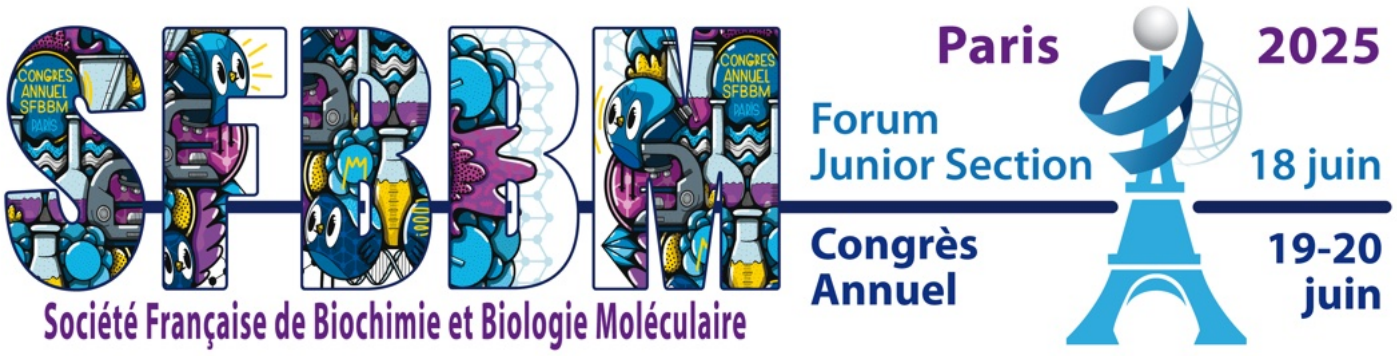


SFBBM
Société Française de Biochimie et Biologie Moléculaire

Chers collègues, chers amis,

C'est un vrai plaisir de vous retrouver pour ce nouveau Congrès Annuel de la SFBBM !

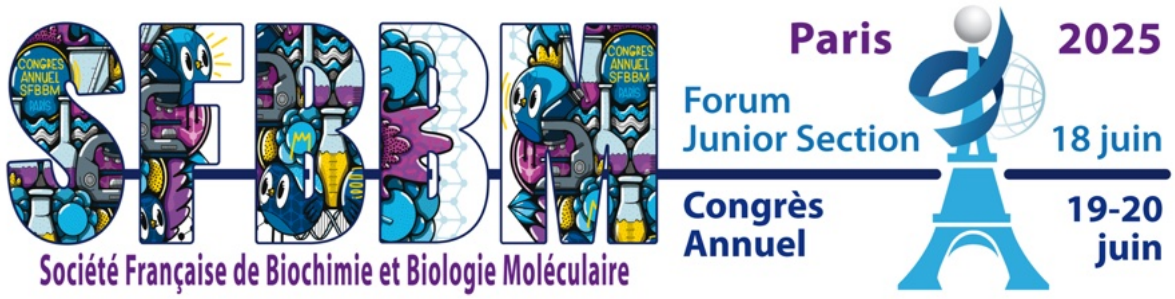
Ce congrès est l'occasion de partager ensemble notre passion pour la science, notre volonté collective de la faire vivre et progresser, avec enthousiasme et curiosité. Pendant ces deux jours, nous aurons le privilège de découvrir des travaux à la pointe de la biochimie et de la biologie moléculaire, portés par des chercheuses et chercheurs de tous horizons, de la recherche fondamentale aux applications biotechnologiques, de la biologie structurale à l'épigénomique, en passant par l'innovation pédagogique.

Le programme de cette édition reflète l'extraordinaire vitalité de notre communauté : des conférenciers et conférencières de renom, des jeunes talents récompensés par les prix de la SFBBM, des sessions riches et diversifiées allant des membranes aux plantes, des acides nucléiques à la biologie synthétique. C'est aussi un congrès tourné vers l'avenir, comme en témoignent les conférences sur l'intelligence artificielle ou la valorisation industrielle de nos travaux.

Je tiens à remercier chaleureusement les orateurs invités, les lauréats, les nombreux participants, les sponsors, le comité local d'organisation, ainsi que le conseil scientifique. Grâce à votre engagement, ce congrès est à nouveau une réussite collective et un moment de respiration et d'inspiration pour notre belle société.

Au nom de la SFBBM, je vous souhaite un excellent congrès, plein d'échanges stimulants, d'idées neuves, de beaux moments… et de bonne humeur partagée !

Martin Picard
Président de la SFBBM



Présentation de la SFBBM
Société Française de Biochimie et Biologie Moléculaire

La Société Française de Biochimie et Biologie Moléculaire (SFBBM) est une société savante fondée par le Professeur Maurice Nicloux en 1914 au Collège de France à Paris. Association loi 1901, la SFBBM a été reconnue comme établissement d'utilité publique par décret le 27 avril 1933.

Ses missions permettent :
- de rassembler les biochimistes et biologistes moléculaires de toute la France
- d'animer via ses groupes thématiques des actions auprès de sa communauté aussi bien dans la recherche que dans l'enseignement
- de représenter sa communauté auprès des instances politiques et scientifiques nationales et internationales comme la FEBS.

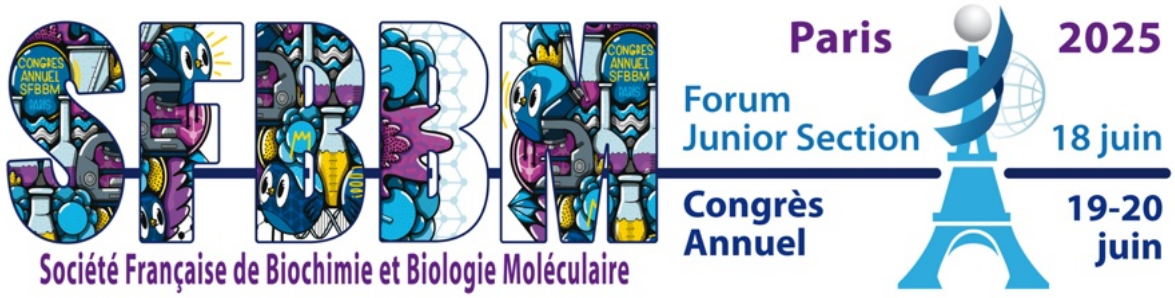
La SFBBM organise des congrès, des réunions scientifiques et des journées sur l'innovation pédagogique.Elle finance ces actions au travers de prix scientifiques, de bourses et aides financières pour la participation à des congrès en France comme à l'étranger.

La SFBBM est impliquée dans des publications scientifiques. Le journal BIOCHIMIE est le journal de la SFBBM. En plus de 40 ans il a accueilli près de 5500 articles originaux ou de revues, et compte parmi les mieux connus et plus respectés dans le domaine.

BIOCHIMIE publie des travaux originaux, articles de revue et mini-revues dans le domaine large de la biologie, couvrant biochimie, biologie moléculaire et cellulaire, régulations métaboliques, génétique, immunologie, microbiologie, biologie structurale, génomique, protéomique, et mécanismes moléculaires des maladies. Le facteur d'impact sur 5 ans est de 3,124. Le journal publie régulièrement des numéros spéciaux dédiés à des sujets de recherche d'un intérêt particulier.

Cette année notre société a su fédérer nos jeunes chercheurs autour d'une « Junior Section ». Il s'agit d'un groupe dédié aux doctorants, post-doctorants et jeunes chercheurs dans le domaine de la biochimie et de la biologie moléculaire qui a pour missions de :

- Permettre une participation active à la communauté scientifique
- Répondre aux besoins spécifiques des jeunes chercheurs
- Rejoindre un espace dédié sur le site web de la SFBBM et "FEBS network" pour la collaboration et le partage des ressources
- Fournir un cadre pour développer des compétences et permettre la formation d'un réseau collaboratif
- Organisation d'un forum adossé aux congrès annuels de la SFBBM et de la FEBS
- Activités des groupes thématiques de la SFBBM
- Conférences en ligne : recherche, pédagogie et plan de carrière de la junior section SFBBM et des juniors sections de la FEBS



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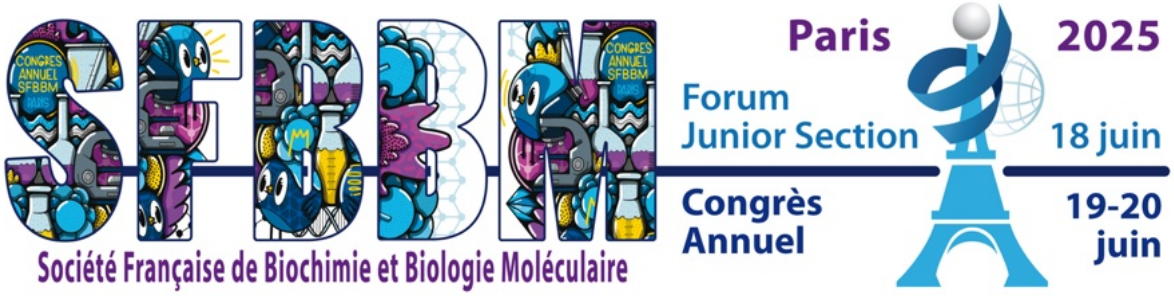
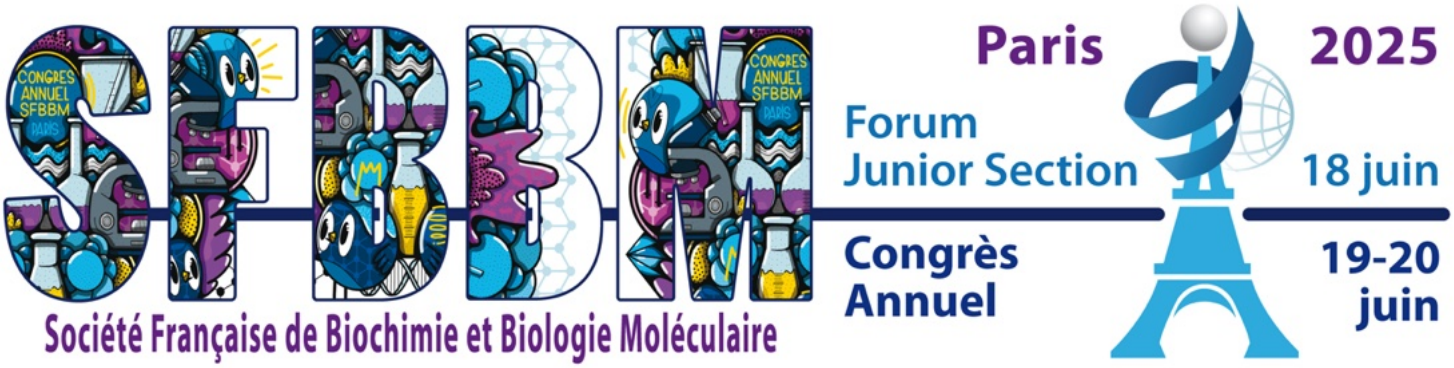


NOS PARTENAIRES

Nous tenons à remercier chaleureusement nos partenaires et sponsors : les éditions Dunod qui soutiennent depuis de nombreuses années le travail du groupe sur les innovations en pédagogie, la Faculté de Pharmacie de Paris de l'Université Paris Cité, Cytiva, Macrogen et NEB « New England Biolabs ».
Vos contributions à ce congrès nous ont permis de réaliser cet évènement dans les meilleures conditions pour l'accueil des participants en plein centre de Paris.



Forum Junior Section: SCIENTIFIC PROGRAM

Wednesday 18th June, 2025

13h30 Participant Welcome

14h **SFBBM presentation by Martin Picard (President) & JS members**
Introduction to SFBBM JS, FEBS JS, YSF & ENABLE

14h30 SESSION 1: Early-Career Researchers in Academia

INVITED SPEAKERS:

14h30 Elise Kaplan (CR CNRS, MMSB, Lyon)
15h20 Nicolas Kint (MCU, Sorbonne University, Paris)

15h50 COFFEE BREAK

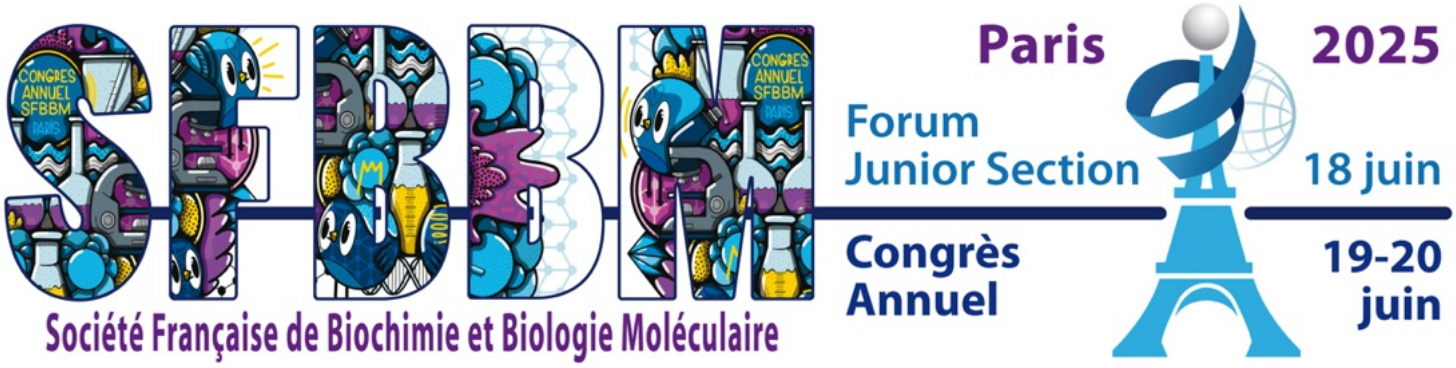
16h20 SESSION 2: Early-Career Researchers in Industry

INVITED SPEAKERS:

16h20 Delphine Allouche (Scientist, Sanofi, Lyon)
17h10 Thomas Alexandre (Microbiology expert, InterScience, Paris)

17h40 Panel Discussion: Career Development and Future of Biochemistry and Molecular Biology

2025 JS SFBBM cocktail
Faculty of Pharmacy



SCIENTIFIC PROGRAM OF THE ANNUAL SFBBM CONGRESS

DAY 1 – Thursday 19th June, 2025

8h Welcome, coffee and poster installation

9h Opening of the congress

9h15 **Plenary lecture by Claire HELLIO**
" Fostering innovation in marine biotechnologies (R&D and higher education): from chemical ecology to marine biotechnology using bioinspirated approaches "

10h15 **SESSION 1: RNA MODIFICATIONS AND EPIGENOMICS**
Chairs Sylvain Maenner & Philippe Fossé

INVITED SPEAKER: Youri Motorin
" Challenges in RNA modification mappping by deep sequencing "

10h45 **Grunberg-Manago 2024 Prize: Pascale LESAGE**
" Strategies for targeting retrotransposon integration to safe harbors "

11h05 **Maurice Nicloux 2024 Prize: Maxime WERY**
" Pervasive translation of cryptic cytoplasmic long noncoding RNAs "

11h25 **Clément CARRE**
" The RNA methyl-transferase enzyme FTSJ1: conserved role in neuron morphology & learning performance "

11h40 **Zeynep BAHAROGLU**
" Epitranscriptomic response to antibiotics in *Vibrio cholerae* "

11h55 **Partho Sarothi RAY**
" Transcriptomic analysis reveals regulation of miRNA and RBP binding by mRNA m6A modifications "

12h15 **LUNCH BREAK**

13h30 **Presentation of the SFBBM Honorary Membership Medal to Alain Krol in recognition of his commitment to our society**

14h **SESSION 2: MEMBRANE**
Chairs Isabelle Broutin & Martin Picard

INVITED SPEAKER: Nadia IZADI-PRUNEYRE
" Integrative structural biology of bacterial membrane nanomachines "

14h30 **Maurice Nicloux 2022 Prize: Ludovic PELOSI**
" Ubiquinone biosynthesis in *Escherichia coli*: characterization of an O2-independent pathway "

14h50 **Gilles PHAN**
" Structure-function study of pseudopaline secretion in *Pseudomonas aeruginosa* "

15h05 **Damila MILHOVILCEVIC**
" Structural and functional characterization of the sensor/transducer MecR1 protein of *Staphylococcus aureus* "

