound healing. In this project, we identified which plasma component induce a positive effect on cell migration.

Session 7.

Chips and Tissue

Mechanical regulation of growth by FERONIA in Marchantia gemmae

Speaker : Elise MULLER
Laboratory of Hydrodynamics (LadHyx)

Plant cells and tissues are subjected to internal and external mechanical stress which impact several processes such as growth or tissue patterning (Nakayama et al., 2012; Landrein and Ingram, 2019; Cosgrove, 2016). Response to mechanical stress can be passive but is also active and mediated by mechanosensors or cell wall integrity sensors. If impaired sensing has been associated with defects as in cell wall integrity, cell-cell adhesion and tissue cohesion (Fruleux et al., 2019), the mechanisms linking mechanical stresses to morphogenesis are poorly understood. We thus focus on the role of integrity sensing and cell wall mechanics in plant morphogenesis.

We use the plant Marchantia polymorpha as a model system. We take advantage of its low genetic redundancy and its simple organization to analyze its early morphogenesis in a high-throughput way thanks to a microfluidic device (Laplaud et al., 2024).

We show that the transmembrane integrity sensor FERONIA (Mecchia et al., 2020), as well as a downstream kinase MARIS (Boisson-Dernier et al., 2015), affect Marchantia's early growth kinetics and spatial patterning. Indeed, compared to wilde-type, feronia loss-of-function mutant early growth is delayed and more localized to the stem-cell regions.

Additionally, we probed the plant mechanical properties thanks to osmotic steps and compared those parameters with growth kinetics properties. We showed that FERONIA regulates the plant mechanical state by promoting a high turgor and a high cell wall stiffness. However, we showed that mechanical regulation cannot explain the growth regulation by FERONIA.

Therefor and thanks to mechanical perturbations of the systems by osmolytes treatments, we postulate that FERONIA regulates growth through cell extensibility modification in an incoherent feed-forward loops and response to pressure.

Altogether, our work sheds light on the complex interplay between integrity sensing and cell mechanics during morphogenesis.

Monitoring complex kidney pathophysiological processes by quantitative optical imaging in glomeruli-on-chip devices

Speaker : Aïchata SIDI ABDEL JELIL
Laboratory for optics and biosciences (LOB)

To effectively monitor complex pathologies, it is crucial to develop high-resolution technologies capable of quantitatively assessing biological processes across scales, from the molecular to the organism level. This project focuses on two major kidney diseases: Rapidly Progressive Glomerulonephritis (RPGN) and Focal Segmental Glomerulosclerosis (FSGS). Both are characterized by abnormal cell de-differentiation and migration driven by complex local signals (such as PDGF, EGF, and shear stress) and Reactive Oxygen Species (ROS) production. Due to the lack of quantitative tools for assessing cell responses in multicellular environments and ROS activity, understanding of these mechanisms remains limited.
In this work, we aim to develop (i) biomimetic *in vitro* devices (organ-on-chip) that replicate relevant pathophysiological conditions, and (ii) advanced imaging approaches to study receptor organization and single-particle ROS detection. These technologies will enable comprehensive molecular and functional analyses to elucidate mechanisms related to RPGN and FSGS.
Ultimately, this project will facilitate the profiling of human glomeruli-on-chip models and their regulation by biological and mechanical stimuli, advancing our understanding of pathological transitions. This approach lays the groundwork for robust tools to analyze molecular signaling in both healthy and diseased states, model pathological progressions, and develop strategies for non-invasive profiling of complex pathologies and essential steps for designing personalized treatments.

