Congrès annuel de la Société Française de Biochimie et Biologie Moléculaire

A la Faculté de Pharmacie de Paris

SFBBM

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Société Française de Biochimie et Biologie Moléculaire

Chers collègues, chers amis,

C'est avec grand plaisir que nous vous souhaitons la bienvenue pour le Congrès Annuel 2022 de la SFBBM !

Après le succès de l'édition 2021, ce congrès sera à nouveau l'occasion d'offrir à notre Société un moment d'échanges et de convivialité. Nous aurons le plaisir d'accueillir des conférenciers de renom, les lauréats des prix de notre société (prix Maurice Nicloux, prix de la fondation Dina Surdin, prix Biochimie, …) mais aussi des jeunes chercheurs dont les résumés ont été sélectionnés.

Merci à nos prestigieux orateurs invités, à tous les jeunes chercheuses et chercheurs pour leurs nombreuses contributions, aux sponsors mais aussi au comité local d'organisation et au conseil scientifique du congrès, dont le dynamisme et l'efficacité ont permis d'établir un programme passionnant, divers et riche, à l'image de notre chère société.

Organisé pour la deuxième année dans le cadre unique de la Faculté de Pharmacie, notre congrès bénéficiera à nouveau d'un environnement propice aux échanges et aux débats inspirants dans une atmosphère décontractée et amicale.

Au nom de la SFBBM, je vous souhaite à toutes et tous un excellent congrès !

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 financière pour la participation à des congrès en France comme à l'étranger.

La SFBBM est impliquée dans des publications scientifiques. BIOCHIMIE (revue de la SFBBM) a succédé en 1971 au Bulletin de la Société de Chimie Biologique, fondé en 1914.

En un demi-siècle, BIOCHIMIE a publié plus de 9000 articles originaux ou de revues, et compte parmi les journaux scientifiques les mieux connus et les plus respectés dans le domaine. BIOCHIMIE est une revue internationale publiée en Anglais par Elsevier.

BIOCHIMIE publie des travaux originaux, articles de revue critiques de la littérature, et mini-revues dans le domaine large de la biologie, englobant biochimie, enzymologie, biologie moléculaire et cellulaire, régulations métaboliques, génétique, immunologie, microbiologie, biologie structurale, études « omiques » avec validation fonctionnelle, pharmacotoxicologie et mécanismes moléculaires des maladies. Son facteur d'impact sur 5 ans est de 4.104.

Le journal publie régulièrement des numéros spéciaux dédiés à des thèmes de recherche d'un intérêt particulier.

Membres du Comité Local d'Organisation

Magali Blaud
magali.blaud@u-paris.fr
Julien Dairou
Julien.dairou@u-paris.fr
Philippe Fossé
philippe.fosse@ens-paris-saclay.fr
Julien Henri
Julien.henri@sorbonne-université.fr
Zaineb Kammoun
zaineb.kammoun@parisdescartes.fr
Hélène Munier-Lehmann
helene.munier-lehmann@pasteur.fr
Ariane Pachins
sfbbm@sfbbm.fr
Jean-Christophe Paillart
jc.paillart@ibmc-cnrs.unistra.fr
Martin Picard
martin.picard@ibpc.fr
Julie Soutourina
Julie.Soutourina@cea.fr

Membres du Comité Scientifique

Magali Blaud
magali.blaud@u-paris.fr
Béatrice Clouet D'Orval
Beatrice.Clouet-dOrval@ibcg.biotoul.fr
Bernard Dujon
bdujon@pasteur.fr
Laurence Drouard
laurence.drouard@ibmp-cnrs.unistra.fr
Christine Ebel
christine.ebel@ibs.fr
Bertrand Friguet
bertrand.friguet@upmc.fr
Hélène Munier-Lehmann
helene.munier-lehmann@pasteur.fr
Fernando Rodrigues-Lima
Fernando.rodrigues-lima@u-paris.fr
Emmanuelle Schmitt
emmanuelle.schmitt@polytechnique.edu

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, les sociétés Cytiva, Microsynth et Eurofins. Vos contributions à ce congrès nous ont permis de pouvoir réaliser cet évènement dans les meilleures conditions et d'offrir des prix pour le meilleur poster et la meilleure présentation orale. Nous tenons à remercier la Faculté de Pharmacie de Paris pour son accueil et spécialement Samantha Conti (communication et organisation), Mélanie Martin (installation et organisation) et Stéphane Carraz (TICE).

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PROGRAMME LUNDI 4 JUILLET 2022

8h30 Accueil des participants
Faculté de Pharmacie de Paris – 6 avenue de l'Observatoire/Paris

9h30 Ouverture du congrès

10h00 Conférence plénière par Chris Bowler
« Tara Oceans: ecosystems biology at planetary scale »

11h00 Session 1 : « RNA »
Conférence par Franck MARTIN
Cyril Bourgeois : prix Maurice Nicloux 2021
Jean-Louis MERGNY
Claire Husser

12H30 PAUSE DEJEUNER

13h45 Session 2: « Chemobiology »
Conférence par Alice LEBRETON
CD MOHAN : prix de l'article de l'année Biochimie
Corinne LIONNE

15H00 SESSION POSTERS ET PAUSE CAFE

16H00 Présentations par les sponsors
Mycrosynth, Cytiva, Eurofins et Dunod

16h30 Session 3: « Cellular proteolysis »
Conférence par Catherine MOALI
Yoann SANTIN : prix Dina Surdin
Paul BIGOT
Nicolas MATHAS

18h00 Assemblée Générale de la SFBBM

18h30-22h COCKTAIL

PROGRAMME MARDI 5 JUILLET 2021

8h30 Session 4: « Archaea »
Conférence par Tristan WAGNER
Solenne ITHURBIDE : prix de l'article de l'année de la SFBBM
Clément MADRU
Roxane LESTINI

10h00 SESSION POSTERS ET PAUSE CAFE

11h00 Session 5 : « Genetic »
Conférence par Claire Rougeulle
Maxime WERY
Louise le COADOU
Inge KÜHL

12H30 PAUSE DEJEUNER

13h30 Session 6: « Enzymes »
Conférence par Alain MARTY
Damien SORIGUE : prix article de l'année SFBBM 2018
Nicolas JOLY : prix Maurice Nicloux 2021
Claire CÉRÉ
Paolo ZECCHIN

15h15 Session 7: « Education »
Wilfried GRANGE, Florent BUSI et Caroline CHAUVET

15h45 Remise des prix et conclusions

16h30 Session spéciale du Groupe de Travail Enzymes

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Merci à nos sponsors pour leur soutien dans l'organisation du congrès

Conférence plénière d'ouverture du congrès

Animée par
Christine Ebel
Institut de Biologie Structurale– Grenoble

«Tara Oceans: ecosystems biology at planetary scale»

par Chris Bowler

Ecology and Evolutionary Biology Section, Institut de Biologie de l'Ecole normale supérieure (IBENS), Paris, FRANCE

The ocean is the largest ecosystem on Earth and yet we know very little about it. This is particularly true for the plankton that drift within, even though they form the base of marine food webs and are key players in Earth's biogeochemical cycles. Ocean plankton are at least as important for the Earth system as the forests on land, but most of them are invisible to the naked eye and thus are largely uncharacterized. To increase our understanding of this underexplored world, a multidisciplinary consortium, *Tara* Oceans, was formed around the 36m research schooner *Tara,* which sampled plankton at more than 210 sites and multiple depth layers in all the major oceanic regions during expeditions from 2009-2013 (Karsenti et al. Plos Biol., 2011). This talk will summarize the foundational resources from the project, which collectively represent the largest DNA sequencing effort for the oceans (see Science special issue May 22, 2015 and Cell, Nov 14, 2019), and analyses that illustrate several aspects of the *Tara* Oceans' eco-systems biology approach to address microbial contributions to ecological and evolutionary processes. The project provides unique resources for several scientific disciplines that are foundational for mapping ocean biodiversity of a wide range of organisms that are rarely studied together, exploring their interactions, and integrating biology into our physico-chemical understanding of the ocean, as well as for identifying new organisms and genes of biotechnological interest. These resources, and the scientific innovations emerging to understand them, are furthermore critical towards developing baseline ecological context and predictive power needed to track the impact of climate change on the ocean.

SESSION 1

“ RNA ”

- **Conférence par Franck Martin**
- **Cyril Bourgeois : prix Maurice Nicloux 2021**
- **Jean-Louis Mergny**
- **Claire Husser**

Animée par
Laurence Drouard
Institut de biologie moléculaire des plantes – Strasbourg

Conférence invitée session 1

«Viral and cellular translation during SARS-CoV-2 infection»

par Franck MARTIN

Université de Strasbourg, Institut de Biologie Moléculaire et Cellulaire, Architecture et Réactivité de l'ARN, CNRS UPR9002, 2, allée Konrad Roentgen, F-67084 Strasbourg (France)

SARS-CoV-2 is a betacoronavirus that has emerged in China in December 2019 and which is the causative agent of the Covid-19 pandemic. This enveloped virus contains a large positive-sense single-stranded RNA genome. Non-structural proteins are encoded by the genomic RNA and are produced in the early steps of infection. In contrast, the structural proteins are produced from sub-genomics RNA that are translated in the late phase of the infectious program. Non-structural protein 1 (NSP1) is a key molecule that regulates both viral and cellular translation[1]. In addition, NSP1 interferes with multiple steps of in the interferon I pathway and thereby blocks host antiviral responses[1]. Therefore, NSP1 is a drug target of choice for the development of antiviral therapies.

The 5'UTR part of coronavirus genomes plays key roles in the viral replication cycle and the translation of the viral mRNAs. The first 75-80 nucleotides, also called the leader sequence, are identical for the genomic RNA and for the subgenomic RNAs. Recently, it was shown that cooperative actions of a 5'UTR segment and the non-structural protein NSP1 are essential for both the inhibition of host mRNAs and for specific translation of viral mRNAs[2,3]. Sequence analyses of both the 5'UTR RNA segment and the NSP1 protein have been done for several coronaviruses with special attention to the betacoronaviruses. The conclusions are (i) precise specific molecular signatures can be found in both the RNA and the NSP1 protein; (ii) both types of signatures strongly correlate between each other. Indeed, definite sequence motifs in the RNA correlate with sequence motifs in the protein indicating a co-evolution between the 5'UTR and NSP1 in betacoronaviruses. Experimental mutational data on 5'UTR and NSP1 from SARS-CoV-2 using cell-free translation extracts support those conclusions and show that some conserved key residues in the N-terminal half of the NSP1 protein are essential for evasion to the inhibitory effect of NSP1 on translation[4].

[1] Eriani, G. and Martin, F. (2022) Viral and cellular translation during SARS-CoV-2 infection. FEBS Open Bio. DOI: 10.1002/2211-5463.13413.

[2] Miao, Z., Tidu, A., Eriani, G. and Martin, F. (2020) Secondary structure of the SARS-CoV-2 5'UTR. RNA Biology. 23, 1-10. DOI: 10.1080/15476286.2020.1814556.

[3] Tidu, A., Janvier, A., Schaeffer, L., Sosnowski, P., Kuhn, L., Hammann, P., Westhof, E., Eriani, G. and Martin, F. (2021) The viral protein NSP1 acts as a ribosome gatekeeper for shutting down host translation and fostering SARS-CoV-2 translation. RNA. 27, 253-264. DOI: 10.1261/rna.078121.120

[4] Sosnowski, P., Tidu, A., Eriani, G., Westhof, E. and Martin, F. (2022) Correlated sequence signatures are present within the genomic 5'UTR RNA and NSP1 protein in coronaviruses. RNA. DOI: 10.1261/rna.078972.121

Conférence invitée session 1
Prix Maurice Nicloux 2021

«RNA helicase-dependent gene looping impacts messenger RNA processing»

Par Cyrille Bourgeois

Laboratoire de Biologie et Modélisation de la Cellule, Ecole Normale Supérieure de Lyon CNRS UMR5239, INSERM U1293, Université Lyon 1 46 Allée d'Italie, 69007 Lyon

DEAD-box RNA helicases DDX5 and DDX17 regulate several aspects of gene expression, especially transcription and splicing, through incompletely understood mechanisms. A transcriptome analysis of DDX5/DDX17-depleted human cells confirmed the large impact of these RNA helicases on splicing and revealed a widespread deregulation of 3' end processing. In silico analyses and experiments in cultured cells showed the binding and functional contribution of the genome organizing factor CTCF to chromatin sites near a subset of DDX5/DDX17-dependent exons that are characterized by a high GC content and a high density of RNA Polymerase II. We propose that an RNA helicase-dependent relationship between CTCF and the dynamics of transcription across DNA and/or RNA structured regions contributes to the processing of internal and terminal exons. Our work showed that local DDX5/DDX17-dependent chromatin loops spatially connect RNA helicase-regulated exons to their cognate promoter, and we provide the first demonstration that de novo gene looping modifies alternative splicing and polyadenylation. Overall our findings uncovered the impact of DDX5/DDX17-dependent chromatin folding on pre-messenger RNA processing.

«Quadruplexes are everywhere!»

Jean-Louis Mergny[a]

[a] Laboratoire d'Optique et Biosciences, Ecole Polytechnique, CNRS UMR7645 – INSERM U1182, Institut Polytechnique de Paris, 91128 Palaiseau cedex, France

E-mail of corresponding author: jean-louis.mergny@polytechnique.edu

G-quadruplexes are unusual nucleic acid structures which can find applications in biology, medicine, as well as biotech- and nano-technologies [1]. We are developping tools to understand their folding and polymorphism [2]. In parallel, we proposed a new algorithm for prediction of G4 propensity of unprecedented accuracy [3]. We are now applying this G4-Hunter prediction tool to a number of genomes.

We became interested in quadruplexes quadruplex-prone regions conserved in the genome of a number of viruses [4]. We recently demonstrated that viruses regularly causing persistent infections are enriched in G4 motifs, while viruses causing acute infections are significantly depleted in these structures [5]. We performed a genome analysis of SARS-CoV2 [6]. One of the viral proteins, Nsp3, contains a SARS-Unique domain (SUD), essential for replication, which can bind to G4s. These interactions can be disrupted by mutations that prevent the oligonucleotides from folding into G4 structures and, interestingly, by molecules called ligands specific for these G4s. Our results pave the way for further studies on the role of SUD/G4 interactions during SARS-CoV-2 replication and the use of inhibitors of these interactions as potent antiviral compounds. We are performing a global screening of molecules capable of inhibiting this interaction and testing their antiviral properties.

We are also interested in the role of quadruplexes in parasites such as *Plasmodium falciparum* or *Trypanosoma brucei* [7] and, more recently on parasitic helminths [8], which are highly prevalent and infect approximately two *billion* people worldwide. A nematode, *Ascaris lumbricoides*, was found to be highly enriched in stable quadruplexes. We demonstrated that small compounds able to recognize these structures called G-quadruplex ligands were able to selectively recognize G4 found in the *Schistosoma mansoni* genome. Two of these compounds demonstrated potent activity both against larval and adult stages of this helminth, opening new perspectives for the use of G4 ligands to fight diseases caused by these parasites.

Relevant references:

(1) Mergny & Sen, ***Chem. Rev.*** (2019), *119*, 6290-6325. Mergny ***Biochimie*** (2020), *168*, 100-109.

(2) Cheng *et al,* ***Nucleic Acids Res.*** (2018), *46*, 9264. Chen *et al,* ***Nucleic Acids Res***. (2021) *49*, 9548; Luo *et al,* ***Nucleic Acids Res.*** (2022), *accepted*.

(3) Bedrat *et al,* ***Nucleic Acids Res.*** (2016), 44: 1746; Brazda *et al,* ***Bioinformatics*** (2019), 35, 3493 & ***Bioinformatics*** (2020), *36*, 3246.

(4) Jaubert *et al,* ***Sci Adv.*** (2018) *8*: 8120. Abiri *et al,* ***Pharmacol Rev***. (2021) *73*, 897.

(5) Bohálová *et al,* ***Biochimie*** (2021) *186*, 13-27

(6) Lavigne *et al.* ***Nucleic Acids Res***. (2021) *49*, 7695

(7) Belmonte-Reche *et al*, ***Eur J Med Chem.*** (2018), 61: 1231; Guillon *et al*, ***Chem. Biol. Drug Des.*** (2018), *91*: 974 ; Gazanion *et al,* ***PLoS Pathogens*** (2020), *16*, e1008917. Belmonte-Reche *et al*, ***Eur J Med Chem.*** (2022), *232*, 114183.

(8) Cantara *et al,* ***Nucleic Acids Research*** (2022), *50, 2719-2735.*

KEYWORDS: G-quadruplex; Nucleic acid structures; Prediction; RNA ligands

«Development of Fluorogenic RNA-based biosensors by ultra-high throughput microfluidic screening»

Claire Husser[a], Michael Ryckelynck[a]

[a]Architecture et Réactivité de l'ARN (UPR9002 du CNRS), team "Digital Biology of RNA" Institut de Biologie Moléculaire et Cellulaire du CNRS, Université de Strasbourg

Halogene compounds are implied in many environmental pollutions and are classified as Persistent Organic Polluants (POPs) by the Stockholm convention and the World Health Organization. On top of that these compounds are highly toxic to both humans and wildlife. Their persistence, bioaccumulation, and toxicity lead to the need of the discovery or the development of new dehalogenation reaction catalyst.

Some organisms have been described as able to degrade halogenated compounds leading to the idea that enzyme-based biodegradation of halogene coumpounds can be a promising solution for contaminants degradation.

To be able to monitor the degradation of such compounds, one must either be able to sense in real time the amount of the compound of interest or be able to sense its degradation products. In the case of the research of new enzymes or strains able to degrade fluorinated compounds, our choice was to develop a new method of detection of fluoride ions

Our group is specialized in the development of RNA modules called light-up RNA aptamer and in the development of Fluorogenic RNA-based Biosensor (FRB) using µIVC-seq (2) (a technology coupling In Vitro Compartmentalization assisted by microfluidics with Next generation sequencing and bioinformatic).

In this talk, I will present how we used our µIVC-seq technology for a rapid and efficient identification of Fluoride-FRBs for their capacity to emit fluorescence only in the presence of fluoride from a library containing more than 64 000 variants based on the CrcB Fluoride specific riboswitch aptamer domain (1). And how this biosensor can be used to detect Fluoroacetate dehalogenase activity.

1. Breaker, R. R. New Insight on the Response of Bacteria to Fluoride. *Caries Res* **2012**, *46* (1), 78–81.
2. Autour, A.; Bouhedda, F.; Cubi, R.; Ryckelynck, M. Optimization of Fluorogenic RNA-Based
3. Biosensors Using Droplet-Based Microfluidic Ultrahigh-Throughput Screening. Methods 2019, 161, 46–53.
4. KEYWORDS: Fluorogenic RNA-based Biosensor, Hight-throughput screening, Fluoride

SESSION 2

“ Chemobiology ”

- **Conférence par Alice Lebreton**
- **Chakrabhavi Dhananjaya Mohan : prix de l’article de l’année Biochimie**
- **Corinne Lionne**

Animée par

Hélène Munier-Lehmann
Institut Pasteur - Paris

Conférence invitée session 2
«Shedding new light on host-pathogen interactions with chemobiological labelling approaches»
par Alice LEBRETON

Institut de biologie de l'École normale supérieure (IBENS), CNRS, INSERM, Université PSL, 75005 Paris et INRAE, IBENS, 75005 Paris.

Caroline Peron-Cane[a,b], Arnaud Gautier[c], Nicolas Desprat[a,b] and Alice Lebreton[a,d]

[a] Institut de biologie de l'École normale supérieure (IBENS), CNRS, INSERM, Université PSL, 75005 Paris. [b] Laboratoire de Physique de l'École normale supérieure (LPENS), Université PSL, Université Paris Cité, Sorbonne Université, CNRS, 75005 Paris.[c] Sorbonne Université, École normale supérieure, Université PSL, CNRS, LBM, 75005 Paris. [d] INRAE, IBENS, 75005 Paris.

E-mail of corresponding author: alice.lebreton@ens.psl.eu

Objectives: The onset of host-pathogen interactions constitutes a remarkable example of dynamic phenomena in biology, where both the host and pathogen respond to each-other. In this molecular crosstalk, secretion of protein effectors is key to the virulence of microbial pathogens, by allowing them to interact with their host and subvert molecular mechanisms to their benefit. However, tracking the dynamics of secreted virulence factors in real time has long been impaired by a paucity of appropriate fluorescent tools.

Methods: We took advantage of the properties of the fluorogenic probe FAST to analyse by live microscopy and quantify the secretion of virulence factors from *Listeria monocytogenes* during infection. This versatile tool allowed us to monitor infection dynamics in real time among a population of *Listeria*-infected epithelial cells, as well as to assess the residence time of *Listeria* inside internalisation vacuoles.

Results: We unveiled a surprizing heterogeneity in residence time of *Listeria* inside its entry vacuoles. By tracking the localisation of the secreted pore-forming toxin listeriolysin O over time, we observed that it labelled not only the membrane of entry vacuoles, but also compartments that enlarged over time and where the bacteria could multiply as fast as in the cytosol.

Conclusions: We provide evidence that these compartments result from a process analogous to LC3- associated phagocytosis and constitute an alternative replication niche for *Listeria* in epithelial cells.

Relevant references:

1. Peron-Cane C, Fernandez J-C, Leblanc J, Wingertsmann L, Gautier A, Desprat N, Lebreton A. Fluorescent secreted bacterial effectors reveal active intravacuolar proliferation of *Listeria monocytogenes* in epithelial cells. *PLoS Pathog* (2020) 16(10):e1009001.
2. Chekli Y, Peron-Cane C, Dell'Arciprete D, Allemand J-F, Li C, Ghigo J-M, Gautier A, Lebreton A, Desprat N et Beloin C. **2020**. Visualizing the dynamics of exported bacterial proteins with the chemogenetic fluorescent reporter FAST. *Sci Rep.* 10(1):15791.

KEYWORDS: Up to 5

Listeria monocytogenes; Virulence factors; Bacterial secretion; Pore-forming toxin; Fluorogenic labelling.