15h20 **Adria SOGUES**
" Order at the surface: atomic architecture and living dynamics of the *Corynebacterium glutamicum* molecular armour "

15h35 **POSTERS SESSION – COFFEE BREAK**

16h30 **SESSION 3: EDUCATION**
Chairs Zaineb Kammoun & Xavier Coumoul

INVITED SPEAKER: David Smith
" The power and peril of AI in education"

17h10 **Claire STINES-CHAUMEIL**
" Promoting awareness of industrial property in biochemistry education "

17h25 **Xavier COUMOUL**
" Metabolism in motion: rethinking biochemistry through visual storytelling "

17h40 **DUNOD Edition – Lionel GOURAUD et Laëtitia JAMMET**

SFBBM GENERAL ASSEMBLY
Martin Picard, president
Philippe Fossé, treasurer

2025 SFBBM annual congress cocktail
Faculty of Pharmacy of Paris

DAY 2 – Friday 20th June, 2025

8h30 **SESSION 4: STRUCTURAL BIOLOGY & ENZYMES**
Chairs Béatrice Vallée-Méheust, Marlène Vayssières & Sophie Rahuel-Clermont

INVITED SPEAKER: Marcin SUSKIEWICZ
" Of filaments and condensates: the underappreciated realm of protein polymerization "

9h00 **SFBBM article of the year 2024 award: Marlène VAYSSIERES**
" Structural basis of DNA crossover capture by *Escherichia coli* DNA gyrase "

9h20 **Dina Surdin 2024 Prize: Aria GHEERAERT**
" Harnessing enzyme activities with dynamical structural comparisons "

9h40 **Nathalie COLLOC'H**
" Inhibitor binding in rate excited states of Ras revealed by high pressure crystallography "

9h55 **Maria IMEZAR**
" Study of the RNA Ligase Pab1020, responsible for RNA circularization in Archaea "

10h10 **Marah SAD**
" The IDR of PAP acts as a molecular ruler to guide CFIm25 in mRNA polyadenylation site selection "

10h15 POSTERS SESSION – COFFEE BREAK

11h15 **SESSION 5: SYNTHETIC & CHEMICAL BIOLOGY**
Chair Hélène Munier-Lehmann

INVITED SPEAKER: Karsten HAUPT
" Molecularly imprinted polymer nanogels: synthetic mimics of peptide antibodies for biomedical diagnostics and therapy "

11h45 **Batoul MAHCENE**
" Stable SAM analogues as promising scaffolds for RNA methylation inhibition "

12h **Horgan MANIRAKIZA**
" Chemogenetic spectral modulation of bioluminescence for imaging and sensing "

12h15 **Natale Noël SCARAMOZZINO**
" Antibody targeting the anti-parallel topology of human telomeric G- quadruplex DNA "

12h30 **Agathe URVOAS**
" Engineering artificial repeat proteins towards modular assemblies and cellular tools "

12h45 **LUNCH BREAK**

13h30 **SESSION 6: PLANTS**
Chair Claire Rosnoblet

INVITED SPEAKER: Christophe D'HULST
" My sweet tuber: characterization of the molecular basis of the cold-induced-sweetening in potato "

14h **Linda DE BONT**
" The hexameric atypical thioredoxin from poplar, DCC1, possesses a redox holdase activity "

14h15 **Damien INES**
" *Rhizophagus irregularis* DAOM197198 modulates the root ubiquitinome of *Medicago truncatula* in the establishment and functioning of arbuscular mycorrhizal symbiosis "

14h30 **Sara-Alina NEUMANN**
" Methylation of ribosomes and translation in *Arabidopsis* "

14h45 **SESSION 7: STRUCTURE, FUNCTION & DIVERSITY OF NUCLEIC ACIDS**
Chair Nathalie Chamond & Magali Blaud

INVITED SPEAKER: Redmond Smyth
" Sensitive localization of influenza RNA in cells by direct RNA padlock probing and in-situ sequencing "

15h15 **Gwenaëlle ANDRE**
" A new drug targeting the evolvability protein Mfd against ESKAPE infections "

15h30 **Marie IMBERT**
" Structural snapshots of D-loop formation in Archaea revealed by cryo-EM "

15h45 **Nathalie CHAMOND**
" Challenging the paradigm: DDX3X exhibits robust helicase activity on HIV-1 "

16h **Nicolas SOLER**
" Two OB-fold proteins from a Gram-positive ICE modulate relaxase biochemical activities "

Poster and presentation prize ceremony and conclusion

Fostering innovation in marine biotechnologies: from chemical ecology to marine biotechnology using bioinspirated approaches

Claire Hellio

Univ Brest, BIODIMAR/LEMAR, CNRS, UMR 6539, F-29200 Brest, France.

Claire.hellio@univ-brest.fr

The growing demand for the development of new environmentally friendly materials and processes continues to increase. Bioinspiration is an attractive alternative, drawing inspiration from nature's own concepts and solutions and transferring them to solve particular problems. Marine biotechnology exploits the diversity found in marine environments in terms of structure, physiology and chemistry of marine organisms, many of which have no equivalent on land.

Marine biotechnology is an opportunity recognized by policy makers and the enterprise sector as offering significant potential to fill market gaps for new products.

In order to develop new application, the mechanisms and strategies of marine organisms must be elucidated. The work and concepts presented in this presentation are chemical ecology, the role of microflora in the production of defense molecules, the seasonal nature of defense molecule production and the synergy of defenses. Examples of research collaborative projects with companies will be presented

Session 1

RNA modifications

and

epigenomics

Challenges in RNA modification mapping by deep sequencing

Yuri MOTORIN

Ingénierie Moléculaire, Cellulaire et Physiopathologie (IMoPA), UMR 7365 CNRS-Université de Lorraine, Nancy, France

RNA modifications found in almost all types of cellular RNAs are now recognized as key players of RNA metabolism, affecting all steps of RNA life : processing/maturation, folding, recognition by cognate proteins and RNP assembly, trafficking, translation and degradation. Numerous methods are now available for RNA modification mapping in a subset of stable RNA species or transcriptome-wide. Despite substantial efforts for already >10 years in the epitranscriptome field, the consensus map of RNA modifications is only achieved for a few model living species and mostly only for stable RNAs. The number and the exact location of RNA modifications, as well as their stoichiometries for mRNA and other scarce RNA species, are still under debate. Deep sequencing methods are undoubtedly the best suited for extensive mRNA analysis, but their application is not always straightforward, and every method has its own limitations and drawbacks. Newly appearing protocols involving nanopore sequencing are promising, but their application is still very far from routine RNA modification analysis. Most popular second-generation deep sequencing protocols and their application to whole transcriptome are discussed as well as possible general guidelines for protocol validation and application.

Strategies for targeting retrotransposon integration to safe harbors

Grunberg-Manago 2024 Prize

Pascale Lesage

Université Paris Cité, Institut de Recherche Saint-Louis, CNRS, INSERM, Paris, FRANCE

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Retrotransposons constitute a major part of eukaryotic genomes, and exhibit structural and functional similarities to retroviruses. Since their integration can be mutagenic, many elements have evolved the capacity of integrating into specific regions of genomes devoid of essential genes. This strategy helps to maintain host cell survival and ensures the successful propagation of the retrotransposons themselves.

Retrotransposon integration occurs within a complex and heterogenous chromatin environment, where DNA-bound proteins, chromatin remodelers and/or histone modifications modulate the accessibility of the integration machinery. Yet, how integration site selection occurs in this intricate molecular environment remains largely unknown. Unravelling these processes is essential to elucidate the wider implications of retrotransposon activity on genome stability and evolution.

The yeast Ty1 retrotransposon is an excellent model for studying chromatin-guided integration. Ty1 exhibits high integration specificity for the first three nucleosomes upstream of RNA polymerase III (Pol III)-transcribed genes, determined by an interaction between the Ty1 integrase (IN1) and Pol III [1,2]. Disruption of this interaction leads to Ty1 integration into subtelomeres, suggesting that subtelomeric cofactors or chromatin marks play a role in this process. In the past years, we have developed molecular, genomic and structural approaches to resolve the IN1-Pol III interaction at the atomic level [3]. We are now deciphering the nucleosome features that promote Ty1 integration and the factors driving Ty1 integration into subtelomeres.

References:

1. Bridier-Nahmias, A. *et al.* An RNA polymerase III subunit determines sites of retrotransposon integration. *Science (1979)* **348**, 585–588 (2015).
2. Asif-Laidin, A. *et al.* A small targeting domain in Ty1 integrase is sufficient to direct retrotransposon integration upstream of tRNA genes. *EMBO J* **39**, 1–17 (2020).
3. Nguyen, P. Q. *et al.* Structural basis of Ty1 integrase tethering to RNA polymerase III for targeted retrotransposon integration. *Nat Commun* **14**, (2023).

Keywords:

Ty1 retrotransposon, Integration selectivity, RNA polymerase III, subtelomeres, *S. cerevisiae*

Pervasive translation of cryptic cytoplasmic long noncoding RNAs

Maxime Wery[a], Dominika Foretek[a], Sara Andjus[a], Ugo Szachnowski[a], Marc Gabriel[a], Nicolas Vogt[a], Isabelle Hatin[b], Chris Papadopoulos[b], Anne Lopes[b], Edouard Bertrand[c], Olivier Namy[b] and Antonin Morillon[a]

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[b] Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198 Gif-sur-Yvette, France
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Long non-coding (lnc)RNAs regulate multiple cellular processes. Although they were predicted to lack coding potential, recent works revealed that some lncRNAs can be translated, resulting in the production of lncRNA-derived peptides. However, despite the interest they arouse, the potential of these peptides and the mechanisms controlling their synthesis are poorly characterized. Here, we investigated the functional impact of non-canonical translation events on cytoplasmic lncRNAs in yeast and human cells. We show that Xrn1-sensitive cytoplasmic lncRNAs (XUTs) in yeast are mainly targeted by the Nonsense-Mediated mRNA Decay (NMD) pathway, indicating a translation-dependent degradation process. Ribo-seq confirmed ribosomes binding to XUTs and identified ribosome-associated 5'-proximal small ORFs. Mechanistically, the NMD-sensitivity of XUTs mainly depends on the 3'-UTR length. Moreover, we show that the peptide resulting from the translation of an NMD-sensitive XUT exists in NMD-competent cells, suggesting that despite the cryptic nature of the transcript, its translation results in a detectable product. In human cells, we identified DIS3 as the main exonuclease restricting accumulation of lncRNAs in the cytoplasm and revealed thousands of DIS3-sensitive lncRNAs (DISTs). We show that DISTs also display active translation, producing peptides predicted to be high-affinity antigens in multiple myeloma patients carrying DIS3 mutations. Overall, our work highlights the central role of translation in the metabolism of cytoplasmic lncRNAs, with different potential outcomes. While the resulting peptides could constitute raw material exposed to the natural selection in yeast, some of them could be part of the cell-to-cell communication through tumor-specific antigen presentation in human cells.

Reference:

Wery et al (2016) Nonsense-Mediated Decay Restricts LncRNA Levels in Yeast Unless Blocked by Double-Stranded RNA Structure. *Mol. Cell* 61, 379-392.

The RNA methyl-transferase enzyme FTSJ1: conserved role in neuron morphology, learning performance & translation.

Mira Brazane, Dilyana G Dimitrova, Julien Pigeon, Chiara Paolantoni, Tao Ye, Virginie Marchand, Bruno Da Silva, Elise Schaefer, Margarita T Angelova, Zornitza Stark, Martin Delatycki, Tracy Dudding-Byth, Jozef Gecz, Pierre-Yves Placais, Laure Teysset, Thomas Preat, Amélie Piton, Bassem A. Hassan, Jean-Yves Roignant, Yuri Motorin and **Clément Carré**

Sorbonne Université, IBPS, Dev2A, CNRS, INSERM

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Transfer RNAs (tRNAs) are crucial for translation, with their function heavily influenced by modified ribonucleotides. One such modification, 2'-O-methylation (Nm), affects the ribose moiety and is particularly present on the anticodon loop of some tRNAs. In humans, Nm is catalyzed by the SAM-dependent methyltransferase FTSJ1. Loss of FTSJ1 leads to intellectual disability (ID), though the mechanisms are not fully understood. Our studies in human neural progenitor cells showed that inhibiting FTSJ1 increases dendritic spines, a feature common in neurodevelopmental disorders. This phenotype is also observed in Drosophila larvae with mutated FTSJ1 orthologs and mice. Transcriptome analysis revealed deregulation of mRNA and miRNA involved in brain morphogenesis in human cells, suggesting defective gene expression regulation contributes to the observed morphological defects. Additionally, long-term memory is affected in Drosophila mutants of FTSJ1.

Given tRNAs' role in translation, transcriptome-wide profiling of ribosome footprints was conducted on human and Drosophila cells affected by FTSJ1 activity. These analyses are ongoing and recent results will be presented. Those results indicate significant regulation of brain-specific genes, morphological defects in neuronal cells lacking FTSJ1 and codon bias. The goal is to identify genes involved in the defective morphology of neuronal tissues without tRNA Nm, determine if the regulation occurs at the translational level, and understand the mechanisms behind FTSJ1-related intellectual disability.

Keywords:

2'O-methylation, Epitranscriptomics, translation, Intellectual Disability

Epitranscriptomic response to antibiotics in Vibrio cholerae.

Zeynep Baharoglu

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[b] Epitranscriptomic and translational responses to antibacterial stress Team, Institut Pasteur, Université Paris Cité, 75015, Paris, France

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Antimicrobial resistance poses a growing threat to the treatment of infectious diseases. To uncover mechanisms underlying bacterial antibiotic responses, we performed a genome-wide transposon insertion screen in *Vibrio cholerae*, followed by TN-seq under antibiotic stress. This approach identified 23 tRNA and rRNA modification enzymes linked to differential antibiotic susceptibility. Notably, deletion of the tRNA guanine transglycosylase *tgt*, which catalyzes queuosine (Q) modification at the wobble position of GUN anticodon tRNAs, caused marked hypersensitivity to aminoglycosides.
Using translation fidelity and efficiency reporters alongside quantitative proteomics, we dissected the molecular basis of this phenotype. We show that:
(i) loss of Q modification impairs decoding of tyrosine codons and induces stress-responsive translational reprogramming;
(ii) protein translation is modulated by codon usage bias in a Q-dependent manner;
(iii) candidate transcripts subject to Q-sensitive translation can be predicted in silico based on codon composition.
Our findings reveal an epitranscriptomic layer of regulation that fine-tunes bacterial translation under antibiotic stress and suggest new avenues for targeting translation control in antimicrobial strategies.

Relevant references:

doi.org/10.1093/femsml/uqac019
doi.org/10.7554/eLife.96317.3

Keywords:

tRNA modifications, antibiotic tolerance, *Vibrio cholerae*, tgt

Transcriptomic analysis reveals regulation of miRNA and RBP binding by mRNA m6A modifications

Partho Sarothi Ray[a] and Sukhen Das Mandal[b]

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[b]Ghani Khan Choudhury Institute of Engineering and Technology, Narayanpur, Malda, West Bengal 732141, India

N6-methyladenosine (m6A), the most prevalent epitranscriptomic modification in eukaryotes, is enriched in 3'- untranslated regions (3' UTRs) of mRNAs. m6A modifications directly determine the binding of a class of RNA-binding proteins (RBPs) called as m6A "readers" to RNA. However, as 3' UTRs are major binding sites of RBPs and microRNAs (miRNAs), we hypothesized that m6A-dependent local RNA structure changes may further influence the proximal binding of RBPs and miRNAs and regulate mRNA function. Using a human transcriptome-wide computational analysis, we find a strong positive correlation between number of m6A sites, miRNAs and RBPs binding to mRNAs, suggesting m6A-modified mRNAs are more targeted by miRNAs and RBPs. Moreover, m6A sites are located proximally to miRNA target sites and binding sites of multiple RBPs. This indicated three-way interplay between m6A, microRNA and RBP binding, suggesting the influence of mRNA modifications on the miRNA and RBP interactomes. From the subset of mRNAs which show three-way proximity between m6A sites and target sites of the miRNA miR-125b and the RBP, HuR, we investigated COX7A2L, which encodes a crucial component of the mitochondrial respiratory chain and is upregulated in estrogen-treated breast cancer cells. We found that expression of COX7A2L is regulated by both miR-125b and HuR and m6A modification facilitates the binding of miR-125b to COX7A2L mRNA 3'UTR while HuR antagonizes the miR-125b mediated repression COX7A2L expression. Hence, we have computationally determined and experimentally validated that m6A modifications influence the interactions of miRNAs and RBPs with mRNAs beyond the direct recognition of m6A by "readers".