POSTERS

1

Advanced Imaging of Cardioids

Poster presenter : Matheus CÂNDIA-ARAÑA
Laboratory for optics and biosciences (LOB)

Cardiac organoids (or cardioids) are well suited for live imaging with multiphoton microscopy due to their small size (in the mm range) and optical properties. The aim of this interdisciplinary project is to quantitatively investigate morphogenesis and contractile function of cardioids at sub-cellular resolution using advanced optical imaging tools. This task is challenging for several reasons. (i) High-speed imaging over a large field of view and without inducing photodamage is required to capture cardiac dynamic. (ii) The large size of the acquired 4D imaging data prevents the use of manual image analysis, requiring automated approaches. (iii) Despite recent improvements in acquisition speed of fluorescence microscopes, the temporal resolution or signal-to-noise ratio significantly limits the ability to reliably track the fastest cells throughout the cardiac cycle. (iv) Finally, there is a need to replace the fluorescent labeling with live label-free approaches to simplify and speed-up the experimental workflow. To overcome these limitations, we will develop several new strategies for live imaging of beating cardioids. This includes the development of label-free Second Harmonic Generation (SHG) microscopy to capture the contractility of cardiac myofibrils in 3D and without labeling. We will exploit the periodic nature of cardiac motion to improve image quality, automatize image analysis and devise a new computational imaging strategy to speed-up SHG imaging. We have recently obtained some preliminary results using the zebrafish embryonic heart as a model: we were able to capture the contraction dynamics of individual sarcomeres in 3D in a live and unlabeled heart with unprecedented spatial and temporal resolution (1 ms or 1000 image volumes/second).

2

SOD1G93A neurotoxicity initiated by subversion of cellular prion protein signaling in cell and mouse models of Charcot disease

Poster presenter : Florence ROUSSEL
Laboratory of structural biology of the cell (BIOC)

Amyotrophic lateral sclerosis (ALS), also called Charcot disease, is the most common motor neuron degenerative disease in adults. ALS is associated with the selective loss of motor neurons in the spinal cord, brainstem, and cortex, which causes progressive paralysis and death by respiratory failure. ALS belongs to proteinopathies, a family of diseases provoked by protein misfolding and dysfunction. Among other genes, the gene encoding Cu/Zn Superoxide Dismutase 1 (SOD1), an intracellular enzyme implicated in antioxidant processes, is frequently involved in ALS. The toxicity of SOD1 mutants relies on a toxic gain-of-function due to the misfolding, mislocalization, and formation of intracellular inclusions and aggregates of SOD1. The secretion and accumulation of ALS-linked SOD1 mutants in the surrounding milieu of motor neurons raises the unexplored possibility that abnormal SOD1 also triggers neurotoxicity through binding to membrane receptors and alteration of their signaling activity. Exploiting several neuronal cell lines exposed to misfolded human G93A SOD1 (hSOD1G93A) and primary cultures of mouse motor neurons isolated from Tg-hSOD1G93A transgenic ALS mice (referred to as ALS neurons), we show that plasma membrane cellular prion protein (PrPC), a protein well-known for its implication in prion and Alzheimer's diseases, is a neuronal receptor for misfolded hSOD1G93A. hSOD1G93A interaction with PrPC corrupts PrPC signaling function, leading to 3-phosphoinositide-dependent kinase 1 (PDK1) overactivation and downstream under-shedding of plasma membrane TNFα receptors (TNFRs) by ADAM10/17 α-secretases. Cell surface accumulation of TNFRs renders ALS neurons hyper-vulnerable to TNFÎ± inflammation. Infusion of a PDK1 inhibitor by the intraperitoneal route in Tg-hSOD1G93A ALS mice rescues TNFR shedding mediated by ADAMs, protects spinal cord motor neurons from neurodegeneration, improves motor performance, and extends survival. Our work highlights that excessive stimulation of PrPC coupling to PDK1 by hSOD1G93A contributes to ALS and posits PDK1 as a novel target for developing therapeutics to mitigate ALS.

3

Vascular Endothelial Integration of Multiple Biophysical Stimuli

Poster presenter : Louison BLIVET-BAILLY
Laboratory of Hydrodynamics (LadHyx)

Endothelial cells (ECs) that form the innermost lining of blood vessels are constantly subjected to multiple biophysical cues. Dysfunction of the endothelium leads to atherosclerosis whose pathological complications, namely heart attacks and strokes, are the leading cause of mortality worldwide. A particularly interesting in vivo observation is that ECs in atherosclerosis-prone regions are cuboidal, while those in protected regions are elongated and aligned with blood flow. It has traditionally been thought that EC morphology is principally determined by the flow environment, but the vascular basement membrane's micro-scale roughness has recently been shown to greatly impact EC structure and function.