Conférence invitée session 2
Prix de l'article de l'année Biochimie

«Targeting the STAT3 pathway in human cancers by natural compounds»

Par Chakrabhavi Dhananjaya Mohan

Department of Studies in Molecular Biology, University of Mysore, Mysore 570006, India

STAT3 is an oncogenic transcription factor that is overactivated in various types of human cancers. STAT3 controls the expression of genes associated with oncogenic functions such as cell proliferation, apoptosis, angiogenesis, and metastasis. We are involved in the discovery of new small molecule-based (natural and synthetic) inhibitors of the STAT3 signaling pathway in human malignancies. In one of our studies, we examined the effect of vitexin on the constitutive/inducible activation of STAT3 signaling in hepatocellular carcinoma (HCC) cells. Vitexin effectively inhibited sustained activation of JAK1, JAK2, Src, and STAT3 in HCC cells. Interestingly, treatment with tyrosine phosphatase inhibitor altered the vitexin-induced STAT3 phosphorylation, and the attenuation of STAT3 by vitexin was found to be driven through the upregulation of PTPεC. The combinational studies indicated that vitexin can exhibit substantial apoptotic effects with doxorubicin and sorafenib. It also suppressed the CXCL12-induced cell invasion. Overall, this study demonstrated that vitexin can act as a potential blocker of the STAT3 signaling cascade and mitigate the survival as well as invasion of HCC cells.

«Structure-based design of NAD+ analogues targeting bacterial NAD kinases, promising targets for new antibiotics»

Rahila Rahimova[1], Pauline Nogaret[2], Valérie Huteau[3], Clarisse Leseigneur[4], Muriel Gelin[1], David Clément[3], Olivier Dussurget[4], Gilles Labesse[1], Sylvie Pochet[3], Anne Blanc-Potard[2], Corinne Lionne[1]

[1] Centre de Biologie Structurale (CBS), Université Montpellier, CNRS UMR 5048, INSERM U1054, 29 rue de Navacelles, F-34090 Montpellier, France.

[2] Laboratory of Pathogen Host Interactions (LPHI), Université Montpellier, CNRS UMR 5235, Place Eugène Bataillon, CC107, F-34095 Montpellier cedex 5, France

[3] Institut Pasteur, Université de Paris, CNRS UMR3523, Unité de Chimie Biologique Epigénétique, 28 rue du Dr Roux, F-75015 Paris, France.

[4] Institut Pasteur, Université de Paris, Sorbonne Paris Cité, Unité de Recherche Yersinia, 28 rue du Dr Roux, F-75015 Paris, France

E-mail of corresponding author: lionne@cbs.cnrs.fr

Multi-drug resistance is a major public health problem that requires the urgent development of new antibiotics and therefore the identification of novel bacterial targets. The activity of nicotinamide adenine dinucleotide kinase, NADK, is essential in all bacteria tested so far, including many human pathogens that display antibiotic resistance leading to failure of current treatments. Inhibiting NADK is therefore a promising and innovative antibacterial strategy since there is currently no drug on the market targeting this enzyme. Through a drug design approach based on substrate-derived fragments, we have recently developed NAD^+-competitive inhibitors of NADKs, which displayed *in vivo* activity against *Staphylococcus aureus* or *Pseudomonas aeruginosa* in animal models of infection [1-3].

Funding and supports: Agence Nationale de la Recherche (ANR-17-CE18-0011-02), Institut Pasteur, Centre National de la Recherche Scientifique (CNRS), Institut National de la Santé et de la Recherche Médicale (INSERM), University of Montpellier.

Relevant references:

[1] Clément DA, Leseigneur C, Gelin M, Coelho D, Huteau V, Lionne C, Labesse G, Dussurget O, Pochet S (2020) New chemical probe targeting bacterial NAD kinase. Molecules. doi: 10.3390/molecules25214893.
[2] Gelin M, Paoletti J, Nahori MA, Huteau V, Leseigneur C, Jouvion G, Dugué L, Clément D, Pons JL, Assairi L, Pochet S, Labesse G, Dussurget O (2020) From substrate to fragments to inhibitor active *in vivo* against *Staphylococcus aureus*. ACS Infect Dis. doi: 10.1021/acsinfecdis.9b00368.
[3] Rahimova R, Nogaret P, HuteauV, Gelin M, Clément D, Labesse G, Pochet S, Blanc-Potard A, Lionne C (2022) Structure-based design, synthesis and biological evaluation of a NAD^+ analogue targeting *Pseudomonas aeruginosa* NAD kinase. In Preparation.

KEYWORDS: Antibiotic resistance, Drug design, Inhibitors, *Pseudomonas aeruginosa*, X-ray crystallography

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SESSION 3

“ Cellular proteolysis ”

- **Conférence par Catherine Moali**
- **Yoann Santin : prix Dina Surdin**
- **Paul Bigot**
- **Nicolas Mathas**

Animée par
Bertrand Friguet
Sorbonne Université – Paris

Conférence invitée session 3

«A tale of a protease enhancer becoming a protease inhibitor»

par Catherine Moali

Laboratoire de Biologie Tissulaire et Ingénierie Thérapeutique (LBTI), UMR5305 CNRS-Université de Lyon, France

It is a well-accepted paradigm in the protease field that every protease should work with a specific inhibitor to regulate its activity and avoid the deleterious effects of uncontrolled proteolytic activity. BMP-1/tolloid-like proteinases (BTPs) have been described several decades ago and are known to cleave more than 50 extracellular substrates. They play important roles in extracellular matrix assembly, angiogenesis, growth factor activation and peripheral nervous system development but, strikingly, no endogenous inhibitor is presently reported for this enzyme family (apart from the broad-spectrum a2-macroglobulin). In contrast, BTPs seem to be regulated by several substrate-specific enhancers such as PCPE-1 (procollagen maturation), periostin (LOX cleavage), twisted gastrulation (chordin degradation) or WFIKKN1 (myostatin activation).

In my presentation, I will explain how we identified the first endogenous and specific inhibitor of BTPs. This discovery was made completely incidentally and has broad consequences for the functions and regulation of BTPs.

Conférence invitée session 3

Prix Dina Surdin 2021

« From interbacterial competition to endobiotic predation: Size does matter! »

Par Yoann Santin

De Duve Institute - B-1200 Brussels - Belgium

Size regulation is an important process that occurs at all scales of life. Thus, the study of the influence of size on biological systems is a prerequisite for better understanding their functioning. During my Ph.D., I worked on a bacterial macromolecular system, the type VI secretion system (T6SS), which is depicted as a nano-crossbow that is used by some bacteria to inject toxic effectors directly into competitor cells. Since this system spans the entire cell, we wondered how its length is controlled. By using a combination of biochemical assays and live fluorescence microscopy we first determined that T6SS length is dictated by the cell width and then, that TagA, a new T6SS partner, acts as a molecular latch to ensure proper firing events. Recently, I started a post-doc to work on a predatory bacterium, *Bdellovibrio bacteriovorus*, which grows by filamentation inside its prey. Once the prey content is consumed, the filamentous predator cell divides synchronously to produce a variable number of progenies. Interestingly, how the prey cell size influences the proliferation of their predators remains poorly understood. By monitoring growth of *Bdellovibrio* in preys that exhibit different cell sizes, we demonstrated that the initial volume of the prey influences both the predator growth rate and the number of final progenies. Strikingly, although larger preys give rise to higher numbers of newborn predators at the single-cell level, global predator proliferation seems to be negatively impacted by increased average prey volume. Through these two examples, ranging from molecular to cellular scale, I hope to convince you that in biology, size always matters!

« Exposure to cigarette smoke impairs permeability of lung epithelial barrier through proteolytic cleavage of tight junction occludin by cathepsin S during COPD »

Paul Bigot [a], Simon Chesseron [a], Ahlame Saidi [a], Damien Sizaret [b], Agnès Petit-Courty [a], Yves Courty [a], Fabien Lecaille [a], Gilles Lalmanach [a]

[a] Université de Tours, Tours, France & INSERM, UMR 1100, Research Centre for Respiratory Diseases (CEPR), Team: "Proteolytic Mechanisms in Inflammation", Tours, France

[b] The University Hospital (CHU Tours), Pathological Anatomy and Cytology, Tours, France

Corresponding author: paul.bigot-2@etu.univ-tours.fr

Objectives: Smoking is accountable for more than 80% of chronic obstructive pulmonary disease (COPD, 3rd cause of death worldwide), which is characterized by emphysema, chronic bronchitis associated to an impaired epithelial permeability. Exposure of the lung to cigarette smoke elicits the expression of elastinolytic cathepsin S (CatS) (1). Despite an oxidizing environment, the reactivity of the nucleophilic Cys25 within the active site (sharing a thiolate (C^{25})-imidazolium (H^{159}) dyad) remains partially preserved, due to the reversible formation of a sulfenic acid, followed by a slower conversion to sulfinic acid (2-3). Recently, junctional and/or adhesion molecules were pinpointed as putative CatS targets, suggesting that CatS could proteolytically alter epithelial integrity during COPD.

Methods: ***Clinical features*.** Immunochemical analysis of human COPD and non-COPD lung biopsies revealed a decreased expression level of tight junction occludin for smokers (*vs* non-smokers). Statistical analysis demonstrated that occludin level correlates negatively with the smoking history (number of pack-years), COPD grades as well with proteolytic activity of CatS.

Results: ***Molecular mechanisms.*** Exposure of macrophages to cigarette smoke extract (CSE) or nicotine, a major CSE component, triggered expression of secreted and catalytically active CatS through the mTOR/TFEB signaling pathway, while incubation of lung epithelial cells with CatS is associated with an increased proteolysis of occludin, a decreased trans-epithelial electrical resistance and an amplified epithelial permeability. In a model of co-cultured macrophages/epithelial cells, an increase of epithelial permeability was observed following exposure to CSE. Conversely, both pharmacological inhibition of CatS as well its transient transcriptional inhibition by siRNAs restored the basal permeability of lung epithelial cells.

Conclusions: Altogether with its deleterious elastinolytic activity favoring emphysema, the uncontrolled enzymatic activity of CatS displays detrimental effects on the integrity of lung epithelial barriers, therefore strengthening the therapeutic relevance of targeting CatS in COPD (4-5).

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Keywords: cysteine protease, chronic obstructive pulmonary disease (COPD), epithelial barrier, inflammation, tight junction protein.

«A sensitive HPLC assay with fluorescence detection for the DJ-1 activity (park7), a parkinson-associated deglycase»

Nicolas MATHAS[1], Emmanuelle BRAUD[1], Erwan GALARDON[1], Catherine LAURENT[1], Lucie LARIGOT[2], Daniel MANSUY[1], Mélanie ETHEVE-QUELQUEJEU[1], Marie-Agnès SARI[1], Beatrice LE GRAND[2], Etienne BLANC[2], Xavier COUMOUL[2] and Julien DAIROU[1]

1 Université de Paris cité, CNRS, Laboratoire de Chimie et de Biochimie Pharmacologiques et Toxicologiques, F-75006 Paris, France. 45 rue des Saints-Pères, 75006 Paris, France

2 INSERM UMR-S 1124, Université Paris Cité, Toxicologie Pharmacologie et Signalisation cellulaire, 45 rue des Saints-Pères, 75006 Paris, France

E-mail of corresponding author: nicolas.mathas@parisdescartes.fr

Abstract: Glycation is an inevitable nonenzymatic covalent reaction between nucleophilic groups in proteins and nucleotides and endogenous reducing sugars or dicarbonyls (methylglyoxal) that results in protein and/or nucleotides covalent modification. After which a series of dehydrations, oxidations and rearrangements leads to amyriad of products called advanced glycation end products (AGEs). Previously, we showed that DJ-1 (or Park7) is a protein deglycase that repairs methylglyoxal-glycated biomolecules by acting on early glycation intermediates and releases repaired biomolecule and lactate. Moreover, the DJ-1 gene, also known as PARK7, is associated with recessive and sporadic forms of Parkinson's disease so over the last years, the function of DJ-1 has appeared as an important topic for the understanding on the etiology of Parkinson disease. In this context, availability of sensitive and quantitative enzyme assays is of prime importance to understand the role of DJ-1 and to develop specific effectors. Here, we describe a new method to measure DJ-1 activity based on the separation and quantification of glycated and unglycated fluorescent nucleotide by High Pressure Liquid Chromatography (HPLC). Kinetic and mechanistic analyses using recombinant DJ-1 confirmed the reliability of this approach. In addition, this assay was further validated using cellular lysats. Our results indicate that this novel DJ-1a assay is easy, sensitive, and specific.

KEYWORDS: Up to 5
DJ1, Methylglyoxal, Guanosine Fluorescein, enzyme assay, Electrophile stress.

SESSION 4

“ Archaea ”

- **Conférence par Tristan Wagner**
- **Solenne Ithurbide : prix de l’article de l’année de la SFBBM**
- **Clément Madru**
- **Roxane Lestini**

Animée par

Emmanuelle Schmitt

Ecole Polytechnique - Palaiseau

Conférence invitée session 4

«What are the molecular tricks of methanogenic archaea to save energy?» par Tristan Wagner

Max Planck Institute for Marine Microbiology, Celsiusstraβe 1, 28359, Bremen, Germany.

Nevena Maslać[a], Marion Jespersen[a], Marie-Caroline Müller[a], Olivier Lemaire[a], Seigo Shima[b], Sylvain Engilberge[c], and Tristan Wagner[a].

[a]Max Planck Institute for Marine Microbiology, Celsiusstraβe 1, 28359, Bremen, Germany. twagner@mpi-bremen.de

[b]Max Planck Institute for Terrestrial Microbiology, Marburg, Germany.

[c]European Synchrotron Radiation Facility (ESRF), Grenoble, France.

Objectives. Methanogenic archaea drive the final step in anaerobic organic compound mineralization, dictating the carbon flow of Earth's diverse anoxic ecosystems in the absence of inorganic electron acceptors. In other words, the methanogens had, have, and will have a predominant impact on our planet due to their ability to generate methane (CH_4) from different carbon sources. While the molecular process of CH_4 generation has been studied during the past decades, one question prevails: How do hydrogenotrophic methanogens (reduce CO_2 to CH_4 with H_2) grow so efficiently with an energy metabolism providing 60 times less ATP than O_2 respiration? With such a low energy yield, it would be impossible for this organism to fix CO_2 via known conventional strategies. This presentation will illustrate how these archaea avoid ATP-consuming steps and circumvent non-favorable reactions by tunneling effect strategies and regulatory networks [2,3,4,5].

Methods. *Methanothermococcus thermolithotrophicus* is a marine methanogen able to duplicate as fast as *Escherichia coli* in a fermenter gassed with H_2 and CO_2. However, in comparison to *E. coli*, *M. thermolithotrophicus* grows in a minimum mineral medium with stunning chemolithoautotrophic capabilities. To investigate the metabolic pathway of this archaeon, our laboratory developed a pipeline to extract, purify, characterize and crystallize the proteins from the methanogen under an anaerobic atmosphere. Enzymology and biophysical experiments confirm protein functions, while X-ray crystallography provides structural information.

Results. All the reactions involved in methanogenesis are ATP-independent [1], even the CO_2-fixation step. In this process, CO_2 is firstly reduced to formate and then covalently fixed on a carrier. The formate accumulation in the enzyme core drives its fixation on the carrier molecule [2]. The electrons required for CO_2-reduction are provided by another complex, which "energizes" electrons obtained from H_2 via another coupled reaction called electron-bifurcation [3]. Biosynthetic pathways also rely on these tunneling effects. For instance, the unfavorable first step in archaeal lipid biosynthesis is triggered by the next step, which is highly exergonic [4]. Transcriptome analyses combined with the structural/biochemical elucidation of the whole sulfur and nitrogen assimilation pathways were accomplished. This unprecedented success highlighted new catalytic reactions, allosteric regulation nodes, and an overall view of the stress response to starvation.

Conclusions. Hydrogenotrophic methanogens fuel their catabolic and anabolic pathways with H_2, the simplest molecule in the universe. While hydrogenases harness the reducing power of H_2, energy converters energize the captured electrons and distribute them through the metabolism via carriers. A myriad of processes using directly or indirectly these electrons are dispatched through the central metabolism to save cellular energy [5]. Among them are new catalysts, bypass of ATP-consuming reactions, enzymatic coupling, and regulation via allosteric effectors. These results were obtained by native exploration, an unbiased approach that must be applied to unveil the unsuspected.

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Keywords. Methanogenic archaea, Anaerobic metabolism, Biochemistry and structural biology, Enzymatic complex, Energy-saving strategies, Allosteric regulation.

Conférence invitée session 4
Prix de l'article de l'année de la SFBBM

«Cell division in the archaeon Haloferax volcanii relies on two FtsZ proteins with distinct functions in division ring assembly and constriction»

Par Solenne Ithurbide

University of Freiburg, Institute of Biology II, Molecular Biology of Archaea lab.

In bacteria, the tubulin homologue FtsZ assembles a cytokinetic ring, also called the Z ring, and plays a key role in the machinery that constricts to divide the cells. Many archaea encode two FtsZ proteins from distinct families, FtsZ1 and FtsZ2, with previously unclear functions but early observations by immunolabelling of one FtsZ as a band at mid cell predicted of a bacterial-like FtsZ division mechanisms. Our recent studies, in the archaeal model organisms Haloferax volcanii, showed fundamental differences of FtsZ-based archaeal division compared to Bacteria. Indeed, the 2 FtsZs were revealed to have fundamental but different roles in archaeal cell division. The two FtsZs, FtsZ1 and FtsZ2 colocalize to form the dynamic division ring. However, FtsZ1 can assemble rings independently of FtsZ2, and stabilizes FtsZ2 in the ring, whereas FtsZ2 functions primarily in the constriction mechanism. FtsZ1 also influenced cell shape, suggesting it forms a hub-like platform at midcell for the assembly of shape-related systems too. Many archaea also encode for homologues of MinD family proteins which are playing an important role into the mid-cell placement of the Z ring. However, we showed that none of the MinD homologues is involved in the Z ring placement in H. volcanii, highlighting further the differences between FtsZ based cell division between Archaea and Bacteria. Interestingly, both FtsZ1 and FtsZ2 are widespread in archaea with a single S-layer envelope, but the minority of archaea with a pseudomurein wall and division septum only have one FtsZ1. This suggest that an early duplication of FtsZ and then a lost of one FtsZ , concomitant with the pseudomurein apparition in Archaea have led to the development of a 1 FtsZ based cell division.

«Molecular specificities of the Replication Protein A in the third domain of life»

Clément Madru[a], Markel Martinez-Carranza[a], Maelenn Chevreuil[b], Bertrand Raynal[b], Sebastien Laurent[c], Didier Flament[c], Ahmed Haouz[d], Mart Krupovic[e], Marc Delarue[a], Pierre Legrand[f] and Ludovic Sauguet[a].

[a] Unit of Architecture and Dynamics of Biological Macromolecules, CNRS UMR 3528, Institut Pasteur, 75015 Paris, France.
[b] Plateforme de Biophysique Moléculaire, CNRS UMR 3528, Institut Pasteur, 75015 Paris, France
[c] Laboratoire de Microbiologie des Environnements Extrêmes, CNRS, UMR 6197, Ifremer, Université de Brest, F-29280 Plouzané, France.
[d] Plateforme de cristallographie, CNRS UMR 3528, Institut Pasteur, 75015 Paris, France.
[e] Archaeal Virology Unit, Institut Pasteur, Université de Paris, 75015 Paris, France. [f] Synchrotron SOLEIL, L'Orme des Merisiers, Gif-sur-Yvette, France. clement.madru@pasteur.fr; ludovic.sauguet@pasteur.fr

Replication Protein A (RPA) is the major single stranded DNA-binding protein in both eukaryotes and archaea with essential roles in DNA replication, recombination and repair. RPA binds to exposed ssDNA to protect it from nucleases, participates in a myriad of nucleic acid transactions and coordinates the recruitment of other important players. RPA is a heterotrimer and coats long stretches of single-stranded DNA (ssDNA). We present the first structure of an archaeal RPA in both its apo form and bound to ssDNA. It includes a trimeric core that is conserved with eukaryotes and additional domains that are specific to archaea. In its apo form, the RPA adopts a tetrameric assembly that dissociates upon binding to DNA. By using an integrative approach that combines X-ray crystallography, cryo-electron microscopy protein-protein and protein-nucleic acids interaction measurements, we investigated the role of each individual domain of RPA and present a structural model of how the archaeal RPA assembles on long ssDNA.

KEYWORDS: DNA replication; Archaea; ssDNA binding protein

« Understanding replication dynamics and chromosomal organisation in the archaea *Haloferax volcanii*»

C. Cockram[2], D. Noury[1], A. Thierry[2], R. Koszul[2], O. Nicolas[1] and R. Lestini[1]

[1] Laboratoire d'Optique et Biosciences, Ecole Polytechnique, CNRS UMR7645 – INSERM U1182, Institut Polytechnique de Paris, 91128 Palaiseau cedex, France.

[2] Institut Pasteur, Unité Régulation Spatiale des Génomes, CNRS, UMR 3525, F-75015 Paris, France.

DNA replication is essential to all proliferating cells. However, little is known concerning this important process in archaea. Because the archaeal DNA replication machinery is largely homologous to that of eukaryotes, expanding our knowledge of replication to archaea will deepen our understanding of replication dynamics in all domains of life.

Archaeal main chromosome is circular, a bacterial-like feature, but often contains multiple active replication origins, a eukaryotic-like feature. This is notably the case of the main chromosome of the archaea *Haloferax volcanii* which possesses four replication origins. *H. volcanii* is a halophilic archaea easily grown in laboratory (120 minutes generation time at 45°C on rich media) for which powerful tools have been developed. This includes genetic tools and the use of protein labeling by the green fluorescent protein GFP to study their localization in living cells, which are essential to tackle the *in vivo* study of fundamental processes such as DNA replication.

Using wide-field and 3D-SIM super-resolution live cell imaging, we have provided unexpected insight

into the intracellular dynamics of DNA replication in *H. volcanii* cells (1,2). Considering the 4 replication origins of the main chromosome and its high copy number (18 copies of the genome on average), an unexpected low number of replication foci was observed. These results prompted us to investigate the 3D organization of the chromosome in *H. volcanii*. Using HiC method with a resolution of up to 1 kb, we have revealed a chromosomal organisation composed of self-interacting domains and chromatin loops (3). This organisation is regulated by both transcription and the archaeal SMC protein. We could also show that replication is not strongly implicated in the chromosomal structuring in H. volcanii. Thus, it is unlikely that a chromosomal organisation bringing replication forks close to one another accounts for the relatively low number of replication foci observed. Yet, this method also relies on a population of cells, and, as a result, reflects an average of the population which may mask cell to cell variation. And oligoploidy is in itself another limitation, as interchromosomal contacts cannot be distinguished from intrachromosomal contacts. So it could be that replication affects chromosome organisation but that this effect has been averaged out by the heterogeneity of cells in the population. This why we are developing super-resolution imaging to map the spatial organization of chromosomal copies at the cell-to-cell level (4).

