Table of Content

Foreword

Dear colleagues, dear partners, dear friends,

The BSI-2019 organizing committee and the scientific community of Toulouse is happy to welcome you to the campus of the Paul Sabatier University for the first edition of the French congress on Integrative Structural Biology. It is a great honor and pleasure to welcome such an important event for both the Association Française de Cristallographie (AFC) and the Société Française de Biophysique (SFB), which brings together all the players in the field of integrative structural biology, a rapidly expanding field of biology. The first joint meeting between the two societies took place in 2016 in Obernai (as a "GTBio" meeting), and this BSI-2019 meeting will be the opportunity to officialize a new partnership between our societies.

The BSI-2019 congress will thus enable several multidisciplinary communities – combining X-ray crystallography, nuclear magnetic resonance, electron microscopy, mass spectrometry as well as biophysical and computational methods to discuss and exchange on the latest knowledge and state-of-the-art advances concerning important biological topics at the heart of the most innovative instrumental developments. We also hope that it will provide a unique opportunity to foster exchanges and forge new links between researchers, reflecting the dynamism and vitality of our communities.

We are very grateful to the Councils of the AFC and SFB for trusting us at all times and to our institutions and sponsors for their support. We would like to thank very warmly the members of the Scientific Committee for their availability and responsiveness. We would also like to thank the speakers and those involved in the preparation of the sessions. They have all significantly contributed to the quality of the scientific program, which we hope will prove to be exciting. We really wanted to deliver a rich program while keeping moments of exchange and conviviality.

Finally, we are sincerely grateful to the administrative staff of the Paul Sabatier University, the Délégation Régionale CNRS Occitanie Ouest and the Institute of Pharmacology and Structural Biology for their precious help.

On behalf of the BSI-2019 organizing committee, I wish you an excellent conference.