The goal of the present study is to investigate the response of ECs to a combination of flow on the cells' apical surface and topographical cues on their basal surface. We use PDMS surfaces patterned with microgrooves having a width and spacing of 5 µm and depth ranging from 0.5 to 5 µm as an idealized in vitro model of basement membrane topography on which we culture monolayers of human umbilical vein ECs. These substrates are placed into parallel-plate flow chambers to provide a steady wall shear stress of 2 Pa for 48h.

Our results show that substrate topography and flow have, in most cases, a synergistic effect, promoting cell elongation and alignment in the groove direction. The flow reinforcement of EC response to topography is more pronounced in deeper grooves. These findings underscore the importance of groove depth in modulating EC responses to combined topography and flow stimuli.

4

Characterization of Coronin-7, a novel Arp2/3 binding protein

Poster presenter : Rouba HILAL
Laboratory of structural biology of the cell (BIOC)

The actin cytoskeleton is a dynamic network of proteins that plays a crucial role in maintaining cell shape and motile function. At the essence of this cytoskeleton is actin, constantly fluctuating between monomeric and filamentous forms, creating various networks of discrete architectures. Understanding the processes regulating actin dynamics is crucial for unraveling the complexity of cell structure, function, and motility. Coronin-7, this study's protein of interest, has been recently implicated in the context of cytoskeletal dynamics, given its unique structural characteristics and interactions with central mediators of actin dynamics. Coronin-7 is a distinctive Type III coronin family protein, that stand out as a family of their own structurally, and more recently functionally. Studies on Coronin-7 and its homologs have revealed distinct functions for these proteins in maintaining cellular dynamics. Evidence on the role of this protein has emerged to reveal its necessity in Golgi maintenance and integrity. Moreover, recent emerging evidence has implicated its *C. elegans* homolog, POD-1, in the regulation of actin filament debranching during migration. The role of Coronin-7 in actin dynamics in mammalian cells is, however, poorly understood. In fact, preliminary data shows that a structural-functional interaction exists between Coronin-7 and Arp2/3. In addition, we have observed that knockdown of Coronin-7 resulted in a striking "fried-egg" phenotype, with extensive spreading and defective migration. Altogether, our study aims at characterizing the less understood roles of Coronin-7 in cell dynamics and morphology. We hypothesize that Coronin-7 is required for proper vesicular trafficking along the ER-Golgi-plasma membrane axis as well as in the regulation of debranching events at the leading edges during cell migration. Here, we assess the role of Coronin-7 in vesicular trafficking to show that Coronin-7 is required for proper anterograde trafficking to the Golgi. Additionally, we optimize a protocol for the electroporation of the purified protein, at endogenous levels, into mammalian cells to establish rescues of the phenotype observed.

5

Leaderless mRNA translation initiation in Deinococcus deserti

Poster presenter : Valentina VARRIALE
Laboratory of structural biology of the cell (BIOC)

Translation initiation is the primary rate-limiting step of translation and a critical regulatory phase of gene expression. This event kickstarts protein synthesis thanks to the formation of a stable initiation complex. Our understanding of translation initiation mostly reflects canonical or leadered mRNAs, which possess a regulatory 5' untranslated region (5'-UTR). This region harbors sequences, such as the Shine Dalgarno (SD) sequence in prokaryotes, that anchor the small ribosomal subunit to the mRNA. However, there exists another class of mRNAs – known as leaderless mRNAs (lmRNAs) – that either lack or possess a very short 5'-UTR. These transcripts do not carry a typical SD and their dynamics of recognition and binding to the translational machinery are not fully characterized.