References:

Das Mandal S and Ray P.S. (2020) Transcriptome-wide Analysis Reveals Spatial Correlation between N6-methyladenosine and binding sites of microRNAs and RNA-binding Proteins. Genomics 113, 205-216.
K.D. Meyer, Y. Saletore, P. Zumbo, O. Elemento, C.E. Mason, S.R. Jaffrey (2012) Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons, Cell. 149, 1635–1646.
Pérez-Pérez,R., Lobo-Jarne,T., Milenkovic,D., Mourier,A., Bratic,A., García-Bartolomé,A., Fernández-Vizarra,E., Cadenas,S., Delmiro,A., García-Consuegra,I., et al. (2016) COX7A2L Is a Mitochondrial Complex III Binding Protein that Stabilizes the III2+IV Supercomplex without Affecting Respirasome Formation. Cell Rep., 16, 2387–2398.

Keywords:

m6A modifications, RNA-binding proteins, microRNAs, HuR, miR-125b

Poster 1

Structural study of the RSC chroma3n remodeling complex in *Candida albicans*

Raphaël Dupeyron, Laura Belota, Camille Sayouc, Eleherios Zarkadasa, Guy Schoehna, Sylvie Kieffer-Jaquinodc, Julia Novion Ducassouc, Annie Adraitc, Yohann Coutéc, Félix Weisa, Jérôme Govinb, Carlo Petosaa

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Invasive fungal infec8ons cause ~3.8 million deaths annually, with ~1 million aUributed to invasive candidiasis [1]. SWI/SNF-family chroma8n remodelling complexes are key transcrip8onal regulators across eukaryotes. The RSC (Remodelling the Structure of Chroma8n) complex, extensively studied in *Saccharomyces cerevisiae*, facilitates nucleosome reposi8oning near transcrip8on start sites and is essen8al for viability [2]. Cryo-EM structures of the 16-subunit *S. cerevisiae* complex (ScRSC) revealed its modular architecture and nucleosome-binding mode [3]. However, liUle is known about this complex in pathogenic fungi, despite its poten8al relevance to virulence and an8fungal targe8ng. We inves8gated the RSC complex in *Candida albicans* (CaRSC), the leading cause of invasive candidiasis. Mass spectrometry revealed a divergent 14-subunit composi8on compared to ScRSC, with CaRSC lacking four S. cerevisiae subunits and incorpora8ng two novel, *Candida*-specific proteins (Nri1 and Nri2) of unknown func8on [4]. We used tandem affinity purifica8on (TAP) to isolate endogenous CaRSC and solved its core structure at 3 Å resolu8on by cryo-EM. While the overall architecture is conserved, we iden8fied key structural adapta8ons likely reflec8ng evolu8onary divergence. Nri2 exhibits structural homology to stabilizing elements in other polybromodomain SWI/SNF members, and repressive *NRI2* mutants confirmed its role in suppor8ng yeast growth at elevated temperatures. Addi8onally, cryo-EM and AlphaFold modelling suggest a transcrip8onal ac8vator role for Nri1. Together, these findings underscore the evolu8onary plas8city of SWI/SNF complexes and establish a founda8on for exploring CaRSC's role in *Candida* pathogenesis and its poten8al as a target for an8fungal therapy.

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Keywords:

Chromatin, Epigenetics, Fungal infection, *Candida albicans*, CryoEM

Poster 2

Impact of pathological variants in the LRPPRC/SLIRP complex

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Mitochondria are crucial cell organelles containing their own circular genome, the mitochondrial DNA, which encodes key subunits of the oxidative phosphorylation system. After transcription, it is important for mitochondrial messengers RNAs to be correctly processed in order to ensure their stability until translation. Several proteins are involved in mt-mRNAs processing, and we are particularly interested in the protein complex composed of the Leucin Rich Pentatricopeptide Repeat (LRPPRC) and the Stem-loop-interacting RNA-binding protein (SLIRP). Deletions or pathological variants of these two proteins cause serious disease; neurodevelopmental problems, cardiomyopathy, or congenital malformations. This complex is proposed to stabilize mt-RNAs, through its involvement in the mRNA polyadenylation process and the channeling of mt-RNAs to the mitoribosomes. However, the precise mechanism involved remains unclear and our goal is to understand these interactions at the single-molecule level.

First, we used Electrophoretic Mobility Shift Assays to study the interactions between the LRPPRC/SLIRP complex or its pathological variants with various mt-RNAs. In parallel, we investigate using Acoustic Force Spectroscopy and Total Internal Reflection Fluorescence microscopy, two single-molecule biophysics approaches, the effect of the complex on mRNA compaction. Finally, Cryogenic Electron Microscopy will give us insight into the molecular mechanisms of LRPPRC/SLIRP binding to mt-mRNAs.

Keywords:

mitochondria, mtRNA, single-molecule, LRPPRC, SLIRP

Poster 3

Characterization of two RNA modification enzymes involved in human pathologies

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RNA modification enzymes have become 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). In my thesis, we use biochemistry and mass spectrometry (MS)-based approaches to characterize two RNA modification proteins, ALKBH8 and TRMT9B. ALKBH8 is a tRNA modification enzyme associated with intellectual disorder that modifies the wobble uridine. The first aim of my thesis is to study the effect of pathogenic ALKBH8 mutations on enzyme function and stability. TRMT9B is a putative tumor suppressor protein with a strong structural similarity to ALKBH8. Thus, it is proposed to also be an RNA modification enzyme, however, its substrate and therefore function in disease, especially cancer, is still unknown. The second aim is hence to identify the substrate of TRMT9B and the modification it catalyses.

A multi-pronged approach utilizing *in vitro*, *in vivo,* and structural studies is in place to achieve these goals. Biology-wise, techniques used include the development of a methylation activity assay, phenotypic studies on knockout cell lines, and cryo-EM.

In the interest of studying modified RNA for characterization of pathogenic ALKBH8 mutants and potentially the TRMT9B substrate, we are also implementing relevant MS-based approaches including: LC-MS and MS/MS for identification and quantification of modified nucleosides, modification mapping on partially digested RNA samples using MALDI FT-ICR, chemical derivatization to increase sensitivity of uridine detection, and separation and analysis of oligonucleotides by using ion-pairing reagent-free methods.

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Keywords:
Epitranscriptomics, mass spectrometry, intellectual disorders, cancer, RNA modification enzymes

Poster 4

Impact of oxidative stress on the epigenetic regulation of non-coding RNAs: a biochemical and transcriptomic investigation

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Oxidative stress is known to affect gene expression regulation through epigenetic modifications. While the impact on DNA and histones is well documented, less is known about the modifications of non-coding RNAs (ncRNAs) under oxidative conditions. In this study, we aim to investigate the effects of hydrogen peroxide-induced oxidative stress on RNA epigenetic marks, with a focus on m6A methylation and pseudouridylation of ncRNAs.
Our planned experimental approach will involve HeLa cells and rat hepatocytes (H4IIE), which will be treated with 200 µM H_2O_2 for 1 hour to induce oxidative stress. Total RNA will be extracted using the TRIzol method followed by column-based purification. m6A levels will be quantified using methylated RNA immunoprecipitation followed by RT-qPCR (MeRIP-qPCR), while pseudouridylation will be detected via CMC-derivatization and primer extension assays. High-throughput RNA sequencing will be conducted, and the resulting data will be analyzed using the Galaxy platform and m6A-Finder to identify differential epitranscriptomic profiles.
We expect that oxidative stress will induce significant changes in RNA methylation patterns, particularly in long non-coding RNAs involved in antioxidant response pathways. Moreover, we hypothesize that pseudouridylation levels will increase in small nuclear RNAs (snRNAs), suggesting a potential compensatory stabilization mechanism.
These anticipated results will help to highlight the potential role of epitranscriptomic modifications in the cellular response to oxidative stress and will support the hypothesis that non-coding RNA regulation is a key component of stress adaptation. This study could open new avenues for the development of RNA-based biomarkers and therapeutic strategies targeting oxidative-stress-related pathologies.

Keywords:

Oxidative stress; Non-coding RNAs; Epitranscriptomics; m6A methylation; Pseudouridylation

Session 2

Membrane

Integrative Structural Biology of Bacterial Membrane Nanomachines

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Bacterial membrane machineries mediate exchanges with the host and environment, influencing colonization and pathogenesis. Their essential roles and accessibility make them promising antimicrobial targets.

However, their membrane integration and transenvelope nature make molecular and atomic-level studies challenging. Many also have long, disordered regions that span the envelope to interact across cell compartments. An integrative strategy is therefore required to study them.

Two main systems will be presented. The first one is related to bacterial pili, which are responsible for the secretion of enzymes and toxins through an unknown mechanism. We have investigated the structure, dynamics, and assembly mechanism of the pilus using a combination of NMR, X-ray and cryoEM, with *in vivo* tests, in order to propose a secretion mechanism coupled with pilus polymerization. [1, 2, 3.]

The second one is crucial for importing scarce nutrients such as metals, vitamins and some specific sugars. A specific outer membrane transporter senses, binds and internalizes the nutrient by an energized process, powered by a proton channel molecular motor in the inner membrane. However, how the energy is transmitted across the envelope remains understood. Using NMR, we recently resolved the structure and conformational switch of a previously missing component of the proton channel. We demonstrated that this switch is crucial for nutrient uptake in vivo and sheds light on the energy transfer mechanism. Notably, we also revealed—for the first time—the catalytic role of the cell wall in this process. These findings address key gaps in our understanding of bacterial motor function.

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Ubiquinone biosynthesis in *Escherichia coli*: characterization of an O_2-independent pathway

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Ubiquinone (UQ) is a crucial component of membrane electron transfer chains in proteobacteria and eukaryotes. Its biosynthesis involves multiple steps, most of which are conserved between bacteria and eukaryotes and typically rely on O_2-dependent hydroxylation reactions. However, recent discoveries have revealed that UQ can also be synthesized via an O_2-independent pathway, suggesting that UQ may play a role in anaerobic metabolism in addition to its well-established function in aerobic conditions[1].

The hypothesis of an O_2-independent UQ biosynthesis pathway emerged from the observation that *Escherichia coli* can produce UQ under anoxic conditions. Nevertheless, this pathway remained uncharacterized until 2019, when three genes - *ubiT*, *ubiU*, and u*biV* - were identified as essential for UQ biosynthesis in the absence of oxygen, but dispensable under oxic conditions[2]. These genes are widespread among proteobacterial genomes that encode the O_2-dependent UQ pathway, indicating that many bacteria possess an unrecognized ability to synthesize UQ across a full range of oxygen concentrations. UbiU and UbiV, each coordinating a [4Fe-4S] cluster through conserved cysteine motifs, form a heterodimer that catalyzes hydroxylation steps in the anaerobic UQ biosynthesis pathway[2,3]. While biological hydroxylation reactions typically employ inorganic oxygen donors such as O_2, H_2O_2, or H_2O, UbiU/UbiV instead utilize an organic compound, prephenate - an intermediate of the aromatic amino acid biosynthesis pathway - as the oxygen source for the three necessary hydroxylation reactions[4]. This prephenate-dependent hydroxylation mechanism represents a novel biochemical strategy for adaptation to anaerobic environments, as further evidenced by its involvement in the pathogenicity of *Pseudomonas aeruginosa*[5,6].

Keywords:

Ubiquinone biosynthesis, isoprenoid quinone, protein complex, anaerobic metabolism, bacteria

Structure-function study of pseudopaline secretion by *Pseudomonas aeruginosa*

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Context and objectives: Metal homeostasis is essential for bacterial metabolism. In the Gram-negative pathogen *Pseudomonas aeruginosa*, pseudopaline (Pp) has been discovered as an essential metallophore **[1]**. Because Pp trafficking is a promising antibacterial target, we decided to focus our work on understanding of the molecular mechanism behind Pp secretion. This involves the inner membrane exporter CntI that belongs to the drug/metabolite transporter superfamily (DMT), and the MexAB-OprM efflux pump of the Resistance-Nodulation-Division (RND) family, known to expulse antibiotics through periplasm and outer-membrane. In order to understand how Pp is transported by CntI, and how it is taken over by MexB for final secretion, we aim at quantifying the different interactions and solving the 3D structures of the involved partners.
Results: The purification of CntI was optimized using multi-angle light scattering coupled with size-exclusion chromatography (SEC-MALS) and thermofluor assays, leading to promising crystallization trials. We also identified a dimeric form of CntI sensitive to pH and salt concentration **[2]**. Docking analysis of Pp with 3D models of CntI have suggested important residues involved in the binding pocket, as confirmed by site-directed mutagenesis and complementation assays. Finally, the quantification of Pp binding affinities with both CntI and MexB supports the involvement of MexAB-OprM efflux pump in Pp secretion.

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Keywords:

Membrane transporter, efflux pump, metallophore, pseudopaline, *P. aeruginosa*.

Structural and functional characterization of the sensor/transducer MecR1 protein of *Staphylococcus aureus*

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a global health threat. One of its resistance mechanisms involves MecR1, an integral membrane metalloprotease activated by covalent binding of β-lactam antibiotics to its extracellular sensor domain. This activation is proposed to trigger the cleavage of the cytoplasmic repressor MecI, resulting in the expression of resistance genes. Our objective is to understand how β-lactam binding activates MecR1 and how the signal is transduced across the membrane. However, no high-resolution structure of full-length MecR1 is currently available.

We established an *E. coli* expression system to purify full-length MecR1 using a Mistic fusion, in both detergent micelles and SMALPs nanodiscs. Binding of the fluorescent β-lactam analogue Bocillin™ FL confirmed that the sensor domain remains functional in both environments. Circular dichroism showed similar secondary structure in detergent and nanodiscs. SEC-MALS analysis revealed different oligomeric states in detergent, suggesting possible dimerization.

To further investigate the oligomerization state, we used Gd(III) as a spin probe for EPR spectroscopy. RIDME (Relaxation Induced Dipolar Modulation Enhancement) signals suggested dimer formation. We plan to repeat distance measurements in the presence of β-lactam and MecI to study signal transduction.

We also engineered MecR1 variants with single or double lanthanide-binding tags (LBTs) at various positions to monitor conformational changes. Luminescence confirmed Tb(III) binding, indicating proper folding. RIDME measurements of doubly labeled Gd(III)-LBT-MisticMecR1 showed a distance of ~2.9 nm, consistent with the distance estimated from the BlaR1 structure and AlphaFold2 monomer MecR1 model.

Keywords:

Staphylococcus aureus, membrane proteins, antibiotics, EPR, nanodiscs

Order at the surface: Atomic architecture and living dynamics of the *Corynebacterium glutamicum* molecular armour

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The proteinaceous surface layer (S-layer) forms the outermost component of many bacterial and archaeal cell envelopes. In Actinobacteria (one of the largest and most diverse bacterial phyla) the structure, function, and spatiotemporal assembly of S-layers remain poorly understood. Here, we investigate the S-layer of the biotechnologically relevant organism *Corynebacterium glutamicum*, which possesses a complex cell envelope consisting of a mycomembrane supported by an S-layer composed of the self-assembling PS2 protein. We isolated ex vivo PS2 S-layer fragments and solved the atomic structure using single-particle cryo-electron microscopy. Structural analysis revealed that PS2 monomers form hexameric units anchored to the mycomembrane via coiled-coil transmembrane helices. These hexamers assemble into a two-dimensional semipermeable lattice through trimeric lateral interactions mediated by extended PS2 arms. To investigate S-layer assembly during growth and division, we leveraged the structural information to perform pulse-chase live-cell imaging, which revealed that new PS2 subunits are incorporated at the cell poles, coinciding with sites of peptidoglycan biosynthesis. Functionally, the PS2 S-layer enhances cell envelope integrity, contributing mechanical support under stress conditions and stabilizing the cell wall. Together, these findings provide molecular insight into the architecture and biogenesis of S-layers in Actinobacteria and lay the groundwork for future applications of PS2 as a platform for engineering membrane-anchored, self-assembling biomaterials.