Lionel Mourey

Committees

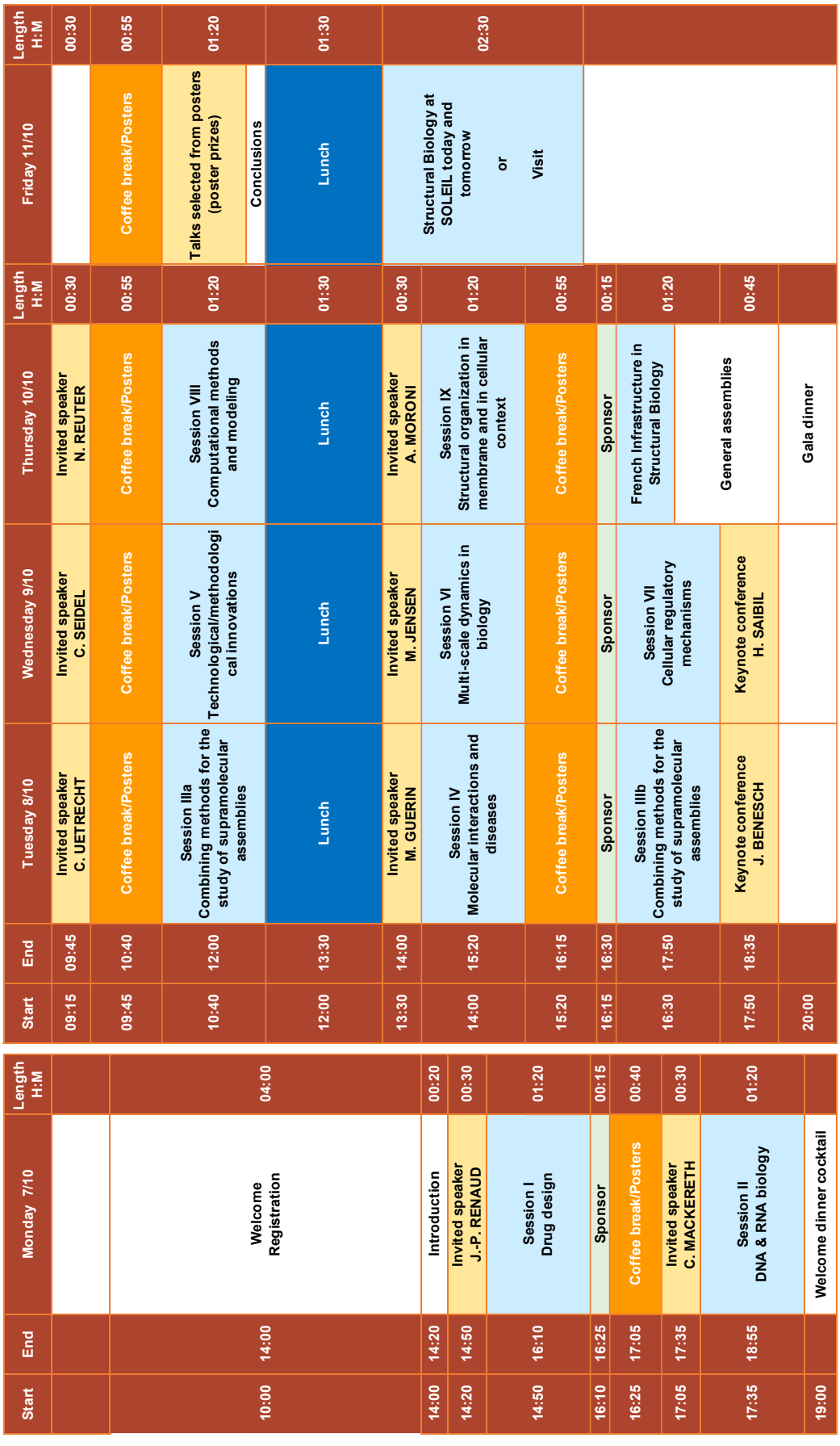
Scientific Committee

Lionel Mourey (IPBS, Toulouse), President
Sarah Cianferani, (IPHC, Strasbourg)
Bruno Kieffer (IGBMC, Illkirch)
Pierre Legrand (SOLEIL, Gif-sur-Yvette)
Emmanuel Margeat (CBS, Montpellier)
Elisabetta Mileo (BIP, Marseille)
Jean-Denis Pedelacq (IPBS, Toulouse)
Eva Pebay-Peyroula (IBS, Grenoble)
Claude Sauter (IBMC, Strasbourg)
Emmanuelle Schmitt (Ecole Polytechnique, Palaiseau)
Joanna Timmins (IBS, Grenoble)

Organizing Committee

Cécile Bon (IPBS, Toulouse)
Matthieu Chavent (IPBS, Toulouse)
Gianluca Cioci (TBI, Toulouse)
Pascal Demange (IPBS, Toulouse)
Virginie Gervais (IPBS, Toulouse)
Valérie Guillet (IPBS, Toulouse)
Julien Marcoux (IPBS, Toulouse)
Laurent Maveyraud (IPBS, Toulouse)
Alain Milon (IPBS, Toulouse)
Lionel Mourey (IPBS, Toulouse)
Jean-Denis Pedelacq (IPBS, Toulouse)
Célia Plisson-Chastang (CBI-LBME, Toulouse)
Samuel Tranier (IPBS, Toulouse)

Program at a glance



Start	End	Monday 7/10	Length H:M
10:00	14:00	Welcome Registration	04:00
14:00	14:20	Introduction	00:20
14:20	14:50	Invited speaker J.-P. RENAUD	00:30
14:50	16:10	Session I Drug design	01:20
16:10	16:25	Sponsor	00:15
16:25	17:05	Coffee break/Posters	00:40
17:05	17:35	Invited speaker C. MACKERETH	00:30
17:35	18:55	Session II DNA & RNA biology	01:20
19:00		Welcome dinner cocktail	