Recent advances in cryo-electron microscopy (cryo-EM) have enabled the acquisition of near-atomic resolution structures of initiation complexes, significantly enhancing our understanding of translation across all domains of life. We applied structural and molecular biology techniques to the study of leaderless mRNA translation in *Deinococcus deserti* and successfully reconstructed the first cryo-EM structure of a 30S translation initiation complex in this species. Surprisingly, the leadered mRNA used for *in vitro* reconstitution was not observed at the decoding center of the 30S ribosome. Instead, we observed a codon-anticodon interaction involving an mRNA with a 5'-triphosphate group, a feature that is typical of leaderless mRNAs. These results raise questions on the differential conditions for successful leadered and leaderless mRNA translation initiation complex formation both *in vitro* and *in vivo*.

6

Development of orthogonal biosynthetic ribosomes for the targeted production of biomolecules in archaea

Poster presenter : Mingjing YU
Laboratory of structural biology of the cell (BIOC)

Archaea are microorganisms capable of thriving in extreme environments, making them valuable sources of extremozymes and biomolecules with potential applications in biopharmaceuticals, environmental protection, and industrial processes. However, their complex translation machinery limits their use in biotechnology. Synthetic biology offers solutions by engineering biological systems for targeted biomolecule production. This project aims to construct an efficient archaeal translation system through the engineering of synthetic ribosomal subunits, enabling the targeted synthesis of high-value biomolecules. In WP1, we will focus on the *in vitro* reconstitution of functional archaeal ribosomes and develop an advanced iSAT system (*in vitro* translation with synthetic archaeal translation) for efficient translation regulation. In WP2, we will explore genetic scaffolds for thermophilic archaea and apply Ribo-T-like technologies (where ribosomal subunits are chemically or genetically linked to expand functionality) to engineer archaeal ribosomes. Ultimately, the project will utilize engineered ribosomal subunits to synthesize extremolytes, driving biotechnological innovations for applications in extreme environments and industrial sectors.

7

Visualizing G-quadruplexes in Archaea using Super-Resolution Microscopy

Poster presenter : Kate SORG
Laboratory for optics and biosciences (LOB)

G-quadruplexes (G4) are non-canonical nucleic acids which may form in guanine-rich sequences. Interest in G4 research has grown over the past decades to understand more about what cellular functions these G4 may have; G4 have been largely regarded as regulators of processes such as DNA replication, transcription, translation, and genome organization. G4 are extensively studied in eukaryotes, but very little is known about the presence of G4 in the third domain of life: Archaea. Particularly with respect to nucleic acid metabolism, archaea tend to more closely resemble eukaryotes than bacteria. Bioinformatic searches have indicated a likely presence of G4 in different archaeal species, but this work presents the first in cellulo evidence that G4 exist in archaea, specifically Haloferax volcanii. Using super-resolution microscopy techniques such as SIM (Structured Illumination Microscopy) in combination with immunofluorescence, we can visualize G4 structures in archaeal cells under different conditions such as G4-binding ligands, stressful growth conditions, and different stages of growth. This continuing work will not only shed more light on the general role of G-quadruplexes, but will also highlight evolutionary similarities and key differences between archaea and eukaryotes.

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SubG4s: A Novel Class of Bulge-Riched G-Quadruplexes Induced by Dehydration

Poster presenter : Yun CHEN
Laboratory for optics and biosciences (LOB)

The classical criteria for screening potential G-quadruplex (G4) sequences have traditionally focused on the motif G3-5NL1G3-5NL2G3-5NL3G3-5, where NL1-3 represent loops of unknown lengths, generally constrained to 1 < NL1-3 < 7 nucleotides (nt). However, compared to model G4 structures, natural G4 sequences often exhibit more complexity and irregularity. In recent years, an increasing number of unconventional G4s have been discovered, such as those containing overlong loops, G-vacancies, bulges, double-stranded regions, and non-G·G·G·G quartets. These studies have significantly expanded our understanding of G4s. For instance, in contrast to the first bioinformatics approach from 2005, which estimated over 300,000 G4-prone sequences in the human genome, more recent *in vitro* studies have increased this number to 736,689.