Keywords:

S-layer, Cryo-EM, Mycomembrane, Protein self-assembly & Polar growth

Poster 5

Purification pipeline for *Corynebacterial* membrane protein complexes

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Cell division is critical to bacterial physiology. At mid-cell, the division machinery (divisome) orchestrates the various steps required for division, leading to cytokinesis and the formation of two new cell poles. Our model organism *Corynebacterium glutamicum* has a polar growth mode typical for *Mycobacteriales*, where elongation is performed by the elongasome, organised around the scaffolding protein Wag31. Wag31 starts to localise at the septum early during the cell cycle, where it is thought to prepare the transition from septum to pole. Martinez et al. identified and characterised Glp and its membrane receptor GlpR, two proteins at the heart of this transition, which interact with both the divisome and elongasome cytoskeleta, but further drivers of the transition remain unknown. Recently, we discovered the hypothetical membrane protein Wip1 (Wag31 interacting protein 1), required for the localisation of Wag31 at the cell poles. To understand the function of these proteins we need to obtain high quality protein material for biophysical and structural characterization. Here, we present a screening pipeline to screen solubilization conditions and affinity tag selection for the purification of membrane complexes for structural studies. Using a native expression system of fluorescently labelled and affinity tagged Wip1, we can detect nanograms of target protein in heterogeneous solutions, while retaining protein function. Our initial biochemical analysis indicates that solubilization and tag selection are critical to isolate two distinct divisome or elongasome Wip1 containing sub-complexes, a milestone to understand the role of this intriguing protein.

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Keywords:

bacterial cell cycle, membrane protein biochemistry, F-SEC, structural biology

Poster 6

Proteomic surface analysis of *Legionella pneumophila*

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Bacterial respiratory infections are a major global health concern, accounting for an estimated 4 million deaths in 2019 (K. S. Ikuta et al., 2022). A critical factor in bacterial pathogenicity is the ability to dynamically remodel the cell surface in response to host-derived signals. In *Legionella pneumophila*, the agent of Legionnaires' disease, pulmonary collectins such as SP-A and SP-D can bind the bacterial surface, altering its physiology and potentially its virulence (K. Sawada et al., 2010). Remarkably, *L. pneumophila* actively counteracts this immune recognition by secreting the zinc metalloprotease ProA, which cleaves SP-D. Degradomics analyses performed on infected lung tissues have also identified additional ProA substrates, suggesting a broader role in modulating host-pathogen interactions.

To explore whether other host proteins may interact with *Legionella*, we performed an unbiased chemical screen using broncho-pulmonary samples from patients with confirmed legionellosis. This approach led to the identification of several previously unrecognized ligands in *vibrio cholerae*, named Host-Derived Binding Bacterial Proteins (HBBPs)(A. Zoued et al., 2021). Importantly, exposure of *L. pneumophila* to these clinical samples resulted in substantial remodeling of its surface proteome, indicating that the lung environment plays an active role in shaping bacterial adaptation.
Together, these findings uncover a novel layer of host-pathogen interaction and suggest that *L. pneumophila* surface remodeling in response to host cues is more dynamic and complex than previously appreciated, offering new perspectives for therapeutic strategies targeting early host–bacteria contact.

Keywords:

Host-pathogen Interaction, Surface proteome, host-derived bacterial binding proteins.

Poster 7

A 7-proteins complex neutralizes the pore-forming TelE toxin in *Streptococcus galloly/cus*

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The Type VII Secretion System (T7SS) is a specialized protein export machinery present in many Gram-positive bacteria (1). In Firmicutes, it allows the delivery of toxic effectors modulating immune response in the host as well as interbacterial competition by secreting polymorphic 'LXG' toxins (2). We recently identified TelE, novel pore-forming LXG toxin from *Streptococcus gallolyticus subsp. gallolyticus* (SGG) (3). TelE is encoded by a conserved ('*Lxg*') operon including 6 other genes of previously unknown function. Induction of TelE in *Escherichia coli* results in protein instability and cellular toxicity. This is partially reduced by co-expression of its immunity protein TipE, encoded in the same operon. Interestingly, expression of the whole *Lxg* operon results in a fully non-toxic and soluble protein complex, including TelE. We investigated this 280 kDa assembly by combining Mass Photometry, Mass Spectrometry, X-ray Crystallography, and single-particle Cryo-Electron Microscopy (cryo-EM). We also set up a functional assay using large unilamellar vesicles (LUVs) demonstrating that this complex suppresses TelE's pore-forming activity and remains intact in the presence of lipids. Furthermore, *in vivo* studies on SGG mutants confirmed that only two proteins of this complex are co-secreted with TelE, supporting their direct role in the effector's stabilization and delivery to the T7SS machinery. This work reveals a novel complex that stabilizes the LXG-effector TelE, reducing its toxicity and increasing its stability for optimal delivery through the T7SS machinery.

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Keywords:

Type VII Secretion System, LXG toxin, pore-forming, *Streptococcus gallolyticus*, protein complex.

Poster 8

Investigating the structure and interaction of VirD4$_{/TrwB}$ within the T4SS

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The R388 T4SS is a complex machinery that transfers DNA through bacterial conjugation. It plays a key role in spreading antibiotic resistance genes. VirD4$_{/TrwB}$ is an essential transmembrane ATPase in the T4SS, crucial for driving DNA translocation during conjugation. VirD4$_{/TrwB}$ is believed to interact with other T4SS components in the inner membrane, ensuring efficient DNA transfer [1]. However, its location within the T4SS still needs further investigation, as no clear structural evidence exists. We and others have identified interactions between VirD4$_{/TrwB}$, VirB10$_{/TrwE}$ and VirB4$_{/TrwK}$ that could help better understand the involvement of VirD4$_{/TrwB}$ in the complex [2,3].
This project aims to solve the structure of the T4SS associated with VirD4$_{/TrwB}$ by understanding specific interactions between the different components and stabilising the protein in place. To do so, we investigate different hypothesis using structure predictions, interaction assays, cryo-EM and crystallography. Crosslinking experiments and structure predictions showed that VirD4$_{/TrwB}$ interacts with VirB10$_{/TrwE}$. However, further experiments are necessary to better understand and characterise this interaction. To do so, we are using biophysical and structural approaches. We intend to validate the minimal motif of VirB10$_{/TrwE}$ involved in the interaction with VirD4$_{/TrwB}$ using SPR. In the meantime, we aim to solve the crystal structure of VirD4$_{/TrwB}$ in the presence of VirB10$_{/TrwE}$ motif. Moreover, to localise VirD4$_{/TrwB}$ and solve its structure within the T4SS, we have fused a bulky domain to the protein that should help us identify its location using cryo-EM. We also investigate a manner to stabilise VirD4$_{/TrwB}$ with the complex during the purification.

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Keywords:

T4SS, Conjugation, Structural Biology, Membrane proteins, ATPase

Poster 9

Regulation of the leukemia-related BCR protein at the membrane interface

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The BCR (Breakpoint Cluster Region) protein was proposed to be implicated in the regulaPon of Rho GTPases, a small GTPases family controlling acPn cytoskeleton dynamics, during direcPonal cell migraPon. Small GTPases act as molecular switches by cycling between an inacPve GDP-bound form in the cytosol and an acPve GTP-bound form at the membrane. This cycle is Pghtly regulated by a set of proteins including Guanine nucleoPde Exchange Factors (GEFs), which acPvate Rho GTPases, and GTPase AcPvaPng Proteins (GAPs), which turn them off by acceleraPng GTP hydrolysis. BCR is a unique protein as it contains both a GEF and a GAP domain, each adjacent to membrane-binding regions. While BCR is extensively studied as a part of the BCR-ABL1 fusion oncogene — parPcularly in lymphocytes — its naPve role remains unclear. Notably, several publicaPons have reported complex Rho GTPases regulaPon by BCR, suggesPng that it may fine-tune the acPvity of the antagonist GTPases Rac1 and RhoA [1], [2].

To clarify the BCR funcPon, we invesPgated GEF and GAP acPviPes of BCR toward Rho GTPases *in vitro* and how membrane interacPon modulates these acPviPes. FlotaPon assays showed that a truncated BCR construct (GEF region), binds strongly to negaPvely charged lipids, whereas full-length BCR exhibits weaker binding but increased specificity for PI(3,4)P2. Next, BCR acPviPes in soluPon using fluorescence-based assays reveals no GEF acPvity but strong GAP acPvity toward Rho GTPases of Rac subfamily. Moreover, the GAP acPvity toward Rac1 increases in the presence of liposomes containing PI(3,4)P2 but not with neutral ones.

Our results demonstrate that BCR lacks detectable GEF acPvity under condiPons where it exhibits GAP acPvity enhanced by the membrane associaPon. Thus, we hypothesis that the main role of the GEF region is to modulate a membrane recruitment. Furthermore, the structural characterizaPon of both soluble and membrane associated forms is in progress.

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Keywords:

small GTPase, membrane, cytoskeleton, direcPonal cell migraPon.

Poster 10

Elucidating a potential novel player in mitochondrial OXPHOS biogenesis

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Mitochondria are essential for mammalian life due to their central role in metabolism and their primary function of producing cellular energy through oxidative phosphorylation OXPHOS), which occurs in the inner mitochondrial membrane. The OXPHOS system has a dual genetic origin: thirteen core subunits are encoded by mitochondrial DNA (mtDNA), while the remaining components are encoded by nuclear DNA (1). Consequently, proper expression and maintenance of mtDNA are crucial for cellular energy balance. However, the regulation of mtDNA expression remains poorly understood (2). Mitochondria have like the prokaryotic cells no compartmentalization between the different steps of mtDNA gene expression. Additionally, mitochondrial transcription and replication are tightly linked processes, both dependent on the sole mammalian mitochondrial RNA polymerase, POLRMT (3). While certain factors are thought to influence mtDNA expression through interactions with POLRMT, the mechanisms remain unclear, and it is likely that not all POLRMT- associated proteins have been identified (2). In a multi-omics approach we identified an uncharacterized candidate mitochondrial protein, MTPPIF, which was enriched in the absence of POLRMT, suggesting a potential functional link between the two proteins. Our data indicate that MTPPIF locates to the inner mitochondrial membrane and matrix, where it may play a critical role in OXPHOS biogenesis. Furthermore, co-immunoprecipitationexperiments revealed several potential protein interactors. In this presentation, I will share our initial findings that point to a novel factor potentially involved in mitochondrial OXPHOS biogenesis.

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Keywords:

Mitochondria, mtDNA, mitochondrial gene expression, OXPHOS biogenesis

Poster 11

Rational approach guided by structural biology in the design of peptidomimetics inhibiting efflux pumps in *Pseudomonas aeruginosa*

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Inhibiting efflux pumps in the pathogen Pseudomonas aeruginosa is currently one of the most promising strategies to combat multiple antibiotic resistance. My project focuses on developing new peptide inhibitors specifically targeting RND-type efflux pumps, such as MexXY-OprM and MexAB-OprM, which play a crucial role in the resistance of P. aeruginosa.
By leveraging our combined expertise in peptidomimetic design and structural studies of efflux pumps through X-ray crystallography and electron microscopy, we are adopting a rational approach guided by high-resolution structures to design these inhibitors. These compounds will then be evaluated using biophysical interaction techniques, as well as Minimum Inhibitory Concentration (MIC) tests and efflux assays, to determine their effectiveness in blocking the pumps and restoring antibiotic sensitivity.
Moreover, considering the devastating impact of Pseudomonas aeruginosa infections in patients with cystic fibrosis, our work could pave the way for new therapies capable of overcoming antibiotic resistance. In this way, we hope to offer new treatment options and improve the quality of life for these patients.

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Keywords:

Drug design, structural biology, efflux pumps, antibiotic resistance,

Poster 12

Investigate the dynamics and molecular mechanism of a bacterial molecular motor involved in Iron Transport

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Molecular motor formed by ExbB-ExbD protein complex is located in the inner membrane of bacteria. It energizes the import of scarce nutrients (metals, vitamins, sugars) through the bacterial envelope via a specific outer membrane transporter. The energy is generated by the proton-motive force or the proton gradient of the inner membrane, and is transferred to the transporter via a third protein TonB/HasB, to open a channel allowing the nutrient entry through the transporter [1].
The complex of ExbB-ExbD and a TonB paralog HasB is involved in the heme acquisition system (Has). To import external heme being the major source of iron for bacteria such as S. marcescens, this system transfers energy by a coupled physical motion between ExbB-ExbD, HasB and transporter.
Recent studies have identified the interface between the periplasmic domain of the molecular motor and HasB, which overlaps with the region recognizing bacterial peptidoglycan [2]. However, how the ExbB-ExbD-HasB complex energizes the whole process and the role of the peptidoglycan remain unknown.
Understanding these processes necessitate a scrupulous molecular and atomic-level investigation of the interactions between proteins of the Has system and peptidoglycan, as well as their dynamics. We will use a combination of experimental approaches (NMR and Cryo-EM) and molecular dynamics (MD) simulations in a model envelope with peptidoglycan. We have simulated the sequential binding of HasB and peptidoglycan to the molecular motor, further experimental validations will follow.
The resulting knowledge could shed light on the regulation of bacterial nutrient uptake and the development of novel antibacterial strategies.

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Keywords: Molecular motor, ExbD, Has System, MD simulation

Session 3

Education

The power and peril of AI in education

David Smith

Email:

Abstract:

Promoting Awareness of Industrial Property in Biochemistry Education

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Raising awareness of intellectual property (IP), particularly industrial property, is essential worldwide. Filing patents, trademarks, or design rights protects research outcomes and fosters innovation, recognition, and financial returns for inventors. These intangible assets are valuable for both companies and academic institutions. A landmark example in biochemistry is the Cohen-Boyer patent on recombinant DNA[1]. In the U.S., inventors benefit from a one-year grace period after disclosure to file a patent, which allowed them—and Stanford University—to earn billions through licensing and royalties. In contrast, France does not offer such a grace period: any public disclosure, even oral, can prevent patent protection. This highlights the importance of educating students about industrial property early in their careers. They must understand key concepts: What is an invention? How long does protection last? What institutions govern IP—national, European, or international? How do you read the Intellectual Property Code? At the University of Bordeaux, we address these questions over 19 hours of lectures, conferences, tutorials, and practical sessions. This course is integrated into the Master's programs in "Biochemistry and Molecular Biology" and "Biomaterials and Medical Devices."

A survey of all student cohorts from 2012 to 2024 was conducted, and a quantitative and qualitative study of the data was carried out to assess the impact of this course on career choices and even on a personal level. The results will be discussed and presented.

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Keywords:

Industrial Property, Innovation, Patent, Biochemistry, Higher education

Metabolism in Motion: Rethinking Biochemistry Through Visual Storytelling

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Can the complexity of metabolic pathways become accessible—and even enjoyable—for students? In this talk, I will share the concept and pedagogical motivations behind *"24 Hours in the Heart of Metabolism"*, a biochemistry comic book designed to help learners revisit core biochemical concepts in an engaging and memorable format. By following molecules through a 24-hour journey inside the human body, the comic blends scientific accuracy with narrative immersion. I will discuss how visual storytelling fosters deeper understanding, improves retention, and reduces the intimidation often associated with biochemistry. This approach may inspire educators to rethink how we teach life's most fundamental processes.

Relevant references:

https://www.dunod.com/sciences-techniques/24-h-au-coeur-du-metabolisme-reviser-biochimie-en-bd

Keywords:

Metabolism, comics, education

Session 4

Structural biology

and

enzymes

Of filaments and condensates: the underappreciated realm of protein polymerisation

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In this talk, I will discuss noncovalent protein polymerisation as an underappreciated but potentially relatively common phenomenon. Recent research suggests that an ability to polymerise into filaments arises relatively 'easily'. It takes a small number of mutations to transform a protein that does not polymerise into a one that does, implying it can occur by chance during protein evolution and be retained if beneficial to the organism. Indeed, polymerisation is advantageous in certain contexts by clustering proteins together, thus contributing to the formation of biomolecular condensates, and by facilitating high-avidity binding to multivalent ligands such as other protein polymers or DNA. I will illustrate these topics by discussing our recent accidental discovery of protein polymerisation in a family of transcription factors called ZBTB [1], as well as other examples from our recent and ongoing work. The talk will combine structural and cell biology, as well as insights that can be obtained through bioinformatic analysis and computational structure prediction.