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SESSION 5

“ Genetic ”

- **Conférence par Claire Rougeulle**
- **Maxime Wery**
- **Louise Le Coadou**
- **Inge Kühl**

Animée par
Bernard Dujon
Institut Pasteur - Paris

Conférence invitée session 5

«X chromosome inactivation, at the interface between development, chromatin and the noncoding genome»

par Claire Rougeulle

Université Paris Cité, CNRS, Epigenetics and Cell Fate, F-75013 Paris, France

X chromosome inactivation (XCI) in mammals is an essential epigenetic process which compensates for X chromosome imbalance between sexes. XCI is established early during female development, at peri-implantation stages, and is triggered by the accumulation of the long noncoding RNA XIST. This process has been mainly studied in the mouse where embryonic stem cells (ESCs) have been instrumental to characterize the actors of the process, and to unravel the kinetics of the molecular events leading to the transcriptional silencing of one of the two X chromosomes. However, it is now known that X-inactivation initiates through remarkable diverse strategies in different species. We are using primate ESCs as a model system for early primate development, to characterize the early stages of X chromosome inactivation and to identify regulators of the process in primates. We are in particular exploring the extent to which long noncoding RNA contribute to the variation in XCI strategies between species.

«Translation is a key determinant controlling the fate of cytoplasmic long non- coding RNAs»

Sara Andjus[1,4], Ugo Szachnowski[1,4], Nicolas Vogt[1], Isabelle Hatin[2], Chris Papadopoulos[3], Anne Lopes[3], Olivier Namy[2], Maxime Wery[1,5] & Antonin Morillon[1,5]

[1] ncRNA, epigenetic and genome fluidity, Institut Curie, PSL University, Sorbonne Université, CNRS UMR3244, 26 rue d'Ulm, F-75248 Paris Cedex 05, France
[2] Genomics, Structure and Translation, Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris-Sud, Université Paris-Saclay, 91198 Gif-sur-Yvette cedex, France
[3] Molecular Bio-informatics, Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris-Sud, Université Paris-Saclay, 91198 Gif-sur-Yvette cedex, France
[4] equal contributions
[5] corresponding authors: maxime.wery@curie.fr; antonin.morillon@curie.fr

The hidden face of genomes produces thousands of long non-coding (lnc)RNAs. Although they were initially presumed to lack coding potential, recent works revealed that lncRNAs can be translated into micropeptides, which might represent proteins "in progress" throughout evolutionary constraints, or have actual intra/extra-cellular roles, or be the source of neoantigens driving the immune response. However, despite the interest they arouse, the *cis*- and *trans*-acting mechanisms controlling the synthesis of these peptides remain poorly characterized. In yeast, we previously showed that the cytoplasmic Xrn1-sensitive lncRNAs are degraded *via* the translation-dependent Nonsense Mediated mRNA Decay (NMD) pathway, suggesting that translation determines their degradation. Here, we show that most cytoplasmic lncRNAs are actively translated, modulating their cellular abundance and providing an opportunity for the cell to produce novel peptides. We found that NMD-sensitive lncRNAs accumulate in wild-type (WT) cells treated with translation elongation inhibitors. Our data indicate that translation also affects lncRNAs decay independently of NMD, by interfering directly with their decapping. Ribo-Seq analyses confirmed ribosomes binding to a substantial fraction of lncRNAs and identified actively translated small (sm)ORFs in their 5'-proximal region. Mechanistic analyses revealed that the NMD sensitivity of lncRNAs depends on the length of the 3' untranslated region, following the smORFs. Finally, we show that translation of an NMD-sensitive lncRNA reporter gives rise to a detectable peptide in WT cells. Our work highlights the role of translation in the metabolism of lncRNAs. We propose that the translation of lncRNAs could contribute to expose genetic novelty to the natural selection, while NMD would restrict their expression.

KEYWORDS: lncRNA/Xrn1/NMD/translation

«Enzymatic and structural characterization of the recurrent S1624C oncogenic mutation of the histone lysine methyltransferase SETD2»

Louise LE COADOU[a], Jérémy BERTHELET[b], Ariel MECHALY[c], Christina MICHAIL[a], Linh-Chi BUI[a], Ahmed HAOUZ[c], Jean-Marie DUPRET[a], Fernando RODRIGUES LIMA[a]

[a] Université Paris Cité, Unité de Biologie Fonctionnelle et Adaptative (BFA), UMR 8251, CNRS, Paris, France; [b] Université Paris Cité, Centre Epigénétique et Destin Cellulaire (CEDC), UMR 7216, CNRS, Paris; [c] Institut Pasteur, Plate-forme de Cristallographie-C2RT, CNRS UMR 3528, Paris, France

E-mail of corresponding author: louise.le-coadou@etu.u-paris.fr

Objectives: The histone methyltransferase SETD2 is responsible for the trimethylation of lysine 36 on histone H3 (H3K36me3) [1]. This epigenetic mark is crucial for transcriptional regulation, splicing and DNA damage repair [2]. Many studies have shown that SETD2 is a tumor suppressor and a cancer diver gene, frequently mutated in cancer [2],[3]. In particular, the S1624C recurrent oncogenic mutation has been identified in patients with T-cell lymphoma [4], adenocarcinoma or adrenal cortical carcinoma [5],[6]. So far, this mutation has not been characterized. Interestingly, it is located in the SET catalytic domain of SETD2 and may thus affect the enzymatic properties of SETD2. The objective of this project is to characterize the structural and functional impact of this mutation on SETD2.

Methods: Different biochemical, enzymatic and structural approaches were used such as: methylation assays (western blot, radioactivity and HPLC), crystallography, thermal shift assay (TSA), cell transfection and cellular enzymology.

Results: Methylation assays (HPLC, western blot/radioactivity) showed that the lysine methyltransferase activity of SETD2 S1624C mutant is drastically reduced (residual activity around 10%). Strong decrease of H3K36me3 mark is also observed on histones extracted from cells transfected with S1634C mutant. On the other hand, a crystallographic structure of the S1624C mutant in complex with the cofactor SAM (S-adenosylmethionine) and a histone H3 substrate peptide was obtained and showed that the mutation does not impair co-factor and substrate binding. Local and very slight deformations can be observed compared to the structure of SETD2 WT. Further results indicate that the replacement of the Ser residue by a bulkier cysteine reduces the stability of the mutant thus increasing its tendency to form aggregates. In addition, increased zinc release from the active-site zinc fingers of the enzyme is observed for the S1624C form thus further contributing to the formation of aggregates. Formation of reversible disulfide-dependent aggregates is also increased for the S1624C mutant thus supporting that the replacement of the Ser residue by a Cys increases redox sensitivity of the mutant form compared to WT SETD2.

Conclusions: Our study provides the molecular basis for the altered lysine methyltransferase activity of the oncogenic SETD2 S1624C mutant form. This mutation leads to a poorly active enzyme that retains the ability to bind its SAM cofactor and a H3 peptide substrate. The bulkier Cys compared to Ser leads to increased instability of the enzyme and aggregation. Moreover, increased disulfide- dependent aggregation of the mutant is observed. Ongoing studies (mass spectrometry and molecular dynamics) will provide further understanding of the molecular impact of the SETD2 S1624C mutation.

Keywords: Histone methyltransferase, SETD2, Mutant, Cancer, H3K36 trimethylation

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«Impact on mitochondrial function by increased levels of mitochondrial RNA polymerase»

Maria Miranda[a], Andrea Mesaros[a], Irina Kurnezova[b], Arnaud Mourier[c], Aleksandra Filipovska[b], Nils-Göran Larsson[a,d,*] and Inge KÜHL[e,*]

[a]Max Planck Institute for Biology of Ageing - Germany
[b] Harry Perkins Institute of Medical Research -The University of Western Australia - Australia
[c] CNRS - Institut de Biochimie et Génétique Cellulaires (UMR 5095) - France
[d] Karolinska Institutet - Sweden
[e] CEA, CNRS, Université Paris-Saclay - Institute of Integrative Biology of the Cell (UMR9198) – France

E-mail of corresponding author*: inge.kuhl@i2bc.paris-sacaly.fr

Objectives:
POLRMT is the sole RNA polymerase in mammalian mitochondria where it transcribes the mitochondrial genome (mtDNA) encoding essential components of the oxidative phosphorylation system which is of key importance for mitochondrial function[1,2]. POLRMT generates also the RNA primers to initiate replication of the mtDNA, placing this enzyme at the heart of both mtDNA expression and replication[3]. We previously showed that POLRMT levels play a role in balancing mtDNA replication and transcription, and elevated POLRMT levels were found in patients with lung cancer. Consistently, POLRMT has been proposed as a therapeutic target to treat some cancers[4].

Methods:
Here, we generated and characterized a mouse over-expressing *Polrmt* via a bacterial artificial chromosome into the germline to investigate the physiological and molecular consequences of elevated POLRMT levels in non-pathogenic conditions.

Results:
We present a first analysis of these mice that have a 30-50% increase of POLRMT protein levels. Our Polrmt overexpressor mice are viable and have no evident aberrant phenotype. Remarkably, this overexpression of Polrmt is accompanied by a strong increase in mitochondrial transcription in mouse heart.

Conclusions:
POLRMT is the limiting factor for mtDNA transcription since POLRMT levels result in an increased L-strand transcription, transcription capacity and mtDNA replication.

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KEYWORDS: mitochondria, mitochondrial RNA polymerase, mitochondrial transcription, mtDNA, mouse

SESSION 6

“ Enzymes ”

- **Conférence par Alain Marty**
- **Damien Sorigue : prix article de l’année SFBBM 2018**
- **Nicolas Joly : prix Maurice Nicloux 2021**
- **Claire Céré**
- **Paolo Zecchin**

Animée par
Fernando Rodrigues-Lima
Université Paris Cité – Paris

Conférence invitée session 6

«Pet recycling : from enzyme and process optimization to an industrial unit»

par Alain Marty

Carbios, Clermont-Ferrand alain.marty@carbios.com

Vincent Tournier; Nicolas Chabot, Carbios, Clermont-Ferrand
Sophie Duquesne; Guy Lippens; Isabelle André, Toulouse Biotechnology Institute (TBI), Toulouse

Plastics are found everywhere in our daily-life, they are exceptional materials and represent an annual world production of 322 million tons. However, their lifetime is often limited and nowadays they represent a major environmental issue with 125 million tons of generated plastic waste annually. Only 10% of collected plastics are recycled, and, at best, plastic wastes are incinerated but an unacceptable quantity are lost in nature, with for instance 9 million tons ending each year in the oceans.

Carbios (http://www.carbios.com), a young innovative green chemistry company, in collaboration with the laboratory TBI (Toulouse Biotechnology institute; INSA/CNRS/INRAE; http://www.toulouse-biotechnology-institute.fr), developed an extraordinary enzyme used to break down PET to return to monomers. A purification scheme was developed at pilot scale (1m3) to produce monomers with the same quality than petrochemical ones. It enables the material to be recycled ad infinitum and creates a virtuous circular economy scheme. The work of enzyme evolution leading to the possibility to make a new bottle from plastic waste was published in Nature (Volume 580 Issue 7802, 9 April 2020) and new development in enzyme engineering will be presented. A demo plant (reactor of 20m3) is operational since September 2021 and validates the technology. Carbios has announced the construction of a first industrial unit which will process 50,000 tonnes of PET waste in France which will be operational in early 2025

Key Words: PET recycling, enzyme, PETase, industrial unit

Conférence invitée session 6
Prix de l'article de l'année de la SFBBM 2018

«Mechanism and dynamic of Fatty acid photodecarboxylase.»
Par Damien Sorigue

Biosciences and Biotechnologies Institute Cadarache CEA Research Center CEA Cadarache 13108 Saint Paul les Durance
D. Sorigué, C. Berthomieu, M. Weik, M. H. Vos, P. Arnoux, P. Müller, F. Beisson.

Microorganisms are considered promising platforms for the production of lipid-based biofuels. In the past 20 years while enzymes allowing accumulation of oil (triacylglycerols) inside microbial cells have been intensively researched, an alternative strategy consisting in producing volatile hydrocarbons that are excreted outside cells has also been explored.

We have previously shown that microalgae have the ability to convert C_{16} and C_{18} fatty acids into alka(e)nes by a light- dependent pathway (1).

The enzyme responsible for this conversion has been identified in the microalgae *Chlorella variabilis* NC64A and has been shown to be a photoenzyme, i.e. an enzyme that is able to use light energy to perform its reaction at each catalytic cycle (2). This photoenzyme was named FAP for Fatty Acid Photodecarboxylase. FAP belongs to an algae-specific clade of the glucose-methanol-choline oxidoreductase family and catalyzes the decarboxylation of free fatty acids to n-alkanes or n-alkenes in response to blue light.

Recently, by combining static, time-resolved spectroscopy and crystallography, we finally provide a comprehensive understanding of the strikingly complex catalytic cycle of FAP.

Here we will present the discovery of FAP (1,2), well as new insights into the mechanism and dynamics of FAP (3). We anticipate these insights will guide future engineering of this unique photoenzyme tailored to specific applications in green chemistry.

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(1) D. Sorigué, *et al.* **Microalgae Synthesize Hydrocarbons from Long-Chain Fatty Acids via a Light-Dependent Pathway.** *Plant Physiol*. 171, 2393–2405 (2016).
(2) D. Sorigué, *et al.* **An algal photoenzyme converts fatty acids to hydrocarbons**. *Science*. **357**, 903–907 (2017).
(3) D. Sorigué, *et al.* **Mechanism and dynamics of fatty acid photodecarboxylase**. *Science*. accepted (2021)

Conférence invitée session 6
Prix Maurice Nicloux 2021

«Regulation and mode of action of AAA+ proteins: the Katanin example»

Par Nicolas Joly

Université de Paris, CNRS, Institut Jacques Monod, F-75013 Paris, France.

Nicolas Joly@, Eva Beaumale, Lucie Van Hove, Lionel Pintard
@nicolas.joly@ijm.fr

AAA+ proteins (ATPases associated with diverse cellular activities) are involved in many cellular activities including transcription, vesicular transport, proteolysis, DNA replication, meiosis and mitosis. They are defined by a conserved AAA+ domain (about 250 amino acids) which allows protein hexamerisation and ATP hydrolysis. This functional ATPase domain is the key element allowing these enzymes to convert chemical energy (ATP) to mechanical energy leading to substrate remodeling. Nevertheless, how this domain is allowing such conversion or how its activity is controlled in space and time for optimal cellular function are still poorly understood.

To address these questions, we are focusing on the structure-function study of Katanin which is an AAA+ enzyme specialized in microtubule severing. Interestingly, mutations of this enzyme which is essential for the control of microtubule organisation in different cellular contexts including cell division or neuron maturation, have been linked to various defects and pathologies including neurodegenerative disorders prostate and breast cancer and male sterility. Very recently, the model concerning this enzyme activity has been revisited. It has been proposed that instead of severing the microtubules, Katanin was damaging microtubule lattice by “extracting” tubulin dimers, leading to the formation of hole, which depending on the cellular context could be amplified (leading to severing) or repaired (stabilizing the microtubules).

Here, we will discuss the recent advances we obtained using complementary biochemical and genetic approaches and provide new insights into the molecular mechanisms which modulate Katanin’s microtubule severing activity depending on the cellular context.

«Antimicrobial and enzymatic properties of a new bacterial homologue of mammalian peroxidases»

Claire Céré[a,b], Brigitte Delord[a], Ænora Letourneur[a], Léa Vimbert[a], Jean-Paul Chapel[a], Claire Stines-Chaumeil[a]

[a]Centre de Recherche Paul Pascal UMR5031

[b]Present address INRAE Villenave d'Ordon

E-mail of corresponding authors: claire.cere@inra.fr; claire.stines@crpp.cnrs.fr

Objectives: Mono- or multi-species biofilms give rise to a wide range of chronic infections difficult to eradicate and negatively impacting on the quality of life of patients and mortality rates. Therefore it is desirable to generate *in situ* a broader spectrum antimicrobial activity, without resistance developed by microorganisms. Myeloperoxidase belongs to the heme dependent peroxidase super family and in addition to convert classical substrate of peroxidase into radical intermediate, has the unique properties to catalyze the formation of hypochloric acid from hydrogen peroxide and chloride (eq. 1) which confers bleach its antimicrobial activity. Eq. 1 $H_2O_2 + Cl^- + H_3O^+ = HOCl + 2H_2O$. The commercial human MPO (hMPO) is obtained from human blood which is precious and rare. We identified a bacterial homologue of mammalian peroxidase from *Rhodopirellula baltica* (RbMPO) by bioinformatic analysis. The aim of the study was to prove experimentally the homology of the bacterial enzyme with mammalian peroxidases to use it and decrease the costs of applications.

Methods: The first step was to clone, produce, purify and reconstitute enzyme with heme. The second step was to extensively characterize the biochemical and enzymatic properties of the bacterial enzyme with pre-steady-state and steady-state kinetics. The last step was to test the antimicrobial properties of the enzyme on ATCC 25922 *E. coli* strain.

Results: The new peroxidase was purified from inclusion bodies with good yield. It is a heme peroxidase with broad substrate specificity. It catalysis the pseudo- and halogenation of SCN^-, Cl^-, Br^- and I^-, and also presents catalase and classic peroxidase activities. Microbicidal effects were observed at least towards SCN^-.

Conclusions: In this study, a novel peroxidase was extensively characterized from biochemical and enzymatic approaches. Comparison with leucocyte hMPO leads to the conclusion that RbMPO is a homologue of hMPO with the same substrates specificity.

R**elevant references:** European Patent EP22305670 BACTERIAL MYELOPEROXIDASE-CATALASE AND APPLICATIONS THEREOF. Deposited the 05052022. Inventors: Stines-Chaumeil Claire, Céré Claire and Delord Brigitte

KEYWORDS: myeloperoxidase; heme; hypohalide and pseudo-hypohalide products; microbicidy

« Structural insights into archaeal [Fe-S]–dependent cysteine desulfidase»

Paolo Zecchin[1], Nisha He[1], Ludovic Pecqueur[1], Ornella Bimai[1], Jean-Luc Ravanat[2], Nadia Touati[3], Christophe Velours[4], Marc Fontecave[1], Béatrice Golinelli-Pimpaneau[1]

[1] Laboratoire de Chimie des Processus Biologiques, UMR 8229 CNRS, Collège de France, Sorbonne Universités, 11 Place Marcelin Berthelot, 75231 Paris cedex 05, France

[2] University of Grenoble Alpes, CEA, CNRS, iNAC, SyMMES, F-38000 Grenoble, France

[3] IR CNRS Renard, Chimie-ParisTech, 11 rue Pierre et Marie Curie, 75005 Paris, France

E-mail of corresponding author: paolo.zecchin@college-de-france.fr

Objectives: Sulfur is an essential element of life, which is stored within compounds such as L- cysteine and inorganic sulfide. Different classes of pyridoxal-phosphate-dependent (PLP)-L-cysteine desulfurases have been shown to catalyze the first step of sulfur mobilization from L-cysteine for the biosynthesis of sulfur-containing cofactors [1] or nucleosides [2]. However, genes encoding cysteine desulfurase are absent in several archaea [3], raising the possibility that, in these organisms, the first step of sulfur transfer from L-cysteine is carried out by another enzyme. A likely candidate is the [Fe- S]-dependent L-cysteine desulfidase (CyuA) [4], which would catalyze the transfer of sulfur from L- cysteine to an acceptor protein. Our project aims at questioning this hypothesis through the structural and biochemical study of CyuA from the archaeum *Methanococcus maripaludis* (MmCyuA).

Methods: X-ray crystallography, EPR spectroscopy, enzymatic assay, FPLC, HPLC, SEC-MALS

Results: We report the purification, and biochemical and biophysical characterization of MmCyuA. UV-visible and EPR spectroscopies together with enzymatic assays indicate that MmCyuA binds a [4Fe-4S] cluster that is required for catalytic activity. We solved the first crystal structure of a cysteine desulfidase, which reveals that it adopts a new fold. The structure of MmCyuA in complex with the L- serine inhibitor at 2.6 Å resolution shows that the [4Fe-4S] cluster is linked by only three cysteines and that the C-terminal catalytic domain displays structural homology to that of [4Fe-4S]-dependent L-serine dehydratase [5].

Conclusions: We have carried out the first spectroscopic and structural characterization of a CyuA enzyme, which leads us to propose a mechanism in which the [4Fe-4S]-cluster is used as a cofactor to transfer the sulfur atom from L-cysteine to an acceptor protein. *In vivo* assays are underway to test the hypothesis that cysteine desulfidase would fulfill the same sulfur mobilization function as PLP- dependent cysteine desulfurases in organisms lacking the latter enzymes.

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KEYWORDS: Enzymology, Protein crystallography, iron-sulfur cluster, Archaea, Sulfur metabolism.

SESSION 7

“ Education ”

- **Wilfried Grange, Florent Busi et Caroline Chauvet**

Animée par

Magali Blaud

Université Paris Cité – Paris

Conférence invitée session 7

« Environnement interactif numérique de simulation de techniques expérimentales »
par Wilfried Grange

Université Paris Cité, UFR Sciences du Vivant & UFR des Sciences Fondamentales et Biomédicales, F-75006 Paris

F. Busi, V. Arluison, N. Janel et W. Grange, C. Chauvet et E. Blanc

Mots-clés. Travaux pratiques, Enzymologie, R

Aujourd'hui des plate-formes telles que Moodle permettent de développer et consolider l'apprentissage à distance. Que ce soit sous forme de Questionnaire à Choix Multiples, de devoirs et/ou d'activités synchrones, les étudiant.e.s peuvent ainsi facilement revoir et approfondir des notions enseignées lors des Cours Magistraux (CM) ou des Travaux Dirigés (TD). Dans le cadre de travaux pratiques (TP), la situation est différente pour la raison simple que les étudiant.e.s ne peuvent disposer *a posteriori* de l'infrastructure utilisée lors des TP. Parce que les notions vues en CM, TD et TP sont intimement liées (une technique de mesure peut être présentée en CM, les données expérimentales résultantes peuvent être analysées en TD), l'impossibilité pour les étudiant.e.s de refaire une ou partie des expériences de TP nuit à leur compréhension et, de fait, à leur réussite.