Nevertheless, we hypothesize that many G4-prone sequences remain undiscovered, particularly due to the disparity between *in vivo* and *in vitro* experimental environments. *In vitro* tests do not replicate the full complexity of the cellular environment, which includes factors such as macromolecular crowding, DNA supercoiling, protein binding, and heterogeneous hydration conditions.

In this project, we have identified a completely new class of G4s, termed subG4s. Unlike standard G4s, subG4s require the presence of an unusually large number of short bulges and a dehydrating environment to form.

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G4 binding viral protein with RG-motif homology

Poster presenter : Jiawei WANG
Laboratory for optics and biosciences (LOB)

G4 binding proteins (G4BP) specifically recognize the unique topology of G-quadruplex structures and bind to them. The binding is often mediated through specific domains or motifs within the proteins. Many G4BP feature an arginine and glycine-rich motif known as the RGG or RG-rich motif. A common 20 amino acid-long RG-rich motif of sequence RGRGRGRGGGSGGSGGRGRG named NIQI was identified by comparing 77 human G4-binding proteins via previous researcher. Based on NIQI, we predict homologous G4BP on 7 known viral proteomes. In the order of short peptides followed by the proteins to which they belong, through several rounds of biophysical and molecular biology experiments, we finally found that certain proteins in Yellow Fever Virus (YFV) and Hantaan (HAN) bind to G4 structure in different extents. We also obtained the details of interactions between G4 and the protein belonging to YFV through MD simulation. The discovery that G4 interacts with viral proteins has led to a better understanding of some of the physiological activities of viruses, and at the same time, these proteins can also be developed as therapeutic targets.

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Diversity and evolution of archaeal thymidylate synthases

Poster presenter : Wenlu YIN
Laboratory for optics and biosciences (LOB)

Thymidylate synthases (TS) ThyA and ThyX are structurally and evolutionary unrelated enzymes, essential for DNA replication and cell viability, that catalyze dTMP (thymidylate) synthesis from dUMP. ThyX family of thymidylate synthases are flavoproteins and were initially identified in Pyrococcus. As thymidylate does not have a direct counterpart in the RNA world, the evolution of TS families is intimately linked with the evolution of DNA as a genetic material. Here we utilized (meta)genomics sequence information to investigate the diversity and evolution of ThyA and ThyX in archaea. Our phylogenetic analysis reveals that the ancestor of archaeal ThyX originates from bacteria, while the ancestor of archaeal ThyA demonstrates a polydomain origin. Moreover, we have experimentally characterized a novel archaeal variant of ThyA that is distinct from the conventional ThyA proteins found in bacteria and eukarya. In particular, we show that this ThyA variant from Candidatus Lokiarchaeota archaea and Candidatus Thorarchaeota, differently from bacterial and eukaryotic ThyA, do not require the formation of a ternary complex with additional co-factors/substrates (methylenetetrahydrofolate) for the chemical activation of dUMP. This indicates that the different branches between archaeal and bacterial ThyA proteins and elucidates the complex evolutionary history of ThyA and ThyX proteins in archaea. Moreover, our phylogenetic analyses suggest that the eukaryotic thymidylate synthase, required for de novo DNA synthesis, is not closely related to archaeal enzymes and may have been transferred from bacteria to eukaryotes during eukaryogenesis.

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Atomistic Modeling and Engineering of Fatty Acid Photodecarboxylase

Poster presenter : Elsa BALDUZZI
Laboratory for optics and biosciences (LOB)