Start	End	Tuesday 8/10	Wednesday 9/10	Thursday 10/10	Length H:M	Friday 11/10	Length H:M
09:15	09:45	Invited speaker C. UETRECHT	Invited speaker C. SEIDEL	Invited speaker N. REUTER	00:30		00:30
09:45	10:40	Coffee break/Posters	Coffee break/Posters	Coffee break/Posters	00:55	Coffee break/Posters	00:55
10:40	12:00	Session IIIa Combining methods for the study of supramolecular assemblies	Session V Technological/methodological innovations	Session VIII Computational methods and modeling	01:20	Talks selected from posters (poster prizes) Conclusions	01:20
12:00	13:30	Lunch	Lunch	Lunch	01:30	Lunch	01:30
13:30	14:00	Invited speaker M. GUERIN	Invited speaker M. JENSEN	Invited speaker A. MORONI	00:30	Structural Biology at SOLEIL today and tomorrow or Visit	02:30
14:00	15:20	Session IV Molecular interactions and diseases	Session VI Multi-scale dynamics in biology	Session IX Structural organization in membrane and in cellular context	01:20		
15:20	16:15	Coffee break/Posters	Coffee break/Posters	Coffee break/Posters	00:55		
16:15	16:30	Sponsor	Sponsor	Sponsor	00:15		
16:30	17:50	Session IIIb Combining methods for the study of supramolecular assemblies	Session VII Cellular regulatory mechanisms	French Infrastructure in Structural Biology General assemblies	01:20		
17:50	18:35	Keynote conference J. BENESCH	Keynote conference H. SAIBIL		00:45		
20:00				Gala dinner			

Detailed program

Monday 7 October

10:00 – 14:00 Welcome and registration

14:00 – 14:20 Inaugural and welcome speeches

Invited Speaker

Chairperson: Lionel Mourey (IPBS, Toulouse)

14:20 – 14:50 Jean-Paul Renaud, Urania Therapeutics, Strasbourg
The evolving role of structural biology and biophysics in drug discovery

Session I: Drug design

Chairpersons: Vanessa Delfosse (CBS, Montpellier), Laurent Maveyraud (IPBS, Toulouse)

14:50 – 15:10 Marc Ruff, IGBMC, Illkirch
HIV-1 pre-integration complexes. Structures, functions and drug design

15:10 – 15:30 Elizabeth Ramos Morales, IGBMC, Illkirch
The challenge of inhibiting selectively histone deacetylases (HDAC) from eukaryotic pathogens

15:30 – 15:50 Gilles Labesse, CBS, Montpellier
Fragment linking yields the first NAD kinase inhibitor active against *Staphylococcus aureus* in vivo

15:50 – 16:10 Simon Veyron, McGill Center for Structural Biology and Department of Pharmacology and Therapeutics, Montreal, Canada
Structure-based design of small-molecule activators of parkin

16:10 – 16:25 Sponsor: Jerome Boutant, Xenocs Laboratory
Accelerating biostructural research with Xenocs laboratory X-ray scattering instruments

16:25 – 17:05 **Coffee break/Posters**

Invited Speaker

Chairperson: Bruno Kieffer (IGBMC, Illkirch)

17:05 – 17:35 Cameron Mackereth, IECB, Bordeaux
Protein recognition of RNA by sequence and shape

Session II: DNA & RNA biology

Chairpersons: Virginie Gervais (IPBS, Toulouse), Jean-Baptiste Charbonnier (I2BC, Gif-sur-Yvette)

17:35 – 17:55 Joanna Timmins, IBS, Grenoble
HU: a key player for nucleoid organization and compaction in *Deinococcus radiodurans*

17:55 – 18:15 Clément Charenton, MRC-LMB, Cambridge, UK
Mechanism of 5’ splice site transfer for human spliceosome activation

18:15 – 18:35 Carine Tisné, IBPC, Paris
m1A modification in tRNAs

18:35 – 18:55 Pierre Damien Coureux, École Polytechnique, Palaiseau
Cryo-EM structure of an archaeal translation initiation complex at 3.1 Å resolution

19:00 – 22:00 **Welcome dinner cocktail**

Tuesday 8 October

Invited Speaker

Chairperson: Claude Sauter (IBMC, Strasbourg)

09:15 – 09:45 Charlotte Uetrecht, Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany
Flying viruses – from biophysical to structural characterization

09:45 – 10:40 **Coffee break/Posters**

Session IIIa: Combining methods for the study of supramolecular assemblies

Chairpersons: Aurélie Albertini (I2BC, Gif-sur-Yvette), Wim Burmeister (IBS, Grenoble)