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Keywords:

Protein polymerisation; protein filaments; protein oligomerisation; condensate formation; transcription factors.

Structural basis of DNA crossover capture by *Escherichia coli* DNA gyrase

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DNA supercoiling must be precisely regulated by topoisomerases to prevent DNA entanglement. The interaction of type IIA DNA topoisomerases with two DNA molecules, enabling the transport of one duplex through the transient double-stranded break of the other, remains elusive owing to structures derived solely from single linear duplex DNAs lacking topological constraints. Using cryo-electron microscopy, we solved the structure of *Escherichia coli* DNA gyrase bound to a negatively supercoiled minicircle DNA. We show how DNA gyrase captures a DNA crossover, revealing both conserved molecular grooves that accommodate the DNA helices. Together with molecular tweezer experiments, the structure shows that the DNA crossover is of positive chirality, reconciling the binding step of gyrase-mediated DNA relaxation and supercoiling in a single structure.

Relevant reference:

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Keywords:

Topoisomerase, DNA topology, cryo-EM, single-molecule

Harnessing Enzyme Activities with Dynamical Structural Comparisons

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Dynamical conformational changes are essential for catalysis, enabling conversions between conformational ensembles favoring substrate binding, chemical transformation, and product release. Atomistic Molecular Dynamics (MD) simulations provide a powerful way to sample these ensembles *in silico*. However, efficiently analyzing those simulations remains a major challenge.

In this study, we extend the use of amino acid contact networks (AANs) to capture and compare the structural signatures of conformational transitions which can be linked to modulation of enzymatic activity. Comparing AANs derived from MD trajectories of *reference* and *perturbed* systems provides insights into population shifts that are critical for enzyme function.

We first applied this methodology to elucidate the allosteric pathways of a prototypical V-type allosteric enzyme: IGPS. The identified pathways are consistent with previous findings and also revealed new residues potentially important for allosteric communication. We then demonstrate the broader applicability of this approach to biological questions that can be framed as population shift problem, namely enzyme engineering.

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Keywords:

allostery, protein design, structural bioinformatics, graph theory

Inhibitor binding in rate excited states of Ras revealed by high pressure crystallography

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Pressure is an ideal tool to explore the conformational landscape of proteins, since it allows to increase the population of functionally relevant high-energy states, but rare at ambient pressure. High Pressure Macromolecular Crystallography (HPMX) is thus an ideal tool to study excited states of proteins with a high precision[1-3].
Moreover, HPMX is a powerful method to induce transitions allowing drug binding in excited states of proteins that are low-populated at ambient conditions, enabling the design of specific inhibitors. These properties will be illustrated with the example of Ras.
Ras in an oncogenic protein involved in a large number of cancers, however the development of efficient inhibitors of Ras is still challenging, since Ras proteins possess multiple conformational states.
Using HPMX, we have been able to induce an in-crystallo transition which drives Ras toward an excited state where an allosteric inhibitor targeted for this rare but functionally important state can bind, while no binding was observed at ambient conditions. We have thus obtain a precise description of the inhibitor binding site, enabling the design of more specific and efficient inhibitors[4-5].

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Keywords:

High Pressure macromolecular crystallography (HPMX); excited state; in crystallo transition; drug design

Study of the RNA Ligase Pab1020, Responsible for RNA Circularization in *archaea*

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Circular RNAs (circRNAs) are a recently discovered class of RNA molecules, abundant and conserved across all kingdoms of life. Depending on the organism, they are generated *via* non-canonical splicing or by the action of RNA ligases that covalently join the 5' and 3' ends of linear RNAs, which makes them more resistant to exonucleases and therefore enhances their stability.

Our model organism, the hyperthermophilic *Pyrococcus abyssi*, has been instrumental in elucidating the RNA circularization mechanism in archaea. Previously, our team identified **Pab1020**, a homodimeric, ATP-dependent RNA ligase, as responsible for RNA circularization in *P. abyssi*, and characterized its circular transcriptome *via* RNA-seq.

We are currently investigating the relationship between **Pab1020's atypical homodimeric structure** and its catalytic mechanism. To better understand the role of dimerization and different protein domains in catalysis, we performed ***in vitro* RNA circularization assays** using a range of **Pab1020 catalytic and structural mutants**. Additionally, our **cryo-EM study** (in collaboration with BIOC, École Polytechnique) has revealed conformational changes upon RNA binding. Ongoing analyses of new **RNA-Pab1020 structures** aim to further elucidate the RNA circularization mechanism.

To elucidate processes related to circRNAs, we have also identified Pab1020's protein partners through *in-cellulo* immunoprecipitation and mass spectrometry. These analyses revealed interactions with components of the RNA degradation machinery, suggesting a link between RNA circularization and degradation, and challenging the view of circRNAs solely involved in RNA stability. To explore this further, we are conducting protein localization studies *via* immunofluorescence and *in vitro* pull-down assays to map these interactions in greater detail.

Keywords:

RNA ligase, circRNAs, Pab1020, archaea

The IDR of PAP Acts as a Molecular Ruler to Guide CFIm25 in mRNA Polyadenylation Site Selection

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Human polyadenosine polymerase alpha (PAP α) plays a crucial role in the maturation of 3'end pre-mRNA by catalysing the addition of a polyadenosine (poly-A) tail, which provides mRNA stability, facilitate nuclear export and translation efficiency. PAP α co-operates with the cleavage and polyadenylation (CPA) machinery and mediates regulation of mRNA poly-A tail length, directly impacting gene expression. Aberrations in PAP α function lead to defective mRNA maturation, contributing to pathological conditions, including cancer 1 2. Yet, no inhibitors of PAP α exist to date and how PAP α assembles with the CPA machinery to promote pre-mRNA cleavage and polyadenylation remains unclear. Here, I will present a detailed characterization of full-length human PAP α, including the N-terminal catalytic core and the C-terminal intrinsically disordered region (IDR). We unveil structures of PAP α, including a complex with CFIm25, a key protein for i) the selection of mRNA polyadenylation sites; and ii) regulation of alternative polyadenylation 3, a process heavily misregulated in cancer 4. Our data show how the IDR of PAP α telescopically links CFIm25's recognition of the upstream UGUA pre-mRNA motif with the downstream polyadenylation site used by the catalytic core of PAP α, providing a physical explanation to the preferential selection of distal polyadenylation sites.

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Keywords:

Polyadenylation, Poly (A) polymerase, IDR, Alternative polyadenylation, CFIm25

Poster 13

Structural and functional characterization of recurrent oncogenic R1523H/C mutations in the AWS domain of in SETD2 methyltransferase

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SETD2 is the sole histone methyltransferase responsible for the trimethylation of histone H3 at lysine 36 (H3K36me3), a key epigenetic mark linked to transcriptional regulation and genome integrity. SETD2 functions as a tumor suppressor and is frequently mutated in various cancers. However, the molecular and structural consequences of these mutations remain poorly understood. While most cancer-associated mutations cluster within the catalytic SET domain, mutations have also been identified in the upstream "Associated With SET" (AWS) domain. The AWS domain is a Zn^{2+}-coordinating structural motif located N-terminal to the SET domain and contributes to the formation and stability of the catalytic core.
We investigated the oncogenic R1523C and R1523H mutations within the AWS domain using biochemical and cellular approaches. Both mutant proteins were successfully expressed and purified from E. coli, albeit with reduced yields compared to the wild-type enzyme. In vitro H3K36 methyltransferase activity, assessed by Ultra-Fast Liquid Chromatography (UFLC) and western blotting, was markedly diminished for both mutants. Thermal shift assays and SDS-PAGE analyses indicated reduced protein stability and increased aggregation propensity.
In SETD2-knockout HEK293T cells, expression of the R1523C/H mutants failed to restore global H3K36me3 levels, consistent with loss of enzymatic function. Additionally, both mutant proteins formed nuclear, ubiquitinated aggregates.
These findings indicate that the R1523C/H mutations impair both the enzymatic activity and structural stability of SETD2, resulting in loss-of-function variants. Ongoing X-ray crystallographic studies aim to elucidate the structural basis by which these cancer-associated mutations disrupt SETD2 function.

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Keywords:

SETD2, H3K36me3, methyltransferase activity, oncogenic mutations, structure, epigenetics

Poster 14

Interplay between mRNA translation and decay in Archaea

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Archaea are critical research models for gaining insights into the origin of the eukaryotic cell and the evolution of life, as well as for studying biogeochemical processes (1). Post-transcriptional regulation of gene expression requires accurate and timely RNA processing and decay to ensure coordinated cellular behaviors and fate decisions. Therefore, understanding RNA metabolic pathways and identifying RNA processing machineries are major challenges in RNA biology. Currently, the best-understood RNA-dedicated pathways at the molecular level are those of Bacteria and Eukarya. In contrast, in Archaea, these molecular processes have been overlooked and are far from being understood.

Proteomic landscapes, direct protein–protein interaction analyses and phylogenomic data support that aRNase J, a 5'-3' exoribonuclease of the β CASP family conserved in Euryarchaeota, engages specifically with a Ski2-like helicase and the RNA exosome to potentially exert control over RNA surveillance, at the vicinity of the ribosome (2). In Eukarya and Bacteria, as a general common thread, both mRNA decay and translation are intimately coordinated (3, 4). While aRNase J homologs are found in bacteria, the RNA exosome and the Ski2-like RNA helicase have eukaryotic homologs, underlining the mosaic aspect of archaeal RNA machines.

Our current work aims to study the aRNaseJ/Ski2-like helicase/exosome/ribosome complex by cryo-EM after *in vitro* reconstitution.

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Keywords:

Archaea, RNA, Decay, Translation, Cryo-EM

Poster 15

Studies of recurrent PTPN2 phosphatase mutations identified in human cancers

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PTPN2 (protein tyrosine phosphatase non-receptor type 2) is an enzyme belonging to the PTP family which regulates the level of phosphorylation of protein tyrosine residues in various signalling pathways. In tissues, PTPs and their respective substrates form a complex signal transduction network. This network controls various cellular processes such as the cell cycle and cell migration and is implicated in the development of cancer pathologies.
The aim of this project is to characterize, at molecular, cellular and structural levels, recurrent PTPN2 mutations identified in human tumors and affecting either the catalytic domain (F195L) or the regulatory domain (R350W/R350G). This regulatory domain and its role in modulating the enzyme's activity have been identified very recently and similar to what has been observed with the SHP2 tyrosine phosphatase, it will be important to determine the existence of oncogenic loss- and gain-of-function mutations affecting these different domains.
Preliminary results already shows that the F195L mutation decreases the catalytic activity of the phosphatase as measured by pNPP assay without altering the protein's nuclear localization.
The structure of the F195L mutations is currently being resolved since a resolution of 2.3 Å has been obtained by crystallography. This is the second structure of PTPN2 obtained in our laboratory. Given the limited structural data available on PTPN2, this study will enhance our understanding of its catalytic and regulatory mechanisms.
Furthermore, it will shed light on the pathological consequences of tumor-associated mutations and support the evaluation of PTPN2 as a potential therapeutic target.

Keywords:

PTPN2, Signaling, enzymatic activity, structure.

Poster 16

Study of p53 regulation by ribosomal proteins

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Ribosome biogenesis is a complex and conserved cellular process. One of the early steps of ribosome biogenesis is the assembly of the 5S ribonucleoprotein particle (5S RNP) to the large ribosomal subunit. In eukaryotes, the 5S RNP is composed of 5S rRNA, ribosomal proteins L5 and L11, and assembly factors Rpf2 and Rrs1 [1]. Before its assembly, 5S rRNA is transcribed in the nucleus. Transcription factor IIIA (TFIIIA) is specifically required for transcription of 5S rRNA genes [2]. Rpf2 and Rrs1 are essential proteins involved in the assembly of the 5S RNP and these two assembly factors function as a chaperone complex, guiding the incorporation of 5S RNP into the ribosome [3]. Cancer cells are associated to strong ribosome production to sustain their proliferation rate. Disassembly or failure in 5S RNP can lead to nucleolar stress (NS), triggering p53-dependent and independent response pathways leading to cell cycle arrest and/or apoptosis. This occurs because the disrupted 5S RNP binds to Mdm2, preventing Mdm2 from degrading p53 [4]. In parallel, SURF2 is a protein involved in cell membrane trafficking and regulation. It's overexpression in most cancers and recent data demonstrate that SURF2 interacts with free-5S RNP particles and can modulate its activity. SURF2 competes with MDM2 to bind with free-5S RNPs, and their alternative binding can modulate the cell response to nucleolar stress [5].

Superposition of the structures of TFIIIA and Rpf2 bound to the 5S rRNA shows that the binding surfaces of the two proteins overlap on the RNA [6]. The TFIIIA and Rpf2-Rrs1 proteins will be expressed and purified and then use fluorescence polarization anisotropy (FPA) to explore and compare their binding affinity with 5S rRNA. With this result we'll understand the regulation of ribosome biogenesis process linked to p53 regulation pathway. The next step will consist in the screen and the design of small molecules to inhibit the binding of Rpf2-Rrs1 complex with 5S rRNA.

With the SURF2 protein I'll do structural biology to solve the SURF2-h5S rRNA structure, employing cryo-electron microscopy to further explore the structure to develop potential therapeutic drugs for p53-related cancers.

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Keywords:

Ribosome biogenesis, 5S RNP, Rpf2-Rrs1, TFIIIA, SURF2

Poster 17

Identification and characterization of a potential CoM transporter

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Methanogens are anaerobic Archaea that generates methane as a by-product of their energy metabolism. Different methanogenesis pathway have been described and all depends on an enzyme complex called the methyl coenzyme M reductase (MCR). This enzyme reduces the methyl associated with the coenzyme M (CoM) cofactor to methane. Therefore, CoM is an essential cofactor for methanogens and needs to be either produced or imported. Not all methanogens have the pathway for CoM synthesis and thus depends on an external source of CoM, but how CoM is imported is still unknown. Bioinformatic and phylogenetic studies allowed us to identify uncharacterized genes associated with methane metabolism and coding for a potential CoM transporter in methanogens. This cluster of genes codes for an ABC transporter composed of three proteins: an ATPase, a transmembrane protein and a ligand-binding protein. We tentatively named these genes *cmtABC* (for coenzyme M transporter).
The aim of this study is to confirm the role of these proteins as CoM transporter. First by expressing the CmtABC of *Methanobrevibacter ruminantium* in *E. coli* to test its activity. Second, by testing if the ligand binding protein (CmtA) is specifically binding CoM by comparing the quenching of its fluorescence in presence of CoM and of sulfonate analogues. Using this approach, we validated that CmtA was binding to CoM with a good specificity compared to another sulfonate. Finally, using AlphaFold3 we have modelized the structure of CmtA together with CoM and determined amino acid conserved in all CmtA, potentially controlling the specificity.