Pour pallier ces insuffisances et ces possibles échecs, nous avons mis en œuvre une plate-forme (un site web) permettant de simuler des instruments et techniques utilisées en travaux pratiques. Cette plate- forme n'a pas vocation à se substituer aux TP en présentiel mais doit permettre aux étudiant.e.s de refaire des expériences, d'aborder des notions nouvelles et/ou de participer à l'auto-évaluation. Eventuellement, elle permettra un contrôle des connaissances sécurisé.

Comme preuve de concept, nous présentons un outil utilisé depuis la rentrée 2021 dans le cadre de TP d'Enzymologie en Licence à *Université de Paris* à plusieurs centaines d'étudiant.e.s [1]. L'intérêt de cet outil est multiple car il est (entre autre):

- sécurisé (l'enregistrement est fait *via* le courriel et le numéro d'étudiant.e),
- très facilement essaimable,
- journalisé,
- utilisable sur un smartphone et
- convivial pour les étudiant.e.s et les enseignant.e.s.

Soulignons enfin que des calculs avancés sont effectués à la demande sur un serveur OpenCPU (open source). Ce dernier fournit une Interface de Programmation Applicative (API) qui permet d'exécuter un script R (un langage de programmation). Les données sont calculées puis affichées de manière asynchrone afin de limiter l'accès au serveur et de rendre compte au mieux de l'expérience (les données s'affichent à la même vitesse que dans la réalité avec un bruit expérimental).

[1] Ce projet a bénéficié des fonds de l'IdEx *Université de Paris* « Innovations pédagogiques, hybridation des formations et pédagogies innovantes » et est soutenu par l'UFR Sciences du Vivant

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- **Christina Michail (Poster 33)**
- **Théo Paris (Poster 36)**

Animée par
Sandrine Boschi
& Sophie Rahuel-Clermont
Université de Lorraine – Nancy

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POSTER 1

Study of higher order nuclear cap-binding complexes mediating Pol II transcripts sorting

Dubiez Etienne[a], Erika Pellegrini[a], Anne-Emmanuelle Foucher[b], Jan Kadlec[b] & Stephen Cusack[a]

[a]European Molecular Biology Laboratory (EMBL), Grenoble

[b]IBS, Grenoble

E-mail of corresponding author: dubieze@embl.fr

Objectives: The nuclear cap-binding complex (CBC, composed of CBP20/NCBP1 and CBP80/NCBP2) binds to the 5' cap of nascent Pol II transcripts and co-ordinates their co-transcriptional maturation, export or degradation. To mediate these functions, CBC forms mutually exclusive, higher order complexes with different event or pathway-specific factors. We aim to characterize these complexes and inform on how they determine mRNA fate.

Methods: Cryo-electron microscopy, biochemistry

Results: To regulate processing and export of mature transcripts or specific degradation of aberrant RNAs, CBC functions together with its partner ARS2. The CBC-ARS2 complex links capped RNAs to protein factors that promote either a degradative (eg: PAXT/NEXT complexes) or productive fate (eg: PHAX, NCBP3). Our recent *in silico*, biochemical and structural studies unveil the basis for the molecular interactions involved in higher order human cap-binding complexes.

Conclusions: Our work reveals how different CBC partners form previously uncharacterized, mutually exclusive interactions on its surface and shed light on how RNA fate is monitored co-transcriptionaly in eukaryotes.

Relevant references:

Mazza *et al.*, 2002; Gonatopoulos-Pournatzis and Cowling, 2014; Schulze and Cusack, 2017

KEYWORDS: Up to 5

Co-transcriptional processing, m^7G cap, nuclear cap-binding complex, ARS2, cryoEM

POSTER 2

Modelling the active SARS-CoV-2 helicase complex as a basis for structure-based inhibitor design and for unveiling the mechanism of its replication

Dénes Berta[ab], Magd Badaoui[ab], Sam Alexander Martino[ab], Pedro J. Buigues[ab], Geoff Wells*[c], Sarah A. Harris*[d], Elisa Frezza*[f] and Edina Rosta *[ab]

[a]Department of Physics and Astronomy, University College London, London, WC1E 6BT, UK. [b]Department of Chemistry, King's College London, London, SE1 1DB, UK.
[c]School of Pharmacy, University College London, 29/39 Brunswick Square, London, WC1N 1AX, UK.
[d]School of Physics & Astronomy, University of Leeds, Leeds, LS2 9JT, UK.
[e]Université Paris Cité, CiTCoM, CNRS, F-75006, Paris, France.
E-mail of corresponding author: elisa.frezza@u-paris.fr

Objectives: Our first aim is to determine the structure of catalytically active complexes of the SARS- CoV-2 RNA helicase, also known as non-structural protein 13 (NSP 13). Second, we aim to characterize its dynamics and to get dynamics and structural insights on its catalytic function. Thirdly, our objectives are to characterize the RTC complex and to unveil the mechanism of translocation.

Methods: We performed a thorough analysis of homologous sequences and existing experimental structures to determine a set of structural models of NSP13 in complex with its native substrates (ATP and ssRNA). We performed microsecond molecular dynamics (MD) simulations using different state- of-the-art protocols and we conducted an extensive analysis of the simulations. To study translocation, we are setting up a new enhanced sampling approach which will allow us to determine a free energy profiles based on the direction of translocation.

Results: The analysis of homologous sequences sheds light upon the specificity of the domain structure of the viral helicase yielding no match over 20% except close relatives from the coronaviridae family. Our MD simulations of both the apo and the holo monomer in comparison with those of the dimer allowed us to address the fexibility and the stability of our catalytically competent structures. Our model provides valuable insights on the binding of the ATP and ssRNA at the atomic level. We identify the principal motions characterising the enzyme and highlight the effect of natural substrates on this dynamics. Furthermore, allosteric binding sites are suggested by our pocket analysis.

Conclusions: We proposed the first model of catalytically active complexes of the SARS-CoV-2 RNA helicase. The obtained structural and dynamical insights are important for subsequent studies of the catalytic function and for the development of specific inhibitors at our characterised binding pockets for this promising COVID-19 drug target.

Relevant references: [1] F. Wu et al., Nature, 2020, 579, 265–269. [2] J. A. Tanner, et al J. Biol. Chem., 2003, 278, 39578–39582. [3] K. A. Ivanov, et al. Nat. Rev. Drug Discovery, 2005, 4, 845–853. [4] I.
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KEYWORDS: RNA helicase, protein-RNA interactions, molecular modelling, allostery

POSTER 3

Nucleic acid-based biosensors for extracellular applications

Janine Kehrli, Farah Bouhedda, Claire Husser and Michael Ryckelynck

Université de Strasbourg, CNRS, Architecture et Réactivité de l'ARN, UPR 9002, Institut de Biologie Moléculaire et Cellulaire, 67084 Strasbourg

E-ail: kehrli@unistra.fr

Live detection of small molecules by RNA-based biosensors is a trending topic. Some well- characterized examples are the Spinach-based sensors that can sense, *in cellulo*, concentration variations of diverse metabolites such as adenosine diphosphate, guanine, adenine, guanosine triphosphate or cyclic di-AMP [1,2]. These biosensors are composed of three domains: a sensing aptamer dedicated to specific ligand recognition, a reporter domain that specifically binds a fluorogen and activate its fluorescence, and a communication module (CM) that transmits the ligand binding event to the reporter. We decided to develop an RNA-based biosensor of S-adenosylmethionine (SAM), a co-factor involved in cell cycle, autophagy, cell differentiation and metabolism. The obtention of a SAM biosensor is instrumental to monitor bacterial growth, and has additional applications in eucaryotic cell biology, as SAM is involved in multiple cellular mechanisms [3], or diagnostic use since SAM is a prime marker for the detection of certain cancers.

For applications going beyond intracellular detections (e.g., in patient-derived fluids), the intrinsic instability of RNA in complex medium is a major limitation. Previous studies on Spinach aptamer have demonstrated that the incorporation of 2' fluorinated (2'F) nucleotides significantly increased its chemical stability without disturbing its fluorescence emission capacity and overall structural organization [4]. Hence, this motivated us to use fluorinated nucleotides to develop a SAM biosensor capable of resisting premature degradation in complex environments.

Our group is specialized in the development of RNA-based fluorescent tools such as light-up RNA aptamers and fluorogenic biosensors by combining the use of droplet-based microfluidics, especially the ultrahigh-throughput functional screening "microfluidic assisted *in vitro* compartmentalization" (µIVC), with Next Generation Sequencing (NGS) and bioinformatics [5]. Iterative positive and negative rounds of µIVC enable the selection of candidate biosensors that generate fluorescence predominantly in the presence of their target molecule, while remaining mainly non-fluorescent in its absence. To isolate a SAM biosensor, we prepared a starting library of more than 65000 (4^8) variants by coupling the SAM-sensing aptamer to a light-up aptamer *via* a fully randomized twice four bases communication module (CM). This library will then be expressed in droplets as 2'F transcripts prior to screening them for their capacity to report on the presence of SAM.

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KEYWORDS: Biosensors – Nucleic acid modifications – S-adenosylmethionine

POSTER 4

Production, purification and characterization of CntI, the inner membrane exporter of pseudopaline from *Pseudomonas aeruginosa*

Maxime Mégret[a], Pascal Arnoux[b], Romé Voulhoux[c], Gilles Phan[a] and Isabelle Broutin[a]

[a]Laboratoire Cibles Thérapeutiques et Conception de Médicaments (CiTCoM), CNRS UMR 8038, Faculté de Pharmacie, Université Paris Cité, 75006 Paris, France.
[b]Institut de Biosciences et Biotechnologies (BIAM), CNRS UMR 7265, CEA Cadarache, Université Aix-Marseille, Saint-Paul-lez-Durance, France.
[c]Laboratoire de Chimie Bactérienne (LCB), CNRS UMR7283, Institut de Microbiologie de la Méditerranée (IMM), Université Aix-Marseille, Marseille, France.
maxime.megret@etu.u-paris.fr (M.M.) ; gilles.phan@u-paris.fr (G.P.) ; isabelle.broutin@u-paris.fr (I.B.).

Objectives: Metal homeostasis is essential for bacterial metabolism. Metal capture is secured by secondary metabolites called metallophores. In *Pseudomonas aeruginosa*, pseudopaline (Pp) has been recently discovered as an essential metallophore in chelating environment [1]. The objective of my PhD project is to understand the molecular basis behind Pp transport, in particular the mechanism of the inner membrane transporter CntI that belongs to the drug/metabolite superfamily (DMT) and that plays a role in airway infection [2]. In order to solve the 3D structure of CntI, we first need to produce, to purify and to stabilize the membrane transporter in a native conformation and high quantity. We will present preliminary data about CntI production, purification and biochemical characterizations.

Methods: The *cntI* gene was cloned in two expression vectors: pET22b with a C-ter His_6 Tag, and pET- TEV with a His_6 Tag and a TEV protease (TEVp) cleavage site at the N-terminus. For the two constructs, we screened different conditions of expression *in E. coli* and analyzed CntI production by dot-blot to identify the best condition. CntI was then extracted from the bacterial membrane with dodecylmaltoside (DDM) detergent and further purified in three steps by SP cation exchange, affinity Ni-NTA and S200 increase size exclusion chromatographies (SEC). Oligomeric state and protein- detergent ratio were analyzed by SEC-MALS (Multi Angle Light Scattering). In parallel, comparative homology modeling of CntI was performed using different programs in order to visualize the hypothetic binding cavity of Pp and the electrostatic distribution of CntI.

Results: CntI has been successfully produced and purified, but with a limited yield of 100 µg/L of bacterial culture. SDS-PAGE electrophoresis shows an apparent size of 24 kDa which is lower than expected (i.e. 33 kDa), nevertheless, mass spectrometry analysis confirmed the identity of CntI. Interestingly, SEC elution profile shows a peak corresponding to an apparent size of around 130 kDa which is more than a monomer of CntI. In order to analyze the oligomeric state of CntI, we decided to perform SEC-MALS analysis which revealed a 127 kDa complex including 95.8 kDa of DDM detergent which is in favor of a monomeric state of CntI. A TEVp cleavage assay of N-terminal His_6-tag obtained from the pET-TEV construct was performed, revealing a very partial cleavage suggesting a possible steric occlusion by the detergents or a low accessibility to the N-terminus as shown by the homology model.

Conclusions: We manage to produce and purify CntI in a non-aggregated state. We now need to minimize the amount of detergent around the protein and optimize the yield for structural studies. The stability of CntI will also be tested in different buffers and detergents.

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KEYWORDS: Membrane transporter, metallophore, *Pseudomonas aeruginosa*, structural biology.

POSTER 5

Investigation of the mitochondrial molecular organization using cryo-electron tomography

Florent Waltz [a], Benjamin D. Engel [a]

[a] Biozentrum, University of Basel, Basel 4056, Switzerland

E-mail of corresponding author: florent.waltz@unibas.ch

Mitochondria are essential components of eukaryotic cells that act as metabolic hubs and powerhouses, producing energy through aerobic respiration. Recent studies have highlighted how the main mitochondrial complexes, including mitochondrial ribosomes (mitoribosomes) and respiratory complexes, vary widely between eukaryotes in their composition, structure, and function. Additionally, the overall mitochondria architecture, notably cristae organization, is also different between lineages and probably under different growth conditions[1].

Cryo-electron tomography (cryo-ET) is a cutting-edge technique that unravels the organization of molecular complexes directly inside the cell, in native state. In the Engel lab, we apply cryo-ET to investigate organelle biology (nucleus, ER, Golgi, chloroplast), specifically on photosynthetic organisms[2]. Several studies have highlighted how the main mitochondrial complexes, including mitochondrial ribosomes (mitoribosomes) and respiratory complexes, vary widely between eukaryotes in their composition, structure, and function[3]. Using a combination of biochemistry, high resolution cryo-EM and cryo-ET, we study the molecular diversity of mitochondrial complexes across the photosynthetic lineages and how this diversity impacts mitochondria ultrastructure. Previously, we investigated the structure and organization of the green algae *Chlamydomonas reinhardtii* mitoribosome[4]. Employing a similar approach, we aim to elucidate the compositional and structural diversity of the main mitochondrial complexes in diverse photosynthetic organisms and determine how they shape mitochondria ultrastructure.

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KEYWORDS: Cryo-electron microscopy, tomography, mitochondria, respiratory complexes

POSTER 6

Lyme borreliosis diagnosis: combine *in silico* and *in vitro* approaches

Mickaël Guérin[a], Irene Maffucci[a], Séverine Padiolleau[a]

[a]University of Technology of Compiègne, UPJV, CNRS UMR 7025, Enzyme and Cell Engineering, Centre de recherche Royallieu, 60200, Compiègne, France

E-mail of corresponding author: severine.padiolleau@utc.fr

Objectives: Lyme borreliosis is a tick-borne disease caused by *Borrelia burgdorferi sensu lato*. Patients with Lyme disease suffer from various symptoms, one of which -namely erythema migrans- is characteristic, whereas others induce blurred clinical features such as fatigue, headaches, arthralgia and myalgia (1). The diagnosis of Lyme borreliosis, based on serology, is the subject of many debates and controversies since it lies on indirect approach and suffers from immune escape ability of bacteria inducing a poor immune response of patients. Therefore, a proper detection of Lyme borreliosis is essential to propose an adequate treatment to patients and avoid persistence of pathogens (2,3). The project aims to implement a new direct diagnostic test for Lyme disease by circumventing current limitations.

Methods: The structural data available in the literature on a targeted protein exposed on the surface of the pathogen had been used. This protein called BbCRASP-2 (or CspZ, BBH-06) can bind the factor H-like protein 1 (FHL-1) (4). The hot spots residues of the interaction had been determined using *in silico* alanine scanning mutagenesis. 3 *in silico* peptides had been designed based on the hot spots and bioinformatics methods such as folding prediction, accelerated molecular dynamics and docking prediction. The affinity will be determined *in vitro* using different approaches (ELISA, SPR, ITC, WB, Flow Cytometry) and the structural characterization will be achieved.

Results: Based on the hot spots, the 3 peptides designed *in silico* show promising results in terms of structural stability and docking prediction, almost comparable to the natural interaction between FHL- 1 and BbCRASP-2. The peptides are currently in synthesis. Regarding the target protein BbCRASP-2, the production, purification and characterization of this recombinant protein in *BL21(DE3) E. coli* strain is managed. The confirmation of the peptides interaction with BbCRASP-2 using ELISA and ITC methods is in progress.

Conclusions: The *in silico* results are promising and will be confirmed *in vitro*. Continuous exchanges between *in silico* and *in vitro* data will allow to optimize the definitive peptide ensuring a sensitive and reliable detection of bacteria. By circumventing the traditional phage display approach, which remain a long and fastidious method, the final objective is to pave the way for a multiplexed test and open a few doors for the artificial intelligence. Thus, we will bypass the two main limitations of current diagnostic tests, (i) by allowing direct detection of infection, and (ii) by detecting a wide variety of pathogens involved in Lyme borreliosis.

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KEYWORDS: Lyme borreliosis, Diagnosis, Bioinformatic, Molecular modelling and interaction, Immunotechnology

POSTER 7

Polyphenols-rich extracts of Annonaceae and Zingiberaceae dietary plants show metabolic benefits by lowering lipid accumulation in high-fat-diet- induced obese C57BL/6 mice.

Achille Parfait Nwakiban Atchan[a]*, Shilpa Talkad Shivashankara[b], Uma Venkateswaran Manjappara[2]

[a]Department of Biochemistry, Faculty of science, University of Dschang, P.O. Box 96 Dschang, Cameroon;

[b] Department of Lipid Science, CSIR-Central Food Technological Research Institute (CFTRI), Mysore 570 020, India.

E-mail of corresponding author: nwakiban@gmail.com

Objectives: Metabolic syndrome (MetS) is very complex and is associated with a series of pathologies, with an overall prevalence constantly increasing worldwide. The initial management of this physiopathology involves lifestyle modifications, including changes in diet and exercise habits [1]. Besides, rather than conventional drugs like Orlistat, herbs/spices traditionally used to remedy these diseases have been systematically re-evaluated [2]. In the present work, we chemically characterized and investigated the *in vivo* beneficial effects of *Xylopia parviflora (A. Rich.) Benth* and *Aframomum citratum (Pereira ex Oliv. et Hanb.) K. Shum* extracts, focusing on Obesity-related lipid parameters in high fat-fed C57BL/6 mice.

Methods: Hydro-ethanolic extracts were prepared and characterized by RP-HPLC-PDA and UPLC- Triple TOF-ESI-MS/MS analysis. They were orally administrated for 30 days in different doses (100 $mg.kg^{-1}$ B.W and 200 $mg.kg^{-1}$ B.W) to obese C57BL/6 mice. Food intake and body weight were recorded every day. Anthropometric, plasma biochemical parameters, and lipid content were estimated at the beginning and end of the experiment. Epididymal and inguinal adipose tissues, as well as liver tissue, were subjected to histological examinations and oxidative stress markers were estimated. Lipid content estimation and FAME analysis were performed in the fecal, liver, and adipose tissue samples.

Results: Oral administration of the extracts at 200 mg.kg-1 B.W significantly reduced food intake, body weight. Decreased in liver and white adipose tissue (WAT) weight as well as lipid content in plasma were observed. Plasma enzyme (SGOT, SGPT, ALP) estimation showed there was no damage to vital organs. The chemical analysis suggested that phenolic acids and flavonoids identified in the extracts could potentially justify the biological properties observed

Conclusions: The main findings of this study showed that *Xylopia parviflora (A. Rich.) Benth* and *Aframomum citratum (Pereira ex Oliv. et Hanb.) K. Shum* decreased lipid accumulation in high-fat- diet-induced obese C57BL/6 mice and confirmed, at least in part, our previous *in-vitro* and *ex-vivo* analysis. However, deduction of molecular mechanisms underlying these effects will require further investigations.

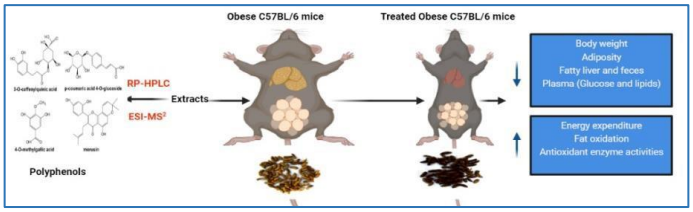

Figure 1: Effect of extracts on high-fat-diet-induced obese C57BL/6 mice.

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Keywords: Polyphenols, High-fat diet, Obesity, C57BL/6 mice, Liver tissues, Lipid accumulation, botanicals

POSTER 8

Lon protease knockdown induces mitochondrial DNA damage

Marie-Paule Hamon*, Bertrand Friguet

Sorbonne Université, CNRS, INSERM, Institut de Biologie Paris-Seine,

E-mail of corresponding author: mariepaulehamon@gmail.com

Objectives: Well known for its important involvement in oxidized protein elimination within the mitochondrial matrix Lon protease also intervenes with the mitochondrial DNA (1). In addition to a maintenance action on this genome, Lon contributes to the regulation of its replication and transcription. Therefore, a Lon depletion can be expected to induce effects on the mitochondrial DNA and, from there, on the mitochondrial function. This is why we decided to deepen the missions provided by Lon by looking for possible damage to mitochondrial DNA associated with its under-expression. We have therefore investigated whether or not the integrity of mitochondrial DNA is preserved in a HeLa cell line stably transfected with an inducible shRNA directed against Lon.

Methods: We carried out qPCR experiments based on the principle that DNA lesions (abasic sites, strand breaks, nucleic acids oxidation, …) can slow down or even block the progression of DNA polymerase. Therefore, the amplification of a damaged sequence will be less efficient than that of an undamaged sequence. To localize the sites of possible lesions, the mitochondrial DNA was divided into nine consecutive sequences and nine pairs of primers were used to amplify these sequences separately.

Results: Lon deficiency results in less amplification of the mitochondrial genome with differences between the nine sequences.