Fatty Acid Photodecarboxylase (FAP), one of a few natural photoenzymes described to date, is a hydrocarbon-forming flavoenzyme with significant industrial potential for green chemistry applications. Prior studies, using a combination of biophysical, biochemical, and computational techniques, have illuminated key aspects of its photocycle, revealing unexpected reaction intermediates and an autocatalytic effect unique to specific medium-chain fatty acids. In this process, the alkane product acts as a cocatalyst, enhancing photodecarboxylation of the initial substrate. This finding suggests that enzyme engineering could increase FAP's efficiency for biofuel-relevant medium-chain fatty acids. The primary focus of this project is to optimize substrate binding and precise positioning within FAP's active site, with the overarching objective of minimizing photoinactivation and maximizing catalytic activity. A major objective is to introduce a competitive photo induced Electron Transfer (ET) reaction, incorporating a tyrosine residue to serve as an electron donor, aiming to mitigate intersystem crossing (ISC) from the excited singlet flavin to the triplet state in the absence of substrate. To realize these aims, we use atomistic modeling with conventional force fields in collaboration with experimental labs. A computational protocol that relies on Rosetta software is developed to identify mutations that allow for the binding of target medium-chain fatty acids in the proper location while reducing the binding of fatty acid substrates in the remote positions. To mitigate intersystem crossing (ISC) I am investigating structural and dynamical properties of the substrate-devoid protein complex. This modeling approach will enable us to explore and understand the molecular interactions involved in the absence of substrates.

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Characterization of two RNA modification enzymes involved in human pathologies

Poster presenter : Laurianne Li En TAY
Laboratory of structural biology of the cell (BIOC), Laboratory of Molecular Chemistry (LCM)

RNA modification enzymes targeting factors involved in mRNA translation have become a topic of interest due to their roles in maintaining the efficiency, fidelity and regulation of protein synthesis by chemically modifying key factors (eg. tRNA, mRNA, rRNA). This thesis focuses on two related proteins: ALKBH8 and TRMT9B. ALKBH8 is considered to be crucial for mRNA decoding fidelity as it is involved in the formation of a complex modification at position 34 of the anticodon loop of some tRNAs. Furthermore, ALKBH8 mutations have been identified in patients suffering from intellectual disorder (ID), emphasizing the importance of this protein for brain development and function. TRMT9B acts as a tumor growth repressor in certain cancers but its biochemical function is yet unclear. Based on its strong homology with the catalytic domain of ALKBH8, it is supposed to modify tRNAs. The aims of this thesis are thus: 1) to characterize *in vitro* and *in vivo* the effect of pathogenic mutations on ALKBH8 function; and 2) to identify the TRMT9B substrate and the chemical modification it is catalyzing. To date, we have shown that the small methyltransferase activator protein TRMT112 increases the solubility of TRMT9B and co-purifies with TRMT9B. Validation of the results of an RNA-crosslinking experiment will soon follow. Efforts are also ongoing to study *in vitro* the enzymatic activity of WT ALKBH8 and pathogenic mutants found in patients diagnosed with ID. In parallel, we have validated the use of LC-MS for nucleoside quantification and MALDI-FT-ICR for modification mapping via a partial digest approach. We are now applying chemical derivatization to improve the ionization of uridines and modified uridines in ESI-MS, to further improve detection and quantification of these nucleosides in biological samples.

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Elucidating the intracellular organization and biogenesis of Haloferax volcanii's translation machinery by microscopy

Poster presenter : Tom GARNIER
Laboratory of structural biology of the cell (BIOC), Laboratory for optics and biosciences (LOB)

Today, the strong phylogenetic proximity between eukaryotes and archaea supports the hypothesis of a potential eukaryotic emergence from an archaeal ancestor. In addition, many proteins, originally thought to be specific to eukaryotes, are now known to be shared between archaea and eukaryotes, including proteins involved in ribosomes and their biogenesis. Eukaryotic ribosome biogenesis is characterized by a high degree of functional compartmentalization, from the nucleolus to cytoplasm. Moreover, the translation process is separated from the stored genetic information. Accordingly, the early formation of intracellular organization in archaea may have facilitated the emergence of eukaryotes and their compartmentalization.
Therefore, to contribute to the resolution of this hypothesis, we aim to characterize the degree of intracellular organization of ribosome biogenesis in different model archaea, through the use of wide-field fluorescence microscopy and super-resolution techniques (dSTORM).