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Keywords:

Methanogens, Ligand-protein interaction, Coenzyme M, ABC transporter

Poster 18

Development of RioK1 kinase inhibitors

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Ribosome biogenesis is a highly regulated, energy-intensive, and sequential process that enables the assembly of functional ribosomal subunits. This complex pathway initiates in the nucleolus and concludes in the cytoplasm, involving over 200 assembly factors. [1] Among these, the atypical kinase RioK1 plays a critical role during the final maturation steps of the 40S ribosomal subunit, facilitating its proper assembly and final maturation. Specifically, RioK1 contributes to the processing of pre-ribosomal RNA into mature ribosomal RNA through the action of the endonuclease Nob1. Following this cleavage, RioK1 undergoes autophosphorylation, triggering conformational rearrangements within the small subunit that ultimately lead to the release of Nob1 and Pno1, finalizing 40S subunit maturation. [2]
Previous studies have shown that RioK1 is overexpressed in cancer such as colorectal and prostate cancer. However, deletion of RioK1 results in reduced proliferation, migration/invasion, colony formation ability, and metastasis formation in various cancer cell lines. It has also been demonstrated that RioK1 is involved in the negative regulation of p53 protein stability in colorectal cancer cells. Therefore, RioK1 may represent a novel potential target for inhibiting ribosome biogenesis in cancer cells. [3-5]

The aim of my PhD is to design and test small molecules capable of inhibiting ATP binding to RioK1. The structure of RioK1 has already been resolved in the presence of ATP, its natural ligand, revealing key interactions within the active site pocket. To enable the testing of novel compounds, I have characterized and optimized the conditions for the expression and purification of RioK1 for use in both functional and structural biology studies. With the purified protein, I will perform X-ray crystallography, and protein–ligand interactions will be investigated using tryptophan fluorescence quenching and thermal shift assays (TSA), which will allow the assessment of binding affinities and protein stability upon ligand binding.

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Keywords:

Ribosome biogenesis, RioK1, enzyme inhibition

Poster 19

Quinone Biosynthesis and Earth's Oxygenation: An Evolutionary perspective

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The Great Oxidation Event (GOE) corresponds to an irreversible rise in the concentration of free oxygen about 2.4 billion years ago. In response, bacteria adapted their energetic metabolism, notably through the diversification of isoprenoid quinones (hereafter simply referred to as quinones), which play a crucial role in transferring electrons within respiratory and photosynthetic chains. Quinones can be classified according to their mid-point redox potential, as low potential (LP) such as menaquinone (MK) or high potential (HP) quinones such as ubiquinone (UQ) or plastoquinone (PQ). LP quinones are usually considered more ancient and mostly involved in anaerobic processes, whereas HP quinones are thought to have emerged to cope with rising O_2 levels. However, our team's discovery of an alternative O_2-independent pathway to produce UQ has challenged some hypotheses (Pelosi et al., mBio 2019).

We used phylogenetic and comparative genomic methods to annotate and trace the evolution of quinone biosynthetic pathways. Our results suggest that the O_2-independent pathway is the ancestral pathway for UQ production. We demonstrate that the common ancestor of the bacterial phylum *Pseudomonadota* relied only on UQ for its energy metabolism and reacquired LP quinones such as MK later on through horizontal transmission (Chobert et al., ISME Journal 2025). In addition, we observe that the quinone repertoire is linked to the O_2 requirement of *Pseudomonadota*. We propose that the ability of *Pseudomonadota* to adjust their energy metabolism by modulating their quinone repertoire contributed to the evolutionary success of this phylum.

In addition, a "new" quinone, the methyl-plastoquinone, was recently discovered in the bacterial phylum *Nitrospirota*. We were able to partially characterize its biosynthetic pathway based on its evolutionary connections with UQ and PQ biosynthetic pathways and experimental validations (Elling et al., PNAS 2025). Overall, these findings prompt us to reconsider the question of the origin and timing of evolution of quinones, particularly in relation to Earth's oxygenation.

Poster 20

Cathepsin V and legumain: new players in ECM accumulation during human lung myofibrogenesis

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Idiopathic pulmonary fibrosis (IPF) is a chronic and lethal disease characterized by irreversible fibrous thickening in the interstitial spaces of the lung. Alterations in alveolar epithelia induce TGF-β1-dependent differentiation and proliferation of fibroblasts into myofibroblasts. Extracellular matrix (ECM) overproduction by myofibroblasts causes a dysregulated remodeling in fibrotic foci, associated with an altered proteolytic balance [1].

Among proteases involved, some cysteine cathepsins are potent collagenases and elastases participating in ECM remodeling. During IPF, the cathepsin/cystatin (i.e., their endogenous inhibitors) balance is impaired in favor of cystatins; especially, the secretion of human Cystatin C (hCC), a fibrotic biomarker, is upregulated during myodifferentiation, promoting collagen deposition [2-4].

In addition, expression levels of both fibronectin and elastin are altered during fibrogenesis. Accordingly, this led us to investigate the involvement of Cathepsin V (CatV, the most potent human elastase) [5] and Legumain (LGMN) (a.k.a., Asparaginyl endopeptidase) in ECM degradation [6] and of Cystatin M/E (CysM) (a dual tight-binding inhibitor of both enzymes) [7] in the differentiation of human lung fibroblasts. Increased expressions of LGMN and CysM were observed in lung biopsies (n=26) and bronchoalveolar lavage fluids (n=18) from patients with IPF, while CatV decreased. Similar transcriptional and translational regulations were found in a model of TGF-β1-dependent myodifferentiation (human lung primary fibroblasts).

Deciphering molecular mechanisms sustains that the overproduction of CysM (besides hCC) could mitigate enzymatic activities of CatV and LGMN. Consequently, impairment of the proteolytic balance during myofibrogenesis leads to the buildup of fibronectin and elastin, promoting ECM deposition and fibrotic phenotype.

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Keywords:

Extracellular matrix, Cysteine proteases, Cystatins, Pulmonary fibrosis, Myofibroblasts

Poster 21

Myeloperoxidase: from fundamental to applications

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Mono- and multi-species biofilms are responsible for a wide range of chronic infections that are difficult to eradicate and significantly impact patients' quality of life and mortality rates. Consequently, there is a strong interest in generating a broader spectrum of antimicrobial activity directly at the site of infection, without inducing microbial resistance.

Myeloperoxidase, a member of the heme-dependent peroxidase superfamily, not only converts classical peroxidase substrates into radical intermediates but also possesses the unique ability to catalyze the formation of hypochlorous acid from hydrogen peroxide and chloride ions (Eq. 1). This reaction underlies the antimicrobial activity of bleach:

Eq. 1: $H_2O_2 + Cl^- + H_3O^+ \rightarrow HOCl + 2H_2O$

Various strategies have been employed to investigate the properties of bacterial myeloperoxidase[1-2]. The results include studies on enzyme production and purification, enzymatic and biophysical characterization, as well as microbicidal assays. Coupled enzymatic systems were compared to a chimeric enzyme in which two catalytic domains are covalently linked. The role and significance of the linker used in such chimeric constructs will also be discussed.

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Keywords:

Myeloperoxidase, chimera, linker, hypochlorous acid, fight infection

Poster 22

Leaderless mRNA translation initiation in *Deinococcus deserti*

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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*.

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Keywords:

biochemistry; cryo-EM; ribosomes; bacteria

Poster 23

Corynebacterium glutamicum as a surrogate for the discovery of new anti-mycobacterial compounds

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Despite global efforts, tuberculosis (TB) remains the leading cause of death from infectious disease, with 1.3 million deaths reported in the latest WHO report, including 167,000 among people with HIV. Multidrug-resistant TB (MDR-TB) is a major public health issue and security threat. Eradicating TB requires a better understanding of *Mycobacterium tuberculosis* (*Mtb*) biology, identification of new therapeutic targets, and the discovery of novel inhibitors with new mechanisms of action (MOA), as well as the development of biochemical screens mimicking the in vivo environment.
In our lab, we aim to develop a unified framework to identify new anti-TB compounds using *Corynebacterium glutamicum* as a non-pathogenic model organism. *C. glutamicum* shares key features with *Mtb,* including a complex cell wall that limits antibiotic permeability. We are establishing a whole-cell screening assay to directly test candidate compounds on this model. As a proof of concept, we focus on DNA gyrase, a validated and essential target for *Mtb*[1]. DNA gyrase is a type IIA topoisomerase involved in DNA topology regulation and is required for bacterial survival.
Our preliminary results show that *Mtb*-targeting gyrase inhibitors also affect *C. glutamicum,* inducing a characteristic morphology[2]. These phenotypic changes can be exploited in our assay to identify active molecules. To further understand their MOA, we are conducting structural analyses using cryo-electron microscopy, which allows us to visualize drug–target interactions at atomic resolution. This integrative approach aims to link structural insights with cellular phenotypes, accelerating the discovery of new anti-TB agents.

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Keywords: Microbiology, Cryo-EM, Drug discovery, Tuberculosis, Antibiotic-Resistance

Session 5

Synthetic

&

chemical

biology

Molecularly Imprinted Polymer Nanogels: Synthetic Mimics of Peptide Antibodies for Biomedical Diagnostics and Therapy

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Molecularly imprinted polymers (MIPs) [1] are synthetic antibodies that specifically recognize molecular targets. They are cross-linked polymers synthesized in the presence of a molecular template, which induces three-dimensional binding sites in the polymer that are complementary to the template in size, shape and chemical functionality. MIPs against proteins are obtained through a rational approach starting with *in silico* epitope design. Chemically synthesized peptide epitopes can then be used as templates in a solid-phase protocol for MIP synthesis [2,3]. We demonstrate the potential of MIP nanogels (~50 nm) for diagnostics, bioimaging [4] and medical therapy, on the example of cell surface protein targets [4], as well as soluble cytokines [5].

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Stable SAM Analogues as Promising Scaffolds for RNA Methylation Inhibition

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Methyltransferases (MTases) are enzymes that methylate biomolecules like proteins, DNA, RNA, lipids, and small molecules, mostly using S-adenosyl-L-methionine (SAM) as a methyl donor. MTases have emerged as promising drug targets, and SAM analogues are widely employed to investigate their involvement in diseases and to develop effective drug therapies. We designed and synthesized stable SAM analogues with a squaramide moiety mimicking the methionine side chain. These compounds were tested on the two human m^{6}A RNA MTases METTL3/14 and METTL16. While these SAM analogues failed to support catalytic activity, they exhibited potent inhibitory effects on the METTL3/14 activity. Surprisingly, some of these compounds demonstrated remarkable specificity, likely attributed to the unique properties of the squaramide motif. Docking studies showed they bind METTL3/14 cofactor pocket similarly to SAM, allowing us to make new hypothesis on the catalytic mechanism. Our synthetic method expands the structural diversity of SAM analogues, providing a foundation for developing selective RNA MTase inhibitors.

Keywords:

RNA methylation, Methyltransferases, m^{6}A modification, SAM analogue**,** METTL3 inhibition.

Chemogenetic spectral modulation of bioluminescence for imaging and sensing

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Luciferases have become essential tools in biotechnology, bioimaging and biosensing. Because of its small size (19 kDa) and high brightness, the luciferase NanoLuc, engineered from the deep-sea shrimp Oplophorusgracilirostris, has become the most popular bioluminescent reporter for a variety of applications. NanoLuc reacts efficiently with furimazine leading to the efficient emission of blue light. The emission maximum of the luciferase Nanoluc has been pushed to higher wavelength more suitable for biological imaging by fusion to fluorescent proteins or self-labeling tags (e.g. HaloTag) enabling bioluminescence resonance energy transfer (BRET), resulting however in a significant increase of its size. Here, we present the engineering of LumiFAST, a fusion of Nanolucand pFAST, a small protein tag (14kDA) that can form tunable fluorescent assemblies with fluorogenic chromophores. Thorough optimization of the fusion topology and linker resulted in up to 90% BRET efficiency, allowing us to push the spectral properties of Nanoluc to higher wavelength while keeping the overall size of the luciferase reasonable. The spectral tunability of pFAST through chromophore change allowed us to optimize the BRET efficiency and generate chimeric luciferase with tunable emission from green to red. We demonstrate that LumiFAST is well suited for multicolor bioluminescence-based cell imaging and for imaging deep into tissues. Our understanding of the parameters leading to high BRET efficiency between NanoLuc and FAST allowed us furthemore design new biosensors and monitor protein-protein interactions.

Keywords:
Bioluminescence, Chemogenetics reporters, BRET, Imaging, Biosensing

Antibody targeting the anti-parallel topology of human telomeric G-quadruplex DNA

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G-quadruplexes (G4s) are four-stranded nucleic acid structures that have gathered a significant attention due to their involvement in key biological processes, including gene regulation, genome stability, and telomere maintenance. Some G4 antibodies have been developed to selectively recognize these structures over duplex DNA; however, most, even the widely studied BG4 and 1H6, bind G4s in a general manner and lack discrimination between distinct topologies, particularly between parallel and antiparallel conformations. In this study, we report the development and characterization of a novel antibody selected via phage display using a constrained antiparallel G4 structure mimicking one of the conformation adopted *in vitro* by the human telomeric sequence. Our findings demonstrate that this new antibody selectively recognizes the antiparallel topology of the telomeric G4 sequence, a property further validated in cellular models.

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BIORXIV/2025/653741;doi: https://doi.org/10.1101/2025.05.13.653741

Keywords:

Antibody, phage-display, G-quadruplex, human telomeric DNA, antiparallel topology

Engineering artificial repeat proteins towards modular assemblies and cellular tools

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A family of artificial repeat proteins, named αRep, was designed from the concatenation of a consensus helical motif with 5 hypervariable positions. αRep proteins from the highly diverse library (10^9 variants) present a concave diversified binding surface and a variable number of repeats. αRep proteins are easily produced and highly stable[1]. Using phage display methods, it is possible to select protein binders with high selectivity and affinity for a variety of protein targets (K_D from nM to µM)[2]. These proteins can be easily produced and purified with high yields, genetically engineered in multidomain proteins, functionalized with chemicals or nanoparticles and functional in living cells. Applications of these artificial proteins have been explored in various contexts through collaborations with biologists, chemists and physicists. αRep can be used as crystallization helpers for structural biology[3], intracellular tracers in living cells[4,5] or antiviral nanoligands[6]. As compared with molecular Lego® bricks, αReps can also be engineered into modular functional assemblies[7].

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Poster 35

Versatile & comprehensive microplate assay to screen inhibitors of human sialyltransferases

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Aberrant cellular glycosylation has been established as a hallmark for the development and progression of several human pathologies including immune dysregulation, and tumour metastasis. Alteration in the expression of cell surface sialylated glycans, known as Tumour Associated Carbohydrate Antigens (TACAs), is widely observed among the glycosylation changes that occur in cancer

We are interested in human sialyltransferases (STs), particularly ST6GalI and ST6GalNAcI, for their roles in aberrant glycosylation in cancer. While ST6Gal1 catalyzes the transfer of Neu5Ac residue onto Gal residue on lactosamine on N-glycoproteins, the ST6GalNAc1 catalyzes the transfer of Neu5Ac onto the GalNAc residue found on mucin type O-glycoproteins to generate sialylTn (sTn) antigen.

We had developed a microplate-based sialyltransferase assay (MPSA) (1) that is rapid, sensitive, and can be used to assay the activity and kinetics of STs. CMP-SiaNAl, an alkynyl functionalised sialic acid donor, is used for the sialylation reaction followed by a bioorthogonal labelling step. We expanded this assay to thirteen mammalian STs and determined their optimum conditions of activity and kinetic parameters (2).

We have used the same assay to screen for inhibitors against STs. This assay allows the comparison of inhibitors potency on different STs in conjunction, which enables the identification of specific and selective inhibitors for each of them. We demonstrate the use of this assay for the determination of inhibition kinetics parameters (such as Ki, IC50) and the type of inhibition.

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Keywords:

Glycosyltransferase, sialyltransferase, inhibitor, microplate assay

Session 6

Plants

My sweet tuber: characterization of the molecular basis of the cold-induced-sweetening in potato

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After harvest, potato tubers are stored in the cold to mimic winter conditions, during which germination is stopped. Nevertheless, this method of storage induces a physiological response known as "Cold-Induced Sweetening" (CIS), corresponding to an increase in tuber soluble sugar content caused by partial starch degradation. Physiologically, the accumulation of soluble sugars in the tuber at low temperatures serves two purposes: to protect cell structures from freezing, and to rapidly lift dormancy during germination. However, this phenomenon is undesirable when applied to tubers kept in the cold after harvest, as it promotes the so-called Maillard reaction between reducing sugars and amino acids when the tubers are fried, producing acrylamide among other detrimental molecules.

CIS is a complex, multifactorial trait, and current means of combating it are unsatisfactory. The strategy relies on transgenic varieties in which the expression of enzymes involved in starch and/or sucrose metabolism is altered with mitigated results when grown in the field. Some potato varieties have been bred for their relative resistance to CIS, but the physiological basis of this resistance is unknown, and these varieties are not necessarily suited to all desired uses. Our aim is to determine the physiological basis of this resistance using a non-targeted, global approach based on establishing the proteome and metabolome of different potato varieties using FT-ICR mass spectrometry. The data thus generated, combined with those already available in the literature, will provide a better understanding of the metabolic pathways involved in CIS.