Conclusions: Lon depletion results in mitochondrial DNA alterations that have now to be determined. Due to the oxidative stress (ROS and protein carbonylation) observed in our previous works (2) on the effects of a Lon knockdown, we speculated that nucleotide oxidation could be one of these mtDNA damages. Regarding the guanine tendency to oxidation and the fact that Lon protease binds mt DNA on guanine-rich sequences, we are now working on the evaluation of 8-hydroxy-2'-deoxyguanosine levels with and without Lon.

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KEYWORDS: Lon protease, oxidative stress, HeLa cells, mitochondrial DNA damage and dysfuntion

POSTER 9

The hyperthermophilic archaeon *Thermococcus kodakarensis* is resistant to pervasive negative supercoiling activity of DNA gyrase

Paul Villain[a], Violette da Cunha[a], Etienne Villain[d], Patrick Forterre[a,b], Jacques Oberto[a], Ryan Catchpole[a,c] & Tamara Basta[a]

Affiliation :

[a] Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198, Gif-sur-
Yvette, France

[b] Archaeal Virology Unit, Institut Pasteur, Paris, France

[c] Present address : Department of Biochemistry and Molecular Biology, University of Georgia, Athens,
GA 30602, USA

[d] Current affiliation, Immunology department, Institut Pasteur, Paris, France.

E-mail of corresponding author: tamara.basta@i2bc.paris-saclay.fr

In all cells, DNA topoisomerases dynamically regulate DNA supercoiling allowing essential DNA processes such as transcription and replication to occur. How this complex system emerged in the course of evolution is poorly understood. Intriguingly, a single horizontal gene transfer event led to the successful establishment of bacterial gyrase in Archaea, but its emergent function remains a mystery. To better understand the challenges associated with the establishment of pervasive negative supercoiling activity, we expressed the gyrase of the bacterium Thermotoga maritima in a naïve archaeon Thermococcus kodakarensis which naturally has positively supercoiled DNA. We found that the gyrase was catalytically active in T. kodakarensis leading to strong negative supercoiling of plasmid DNA which was stably maintained over at least eighty generations. An increased sensitivity of gyrase-expressing T. kodakarensis to ciprofloxacin suggested that gyrase also modulated chromosomal topology. Accordingly, global transcriptome analyses revealed large scale gene expression deregulation and identified a subset of genes responding to the negative supercoiling activity of gyrase. Surprisingly, the artificially introduced dominant negative supercoiling activity did not have a measurable effect on T. kodakarensis growth rate. Our data suggest that gyrase can become established in Thermococcales archaea without critically interfering with DNA transaction processes.

KEYWORDS: Archaea, Thermococcus, DNA Topology, DNA topoisomerases, DNA gyrase

POSTER 10

Role of aIF5B in archaeal translation initiation

Ramy Kazan[a], Gabrielle Bourgeois[a], Christine Lazennec-Schurdevin[a], Eric Larquet[b], Yves Mechulam[a], Pierre-Damien Coureux[a] and Emmanuelle Schmitt[a]

[a]Laboratoire de Biologie Structurale de la Cellule, BIOC, Ecole polytechnique, CNRS, Institut Polytechnique de Paris, 91128 Palaiseau cedex, France.

[b] Laboratoire de Physique de la Matière Condensée, PMC, Ecole polytechnique, CNRS, Institut Polytechnique de Paris, 91128 Palaiseau cedex, France.

In eukaryotes and in archaea late steps of translation initiation involve the two initiation factors e/aIF5B and e/aIF1A. In eukaryotes, the role of eIF5B in ribosomal subunit joining is established and structural data showing eIF5B bound to the full ribosome were obtained. To achieve its function, eIF5B collaborates with eIF1A. However, structural data illustrating how these two factors interact on the small ribosomal subunit have long been awaited. The role of the archaeal counterparts, aIF5B and aIF1A, remains to be extensively addressed. We study the late steps of *Pyrococcus abyssi* translation initiation. Using *in vitro* reconstituted initiation complexes and light scattering, we show that aIF5B bound to GTP accelerates subunit joining without the need for GTP hydrolysis. We report the crystallographic structures of aIF5B bound to GDP and GTP and analyze domain movements associated to these two nucleotide states. Finally, we present the cryo-EM structure of an initiation complex containing 30S bound to mRNA, Met-tRNAi^{Met} , aIF5B and aIF1A at 2.7 Å resolution. Structural data shows how archaeal 5B and 1A factors cooperate to induce a conformation of the initiator tRNA favorable to subunit joining. Archaeal and eukaryotic features of late steps of translation initiation are discussed.

KEYWORDS: Translation, Initiation, Ribosome

POSTER 11

Detection of G-quadruplexes (G4) with super-resolution microscopy in *Haloferax volcanii*; a model archaeon for studying G4 functions.

Zackie Aktary[a], Guglielmo Vescoa,[b], Anne Cucchiarini[a], Yu Luo[a], Daniela Verga[c], Patricia Duchambon[c], Pierre Mahou[a], Roxane Lestini[a], Nicolas Olivier[a], Natalia Bohalovad, Otilia Porubiaková[d], Claudia Sissi[g],Luca Nardo[h], Václav Brázda[d], Amal Makrini[e], Jean-Christophe Andrau[e], Jean-Louis Mergny[a] & **Lionel Guittat**[a,f]

[a] Laboratoire d'Optique et Biosciences, Ecole Polytechnique, CNRS UMR7645 – INSERM U1182, Institut Polytechnique de Paris, Palaiseau, France.
[b] Department of Science and High Technology, University of Insubria, 22100 Como, Italy.
[c] Institut Curie, CNRS UMR9187, INSERM U1196, PSL Research University, F-91405 Orsay, France.
[d] Institute of Biophysics of the Czech Academy of Sciences, Královopolská 135, 612 65 Brno, Czech Republic.
[e] Institut de Génétique Moléculaire de Montpellier, CNRS-UMR 5535, Université de Montpellier, Montpellier, France.
[f] Université Sorbonne Paris Nord, UFR SMBH, 93017 Bobigny, France.
[g] Department of Pharmaceutical and Pharmacological Sciences, University of Padova, 35131 Padova, Italy
[h] School of Medicine and Surgery, BioNanoMedicine Center NANOMIB, University of Milano-Bicocca, 20854 Vedano al Lambro (MB), Italy.
E-mail of corresponding author: lionel.guittat@polytechnique.edu

Phylogenetic analyses performed by Carl Woese and other microbiologists in the 70's led to a profound reorganization of the tree of life with the proposal of three primary domains: Eukarya, Bacteria and Archaea. Detailed phylogenomic analyses of Asgard kingdom indicate that these archaea represent the closest known relatives of eukaryotes suggesting an archaeal ancestor of the origin of eukaryotes (1,2).

G-quadruplex structures (G4) formed by guanine rich sequences are among the most intensively studied local DNA/RNA structures that regulate diverse biological processes such as replication and gene expression. A recent seminal analysis of Potential Quadruplex Structure (PQS) occurrences, frequencies and distributions in archaeal genomes indicates that archaea are, like eukaryotes and bacteria, prone to G4 formation (3).

To understand the roles of G4 in archaea, we use *Haloferax volcanii* since a variety of genetic, molecular and biochemical tools have been developed, making it one of the key model organisms within the Archaea. Detailed bioinformatic analyses of the *H. volcanii* genome with our home-made G4Hunter software revealed a large number of G4-prone motifs. Biophysical analyses showed that many of these sequences form stable G4 structures under physiological conditions *in vitro*. To go further and detect G4s *in vivo* at the single-cell level, we used two different super-resolution light microscopy methods: STORM (stochastic optical reconstruction microscopy) and SIM (structured illumination microscopy) (4). Using the BG4 antibody specifically targeting G4, we directly visualized these non-canonical structures within intact single cells. G4 foci are detected both in proliferating cells and in cells in stationary phase, and their number are modulated by the presence of G4 specific ligands.

Our findings constitute, to our knowledge, the first detection of G4 structures with BG4 in prokaryotic cells. Together, with *H. volcanii* as a new model, our work fills the gap between bacteria and eukaryotic organisms for G4 studies and will help to uncover the evolutionary history of G4 structures in the tree of life (5).

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KEYWORDS: Archaea, G-quadruplex, Haloferax volcanii, Super-Resolution microscopy

POSTER 12

From one C2 to two C1: how deep-sea archaea consume ethane without oxygen

Olivier N Lemaire[a], Cedric J Hahna[a], Jörg Kahnt[b], Sylvain Engilberge[c], Gunter Wegener[a,d,e] and Tristan Wagner[a].

[a]Max Planck Institute for Marine Microbiology, Celsiusstraβe 1, 28359, Bremen, Germany.
[b]Max Planck Institute for Terrestrial Microbiology, Marburg, Germany.
[c]European Synchrotron Radiation Facility (ESRF), Grenoble, France.
[d]MARUM, Center for Marine Environmental Sciences, University of Bremen, Bremen, Germany.
[e]Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, Bremerhaven, Germany.
olemaire@mpi-bremen.de

Objectives. Oceanic geothermal seeps perfuse sediments with abiogenic alkanes, partly counterbalanced by their biological oxidation. Anaerobic bacterial alkanotrophs have been described since decades but the recent studies keep highlighting the role of their poorly characterized archaeal counterparts [1]. The archaeon *Candidatus* Ethanoperedens thermophilum is one of the two known organisms able to anaerobically oxidize ethane, the second most abundant emitted alkane. A recently described enrichment of this archaeon depending on bacterial sulfate reduction allowed the study of the ethanotrophic pathway, adapted from the C1-strict methanotrophy but exhibiting several unclear steps.

Methods. To verify the previously proposed ethanotrophic pathway, the enrichment was grown in the presence of specific inhibitors and the protein profile was compared to methanotrophic and methanogenic archaea. In order to ensure an unbiased analysis of such an exotic metabolism, we then directly purified in anaerobic conditions the enzymes from the Ca. E. thermophilum enrichment. The purification was challenging due to the biomass limitations and the microbial complexity of the enrichment. The purified complexes were biochemically characterized and their structure were obtained by x-ray crystallography.

Results. Like archaeal methanotrophy, the process is initiated by the ethane fixation as an ethyl-thiol compound (ethyl-Coenzyme M) catalyzed by a specific Alkyl-CoM reductase (ACR) [1]. We obtained the atomic- resolution structure of the native enzyme and described its specific features for adapting to ethane the C1 chemistry proposed in methane-generating ACR [2]. A specialized gas tunnel, cofactor and post-translational modifications as well as a wider catalytic chamber distinguish this enzyme from other ACRs [1,2]. During ethanotrophy, the ethyl-CoM is converted into acetyl-Coenzyme A (CoA) by a yet uncharacterized pathway and the acetyl group is split into its methyl and carbonyl C1 moieties by the Acetyl-CoA decarbonylase/synthase (ACDS) complex, generating CO_2 and a methyl-group ultimately converted to CO_2 by the formyl-methanofuran dehydrogenase (Fwd) complex during reverse methanogenesis [3]. We biochemically and structurally characterized a native ACDS sub-complex and the native complete Fwd complex from *Ca*. E. thermophilum and showed the presence of a new module in both enzyme, allowing the coupling of the acetyl-CoA and formyl- methanofuran oxidation to the reduction of the electron shuttle F_{420}.

Conclusions. The ethanotroph successfully adapted the specific chemistry of ACR for a C2 compound. The use of the ACDS complex then allows the generation of two C1, ultimately generating CO_2 and reduced F_{420} at the end of the ethanotrophy pathway. This is the first description of such thermodynamically favorable F_{420}- reducing CO_2 generation which explains the efficiency of archaeal ethanotrophs.

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Keywords. Archaea, Microbial Metabolism, Enzymatic complex, Structure, Alkanotrophy.

POSTER 13

A new example of food translocation : characterization of a novel methanogenic archaea isolated from human feces

Virginie PILLIOL[1, 2], Madjid MORSLI[1,3], Laureline TERLIER [1], Yasmine HASSANI[1,3], Ihab MALAT[1,3], Michel DRANCOURT[1,3], Gérard ABOUDHARAM[1,2], Ghiles GRINE[1,3], Elodie TERRER [1,2]*

1. Aix-Marseille Univ., IRD, MEPHI, AP-HM, IHU Méditerranée Infection, Marseille, France.
2. Aix-Marseille Université, Ecole de Médecine Dentaire, Marseille, France.
3. IHU Méditerranée Infection, Marseille, France.

E-mail of corresponding author: elodie.terrer@univ-amu.fr

Objectives: Here we describe a new *Methanosphaera sp.* isolated from a human stool sample and also detected in pig faeces.

Methods: Anonymized stool remains were taken from the clinical microbiology laboratory of the IHU Méditerranée Infection after the routine analysis. A suspension of each left-over stool sample was inoculated in a Hungate tube containing GG medium. Culture following was performed by gas chromatography to measure methane production. Methane positive cultures were inoculated on solid GG medium in anaerobic atmosphere at 37°C. After nine days, colonies were observed after Gram coloration and by confocal and electronic microscopy. MiSeq Illumina sequencing was used to obtain the complete genome. Then, RT-PCR system was designed for screening in animal faeces.

Results: A new species of *Methanosphaera* never described before, was isolated from a human stool sample. It is a Gram-positive coccus, autofluorescent by confocal microscopy at a wavelength of 420 nm. Complete genome was obtained by MiSeq Illumina sequencing and allowed the design of a specific RT-PCR system based on a DNA-polymerase subunit. Screening in animal faeces allowed the detection of this species in pigs with a prevalence of 50%.

Conclusions: The isolation of this unpreviously described *Methanosphaera sp.* from a human left- over stool sample may be an example of food translocation from pigs. This species could be one the most prevalent methanogen in pigs.

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KEYWORDS: methanogen, methanosphaera, anaerobic culture, food translocation

POSTER 14

Evolution, enzymatic activities and biological functions of ASH-Ski2 and Hel308, the two main groups of Ski2-like helicase proteins in Archaea

Manon Batista[1], Petra Langendijk-Genevaux[2°], Mirna Hajj[1],Duy Khanh Phung[3], Laura Plassart[1], Isabelle Canal[1], Régine Capeyrou[1], Marta Kwapisz[1], Christine Gaspin[4], Didier Flament[5], Rémi Dulermo[5], Sébastien Laurent[5], Gwennaele Fichant[2], Marie Bouvier[1], and Béatrice Clouet-d'Orval[1]*

[1] Laboratoire de Biologie Moléculaire, Cellulaire et du Développement, UMR5077, Centre de Biologie Intégrative (CBI), Université de Toulouse, CNRS, Université Paul Sabatier, F-31062 Toulouse, France.

[2] Laboratoire de Microbiologie et de Génétique Moléculaires, UMR5100, Centre de Biologie Intégrative (CBI), Université de Toulouse, CNRS, Université Paul Sabatier, F-31062 Toulouse, France.

[3] University College London, Gower Street, Darwin Building, London WC1E 6BT United Kingdom.

[4] INRAE, UR875 Mathématiques et Informatique Appliquées Toulouse, Chemin de Borde-Rouge BP 52627, Castanet-Tolosan 31326, France.

[5] Laboratoire de Microbiologie des Environnements Extrêmes, UMR6197, Ifremer, Université de Bretagne Occidentale, CNRS, F-29280 Plouzané, France

° Co-first author

E-mail of corresponding author: Beatrice.Clouet-dOrval@univ-tlse3.fr

ABSTRACT: RNA helicases perform essential housekeeping and regulatory functions in all domains of life by binding and unwinding RNA molecules. The Ski2-like RNA helicases are among the most important enzymes in RNA metabolism and are ubiquitous among Eukarya. Eukaryal genomes often encode multiple homologs with specialized functions. The significance of the expansion and diversification of Ski2-like proteins in Archaea has not yet been established. The domain Archaea is a key research model for gaining insights into the origin and evolution of life and it is now proposed that Eukarya arose within Archaea. Indeed, these micro-organisms share a high significant similarity with the basal transcriptional machinery of Eukarya. Here, by studying the diversity of Ski2-like helicases and their phylogenetic distribution among archaeal genomes, we provide evidence for the importance of this protein family in archaeal RNA as well as DNA metabolism. We show that, in the course of evolution, ASH-Ski2 and Hel308, the two main groups of Ski2-like proteins, have diverged in their biological functions and enzymatic activities. Whereas Hel308 has been shown to mainly act on DNA, we show that *Pyrococcus abyssi* ASH-Ski2, previously described to be associated with the 5'-3' aRNase J exonuclease, is an RNA helicase. By characterizing the *in vitro* enzymatic activity of ASH-Ski2 from the thermococcale *Pyrococcus abyssi* archaeon, we show that ASH-Ski2 supports an RNA unwinding as well as efficient annealing activities with a 3'-5' polarity. This activity appeared to be impaired in the presence of its protein partner, aRNase J. In addition, we provide evidences of the importance of ASH-Ski2 in cellular metabolism pathways related to translation by analysing the transcriptome of *Thermococcus barophilus* ΔASH-Ski2 strain.

KEYWORDS: RNA helicase, Ski2-like, *Archaea*

POSTER 15

Are rhodanese family enzymes new promiscuous catalysts ?

Razan ALSOHAIBANI[a], Hortense MAZON[a], François TALFOURNIER[a] & Sandrine BOSCHI-MULLER[a]

[a] Laboratoire IMoPA Equipe Enzymologie Moléculaire et Structurale, UMR 7365 CNRS-Université de Lorraine, Faculté de médecine, Bâtiment Biopôle, 9 Avenue de la Forêt de Haye, BP 20199 54505 Vandœuvre-lès-Nancy, France

E-mail of corresponding author: razan.alsohaibani@univ-lorraine.fr

The rhodanese domain, composed of a central β sheet of 5 strands flanked by 4 or 5 α helices, is found in all major evolutionary phyla and often in several proteins encoded by the same genome. Rhodanese proteins are classified into four families according to the number of rhodanese domains and the presence, or not, of additional domains. Some of the rhodanese-family members exhibit catalytic activities like the sulfurtransferases which are implicated in hydrogen sulfide metabolism and signaling processes as sulfur carriers. These enzymes catalyze a sulfur transfer reaction from a donor substrate like 3-mercaptopyruvate or thiosulfate for 3-mercaptopyruvate sulfurtransferases or thiosulfate sulfurtransferases, respectively. Human CDC25 phosphatases (encoded by the "Cell Division Cycle 25" genes) which catalyze the dephosphorylation of cyclin dependent kinases, belong also to this family. The fact that these enzymes exhibit a side arsenate reductase activity suggest that the rhodanese domains represent new examples of promiscuous catalysts exhibiting sulfurtransferase, phosphatase and arsenate reductase activities in the same active site. Interestingly, all these activities rely on the presence of a Cys-containing catalytic loop, but sulfurtransferases and phosphatases/arsenate reductases mainly differ by the size of the catalytic loop which is one residue longer in the later ones (CX_5R vs CX_4R).

In this context, this project aims to characterize by a structure-function relationship approach the promiscuous activities of rhodanese family enzymes, using the human TSTD1 sulfurtransferase and the catalytic domain of the human CDC25B as model enzymes. Preliminary results will be presented and the role of the length of the active-site loop in the substrate recognition and the catalytic activities will be discussed.

KEYWORDS: Rhodanese family, sulfurtransferases, phosphatases, promiscuity, structure-function

POSTER 16

Discovery and Engineering of bacterial alpha-transglucosylases for the tailor-made synthesis of alpha-glucans and gluco-conjugates

Elodie Bascans, Etienne Severac, David Guieysse, Magali Remaud-Simeon, Claire Moulis

Toulouse Biotechnology Institute, INSA, CNRS, INRAE, Toulouse, France

E-mail : bascans@insa-toulouse.fr

Alpha-transglucosylases produced by lactic acid bacteria are promising tools for the synthesis of
novel carbohydrate structures, as they catalyze the production of high molar mass alpha-glucans from sucrose, a simple and low-cost agroresource. The best-known polysaccharide of this family is dextran, an homopolymer of glucosyl units mainly linked by alpha-1,6 osidic linkages that found various industrial applications, e.g. in food, health, and as biomaterials [1]. However, different alpha- glucans varying in term of osidic linkage content and physico-chemical properties can be produced following the enzyme specificity used, that could open the route for new potential applications.

These sucrose-active enzymes are classified in the family 70 of Glycoside-Hydrolases, which comprises today around 800 sequences for only about sixty enzymes biochemically characterized, that remains very low. However, with the progress in bioinformatics, structure-function studies, screening technologies and enzyme engineering, we recently discovered new enzymes that catalyze the formation of a broad variety of new gluco-oligosaccharides and polymers.

The presentation will first focus on the structure-function relationship studies of these intriguing enzymes, distinguishable by their linkage specificity or ability to control the size of the produced polysaccharides. The resolution of some 3D structures allowed us to decipher structural features playing a key role in polymer elongation, enzyme processivity, and/or linkage specificity [2,3]. These findings open promising strategies for GH70 enzyme engineering aiming at customize the alpha- glucan architectures on purpose, and some examples will be given during the presentation.

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KEYWORDS: enzyme characterization, structure-function studies, polysaccharides, glycoconjugates.

POSTER 17

Enzymatic scaffolding around a protein ring

Alexis Boutilliat1, Marc Quinternet2, Alexandre Kriznik2-3, Marie-Eve Chagot3, Florent Canonico1

[1] SAYENS, 2 rue Jean Zay 54519 Vandœuvre-lès-Nancy

[2] UMS IBSLor 2008/US40 Ingénierie-Biologie-Santé en Lorraine, University of Lorraine

[3] UMR 7365 IMoPA, Biopôle de l'université de Lorraine, University of Lorraine

E-mail of corresponding author: alexis.boutilliat@sayens.fr

Peroxiredoxin (PRX) is an ubiquitous protein physiologically involved in the redox machinery of the cell, many of them have a natural decameric ring structure [1]. In this work, this structural property has been exploited to create a scaffold structure using a non-catalytic variant of the thermally stable PRX coming from the thermophilic organism Pyrococcus furiosus (PfuPRX). More precisely, the N and C ends of the PRX monomer could be used to graft proteins such as enzymes on a robust scaffold resistant to thermal denaturation. The overall scaffold is either obtained by genetically fused protein sequences or by using specific protein adapter pairs. The scaffolded enzymes were produced in E. coli system and purified by simple chromatographic methods (metal affinity and gel filtration). This new scaffold technology [2] allows the creation of soluble and large catalytic objects (0.5 to 1 MDa and about 20 nm) and has potential advantages such as improved stabilization, solubilization, production rate and activity of the scaffolded enzyme. Our method was successfully applied to several enzymes such as proteases (3c, TEV), cellulases (CelCD) [3] and plastic degrading enzymes (LCC PETase) [4]. In all cases, the activity of the scaffolded enzyme was maintained.

The diversity of the PRX family offers a wide range of biophysical properties, allowing tailor-made

scaffolds. It could also allow the co-localization of two or more complementary enzymatic activities scaffolded on the same ring structure.

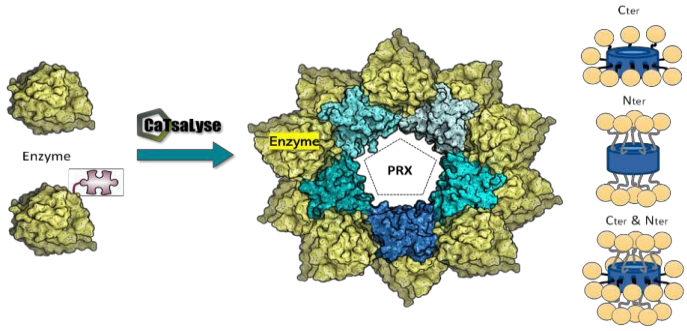

Figure1: Schematic representation of the scaffolding strategy. Direct genetic fusion or protein adaptor allow the grafting of enzyme on the different positions of PRX ring.

Relevant references:

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KEYWORDS: scaffold, enzymes, biocatalyst, peroxyredoxin

POSTER 18

Modulating eIF2α activity for cardiomyocyte rescue

Cristina Cardenal[a], Yves Mechulam[a], Emmanuelle Schmitt[a] and Jérôme Fagart[aa]
Laboratoire de Biologie Structurale de la Cellule, Ecole polytechnique, CNRS, Institut E-mail of corresponding author: cristina.cardenal-peralta@polytechnique.edu

Objectives:

eIF2α is a key effector of the Unfolded Protein Response (UPR), its phosphorylation in serine 52 being the trigger of this pathway. A new post translational modification (PTM) was discovered by our INSERM collaborator, acetylation in lysines 141 and 143. Deacetylation of eIF2α by SIRT1, protected cardiomyocytes from apoptosis. Phosphorylation and acetylation levels of eIF2α seem to be co- dependent, adding an extra step of regulation to this pathway. SIRT1 activity can be modulated by sirtuin-activating compounds (STACs), which could potentially be used in the treatment of cardiac diseases. The aim of my PhD is to understand the mechanism by which SIRT1 deacetylates eIF2α, and how phosphorylation may affect SIRT1 deacetylation rates; and to modulate SIRT1 activity by discovering specific Sirtuin Activating Compounds (STACs)

Methods:

Production of acetylated protein in vitro was achieved using an orthogonal translation system. SIRT1 and acetylated eIF2α interaction was demonstrated using pull-down assays. SIRT1 deacetylation rates were determined by setting up specific enzymatic tests, in which acetylated mimic peptides were used to optimize the test, to compare with the real acetylated substrate. This enzymatic test can also be used to determine the effect of the different STACs in SIRT1 deacetylation rates. Crystallisation experiments were performed in an attempt to obtain a structure of the SIRT: eIF2α complex.

Results:

The Km value for acetylated heIF2α is lower than for the mimic peptides. This strongly suggests that acetylated heIF2α has a higher affinity for SIRT1 than the mimic peptides and validates the use of the full-length acetylated protein to screen for specific SIRT1 activators. To study the interplay between acetylation and phosphorylation, I have produced an heIF2α variant with an S to D mutation to mimic the phosphorylation modification, that is also acetylated in the desired lysines. Finally, I set the ground for heIF2α-143AcK-188 crystallization, successfully crystallizing heIF2α-188.

Conclusions:

The production of acetylated eIF2α enables us to have an in depth look to the SIRT1: eIF2α interaction. Further studies into this pathway will decipher the interplay between acetylation/phosphorylation, by producing heIF2α variants that can mimic phosphorylation while acetylated.

Relevant references:

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KEYWORDS: Up to 5:eIF2α, SIRT1, phosphorylation, acetylation, UPR

POSTER 19

Role of Nitric Oxide Synthases from *Klebsormidium nitens*: first structural characterization and partners identification

Pauline Chatelain[a], Jeremy Astier[a], Agnès Klinguer[a], David Wendehenne[a], Sylvain Jeandroz[a] and Claire Rosnoblet[a]

[a]Agroécologie, Institut Agro, CNRS, INRAE, Université de Bourgogne, Université Bourgogne Franche- Comté, Dijon, France

E-mail of corresponding author: pauline.chatelain@inrae.fr

Objectives: Nitric oxide (NO) is an important cellular signaling molecule regulating various physiological processes, in both animals and plants. In animals, NO synthesis is mainly catalyzed by NO synthase (NOS) enzymes. In plants, NOS-like activities sensitive to mammalian NOS inhibitors have been measured, although no sequences encoding mammalian NOSs have been found in land plants. Interestingly, we identified NOS-like sequences in 20 algae species. These latter include the filamentous charophyte green algae *Klebsormidium nitens*, a biological model to study the early transition step from aquatic algae to land plants. In order to understand the mechanisms governing NO synthesis and signaling in green lineage we initiated the functional characterization of *K. nitens* NOSs (KnNOS) by analyzing their primary sequences as well as their expression levels in response to abiotic stresses.

Methods: KnNOSs nucleotide sequences were verified by RACE-PCR and sequencing, and their mRNA level were monitored by RT-qPCR and protein abundance by western blot. Protein partners were studied, firstly *in sillico* using the BioGrid database and human NOS interaction data, and secondly *in vivo* by immunoprecipitation experiments followed by mass spectrometry analysis.

Results: Currently, two NOSs were identified in *K. nitens* genome: the KnNOS1 which possesses classical mammalian NOS architecture consisting of oxygenase and reductase domains with some specificities as lack of conserved residues in binding domain of BH_4 cofactors; and the KnNOS2 displaying a large C-ter extension containing an ANK motif and a globin domain. The two KnNOSs seem to be regulated in different ways. KnNOS1 exhibited constitutive expression during the conditions tested, whereas KnNOS2 appeared to be transcriptionally regulated during stress. In parallel studies, we also built the *in silico* protein–protein interaction network of human NOSs. Interestingly, genes encoding orthologs of several of these candidates were found in *K. nitens* genome. Some of these conserved partners are known to be involved in mammalian NOSs regulation and represent interesting candidates for further investigation.

Conclusions: Overall these findings open the way for a deeper characterization of KnNOSs and its protein partners and will facilitate further investigation of NO signaling in green lineage.

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KEYWORDS: algae, nitric oxide, nitric oxide synthase, interactome

POSTER 20

Arabidopsis thaliana DGAT3 is a [2Fe-2S] protein involved in TAG Biosynthesis

Laure Aymé[1], Simon Arragain[2], Michel Canonge[1], Sébastien Baud[1], Nadia Touati[3], Ornella Bimai[2], Franjo Jagic[1], Christelle Louis-Mondésir[1], Pierre Briozzo[1], Marc Fontecave[2] Thierry Chardot[1]

[1]Institut Jean-Pierre Bourgin, INRA, AgroParisTech, CNRS, Université Paris-Saclay, 78000, Versailles, France. [2]Laboratoire de Chimie des Processus Biologiques, UMR 8229 CNRS, Collège de France, Université Paris 6, 11 Place Marcelin Berthelot, 75231, Paris, CEDEX 05, France. [3]Chimie ParisTech, PSL Research University, CNRS, Institut de Recherche de Chimie Paris (IRCP), F-75005, Paris, France.
Correspondence and requests for materials should be addressed to M.F. (email: marc.fontecave@college-de-france.fr) or T.C. (email: thierry.chardot@inrae.fr)

E-mail of corresponding author: thierry.chardot@inrae.fr

Summary: Acyl-CoA:diacylglycerol acyltransferases are key enzymes of neutral lipids accumulation. The fall into three different families. DGAT1 and 2 are membrane proteins. DGAT3 are described as cytosolic enzymes found to date only in plants and their protein sequences exhibit a thioredoxin-like ferredoxin domain typical of a class of ferredoxins harboring a [2Fe-2S] cluster. The *Arabidopsis thaliana* DGAT3 (AtDGAT3; At1g48300) protein is detected in germinating seeds. The recombinant purified protein produced from *Escherichia coli*, although very unstable, exhibits DGAT activity *in vitro*.

Objectives: Our goal was to find stable forms of DGAT3 in order to study their catalytic activity and check for the presence of a [2Fe-2S] cluster.

Methods: Site directed mutagenesis, expression of recombinant genes in *E. coli*, S and Fe quantification, EPR, activity assays

Results: Shorter AtDGAT3 versions devoid of its N-terminal putative chloroplast transit peptide, were cloned and expressed successfully in E. coli. ATDGAT3Δ46 was the longest version that was more stable *in vitro* than the full length one, allowing biochemical and spectroscopic characterization. The results obtained demonstrate the presence of a [2Fe-2S] cluster in the protein.

Conclusions: To date, AtDGAT3 is the first metalloprotein described as a DGAT.

Relevant references: Aymé, L., et al., *Arabidopsis thaliana DGAT3 is a [2Fe-2S] protein involved in TAG biosynthesis.* Sci Rep, 2018. **8**(1): p. 17254.

POSTER 21

High pressure macromolecular crystallography revealed equilibria between conformational states of the Ras oncogene protein

Nathalie Colloc'h [a], Pedro Lopes [b], Michael Spoerner [b], Anne-Claire Dhaussy [c], Thierry Prangé [d], Hans Robert Kalbitzer [b] , Eric Girard [e]

[e]ISTCT UMR 6030 CNRS Normandie Univ. Centre Cyceron, Caen, France

[b]Institute of Biophysics and Physical Biochemistry, University of Regensburg, Regensburg, Germany

[c]CRISTMAT UMR 6508 Normandie Univ. Ensicaen, CNRS, Caen, France

[d]CiTCoM UMR 8038 CNRs Université de Paris, Faculté de Pharmacie, Paris, France

[e]IBS Univ. Grenoble Alpes CEA CNRS, Grenoble, France

E-mail of corresponding author: colloch@cyceron.fr

Objectives: Ras protein is a small GTPase involved in central regulatory processes and acts as a molecular switch between active GTP-bound and inactive GDP-bound states, controlling essential transduction pathways. These regulatory processes occur in multiple conformational states. An allosteric network of interactions between the effector binding regions and the membrane interacting regions is involved in Ras cycling. The different conformational states which coexist simultaneously in solution with low occupancies possess higher Gibbs free energy than the ground state. Equilibria between these states can be shifted by applying high hydrostatic pressure favoring conformations with lower molar partial volumes.

Methods: The allosteric transitions between conformational states of Ras have been investigated using high pressure crystallography, which is a powerful tool to characterize at the molecular level the different high-energy conformational states.

Results: We have determined at high resolution the crystallographic structures of Ras(wt).Mg2+.GppNHp and Ras(D33K).Mg2+.GppNHp at pressures up to 900 MPa. We have observed a transition above 300 MPa in the crystal leading to more stable conformers. The comparison of these different structures gives insight to per-residue descriptions of the structural plasticity involved in allosteric equilibria between conformers. The different segments of Ras protein which remains in the ground-state conformation or undergo structural changes, adopting excited- energy conformations corresponding to transient states, have been mapped out at atomic resolution.

Conclusions: Such in-crystallo phase transitions induced by pressure opens the possibility to finely explore the structural determinants related to switching between Ras allosteric sub-states without any mutations nor exogenous partners.

Relevant references: Girard E. *et al*., Chem. Sci. 2022, 13:2001-2010

KEYWORDS: HIGH-PRESSURE CRYSTALLOGRAPHY, EXCITED STATES, ALLOSTERIC TRANSITION

POSTER 22

An original oligosaccharide as inhibitor of heparanase 1 activity and anti-inflammatory activities

Clément Daviaud [1], Chanez Manseur [1], Rémi Cousin [1], Benjamin Musnier [1], Hugo Main [2], Hugo Groult [1], Thierry Maugard [1], Franck Morel [2], Ingrid Fruitier-Arnaudin [1], Kévin Baranger [1,*]

1. LIENSs UMRi 7266 CNRS/Université de La Rochelle, La Rochelle, France
2. Laboratoire Inflammation, Tissus Epithéliaux et Cytokines, UR 15560, Université de Poitiers, Poitiers, France
* E-mail of corresponding author: kevin.baranger@univ-lr.fr

Objectives: Heparanase 1 (HPSE) is an endo-β-D-glucuronidase and the only enzyme, in mouse and human, able to cleave specifically Heparan Sulfate (HS) chains on the proteoglycans (HSPG) like Syndecan-1 (SDC1). Among its numerous activities, it regulates cell signalling, inflammatory and cancerous processes, particularly in breast cancer (Vlodavsky *et al.*, 2012).

Heparin is a well-described endogenous HS inhibitor of HPSE; however, the use of heparin or derivatives as a therapeutic treatment to treat inflammatory or cancer diseases is not possible because of its anticoagulant activities (Israel Vlodavsky *et al.*, 2007). Therefore, using innovative and green biotechnological approaches, we developed a new oligosaccharide obtained by HS λ-carrageenan depolymerisation produced by red algae. Named λ-CO, we demonstrated that this molecule exhibits low anti-coagulant activity while inhibiting HPSE. We recently validated its HPSE-dependent beneficial effects on angiogenesis and migration and invasion assays (Groult *et al.*, 2019; Cousin *et al.*, 2021). **We now want to characterize the possible anti-inflammatory and immunomodulatory effects of λ-CO.**

Methods: To that end, we used LPS stimulated murine RAW 264.7 cell line as inflammatory model and used heparin as gold standard HPSE inhibitor. Anti-inflammatory and immunomodulatory effects of λ-CO were monitored by measuring cytokines production by RT-qPCR, Elisa experiments and by phagocytosis assays.

Results: We first demonstrated that λ-CO was neither cytotoxic nor pro-inflammatory and that *Hpse* expression was correlated to that inflammatory cytokines *Il6* and *Il1b*. Furthermore, λ-CO treatment alleviates inflammatory genes expression and this was correlated with reduced levels of IL-1β, IL-6, TNF-α or MCP-1. However, neither heparin nor λ-CO do control NO production. Using phagocytosis assay we demonstrated that LPS-treatment reduces phagocytosis activity of macrophages. Heparin treatment does not rescue that activity while λ-CO does.

Conclusions: Our preliminary results suggest interesting activities of λ-CO on inflammatory processes and open the way to its use *in vivo* in LPS-injected animals or in other chronic inflammatory models.

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KEYWORDS: Heparanase 1, oligosaccharide, Inflammation

POSTER 23

Amuλett: Super-Fast Targeted Multi-Site Mutagenesis based on Uracilated Single-Stranded Transient Template

Rocio Espada[a], Rémi Sieskind[b], Yannick Rondelez[a], Adèle Dramé-Maigné[c]

[a]ESPCI, CNRS UMR 7083 Gulliver (SPM) [b]Institut Pasteur, UMR 3528 [c]PURPL
Research e-mail of corresponding author: adele.dm@purpl-research.com

Objectives: Tailor-made designed smart libraries based on structural analysis, consensus or phylogenetic studies, but also output of algorithms trained on directed evolution deep sequencing data, all benefits from targeted multi-site mutagenesis to introduce either pre-defined or random mutations at identified distal specific sites[1]. This step can be challenging and time-consuming, limiting the speed of projects and sometimes their feasibility. Single strand templates providing a backbone to anneal mutagenic primers shows some of the best efficiencies. Importantly, the removal of this template strand decreases the number of wild-type contaminants. Two main strategies have been used to generate ssDNA. In the first one, an exonuclease degrades the nicked strand of a plasmid[1,2] while the second one uses uracilated single strand phagemids produced in bacteria[3]. Despite of their convenience these uracil-containing ssDNA are restricted to phagemids and require knowledge in phage production. On the other side, nicked plasmids require either another exonuclease degradation of the template strand or isolation of the mutated strand by beads purification[1,2]. Here, we developed a full *in vitro* multi-site targeted mutagenesis method based on PCR generated uracilated single strand transient template. The output of the Annealing of Mutagenic oligonucleotides on Uracilated λ-exonuclease generated Transient Template (Amuλett) is a fully mutated double strand DNA ready to be inserted in a vector.
Methods: Preparation of the transient template is made in one day by PCR and digestion of one of the two DNA strands. Then, the mutated dsDNA can be obtained in less than 4 hours via mutagenic primers annealing, polymerase/ligase complementation and digestion of the template followed by a PCR amplification. The Amuλett does not require any specificity in the plasmid and uses only regular primers, phosphorylated by a T4KNP phosphatase.
Results: Mutations expected to increase themostbility and turn-over rate of the nicking enzyme Nt.bstNBI were identified by the PEN CSR directed evolution method[4]. We used the Amuλett to introduce these mutations in the wild type sequence in combinations from 2 and up to 5 simultaneous mutations. We obtained 12 mutants validated by Sanger sequencing in one week with very few screening efforts.
Conclusions: The Amuλett offers a fast, efficient and cheap alternative to multi-site mutagenesis techniques. Used here to produce individual mutants, it is very promising for libraries creation.

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KEYWORDS: Mutagenesis, Directed Evolution, Libraries, Protein Engineering, DNA

POSTER 24

Production and biochemical characterization of human FICD, a membrane-associated bifunctional enzyme involved in proteostasis

Pavlina Dubois[a], Gérald Peyroche[a] and Jacqueline Cherfils[a]

[a]Laboratoire de Biologie et Pharmacologie Appliquée, CNRS and Ecole Normale Supérieure Paris- Saclay, 91190 Gif-sur-Yvette, France

E-mail of corresponding author: gerald.peyroche@ens-paris-saclay.fr

Objectives: The Unfolded Protein Response (UPR) is a signaling pathway that participates in the control of proteostasis. UPR is initiated when misfolded proteins accumulate in the endoplasmic reticulum (ER). A defect in the UPR leads to serious pathologies such as neurodegenerative diseases. A key UPR player is FICD, an enzyme associated to the intraluminal layer of the ER. FICD controls the activity of ER Hsp70 chaperone BIP, by catalyzing both its AMPylation (addition of an AMP moiety on a threonine residue) and its deAMPylation. Available *in vitro* studies use truncated and mutant hyperactive forms of FICD, in the absence of membranes, and in the presence of manganese or magnesium ions but never in the presence of calcium ion, yet the prevalent ion in the ER. The objective of our study is to assess *in vitro* AMPylation and deAMPylation activities of the wild type full-length FICD in the presence of membranes and Ca^{2+}.
Methods: The wild type full length FICD protein was recombinantly expressed in *E. coli* and purified by FPLC. The activity of the purified enzyme was assessed in solution and on artificial membranes (liposomes).
Results: Wild type full-length FICD was purified to homogeneity and was inserted into ER-like liposomes. *In vitro* enzymatic assays indicated that purified wt FICD AMPylates the BIP chaperone in solution. Preliminary results suggest a potential stimulating effect of liposomes on BIP AMPylation by FICD.
Conclusions: Our results show for the first time that wild type full-length FICD AMPylates BIP in solution and in the presence of membranes, and suggest that the association of FICD to membranes positively modulates its activity.
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KEYWORDS: AMPylation, Hsp70, FICD, proteostasis, post-translational modification

POSTER 25

DNA gyrase: a drug target, despite all the odds

Antoine Gedeon[a], Émilie Yab[a], Estelle Capton[b], Aurelia Dinut[d,e], Ariel Mechaly[f], Francesca Gubellini[a], Christel Neut[c], Luc Dubreuil[e], Benoît Deprez[c,d], Alexandra Aubry[b], Nicolas Willand[c,d], Stéphanie Petrella[a]

[a]Unité de Microbiologie Structurale, CNRS UMR 3528, Institut Pasteur, Université Paris Cité ; [b]Sorbonne Université, Cimi-Paris, INSERM U1135, Hôpitaux Universitaires Pitié-Salpêtrière, Paris; [c]Drugs and Molecules for Living Systems, Université de Lille, INSERM U1177, Institut Pasteur de Lille ; [d]Pôle de Recherche Interdisciplinaire pour le Médicament, Lille ; [e]Laboratoire de Bactériologie Clinique, Faculté de Pharmacie, Lille ; [f]Plateforme de Cristallographie, Institut Pasteur.

E-mail of corresponding author: agedeon@pasteur.fr

Objectives: Genomic DNA topology is highly dynamic and is modulated by several enzymes, mainly topoisomerases. Gyrase (EC 5.6.2.2) is an ATP-dependent heterotetrametric enzyme that consists of 2 GyrA and 2 GyrB subunits. This enzyme is exclusively prokaryotic and is capable, by generating double-stranded gaps, of DNA supercoil relaxation and decatenation. In bacteria, this later function is mainly coordinated by Topo IV. In the case of the Actinobacteria *Mycobacterium tuberculosis* (*Mtb*), the causing agent for tuberculosis (TB), gyrase is essential in maintaining DNA topology for replication and transcription, as no homolog of Topo IV exists in this bacterium. Concordantly, gyrase's vital role in bacterial physiology aroused interests in developing anti-TB inhibitors altering its activity. Several drugs have been historically characterized and used, such as the molecules belonging to the fluoroquinolone's (FQ) or the novel bacterial topoisomerase inhibitors (NBTI) superfamily. Nonetheless, despite global control efforts to overcome the unfortunate emergence of resistant strains to this class of inhibitors, the latest World Health Organization report documented that TB remains amongst the top 10 causes of death worldwide. Therefore, we focused our interest on two main items; (i) **developing new inhibitors targeting *Mtb* gyrase,** and (ii) developing an **antimicrobial *in cellulo* target-based screening** using *Corynebacterium glutamicum* (*Cgl*), another Actinobacteria, as a model.

Methods: Firstly, we synthesized and characterized a new class of molecules sharing a triazole scaffold that inhibit the wild-type and several fluoroquinolone-insensitive gyrase mutants of *Mtb*. One of the potent hits with MIC value similar to MIC of fluoroquinolones and with the lowest IC_{50} value was selected for further mechanistic characterization including enzyme activity assays and structural investigation by CryoEM. Secondly, in order to setup our *in vivo* model, we initiated a biochemical characterization of *Cgl* gyrase.