Keywords:

Proteomics, Metabolomics, Potato tubers, Starch, Cold-induced sweetening

The hexameric atypical thioredoxin from poplar, DCC1, possesses a redox holdase activity

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Photosynthetic organisms contain more than 150 proteins belonging to the thioredoxin (TRX) superfamily. Many possess one or two redox-active cysteines and a characteristic *cis*-proline at defined positions, along with additional domains or secondary structures. We have identified a new set of TRX-like proteins in photosynthetic organisms, with unknown functions[1]. Among these, phylogenetic studies have identified a distinct family called DCC, characterized by a conserved DXXCXLC motif and a specific C-terminal extension. We have performed the biochemical and structural characterization of DCC1 from poplar and Arabidopsis, one of the three DCC proteins existing in terrestrial plants. Our results indicate that full-length recombinant DCC1 proteins form primarily high order oligomers, which in the case of poplar DCC1 are hexamers as assessed by light scattering[2]. This multimeric conformation correlates with the existence of redox-dependent holdase activity while no or poor oxidoreductase activity have been detected. Indeed, truncated versions of DCC1 devoid of the C-terminal extensions have lost holdase activity. Moreover, truncated and mutated versions mimicking the classical active site motifs present in typical TRX or GRX did not acquire conventional oxidoreductase activity, despite the cysteines are reactive and form an intramolecular disulfide bridge. These results point to the existence of redox chaperones (chaperedoxins) belonging to the TRX superfamily in plant organelles.

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Keywords:

Plants, atypical thioredoxin, chaperone, redox, holdase.

Rhizophagus irregularis DAOM197198 modulates the root ubiquitinome of Medicago truncatula in the establishment and functioning of arbuscular mycorrhizal symbiosis

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The regulation of cellular protein homeostasis involved the ubiquitin-proteasome system (UPS) by selectively targeting misfolded or end-of-life proteins. The involvement of the UPS in biotic stresses has been studied mainly in plant-pathogen interactions and poorly in plant-mutualistic interactions. Here, we studied through proteomic approaches (western blot, pull-down of polyubiquinated proteins and nano-LC-MS-MS analysis), the involvement of the UPS during the establishment of the mutualistic interaction between the arbuscular mycorrhizal fungus *Rhizophagus irregularis* DAOM197198 and the roots of *Medicago truncatula*, as well as in the established symbiosis. Roots of *M. truncatula* seedlings were harvested 0 h, 3 h, 6 h, 9 h, 12 h, 24 h and 15 days post-inoculation. We characterized a short-time and a-long-time response of the root ubiquitinome. Some proteins as flotilins or involved in the translational machinery were less-ubiquitinated, suggesting the facilitation of the *de novo* synthesis of proteins required to the establishment of arbuscular mycorrhizal symbiosis. Other proteins as transporters involved in plant nutrition through the direct pathway (i.e., MtPT5) and some enzymes involved in the lipid and sterol biosynthesis pathways were more-ubiquitinated, suggesting their degradation. In addition, western blot analysis revealed that Cdc48 proteins accumulates in roots from 9 to 24 h post-inoculation as in plant-pathogen interaction, suggesting a similar role of Cdc48 in the transitory immune response during plant-fungal interactions. The activity of the UPS is central in the establishment and functioning of arbuscular mycorrhizal symbiosis by modulating protein ubiquitination.

Keywords:

Mycorrhizal symbiosis, Medicago truncatula, Rhizophagus irregularis DAOM197198, Ubiquitin-Proteasome System, ubiquitinome.

Methylation of ribosomes and translation in Arabidopsis.

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Plant development is determined by critical transitions steps, which are controlled in response to environmental cues. Among these transitions, seedling establishment and flowering are crucial. Both require rapid reprogramming of gene expression to produce specific effector proteins. Herein, translation is a key level of control, regulating the translation efficiency of specific mRNA and the assembly and activity of ribosome. Ribosomes, and more specifically ribosomal RNA (rRNA), are subject to several modifications, including methylation of sugars (2'-*O*-Me), isomerization of uridine to pseudouridine (Ψ) and/or methylation of purine and pyrimidine rings (m1N, m6N; m5N and m7N), in addition, rRNA pyrimidine rings are also aminocarboxypropilated (acp3N) and/or acetylated (ac4N).
The 2'-*O*-Meisa 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 in the C/D snoRNA ribonucleoprotein complex (C/D snoRNP). Previously, we reported the mapping of 117rRNA 2'-*O*-Me sites in *Arabidopsis thaliana* and the identification of most of the corresponding C/D snoRNAs [1]. Here, we show that the 2'-*O*-Me of specific rRNA sites is dynamically regulated during development of *Arabidopsis thaliana* plants. However, 2'-*O*-Me hypomethylation of rRNA seems to be independent of snoRNP expression. We also show that 2'-*O*-Me hypomethylation of ribosomes causes an accumulation of polysome and affects ribosome loading and translation. Our results highlight the importance of rRNA modifications and suggest that ribosome heterogeneity plays an important role in orchestrating ribosome assembly and translation.

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Keywords:

2'-O-Methylation; C/D snoRNA; rRNA; Fibrillarin; rRNA modifications; *Arabidopsisthaliana*

Poster 24

Physiological analysis of *selU2* mutants in *C. reinhardtii* under salt stress

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Thioredoxins (TRX) are ubiquitous oxidoreductases characterized by a CxxC motif containing two cysteines capable of forming a disulfide bond within a thioredoxin fold. In photosynthetic organisms, thioredoxins play key roles in regulating various metabolic and stress-related pathways, particularly in the chloroplast. A predicted chloroplast-localized protein, SELU2, has been annotated as TRX-like, although its function remains unknown. Interestingly, its predicted structure resembles thiol peroxidases, specifically the AhpC_TSA2-like domain[1]. SELU2 is conserved in the green lineage, yet no biochemical characterization has been performed on any of its paralogs. In the unicellular alga *Chlamydomonas reinhardtii*, SELU2 is a selenoprotein with a unique UxxC motif, in which the first cysteine of the CxxC motif is replaced by selenocysteine ("U"). This motif is located in the first α-helix and is surface-exposed, suggesting possible catalytic or interaction roles. Given its atypical active site and chloroplastic localization, SELU2 may contribute to specific redox processes related to environmental stress. However, previous biochemical studies have not clarified a possible reductase function. To explore SELU2's physiological role, we used *Chlamydomonas reinhardtii* insertional mutants on SelU2 gene, obtained from the Chlamydomonas Resource Center. These strains were grown photoautotrophically on rich medium under both control and salt stress conditions. We conducted phenotypic analyses including cell growth, viability, and cell size. In addition, carbon metabolism is being investigated through primary metabolite and carbon storage compound analysis. This study aims to reveal SELU2's potential role in stress adaptation and redox/carbon metabolic regulation.

Reference:

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Keywords:

Thioredoxin, Selenoprotein, Carbon metabolism, Salt stress, *Chlamydomonas reinhardtii*

Poster 25

Search for ligands of plant osmosensor: toward application as biostimulant

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Histidine kinases are membrane receptors involved in a multistep phosphorelay signaling pathway, widely represented in bacteria, known as two-component system, in yeasts and plants. In poplar, in response to drought, the generated osmotic stress seems to be perceived by two Histidine Kinases, HK1a and HK1b. In order to understand the molecular mechanism of this pathway in drought tolerance, this study aims to identify the possible ligands that bind to these receptors. A sequence analysis of these receptors revealed a Cache domain in the extracellular part that is well conserved among numerous plant species. Such domains are known as small-ligand binding domain and comprise the largest superfamily of extracellular sensors in prokaryotes. The well conservation of this domain in osmosensor from yeast to plant may represent a functional importance in osmosensing that led us to hypothesize that this Cache domain is the binding domain of a ligand yet to be determined.
Furthermore, the creation of a fluorescent yeast osmotic biosensor allowed us to screen 24 phytoextracts and identified two positive extracts that may contain HK1s ligands. These extracts could be used for application in agriculture as biostimulant. Assays on plants have been conducted with the two selected leaf extracts and the results demonstrated a drought tolerance enhancement for two plant species.
Finally, the production of these Cache domains have been performed in yeast *S. cerevisiae* and *P. pastoris*. The purified proteins will be used to resolve their 3D structure and will help to guide the ligand finding by docking experiments.

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Keywords:

Histidine-aspartate kinase, yeast biosensor, osmosensing, biostimulant

Poster 26

Functional characterization of chloroplastic FBPases from the alga *Klebsormidium nitens*

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Fructose-1,6-bisphosphatase (FBPase) is a key enzyme in carbohydrate metabolism in all living organisms. In plants, it exists in three isoforms: a cytosolic form (cyFBP) and two chloroplastic forms (cpFBPI and II). CpFBPI plays an essential role in the Calvin cycle, a fundamental process in photosynthesis. It contains a regulatory region with two cysteine residues that enable redox regulation. In the dark, a disulfide bridge forms between the cysteines, rendering the protein inactive. Under light conditions, this bridge is reduced, activating the protein. Recently, a transient regulation involving nitric oxide has been proposed for cpFBPI, although this mechanism remains to be further investigated (Serrato et al 2018). The second chloroplastic isoform, cpFBPII, is less well characterized and, in most green lineage species, lacks a regulatory region comparable to cpFBPI.
In our model*, Klebsormidium nitens*, a charophyte alga, two cpFBPases have also been identified on the basis of genomic and transcriptomic data. However, cpFBPII, contrary to published data in terrestrial plants, has a region including two cysteines, similar to that of cpFBPI, and is therefore likely to be redox-regulated.
After cloning the gene for the two *K. nitens* cpFBPs, the proteins were produced in a heterologous system and purified. Initial experiments verified the functionality of the two cpFBPs by measuring enzymatic activity. The aim of my work is now to characterize the regulation of *K. nitens* cpFBPI by nitric oxide and to assess whether cpFBPII, because of its particular structure, is also subject to redox and/or nitric oxide control.

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Keywords:

Fructose-1,6-bisphosphatase; redox regulation; nitric oxide regulation

Poster 27

How monolignol availability impacts lignin acylation in poplar?

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Lignins are key components of the plant secondary cell wall. Their structure varies widely among species due to differences in the proportions of their two main monomers, the G and S units. In certain botanical families, the incorporation of acylated monomers further modifies lignin structure. This acylation is catalyzed by acyltransferases, which belong to the BAHD family and exhibit a broad range of substrate specificities. For instance, *p*-coumaroyl-CoA monolignol transferase (PMT) acylates monomers with *p*-coumaric acid in grasses, while *p*-hydroxybenzoyl-CoA monolignol transferase (*p*HBMT) acylates monomers with *p*-hydroxybenzoic acid in Salicaceae. The biological function of lignin acylation in plants remains unclear. However, it is known that (i) acylation occurs primarily on sinapyl alcohol [1] and (ii) the incorporation of acylated monomers results in a more fragmented lignin polymer [2], which is of interest for biotechnological applications. In this study, we investigated the effects of overexpressing a grass PMT in poplars without a functional *p*HBMT or poplars depleted in S-lignins on cell wall composition and structure. Analytical chemistry, vibrational spectroscopy (RAMAN, FTIR), and fluorescence microscopy revealed that while PMT can acylate sinapyl alcohol and, to some extent, coniferyl alcohol in a poplar genetic background, *p*HBMT appears to acylate sinapyl alcohol in wild type but not coniferyl alcohol in poplars deficient in S-lignins. Saccharification assays performed on this material highlighted the potential of monolignol acyltransferases with distinct specificities to modify lignin structure and enhance bioethanol production.

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Keywords:

Poplar, lignins, monolignol acyltransferase

Session 7

Structure, function and diversity of nucleic acids

SENSITIVE LOCALIZATION OF INFLUENZA RNA IN CELLS BY DIRECT RNA PADLOCK PROBING AND IN-SITU SEQUENCING

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Influenza A viruses (IAV) contain eight negative-sense single-stranded viral RNA (vRNA) molecules, which are transcribed into mRNA and replicated via complementary RNA (cRNA). These processes are tightly regulated, but the precise molecular mechanisms governing the switch from transcription to replication remain elusive. Here, we introduce multiple direct-RNA assisted padlock probing in combination with in situ sequencing (mudRapp-seq) to visualize the transcription and replication of all eight IAV vRNA and mRNA molecules at the single-cell level. We demonstrate that direct RNA padlock probing is three times more efficient than conventional probes that target cDNA. Individual probes showed variations in efficiency, partly due to the RNA structure of the target, which was mitigated by employing multiple padlock probes per target. Applying mudRapp-seq to an infection time course, we observed early mRNA expression, followed by vRNA accumulation approximately 3 hours later. Individual viral segments exhibited differential expression, particularly in the mRNA population. Both bulk and single-cell analyses revealed a correlation between the expression of 'M' mRNA and the onset of the transcription-to-replication switch. Our findings demonstrate that mudRapp-seq offers significant potential for elucidating viral replication mechanisms and may be applicable to studying other RNA viruses and cellular RNA processes.

A new drug targeting the evolvability protein Mfd against ESKAPE infections

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In the context of rising antimicrobial resistance (AMR), the urgent search for new antimicrobials led us to identify Mfd, a bacterial protein, as an innovative target for drug development. Mfd plays a key role in bacterial virulence and grants resistance to nitric oxide, a crucial antimicrobial defense mechanism by promoting the repair of NO-induced DNA damage [1-3]. Mfd is a non-essential transcription-repair coupling factor ubiquitous in bacteria, recognizes RNA polymerase stalled at non-coding lesions[4]. It hydrolyzes ATP to dismantle the transcription complex, and to recruit components of the nucleotide excision repair machinery. Mfd promotes mutations, thereby accelerating the development of antibiotic resistance [5]. A high-throughput *in silico* screening identified molecules that specifically bind to Mfd's ac0ve site. The molecular mode of action was characterized in *E. coli* and extended to the ESKAPE group of pathogens, priority targets in AMR. We identified and characterized NM102 compound, that displays antimicrobial activity exclusively in the context of infection. NM102 inhibits the activity of the non essential Mutation Frequency Decline (Mfd) protein by competing with ATP binding to its active site. Inhibition of Mfd by NM102 sensitizes pathogenic bacteria to the host immune response and blocks infections caused by the clinically-relevant bacteria *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, without inducing host toxicity. Finally, NM102 inhibits the mutation and evolvability function of Mfd, thus reducing the bacterial capacity to develop antimicrobial resistance. These data, recently published, provide a potential roadmap for the development of drugs to combat antimicrobial resistance[6].

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Keywords:

AntiMicrobial Resistance, Mfd, drug design, ATP-site, NM102

Structural snapshots of D-loop forma2on in Archaea revealed by Cryo-EM

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Genome maintenance in bacteria, archaea and eukaryotes cri9cally depends on homologous recombina9on (HR), which enables the exchange of strands between homologous DNA molecules. During HR, a recombinase binds to ssDNA and forms a righthanded
helical nucleoprotein filament, known as a presynap9c filament, which then searches dsDNA for homology. Upon encountering a homologous sequence, the presynap9c filament pairs with the complementarity strand, resul9ng in the displacement of the noncomplementarity
strand form the duplex to generate a displacement loop (D-loop) and
promote DNA strand exchange. In all forms of life, DNA recombinases share a conserved ATPase domain for ATP binding. ATP is required for the self-associa9on of protomers into an ac9ve nucleoprotein filament. In bacteria, HR relies on RecA while in eukaryotes it relies on Rad51/Dmc1. While Rad51 is the universal recombinase in eukaryotes, meiosis also requires Dmc1, which enables mismatch-tolerant inter-homologue recombina9on. In archaeal cells, the reac9on of HR is performed by RadA. To uncover the specific features of archaeal recombinases, we determined the cryo-EM structures of RadA at dis9nct stages of the homologous recombina9on reac9on: in its apo form, pre-synap9c, synap9c, and post-synap9c
states. These structures highlight the evolu9onary rela9onships between archaeal and eukaryo9c recombinases.
Keywords: Cryo-EM, Homologous Recombina9on, DNA repair, Structural Biology

Challenging the Paradigm: DDX3X Exhibits Robust Helicase Activity on HIV-1 RNA

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DEAD-Box helicases are enzymes that bind and remodel RNA and ribonucleoproteins. They are involved in almost every step of RNA metabolism. DEAD-Box helicases are thus major players of gene expression (dys)-regulation and intracellular parasite invasion such as retroviruses. Among many implications in pathologies, the human DEAD-Box helicase DDX3X has been shown to be hijacked by HIV-1 at various steps including viral RNA export from the nucleus and translation initiation, but little is known about the way it interacts with the viral RNA as well as the structural consequences of this interaction. Here, we show that DDX3X binds to specific regions of HIV-1 5'UTR and dissociates tightly bound dimers of HIV-1 RNA. This unprecedented enzymatic activity for a DEAD-box protein is achieved with a strong efficiency and in multiple turn-over conditions. DDX3X-induced dynamics was followed using time-resolved structure probing, while footprinting revealed DDX3X preferential binding sites. By coupling the biochemical analysis of DDX3X enzymatic activity the systematic probing of HIV-1 derived RNAs structure, we challenge both the accepted structural model of HIV-1 gRNA dimers as well as the dogma considering DEAD box proteins as inefficient and rather promiscuous towards their RNA substrates. An explicative mechanistic model is proposed.