Results: Our molecule collection showed several gyrase inhibitors with IC_{50} ranging from 2 to 25 μM and MICs as low as 0.06 μg/mL. Biochemical and CryoEM experiments revealed that one of the hits, BDM-71403, is capable

of binding within the cleavage site in a pocket that shares several residues with the NBTI-pocket but distal from the FQ-pocket. The high-resolution structure of the complex with the drug allowed to see a stabilization in an inactivated state of the gyrase-DNA complex (with intact double strand-DNA). For *Cgl* gyrase, activity assays showed similar properties as the *Mtb* gyrase, i.e. functional supercoiling and decatenating activities; positive cooperativity towards ATP, and high sensitivity to variable concentrations of bivalent metal ions.

Conclusions: To counterpose the emergence of new mutants linked to conventional gyrase inhibitors, development of new classes of inhibitors and of robust phenotypical screening methods are needed. These biochemical and structural observations seem promising for further correlations to *in vivo* studies of *Cgl* and *Mtb* gyrase activities.

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KEYWORDS: Gyrase, actinobacteria, drug design, enzymology, structural biology

POSTER 26

New function of [Fe-S] clusters in biology: [4Fe-4S] enzymes for non-redox (de)sulfuration reactions

Jingjing Zhou, Paolo Zecchin, Ludovic Pecqueur, Marc Fontecave, Béatrice Golinelli-

Laboratoire de Chimie des Processus Biologiques, Collège de France, CNRS, Sorbonne Universités, 11 Place Marcelin Berthelot, 75231 Paris cedex 05, France

E-mail of corresponding author: beatrice.golinelli@college-de-france.fr

Objectives: Whereas the redox function of [Fe-S] clusters is well known, their role as cofactors in enzymatic catalysis appeared to be limited to dehydration reactions such as that catalyzed by aconitase. We wanted to show that, in the case of [4Fe-4S]-dependent enzymes that catalyze tRNA sulfuration reactions [1], the [4Fe-4S] cluster is also used as a cofactor and not as the sulfur source for the reaction.

Methods: UV-visible, EPR and Mössbauer spectroscopies, X-ray diffraction, enzymatic assays

Results: we have recently shown that several enzymes containing a [4Fe-4S] cluster, bound to three amino acids only, catalyze a non-redox sulfuration/desulfuration reaction. Examples include several sulfuration enzymes that target uridine at positions 8, 34 or 54 in tRNA [2, 3, 4], as well as desulfuration enzymes such as cysteine desulfidase or thiouracil desulfidase [5].

Conclusions: We propose a novel mechanism for tRNA sulfuration or sulfur abstraction, in which the fourth non-protein-bonded iron atom of a [4Fe-4S] cluster binds and activates sulfur atom of the substrate to form a [4Fe-5S] cluster species.

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KEYWORDS: tRNA modification, [4Fe-4S] cluster, sulfuration, desulfidase

POSTER 27

Anaerobic ubiquinone biosynthetic pathway in *Escherchia coli*

Katayoun Kazemzadeh[a], Sophie Abby[a], Emmanuel Sechet[b], Emmanuelle Bouveret[b], Frédéric Barras[b], Ludovic Pelosi[a], Fabien Pierrel[a]

[a] Univ. Grenoble Alpes, CNRS, CHU Grenoble Alpes, Grenoble INP, TIMC, F-38000 Grenoble, France

[b] SAMe Unit, Département de Microbiologie, Institut Pasteur, 25 Rue du Dr Roux, 75015 Paris, France E-mail of corresponding author: fabien.pierrel@univ-grenoble-alpes.fr and ludovic.pelosi@univ-grenoble-alpes.fr

Objectives: Ubiquinone (UQ) is a redox active lipid, which mediates the transfer of electrons in aerobic respiratory chains in eukaryotes and proteobacteria [1]. *Escherichia coli* synthetizes UQ via a well-known multistep O_2-dependent pathway that requires nine biochemical reactions carried out by Ubi-enzymes and accessory proteins. Interestingly, *E. coli* was also reported to synthetize UQ in anaerobic conditions but this O_2-independent biosynthetic pathway needs to be elucidated.

Results and methods: A collection of multiple and single-gene(s) knock out (KO) strains of *E. coli* were cultured under aerobic and anaerobic conditions. After quinone extraction and UQ detection by HPLC-MS analysis, we showed that oxic and anoxic UQ biosynthetic pathways share mainly the same enzymes except hydroxylases [2]. Under aerobic conditions, the three hydroxylation reactions of the pathway are carried out by the flavoprotein monooxygenases UbiI, UbiH and UbiF, which use dioxygen as a source of hydroxyl group. Therefore, they are not involved in the anaerobic pathway. Moreover, we identified three new genes called hereafter *ubiT, ubiU* and *ubiV* as essential for UQ biosynthesis under anoxic conditions [2]. First, we investigated the regulation of the *ubiT-V* genes and we show that *ubiU* and *ubiV* are expressed only under anoxic conditions in an FNR-dependent manner whereas the expression of *ubiT* seems to be constitutive. Next, to explore the functions of UbiU and V, we expressed them under aerobic conditions in single or multiple-*ubiI*, *ubiH* and *ubiF* KO strains, which are impaired for UQ synthesis only under aerobic conditions. Restoration of the UQ level in these later conditions suggested that UbiU and UbiV correspond to hydroxylases. We also propose that they use a metabolite of the shikimate pathway as a hydroxyl donor. In addition, despite the anaerobic expression of UbiU and UbiV and that both proteins exhibit 4Fe-4S clusters, dioxygen is not toxic for their enzymatic activities. The role of the UbiT protein is still unclear but it possesses a sterol carrier protein 2 domain similar to the UbiJ protein, which organizes a soluble Ubi complex in aerobic conditions and binds hydrophilic UQ biosynthetic intermediates [3]. Finally, we were interested in the conservation of the *ubiT-V* genes in proteobacteria as well. The phylogenetic analyses demonstrated that the *ubiT-V* genes are conserved exclusively in proteobacteria containing UQ [2].

Conclusions: We identified a new family of hydroxylases involved in UQ biosynthetic pathway, which may use an organic oxygen donor as a co-substrate.

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KEYWORDS: Ubiquinone, anaerobiosis, hydroxylation, respiration and 4Fe-4S cluster.

POSTER 28

Investigation of human acetylcholinesterase and alpha-amylase inhibition by *Phaeodactylum tricornutum* extracts

Nadia Khelifi[a,b], Sheyma Khemiri[a] Takoua Ben Hlel[a], Farah Triki[a], Issam Smaali[a]

[a]University of Carthage, National Institute of Applied Sciences and Technology, LIP-MB Laboratory,Tunis-Tunisia

[b]Higher Institute of Fisheries and Aquaculture of Bizerte-Tunisia

E-mail of corresponding author: khelifi.nad@gmail.com

Objectives: Microalgae are known as emerging sources of biologically active biomolecules. The current research focused on assessing oh the potential of methanolic extracts derived from *Phaeodactylum tricornutum* to inhibit human acetylcholinesterase and pancreatic alpha-amylase, then contributing to the management of neurodegenerative diseases and diabetes.

Methods: ultrasound assisted methanol extraction were carried out on different lyophilized *Phaeodactylum tricornutum* biomasses, obtained under different culture conditions by varying media composition (conway, ASW-algal and f/2 media) to provoke secondary metabolites production under nutritive stresses. Inhibitions of acetylcholinesterase and pancreatic alpha-amylase were studied in microplate spectrophotometer using respectively acethylthiocholine iodide/5,5dithiobis-2- nitrobenzoic acid and starch as substrates, and by varying the extracts concentrations. Both donepezil and acarbose were used as effective pure inhibitors for positive control reactions. Further characterization was focused on total polyphenols, crotenoids contents and the DPPH antioxidant activity.

Results: Three methanolic extracts were prepared by maceration in methanol (10 % w/v) assisted by ultrasound of three biomasses derived from cultures characterized by different amounts of nitrogen and phosphorus (ASW-algal, f/2 and Conway media). Our results showed that the f/2 extract has interesting inhibition capabilities of both acetylcholinesterase and amylase (IC50 of 0,71 mg/mL and 0,14 mg/mL respectively), compared to bibliographic data and pure drug donepezil and acarbose taken as positive controls (IC50 of 1,17 µg/mL and 37 µg/mL respectively). This extract was further characterized by measuring antioxidant activity by the DPPH scavenging assay (IC50 = 0,43 mg/mL), the total polyphenols (10,58 mg EAG/g dry extract) and the carotenoids contents (7,2 mg/g dry extract). These interesting biochemical properties led us to study their correlation with biomass production. Under the conditions used, a growth rate (µmax) of 0,21 d^{-1} associated with a generation time of 3,3 d of this algae were recorded and considered as acceptable compared to bibliographic data and for the intended application.

Conclusions: The present study showed the potential of *Phaeodactylum tricornutum* extracts as an alternative and sustainable source to manage neurodegenerative diseases and diabetes by inhibition of enzymes involved in. Structural characterization of the chemical composition and the delivery of these microalgae or their extracts as nutraceutical constitute a promising road to be explored.

KEYWORDS: acetylcholinesterase, alpha-amylase, inhibition, microalgal extrcts, *Phaeodactylum tricornutum*

POSTER 29

Structural and functional insights of Hsp90C, the chloroplastic Hsp90 family member

Romain La Rocca[a], Thomas Chenuel[a], Céline Bergonzi[a] and Philippe Meyer[a]

[a]Laboratoire de Biologie Moléculaire et Cellulaire des Eucaryotes (LBMCE), UMR 8226 CNRS Sorbonne Université, Institut de Biologie Physico-Chimique (IBPC), 75005 Paris, France
E-mail of corresponding author: larocca@ibpc.fr

Objectives: The Hsp90 family have been largely studied through the last 30 years, so that now its molecular mechanisms and pathological implications are well known in various species [1]. However, a subset of Hsp90 proteins, namely the stromal Hsp90 proteins, are less described. More specifically, Hsp90C, the main Hsp90 of the chloroplast, is very poorly known despite its crucial role in protein import [2], which is essential for photosynthesis.

Methods: To investigate the function of Hsp90C *in vitro*, we performed ATPase activity assays and luciferase renaturation assays using the bacterial refolding machinery. Both experiments were also performed with HtpG, the bacterial Hsp90 family member, in order to appreciate the effect of Hsp90C. For the structural investigations, we used crystallography and SEC experiments on different truncated forms of Hsp90C, which were designed using molecular biology approaches.

Results: We show that Hsp90C has an ATPase activity comparable to HtpG, its bacterial homolog. The *in vitro* luciferase refolding assays with the full bacterial refolding machinery revealed that Hsp90C can stimulate the renaturation of luciferase induced by DnaK. Interestingly, Hsp90C refolds a significantly higher proportion of luciferase compared to HtpG. Besides, we obtained the first crystallographic structure of the middle domain of Hsp90C. Noticeably, the structure reveals a longer helix located in the end of the domain, which implies that the C-terminal domain may have a different conformation from the other Hsp90 proteins. Further investigation with SEC experiments showed that the Hsp90C dimerization is led by its unique very end of the C-terminal tail among the Hsp90 family.

Conclusions: Our results suggest that Hsp90C is believed to stimulate the refolding of client proteins in the chloroplast and dimerizes through a non-canonical dimerization process.

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KEYWORDS: Hsp90, crystallography, folding, chloroplast

POSTER 30

Overcoming the aminoglycoside resistance of Priority-1 pathogens by designing aminoglycoside-phosphotransferase inhibitors

Julien Kowalewski[a], Jean-François Guichou[a], Muriel Gelin[a], Gilles Labesse[a], Gerard D.Wright[b] & Corinne Lionne[a]

[a] Centre de Biologie Structurale CBS, CNRS UMR 5048, INSERM U 1054, Université de Montpellier, 34090 Montpellier, France.

[b] David Braley Centre for Antibiotic Discovery, M.G. DeGroote Institute for Infectious Disease Research, McMaster University, 1280 Main Street West, Hamilton, ON L8S 4K1, Canada.

E-mail of corresponding author: lionne@cbs.cnrs.fr

Objectives: Multidrug resistance is a major public health problem requiring urgent development of new antibiotics, as pointed out by the World Health Organization. Priority-1 pathogens are Gram- negative bacteria such as *P. aeruginosa*, *K. pneumoniae*, *A. baumannii* and Enterobacteriaceae. This critical group of bacteria presents an important threat in hospitals. Aminoglycosides are broad spectrum antibiotics reserved for severe infections. They are bactericidal, concentration-dependent, and with a marked post-antibiotic effect. However, among the different mechanisms of resistance developed by these bacteria we can notice the importance of modification enzymes able to inactive aminoglycosides leading to a decreased affinity for their ribosomal target [1]. This modification is the major source of resistance against this class of antibiotics [2]. The Aminoglycosides Phosphotransferases (APH) are part of these enzymes. They catalyze the transfer of the gamma- phosphate of a nucleotide (ATP or GTP) on a free hydroxyl group of an aminoglycoside. Structural and biochemical characterization are crucial to better understand this class of enzyme and develop effective inhibitors to circumvent their activity and to thus rejuvenate the antibacterial action of the aminoglycosides [3]. To date, there have been significant advances providing structural, mechanistic, and inhibitory insights into many members of the APH family.

Results: In the present work we present preliminary results on the inhibitory effect of the enzymatic activity of different APH enzymes from different human pathogens. We also present the structural and biochemical characterization of a new APH: the APH(3')-IIb of *P. aeruginosa* and its inhibition. Our results will be discussed from the perspective of the drug resistances observed in clinical therapy, as well as the potential ways for reversing them. This work was supported by ANR (SIAM project, ANR-19-AMRB-0001-01), Fonds de Recherche France-Canada (Programme 2021 "Nouvelles collaborations de recherche") and Mitacs (Globalink Research Award).

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KEYWORDS: Antibiotic resistance, Drug design, Inhibitors, *Pseudomonas aeruginosa*, X-ray crystallography

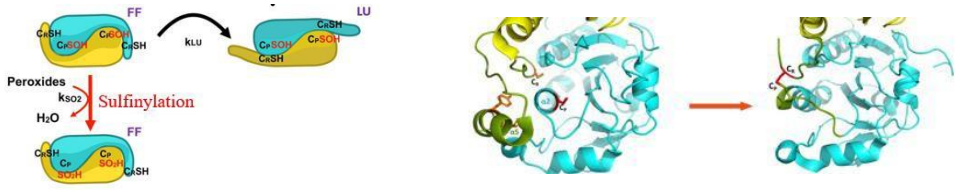
POSTER 31

Comparative analysis of the regulation of Saccharomyces cerevisiae thiol peroxidases Tsa1 and Tsa2 by sulfinylation

MATHIEU Julie, KRIZNIK Alexandre, SELLES Benjamin, RAHUEL-CLERMONT Sophie[a]

[a] ***Université de Lorraine, UMR 7365 CNRS IMoPA,*** *Biopôle, Campus Brabois Santé, 9 Avenue de la Forêt de Haye, BP 20199, 54505 Vandoeuvre Les Nancy, julie.mathieu@univ-lorraine.fr*

Objectives: Peroxiredoxins (Prx) are antioxidant enzymes that reduce peroxides like H_2O_2 using a catalytic Cys and play a role in the response of the cell to various stimuli and (patho)physiological conditions. The hyperoxidation of the catalytic Cys to sulfinic acid is a post translational modification that regulates Prx multiple functions in antioxidant defense, redox signaling, or as a chaperone. The sensitivity of Prx to hyperoxidation, an important determinant of their biological functions, depends



on two steps of the catalytic cycle in competition: conformational change and sulfinylation (Figure 1).

Figure 1. Prx Sulfinylation mechanism

As other eukaryotes including mammals, the yeast *Saccharomyces cerevisiae* possesses two cytosolic Prxs, the constitutive Tsa1 and Tsa2, induced by organic peroxide. The **aim of this study** was to compare hyperoxidation sensitivity of both enzymes with diverse peroxides substrates and identify mechanistic and molecular features responsible for their differences. Specifically, based on the X-ray structures, the active site residue in position 45 is suspected to affect the dynamics of the conformational transition during the catalytic cycle.

Methods: Hyperoxidation sensitivity was measured by using steady state kinetics (1), deconstructing their mechanism using rapid kinetics combined with Trp fluorescence and circular dichroism probes to determine the rate constants of Prx sulfinylation k_{SO2} and conformational change k_{LU} (Figure 1), in combination with interconversion mutants of both Tsa1 and Tsa2 (2).

Results: We show that Tsa2 hyperoxidation sensitivity is 6 times lower than Tsa1 for H_2O_2 and the organic peroxides (CuOOH, tBOOH). Surprisingly, kinetics analysis revealed that interconversion of active site amino acid composition between Tsa1 and Tsa2 (Tsa1 $T_{44}S$ and Tsa2 $S_{44}T$) leads to differential kinetics of sulfinylation, while the dynamics of the conformational change is relatively unaffected.

Conclusions: Despite possessing the specific structural motifs associated with high sensitivity, Tsa2 shows high resistance to hyperoxidation. This difference of sensitivity is mostly linked to the presence of a serine in the active site, which unexpectedly increases its reactivity to sulfinylation. Such behaviour, that remains to be explained at the structural level, likely is linked to Tsa2 higher specificity for organic peroxides and its inducibility under high oxidative pressure conditions.

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KEYWORDS: peroxide, peroxiredoxin, sulfinylation, oxidative stress, redox regulation

POSTER 32

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

Audrey Beaussart[a], Florent Canonico[b], Hortense Mazon[b], Jorge Hidalgo[b], Sarah Cianférani[c], Hélène Le Cordier[b], Alexandre Kriznik[b], Sophie Rahuel-Clermont[b]

[a] Université de Lorraine, CNRS, LIEC, F-54000 Nancy, France.

[b] Université de Lorraine, CNRS, IMoPA, F-54000 Nancy, France.

[c] Laboratoire de Spectrométrie de Masse BioOrganique, Université de Strasbourg, CNRS, IPHC UMR 7178, 67000 Strasbourg, France.

-mail of corresponding author: sophie.rahuel@univ-lorraine.fr

Objectives: Peroxiredoxins from the Prx1 subfamily (Prx) are highly regulated multifunctional proteins involved in oxidative stress response, redox signaling and cell protection. Prx is a homodimer that associates into a decamer. The Prx monomer C-terminus plays intricate roles in Prx catalytic functions, decamer stability and interaction with its redox partner, the small reductase sulfiredoxin (Srx), which regulates the switching between Prx cellular functions. As only static structure of covalent Prx-Srx complexes have been reported, we assessed the non-covalent interaction mechanism and dynamics in the solution of *S. cerevisiae* Srx with the ten subunits of Prx Tsa1 at the decameric level.

Methods: We used a combination of multiscale biophysical approaches (**Figure 1**):

- Native mass spectrometry and atomic force microscopy imaging combined with solution scale techniques (anisotropy fluorescence and dynamic light scattering) determine the oligomeric state of Prx with Srx.
- Protein engineering combined with rapid kinetics determine the mechanism of Srx binding to Prx.
- Single-molecule atomic force microscopy determines the force-induced dissociation of Srx from each subunit of the decameric complex.

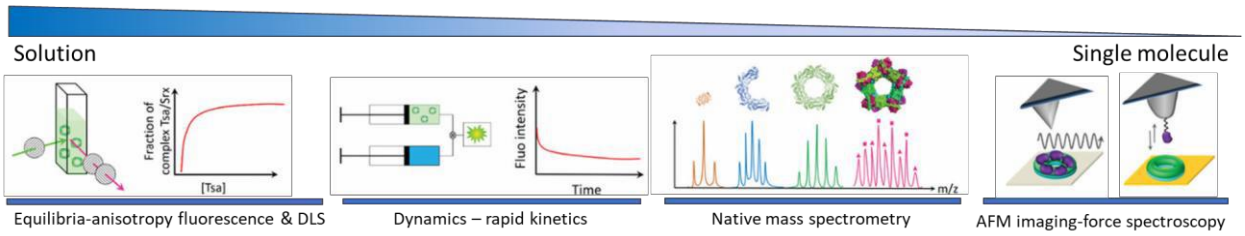

Figure 1: Multiscale strategy.

Results: We show that the ten subunits of Tsa1 decamer can be saturated by ten Srx molecules and that the interaction of Srx does not induce Tsa1 decamer dissociation. The single-molecule atomic force microscopy approach using a tip sized to the decamer dimensions, decorated with multiple Srx molecules, allowed resolving up to five native interactions with Tsa1 subunits within a decamer. Combining protein engineering and rapid kinetics allowed demonstrating a two-step mechanism of Srx binding to Tsa1 and the importance of Tsa1 C-terminus flexibility.

Conclusions: This combined approach from the solution to the single-molecule level offers promising prospects for understanding oligomeric protein interactions with their partners.

Relevant references: A. Beaussart *et al, Nanoscale Horizons*, 2022, **7**, 515-525.

KEYWORDS: Peroxiredoxin, Sulfiredoxin, catalysis, atomic force microscopy, native mass spectrometry

POSTER 33

Understanding the catalytic mechanism of the MOZ (MYST3/KAT6A) histone acetyltransferase through oncogenic mutations analysis

Christina Michail[a], Jeremy Berthelet[b], Ariel Mechaly[c], Linh-Chi Bui[a], Leslie Regad[a], Delphine Flatters[a], HaopengYang[d,e], Valentina Sirri[a], Jean-Marie Dupret[a], Fabien Guidez[f], AhmedHaouz[c], Michael R. Green[d,e], Fernando Rodrigues-Lima[a]

[a] Université de Paris Cité, BFA, UMR 8251, CNRS, F-75013 Paris, France

[b] Université de Paris Cité, CEDC, UMR 7216, CNRS, F-75013 Paris, France

[c] Institut Pasteur, Plateforme de Cristallographie, CNRS UMR 3528, 75015 Paris, France

[d] Department of Lymphoma and Myeloma, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, United States.

[e] Department of Genomic Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, United States.