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2. Chamond N, de Bisschop, G, Faria, L., Laoudi, Y, Martinov, V. and B. Sargueil. DEAD-box Helicase Intrinsically Disordered Domains and Structural Dynamics of HIV-1 RNA are Required to Unmask DDX3X unprecedented Catalytic Efficiency. (under revision)

Keywords:

DDX3X, HIV-1, RNA structure, SHAPE probing, RNA helicase

Two OB-fold proteins from a Gram-positive ICE modulate relaxase biochemical activities

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Integrated and conjugative elements (ICEs) are major genomic islands integrated in bacterial genomes, that propagate by bacterial conjugation. ICEs often spread antibiotic resistance genes or other adaptive genes conferring evolutionary advantages to their hosts. At the initiation of conjugation, the ICE DNA is processed by the relaxase, a transesterase that recognises a peculiar DNA sequence named origin-of-transfer. ICE*St3*/Tn*916*/ICE*Bs1* superfamily of ICEs are widespread in Gram-positive bacteria, and they encode uncanonical relaxases classified within the unique MOB_T family. This study elucidates the critical roles of OrfL and OrfM, two OB-fold proteins encoded by ICE*St3*, in its conjugative transfer. We demonstrated that OrfL and OrfM form a complex and function as relaxosomal auxiliary proteins with RelSt3, the ICE*St3* MOB_T relaxase. The NMR 3D structure of OrfM revealed an OB-fold protein, and 3D models also proposed an OB-fold for OrfL. They both slightly bind DNA without sequence specificity. We also demonstrated that OrfL and OrfM are able to modulate the nicking-closing activity of RelSt3. Our data provide a singular description of essential participation of OB-fold proteins in a relaxosome, contrasting with most of other known relaxosomes both in Gram-negative and in Gram-positive bacteria, which primarily involve ribbon-helix-helix proteins.

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Keywords: DNA processing, OB-fold, bacterial conjugation, relaxase, ICE

Poster 28

Aptamer-based molecular fingerprinting for the diagnosis of neurodegenerative diseases.

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Abstract:
Tauopathies constitute a class of neurodegenerative disorders defined by the accumulation of intracellular aggregates of tau protein. They include different diseases such as corticobasal degeneration, progressive supranuclear palsy, and Alzheimer's disease. Theprogressive accumulation of these aggregates disrupts neuronal function, ultimatelyresulting in neurodegeneration and cell death.
Depending on the type of tauopathy, the predominant isoform of the Tau protein varies.Accurate identification of the specific tau isoform present in a patient would significantlyenhance diagnostic precision and help prevent potential therapeutic mismanagement. To meet this challenge, we have been designing nucleic acid structures, known as aptamers, which are able to bind specifically to different conformations of protein fibers. These aptamers have been used to develop a method called AptaFOOT-Seq, which makes it possible to discriminate different fiber polymorphs by high-throughput sequencing 1,2. The aim of our project is to apply this method to tau fibers in order to discriminate fibers from patients with different tauopathies.
We first performed a selection of aptamers by incubating a random library with one of the two isoforms predominantly present in tauopathies. After several rounds of selection and high throughput sequencing was used to identify sequences that have been enriched in the library. Preliminary results of this study seem to indicate that some of these sequences can have a higher affinity compared to a scramble sequence (used as a negative control). For the next stage of the experiments, we will test these sequences on several isoforms and determine the Kd of the most promising.

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2. Brevet CEA N° B252231FRD41159
Keywords: Aptamers, SELEX, *in vitro* selection, Tauopathies, Alzheimer

Poster 29

Positional mapping of lipid-RNA adduct formation in LNP and structural implications for RNA

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Abstract:

Lipid nanoparticles (LNPs) encapsulate and protect mRNA through self-assembly of four lipid types, facilitating cellular internalization and cytoplasmic protein translation. Recent studies have revealed spontaneous formation of lipid-mRNA adducts within LNPs, potentially impacting mRNA structure and protein expression[1]. Notably, these adduct levels increase over time.

This research investigated an mRNA encoding hemagglutinin subtype H3 (HAH3) from the Singapore strain. The native secondary structure was initially characterized using SHAPE[2] (Selective 2'-Hydroxyl Acylation analyzed by Primer Extension), an efficient method for probing long and dynamic RNAs. SHAPE reveals nucleotide flexibility through selective acylation of 2'OH ribose groups in flexible regions. These adducts block reverse transcription, generating cDNA fragments of various lengths that are analyzed by capillary electrophoresis. IPASUITE[3] software is then used to process this data to generate secondary structure models based on nucleotide reactivity.

Applying similar principles, lipid adduct formation sites on the mRNA were mapped. After forcing adduct formation during increasing incubation time at 25°C, mRNA-LNPs were deformulated, and analyzed as SHAPE samples. IPASUITE analysis identified precise nucleotide positions where adducts formed along the mRNA sequence. Additionally, total RNA-lipid adduct levels were quantified over time using LC-UV analysis.

Analysis of the kinetic data revealed preferential sites for adduct formation when compared to the pre-formulation secondary structure of HAH3 mRNA. The occurrence of adduct formation is influenced by both the type of nucleotide and its position within the RNA's structural elements. This mapping provided insights into how lipid-RNA interactions evolve over time and potentially affect RNA functionality within LNP delivery systems.

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Keywords: lipid nanoparticles, mRNA secondary structure, SHAPE, Lipid-RNA adducts

Poster 30

Bidirectional study of HIV gRNA structure and function of DDX3X RNA helicase on RNA dimerization phenomenon

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Abstract: The human immunodeficiency virus is a retrovirus widely described in the literature with a highly structured and dimerized RNA genome. Dimer RNA is found in newly synthesized virions and is therefore essential for the infection of new cells. DEAD-box helicase DDX3X is involved in many stages of the viral cycle, but its interaction with HIV-1 genomic dimerized RNA is not yet well understood. We have investigated this interaction by studying the gRNA structure and DDX3X function. We were able to demonstrate that this helicase can efficiently undwind gRNA dimers. However, the activity of DDX3X is impaired when it attempted to undo 1636 nucleotides RNA dimers, in other words the Gag coding region. In this study, the helicase activity of DDX3X has been used to identify a region between 942 and 993 nucleotides that would contain a structure capable of stabilizing gRNA dimers. In addition, biochemical probing of these two fragments and studies of nucleotides reactivities demonstrated that the presence of these additional 50 nucleotides was sufficient to induce a structural rearrangement not only within the coding region, but also at the 5'UTR, a region essential for dimerization. This work expands our knowledge about the structure and stabilization of HIV gRNA dimers, but also raises questions about the DDX3X binding site, which could be a therapeutic target.

Relevant references:

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Chamond,N., de Bisschop,G., Faria,L., Laoudi,Y., Martynov,V. and Sargueil,B. (2025) DEAD-box Helicase Intrinsically Disordered Domains and Structural Dynamics of HIV-1 RNA are Required to Unmask DDX3X unprecedented Catalytic Efficiency. submitted

Keywords: DEAD-box Helicase, HIV, enzymatic activity, RNA structure

Poster 31

Identification of Influenza Virus Packaging Motifs via DMS and DVG Analysis

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Abstract:

Influenza virus is a single-stranded negative-sense segmented RNA virus whose packaging mechanism remains incompletely understood. Viral packaging involves distinguishing viral components from host cellular components and organizing segmented viral genomes into virions. In this study, dimethyl sulfate (DMS) modification combined with Nanopore-based DNA sequencing analysis was employed to identify structural changes of viral RNA at different time points and in different contexts (e.g., cells vs. virions), indirectly pinpointing RNA motifs and structures involved in packaging. Additionally, unbiased RNA sequencing was performed to analyze defective viral genomes (DVGs), which are natural products resulting from internal deletions within the influenza viral genome. By comparing the relative abundance of DVGs between cellular and viral fractions, we further evaluated the importance of distinct RNA regions in viral packaging.

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Keywords: Influenza virus, packaging, DVGs, DMS, Nanopore

Poster 32

The in vivo interactome of U2AF2 uncovers unexpected roles in alternative splicing and beyond.

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Abstract:

Splicing factor U2AF2 is known to play a pivotal role for 3' splice site recognition at an early step of spliceosome assembly. Here using proximity labeling and biochemical confirmations, we extend the repertoire of putative functional partners of U2AF2 mainly for splicing, chromatin modification, transcription, 3' end processing and RNA methylation. U2AF2 presents an N-terminal RS domain that drives liquid-liquid phase separation and whose function is poorly characterized (Tari *et al*, 2019). We observed that removal of its RS domain alters numerous interactions of U2AF2 and reduces its localisation in speckles. In parallel we observed that the U2AF2 RS domain impacts alternative splicing genome wide. Further bioinformatics analyses reveals that both 3' splice site context and the overall structure of genes determine the function of the RS domain for cassette exon inclusion. Finally, we show that multiple phosphorylation sites within the U2AF2 RS domain are required for normal splicing, suggesting that its RS domain mediates U2AF2 regulations. Overall, we demonstrate that the low complexity RS domain of splicing factor U2AF2 mediates multiple interactions with its partners that often contain RS domains whereas phosphorylation of serine residues in the RS domain modulates these interactions. Finally, this low complexity domain recruits U2AF2 to speckles to assure the inclusion of alternative exons in regions where the concentration of splicing factors might be limiting.

Tari M, Manceau V, de Matha Salone J, Kobayashi A, Pastré D & Maucuer A (2019) U2AF65 assemblies drive sequence-specific splice site recognition. *EMBO Rep* 20: e47604

Keywords: pre-mRNA splicing, speckles, transcription, chromatin remodeling

Poster 33

Interactions between compaction and repair of oxidized mitochondrial DNA

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Abstract:
Reactive oxygen species (ROS), produced during oxidative phosphorylation, induce a plethora of lesions to mitochondrial DNA (mtDNA), among which 8-oxoguanine (8-oxoG) is the most abundant. 8-oxoG is recognized and excised by the DNA glycosylase OGG1, which initiates the base excision repair pathway. MtDNA repair is less documented than nuclear repair, but it has been shown that an active form of OGG1 within mitochondria is necessary to preserve the mitochondrial network in cells exposed to oxidative stress. A defect in the repair of 8-oxoG lesions can cause mutations leading to metabolic alterations.
MtDNA is organized into a compact DNA-protein complex called the nucleoid. Its main architectural protein is the mitochondrial transcription factor A (TFAM). TFAM-mediated compaction of mitochondrial DNA has been shown to be a highly dynamic process, playing a crucial role in the regulation of mitochondrial gene expression and mtDNA replication. However, the impact of compaction on mtDNA repair mechanisms remains poorly understood.
To address this question, we use biochemical techniques that allow the study of DNA-protein interactions, such as EMSA (Electrophoretic Mobility Shift Assay), and a single-molecule biophysical technique, TIRF (Total Internal Reflection Fluorescence Microscopy), which allows us to analyze protein dynamics on DNA in real-time. Using these techniques, we examine how TFAM and OGG1 interact with DNA and seek to determine whether the two proteins compete for binding sites or cooperate in regulating DNA repair. Our initial results suggest that TFAM dominates OGG1 for nonspecific DNA binding on undamaged DNA, while OGG1 stays strongly bound to oxidized DNA.

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Keywords: mitochondrial DNA, DNA compaction, DNA repair, TFAM, OGG1

Poster 34

Investigating the specificity of MOB_T relaxases *oriT* binding sites

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Abstract:
Integrative and Conjugative Elements (ICEs) are crucial drivers of bacterial evolution, promoting the dissemination of genetic traits such as antibiotic resistance, virulence factors, and novel metabolic capabilities. ICEs encode the machinery necessary for their own excision, horizontal transfer via conjugation, and integration into the host genomes. Relaxase proteins are central to the initiation of this DNA transfer, by recognition of the origin-of-transfer (*oriT*) sequence.
In Gram-positive bacteria, the ICE*St3*/Tn*916* superfamily encodes a unique class of relaxases, known as MOB_T, which are related to *Rep_trans* rolling-circle replication initiators. This study investigates the molecular mechanisms by which MOB_T relaxases recognize and interact with their DNA substrates. Using recent insights into the *oriT* binding site of the ICE*St3* relaxase (RelSt3), we examined the DNA-binding behavior of three related MOB_T relaxases: RelSt3 behavior was compared to the relaxases encoded by Tn*916* from *Enterococcus faecalis* (Orf20) and by ICE_*515*_*tRNA*^{Lys} from *Streptococcus agalactiae* (Rel515). Electrophoretic mobility shift assays and endonuclease activity assays were conducted using *oriT* sequences from these three ICEs.
Despite the structural and genomic similarities among the relaxases RelSt3, Orf20 and Rel515, including an N-terminal helix-turn-helix (HTH) domain, we observed distinct patterns of DNA interaction. However, these relaxases exhibited single-stranded endonuclease activity on various substrates. These findings point out a potential functional divergence within the MOB_T relaxase family, suggesting different DNA recognition mechanisms.
Keywords: *Gram-positive, Integrative Conjugative Elements (ICE), relaxase, MOB_T,* oriT

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Poster 36

Interaction between the RNA-binding protein HuR and microRNA let-7 facilitates their cooperative interplay in regulating pro-oncogenic gene expression

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Abstract:

RNA-binding proteins (RBPs) and microRNAs (miRNAs) are two key mediators of post-transcriptional regulation that demonstrate active interplay to control crucial cellular functions. There is cooperative or competitive interplay between RBPs and miRNAs in the regulation of target mRNAs although the mechanisms underlying such crosstalk are not well understood. The RBP HuR has been shown to act in synergy with let-7 miRNAs to recruit them to their target mRNAs such as the pro-oncogenic mRNA c-Myc, although the mechanism for this cooperative interplay has not been elucidated. We show by RNA-immunoprecipitation that HuR directly binds let-7 miRNAs in cells although binding affinity of HuR to the let-7 isomiRs varies significantly. *in vitro* UV-crosslinking studies show that the interaction between HuR and let-7 mainly involves the RNA recognition motifs (RRM) 1 and 2 of HuR and binding of let-7 miRNAs with HuR RRM 1-2 enhances the dimerization of the protein, which is abrogated by mutations in the seed sequences of let-7. Transcriptome-wide comparison of let-7 target mRNAs with transcripts that bind HuR in eCLIP experiments show multiple genes involved in cell cycle progression. Depletion of HuR results in significant enhancement of cell proliferation and colony formation, which is prevented by overexpression of the let-7 miRNAs. These observations demonstrate a cooperative interplay between HuR and let-7, mediated by direct interaction between HuR and Let-7 miRNAs and dimerization of HuR. The cooperative interplay of HuR and let-7 miRNAs may facilitate the recruitment of let-7 miRNAs to their common pro-oncogenic target transcripts.

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Keywords: RNA-binding protein, HuR, microRNA let-7, RNA-protein interactions, Oncoge