[f] University of Bourgogne-ISITE-BFC-Institut national de la santé et de la recherche médicale (Inserm) UMR1231, Faculty of Medicine, 21079 Dijon, France

E-mail of corresponding author: fernando.rodrigues-lima@u-paris.fr

Objectives: MYST enzymes are one of the three families of histone acetyltransferases. These proteins are frequently found mutated in different pathologies (cancers, neurodevelopmental diseases), but the characterization of these pathogenic mutations remains poorly documented, probably because of the molecular and structural complexity of the enzymes. Our project focuses in particular on the structural and functional characterization of a recurrent mutation of the histone acetyltransferase MOZ observed in colorectal cancers. Interestingly, the mutation studied (T605P) affects a residue of the active site of the enzyme present in a loop possibly involved in the binding of substrates and in the self-acetylation of the enzyme.

Methods: For this work, we used molecular, cellular and also structural enzymology approaches *via* the resolution of 3D crystallographic structures.

Results: Our results have already made it possible to enzymatically and structurally characterize this mutation. Through biochemical approaches, we have demonstrated that MOZ T605P is a "loss of function" mutant that surprisingly still displays autoacetylation activity. In addition, our structural study shows that the mutation causes a significant remodeling of the active site and further support that this mutant is autoacetylated but inactive toward histone H3 and p53 substrates.

Conclusions: Our original work is the first to characterize a pathogenic mutation of MOZ. In order to complete this study, the analysis of the data issued by molecular dynamics will allow us to better understand the catalytic mechanism of the enzyme and the correlation between activity and auto-acetylation of MOZ.

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KEYWORDS: MOZ, cancer, enzyme, mutation, structure

POSTER 34

Nutritional Plants from Cameroon Show Anti-Inflammatory Activity in GES-1 and AGS Human Gastric Epithelial Cells

Achille Parfait Nwakiban Atchan[a], Mario Dell'Agli[b]

[a]Department of Biochemistry, Faculty of science, University of Dschang, P.O. Box 96 Dschang, Cameroon;

[b]Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, 20133 Milan, Italy.

E-mail of corresponding author: nwakiban@gmail.com

Objectives: In Cameroon, many medicinal plants, including spices, are used as herbal medicines and traditionally employed for the treatment of gastric diseases, in which oxidative stress is involved [1,2]. The present work chemically characterizes and investigates the antioxidant and anti- inflammatory effect of hydro-alcoholic extracts of eleven Cameroonian spices at gastric level, focusing on Nuclear Factor (NF)-κB pathway.

Methods: Prepared hydro-ethanolic extracts were screened for their ability to inhibit tumor necrosis factor (TNF)α-induced IL-8 and IL-6 release, in human gastric epithelial cells (GES-1 and AGS), assessing the involvement of NF-κB driven transcription. The antioxidant activity of the extracts was evaluated as well.

Results: The extracts showed antioxidant properties in a cell-free system and reduced H2O2-induced ROS generation in gastric epithelial cells. After preliminary screening on TNFα-induced NF-κB driven transcription, six extracts from *Xylopia parviflora, Xylopia aethiopica, Tetrapleura tetraptera, Dichrostachys glomerata, Aframomum melegueta, and Aframomum citratum* were selected for further studies focusing on the anti-inflammatory activity. The extracts reduced the expression of some NF-κB-dependent pro-inflammatory mediators strictly involved in the gastric inflammatory process, such as IL-8, IL-6, and enzymes such as PTGS2 (COX-2), without affecting PTGS1 (COX-1). **Conclusions:** In conclusion, the selected extracts decreased pro-inflammatory markers by inhibiting the NF-κB signaling in gastric cells, justifying, in part, the traditional use of these spices [3]. Other molecular mechanisms cannot be excluded, and further studies are needed to better clarify their biological activities at the gastric level

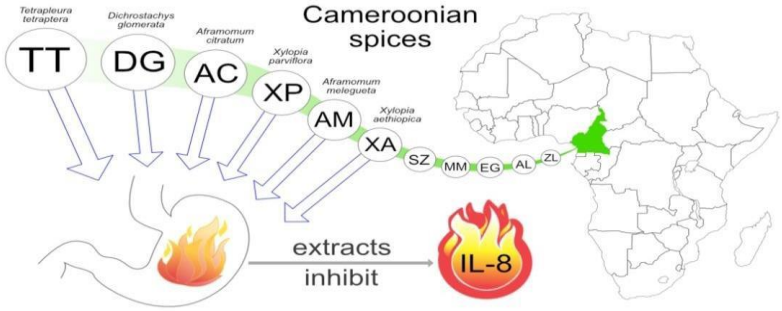

Figure 1: Spice extracts from Cameroon actions against gastric inflammation.

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Keywords: Gastric inflammation; ethnopharmacology; Cameroonian plants; inflammatory markers; antioxidant.

POSTER 35

The Biosynthetic Pathway of Ubiquinone Contributes to Pathogenicity of *Francisella novicida*

Katayoun Kazemzadeh[a], Mahmoud Hajj Chehade[a], Gautier Hourdoir[a], Camille Dorothée Brunet[a], Yvan Caspar[b,c], Laurent Loiseau[d], Frederic Barras[e,f], Fabien Pierrel[a] Ludovic Pelosi[a]

[a]CNRS, CHU Grenoble Alpes, Grenoble INP, TIMC, Université Grenoble Alpes, Grenoble, France
[b]Laboratoire de Bactériologie-Hygiène Hospitalière, Centre National de Référence des Francisella, Centre Hospitalier Universitaire Grenoble Alpes, Grenoble, France
[c]Université Grenoble Alpes, CHU Grenoble Alpes, CEA, CNRS, IBS, Grenoble, France
dAix Marseille Université, CNRS, Laboratoire Chimie Bactérienne, Institut Microbiologie de la Méditerranée, Marseille, France
[e]SAMe Unit, Department of Microbiology, Institut Pasteur, Paris, France
[f]IMM-UMR 2001 CNRS-Institut Pasteur, Paris, France

Corresponding author: ludovic.pelosi@univ-grenoble-alpes.fr

Francisella tularensis is the causative agent of tularemia. Because of its extreme infectivity and high mortality rate, this pathogen was classified as a biothreat agent[1]. *Francisella* spp. are strict aerobes, and ubiquinone (UQ) has been previously identified in these bacteria. While the UQ biosynthetic pathways were extensively studied in *Escherichia coli*[2], allowing the identification of 15 Ubi proteins to date, little is known about *Francisella* spp. In this study, and using *Francisella novicida* as a surrogate organism, we first identified ubiquinone 8 (UQ8) as the major quinone found in the membranes of this bacterium. Next, we characterized the UQ biosynthetic pathway in *F. novicida* using a combination of bioinformatics, genetics, and biochemical approaches. Our analysis disclosed the presence in *Francisella* of 10 putative Ubi proteins, and we confirmed 8 of them by heterologous complementation in *E. coli*. The UQ biosynthetic pathways from *F. novicida* and *E. coli* share similar patterns. However, differences were highlighted: the decarboxylase remains unidentified in *Francisella* spp., and homologs of the Ubi proteins involved in the O_2-independent UQ pathway are not present[3]. This is in agreement with the strictly aerobic niche of this bacterium. Next, *via* two approaches, i.e., the use of an inhibitor (3-amino-4-hydroxybenzoic acid) and a transposon mutant, both of which strongly impair the synthesis of UQ, we demonstrated that UQ is essential for the growth of *F. novicida* in respiratory medium and contributes to its pathogenicity in *Galleria mellonella* used as an alternative animal model[4].

KEYWORDS: Ubiquinone biosynthesis, respiration, metabolism, bacteria

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POSTER 36

The IbeA protein from Adherent-Invasive *Escherichia coli* is a flavoprotein displaying structural homology with FAD-dependent oxidoreductases

Theo Paris[a], Agneta Kiss[b], Luca Signor[b], Georges Lutfalla[a], Elisabetta Boeri-Erba[b], Mickael Blaise[c], Laure Yatime[a]

[a] LPHI, Univ. Montpellier, CNRS, INSERM, Montpellier, France

[b] Univ. Grenoble Alpes, CEA, CNRS, IBS, Grenoble, France

[c] IRIM, Univ. Montpellier, CNRS, Montpellier, France

E-mail of corresponding author: laure.yatime@inserm.fr

Objectives: Adherent-invasive *Escherichia coli* (AIEC) is an intestinal pathogenic *E. coli* that strongly contributes to the high inflammatory burden characterizing Crohn's disease (CD) pathology. Virulence factors from this pathogen are considered as promising drug targets for the development of novel CD treatments. This includes the IbeA protein, specific to pathogenic *E. coli*[1,2], which was recently shown to play an important role in host cell invasion, intramacrophagic survival and replication[3]. How these properties are achieved remains however largely unknown. Our objective is to unravel the structure- function relationships of IbeA from AIEC, in order to precise its biological function and validate it as a relevant drug target for anti-inflammatory strategies in CD.

Methods: using a recombinant IbeA protein, we performed native mass spectrometry and direct infusion ESI-MS analyses to determine the oligomeric state of the protein and identify the co-factor that bound to the protein during heterologous expression in bacteria. We further investigated the binding of this co-factor to IbeA, using size exclusion chromatography and tryptophan fluorescence measurements. Finally, we performed an in silico docking of the co-factor in a 3D-model of IbeA generated with AlphaFold, and we conducted mutational analyses to validate this binding mode.

Results: we here describe an efficient protocol to produce and purify a soluble form of IbeA from the cytosol of *E. coli*, yielding a highly pure recombinant protein suitable for biochemical and structural studies. We identified the co-factor captured by IbeA during bacterial expression as FAD (*flavin- adenine dinucleotide*). We further showed that IbeA associates into homodimers that can incorporate one FAD molecule in each protomer and we measured the binding affinity of IbeA for FAD based on tryptophan fluorescence quenching. 3D-modeling of the IbeA structure with AlphaFold revealed a clear structural homology with FAD-dependent oxidoreductases. Based on this 3D-model and in silico docking, we identified the putative binding pocket for the FAD molecule. Mutational studies are currently ongoing to validate the proposed IbeA:FAD interface.

Conclusions: IbeA is an homodimeric flavoprotein homologous to FAD-dependant oxidoreductase.

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KEYWORDS: IbeA; flavoprotein; FAD-oxidoreductase; AIEC; protein-ligand interactions.

POSTER 37

Cdc48 is a member of Ubiquitin Proteasome System involved in plant immunity

Claire Rosnoblet [a], Pauline Chatelain [a], Hervé Bègue [a], David Wendehenne [a]

[a] UMR 1347 Agroécologie INRAE – Institut Agro Dijon - Université de Bourgogne, 17 rue Sully, 21000 Dijon

E-mail of corresponding author: claire.rosnoblet@u-bourgogne.fr

Objectives: The aim is to highlight the signalization pathways involving the chaperone-like protein Cdc48, an ATPase member of the ubiquitin-proteasome system (UPS), in plant immunity.

Methods: We used Immunoprecipitation of endogenous Cdc48 followed by mass spectrometry analysis in order to identify Cdc48 partners and then characterize the pathways regulated by Cdc48, especially during immunity response. Thanks to transgenic cell lines and plants; we also studied the immunity response and the behavior of Cdc48 partners while Cdc48 is overexpressed.

Results: We have shown that Cdc48 is mobilized and active during immunity response in plants. Its overexpression triggers early programmed cell death in response to biotic stress. Cdc48 is also involved in Redox controle, a pathway particularly regulated in plant immunity. Moreover, while amounts of proteasomal subunits and global protein ubiquitination are affected by biotic stress, those are altered in Cdc48 overexpressing cell lines. The increase of proteolytic activity of the proteasome occurring in response to biotic stress is also not observed in Cdc48 overexpressing cells.

Conclusions: Cdc48 is a involved in the regulation of protein turnover, especially in plant immunity and programmed cell death, through Redox and UPS regulation.

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KEYWORDS: Cdc48, ubiquitin proteasome system, plant immunity

POSTER 38

In cellulo investigation of sulfide oxidation unit component

Yacine Kibouche[a†], Thaldja Amedjoudj[a†], Anne-Lise Claudel[a], Séverine Boutserin[a], François Talfournier[a], Sandrine Boschi-Muller[a] & Benjamin Selles[a]

[a], Laboratoire IMoPA, Equipe Enzymologie Moléculaire et Structurale, UMR 7365 CNRS-Université de Lorraine, Biopôle, campus Biologie-Santé, 9 Avenue de la Forêt de Haye, BP 20199, 54505 Vandœuvre- lès-Nancy Cédex.

[†], Master 2 student.

E-mail of corresponding author: Benjamin.selles@univ-lorraine.fr

Objectives: Hydrogen sulfide (H_2S) is an emerging gazotransmiter recently describe as a pleiotropic modulator of cell's physiology from mammals, plants or bacteria. H_2S is described as a Janus faceted molecule with beneficial or deleterious effects depending on its cell type accumulation and concentration [1]. As a consequence, H_2S cellular concentration has to be tightly regulated. Enzymes that are responsible for H_2S biosynthesis are now identified, with cytosolic (CBS and CSE) or mitochondrial (MPST) protagonists. Regarding H_2S clearance, that occurs in mitochondria, a tripartite protein complex (Sulfide Oxidation Unit, SOU complex), constituted by the SQOR, the ETHE1 and a sulfurtransferase (STR), is proposed [2]. STRs belong to the Rhodanese super family protein and catalyse sulfur transfer from a donor to an acceptor by transient formation of a cysteine persulfide intermediate on its catalytic residue. The human STR equipment is composed of three members, MPST, TST and TSTD1, the two latter being alternatively proposed to be the STR involved in the SOU complex [3]. Hence, the identification of the STR isoform involved in H_2S mitochondrial clearance will be a decisive step forward into the comprehension of the molecular processes responsible for the tight control of H_2S concentration.

Methods: In order to identify the STR involved in the SOU complex *in cellulo* in Hela cells, we have developed **fluorescent reporters** to decipher their respective sub-cellular localization(s) by **Laser Scanning Confocal fluorescence microscopy**. In addition, we have also designed protein / protein interactions molecular tools, based on **Bimolecular Fluorescence Complementation (BiFC)**, and performed cellular biochemistry to validated potential interactions by **immunoprecipitation** coupled to **western-blots**.

Results: The development of an *in cellulo* approaches allowed us to resolve the **subcellular localization of STR isoforms into Hella cells** and to decipher some unexpected **interactions between STR isoforms and SOU partners.**

Conclusions: Our results bring cellular evidences allowing us to identify the TST protein as the STR member involved in H_2S homeostasis within the mitochondrial SOU complex. In addition, our investigation opens new area of investigation regarding the potential physiological function(s) of TSTD1 within human cell.

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KEYWORDS: Rhodanese, persulfide, LSCM, BiFC, protein/protein interaction

POSTER 39

New insights into the regulation of actin cytoskeleton dynamics via the Rho/ROCK/LIMK2/cofilin signalling pathway: a novel mechanism of regulation of cofilin by LIMK2

Elodie Villalonga[a], Célina Chalal[a], Deborah Cassas[b], Michel Doudeau[a], Fabienne Godin[a], Hélène Bénédetti[a], Béatrice Vallée[a]

[a]Centre de Biophysique Moléculaire (CBM), Orléans

[b]Institut de Biologie en Santé (PHB-IRIS), Angers

E-mail of corresponding author: elodie.villalonga@cnrs-orleans.fr

Objectives: LIM kinase 1 (LIMK1) and LIM kinase 2 (LIMK2) are serine/threonine and tyrosine kinases that play a crucial role in cytoskeletal dynamics by inhibiting cofilin, an actin depolymerizing factor [1]. LIMK are involved in many physiological processes including cell division, differentiation, migration, apoptosis and neurite plasticity, but they also play a role in several pathological mechanisms, such as cancers, neurological diseases and viral infections [2]. As they have recently emerged as therapeutic targets of interest [3], it is crucial to have a better understanding of the molecular mechanisms involved in LIMK activity on their main substrate cofilin, in order to develop new innovative therapeutic strategies. In this study, we sought to focus on LIM kinase family member LIMK2.

Methods: LIMK2 activity on its substrate was assessed by *in vitro* kinase assay on purified cofilin and by western-blotting. LIMK2 interactions with cofilin and ROCK were determined by Western-blot following co-immunoprecipitation. ROCK activity on LIMK2 was assessed by *in vitro* kinase assay and by western-blotting. LIMK2 constructions ability to generate stress fibers in HeLa cells was evaluated by immunocytochemistry.

Results: We showed that the entire kinase domain of LIMK2 was not sufficient for the phosphorylation of cofilin, as its C-terminal part is also essential for this process. Moreove r, it appeared that this C- terminal part is phosphorylated, suggesting the existence of a phosphorylation site necessary for LIMK2 activity on cofilin. By site-directed mutagenesis, we have identified an amino acid located in the C-terminal end of LIMK2, tyrosine 630, which is necessary for the phosphorylation of LIMK2 and for its activity on cofilin. Further characterization of this mutant revealed that it is able to interact with cofilin and with ROCK, its upstream activating kinase. However, it was not phosphorylated by ROCK on the canonical phosphorylation site of activation of LIM kinases, threonine 505. Moreover, a constitutively active mutation of LIMK2, T505EE, partially restores LIMK2-Y630A activity on cofilin.

Conclusions: Those results suggest that phosphorylation of T505 is not sufficient for optimal activity on cofilin: Y630 seems also play a crucial part in LIMK2 activity, besides T505 activation by ROCK.

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KEYWORDS: LIMK kinases, cofilin, cytoskeleton, actin, cancer

LISTE DES PARTICIPANTS :

Bachar	ABDOULAYE
Alex	ABHAYA
Salem	ACHOUR
Razan	ALSOHAIBANI
Marjolaine	BALDO
Josette	BANROQUES
Kevin	BARANGER
Elodie	BASCANS
Tamara	BASTA-LE-BERRE
Mélissa	BELHARMI
Charlotte	BELLAMY
Yasmine	BENABDELLAH
Paul	BIGOT
Mickael	BLAISE
Magali	BLAUD
Bruno	BONNETAZ
Sandrine	BOSCHI-MULLER
Serge	BOUAZIZ
Cyril	BOURGEOIS
Gabrielle	BOURGEOIS
Alexis	BOUTILLIAT
Jean-Philippe	BOURSIER
Chris	BOWLER
Isabelle	BROUTIN
Florent	BUSI
Sandrine	CADEL
Florent	CANONICO
Cristina	CARDENAL
Laetitia	CAVELLINI
Claire	CERÉ
Pauline	CHATELAIN
Nathalie	CHAMONT
Thierry	CHARDOT
Caroline	CHAUVET
Clément	CARRÉ
Béatrice	CLOUET D'ORVAL
Nathalie	COLLOC'H
Xavier	COUMOUL
Julien	DAIROU
Clement	DAVIAUD
Alexandre	DE BREVERN
Lila	DELBOS
Agnès	DELMAS
Patrick	DESCHAMPS
Philippe	DESSEN
Adèle	DRAMÉ-MAIGNÉ
Laurence	DROUARD
Etienne	DUBIEZ
Pavlina	DUBOIS
Geoffrey	DUFOUR
Bernard	DUJON
Christine	EBEL
Jérôme	FAGART
Fatemi	FATANEH
Didier	FLAMENT
Philippe	FOSSÉ
Sandrine	FOUCHER
Elisa	FREZZA
Bertrand	FRIGUET
Adeline	GAND
Antoine	GEDEON
Thanos	GHELIS
Beatrice	GOLINELLI-PIMPANEAU
Wilfried	GRANGE
Marie-Pierre	GOLINELLI
Lionel	GOURAUD
Emeric	GUENEAU
Mickael	GUERIN
Lionel	GUITTAT
Emeuraude	HADJ-ATTOU
Marie-Paule	HAMON
Anne	HARDUIN-LEPERS
Valérie	HEURGUÉ-HAMARD
Julien	HENRI
Claire	HUSSER
Solène	ITHURBIDE
Laetitia	JAMMET
Nicolas	JOLY

Inge	KUHL
Zaineb	KAMMOUN
Halil	KAVAK
Katayoun	KAZEMZADEH
Janine	KEHRLI
Safia	KELLOU-TAIRI
Nadia	KHELIFI
Marta	KWAPISZ
Romain	LA ROCCA
Gilles	LALMANACH
Catherine	LAURENT
Alice	LEBRETON
Louise	LE COADOU
Florence	LEDERER
Dominique	LEGRAND
Olivier	LEMAIRE
Théo	LE MOIGNE
Olivier	LEQUIN
Nicolas	LEULLIOT
Roxanne	LESTINI
Corinne	LIONNE
François-Xavier	LYONNET-MOUTIER
Clément	MADRU
Batoul	MAHCENE
Alain	MARTY
Franck	MARTIN
Nicolas	MATHAS
Julie	MATHIEU
Olivier	MAUFFRET
Hortense	MAZON
Yves	MECHULAM
Sarah	MEGHRAOUI
Maxime	MEGRET
Jean-Louis	MERGNY
Christina	MICHAIL
Catherine	MOHALI
CD	MOHAN
Claire	MOULIS
Hélène	MUNIER-LEHMANN
Sylvie	NONIN-LECONTE
Antonin	NOURISSON
Achille	NWAKIBAN ATCHAN
Jean-Christophe	PAILLART
Gilles	PHAN
Théo	PARIS
Ludovic	PELOSI
Louise	PÉRARD
Stéphanie	PETRELLA
Isabelle	PEY BESSON
Martin	PICARD
Virginie	PILLIOL
Odil	PORRUA FUERTE
Sophie	RAHUEL-CLERMONT
Brigitte	RENÉ
Fernando	RODRIGUES LIMA
Cedric	ROMILLY
Claire	ROSNOBLET
Claire	ROUGEULLE
Michael	RYCKELYNCK
Hafra	SABEI
Yoann	SANTIN
Bruno	SARGUEIL
Catherine	SCHLINDWEIN
Emmanuelle	SCHMITT
Benjamin	SELLES
Damien	SORIGUE
Julie	SOUTOURINA
Claire	STINES-CHAUMEIL
François	TALFOURNIER
Xavier	THURU
Béatrice	VALLÉE
Christelle	VASNIER
Reynald	VIDILLI
Elodie	VILLALONGA
Tristan	WAGNER
Florent	WALTZ
Maxime	WERY
Eric	WESTHOF
Paolo	ZECCHIN
Mahel	ZEGHOUF
Émilie	ZÉLIE
Abdelrahim	ZOUED