10:40 – 11:00 Stéphanie Petrella, Institut Pasteur, Paris
Overall structures of *Mycobacterium tuberculosis* DNA gyrase reveal the role of a Corynebacteriales GyrB specific insert in ATPase activity

11:00 – 11:20 Adrien Favier, IBS, Grenoble
Integrated NMR and cryo-EM atomic-resolution structure determination of a half-megadalton enzyme complex

11:20 – 11:40 Benjamin Bardiaux, Institut Pasteur, Paris
Integrative structural biology of bacterial type IV pili and pseudopili

11:40 – 12:00 Guillaume Tétreau, IBS, Grenoble
An integrative approach to unravel the in vivo crystallization pathway and mechanism of toxicity of Cyt1Aa – a naturally crystalline mosquitocidal toxin

12:00 – 13:30 **Lunch**

Invited Speaker

Chairperson: Jean-Denis Pedelacq (IPBS, Toulouse)

13:30 – 14:00 Marcelo E. Guerin, CIC bioGUNE, Derio, Spain
Structural basis of glycosyl transfer reactions

Session IV: Molecular interactions and diseases

Chairpersons: Sonia Fieulaine (I2BC, Gif-sur-Yvette), Laurent Terradot (IBCP, Lyon)

14:00 – 14:20 Paloma Varela, I2BC, Gif-sur-Yvette
Macromolecules interactions in vitro, comparing classical and innovative approaches

14:20 – 14:40 Valérie Guillet, IPBS, Toulouse
Structural basis of the addiction of toxin-antitoxin systems to molecular chaperones

14:40 – 15:00 Florian Malard, ICSN, Gif-sur-Yvette
The molecular mechanism of the interaction between TCTP and the Bcl-2 proteins (Bcl-xL, Mcl-1) in the context of tumor reversion

15:00 – 15:20 Stefan Arold, StruBE/KAUST, Thuwal, Saudi Arabia
HX repeat motifs and their role in a novel neurocognitive syndrom

15:20 – 16:15 **Coffee break/Posters**

16:15 – 16:30 Sponsor: Johnny Faherty, Fluidic Analytics
Diffusional Sizing for protein interaction analysis - latest data and developments

Session IIIb: Combining methods for the study of supramolecular assemblies

Chairpersons: Isabelle Billas (IGBMC, Illkirch), Alain Roussel (AFMB, Marseille)

16:30 – 16:50 Julie Ménétrey, I2BC, Gif-sur-Yvette
Structural characterization of the RH1-LZI tandem of JIP3/4 highlights RH1 domains as a cytoskeletal motor-binding motif

16:50 – 17:10 Maxime Bourguet, LSMBO, Strasbourg
Benefits of MS-based approaches for the structural characterisation of the specific association between the coactivator Med1 with the VDR-RXR heterodimer

17:10 – 17:30 Julien Henri, LBMCE, Paris
Supramolecular organization of the Calvin-Benson cycle

17:30 – 17:50 Antoine Loquet, CBMN, Pessac
Solid-state NMR-based structural biology of functional amyloid fibrils

Keynote conference

Chairperson: Julien Marcoux (IPBS, Toulouse)

17:50 – 18:35 Justin Benesch, University of Oxford, UK
Weighing the evidence for molecular chaperone function

Wednesday 9 October

Invited Speaker

Chairperson: Emmanuel Margeat (CBS, Montpellier)

09:15 – 09:45 Claus A.M. Seidel, Heinrich Heine University, Düsseldorf, Germany
Integrative dynamic structural biology with multi-parameter fluorescence spectroscopy and super-resolution FRET microscopy

09:45 – 10:40 **Coffee break/Posters**

Session V: Technological/methodological innovations

Chairpersons: Nathalie Colloc'h (ISTCT, Caen), Eric Ennifar (IBMC, Strasbourg)

10:40 – 11:00 Dominique Bourgeois, IBS, Grenoble
Mechanistic investigation of a fluorescent protein reveals a strategy to reduce track interruptions in single particle tracking PALM

11:00 – 11:20 Igor Chaussavoine, SOLEIL, Gif-sur-Yvette
Microfluidic positioning of biological macromolecular crystals for serial X-ray diffraction on the PROXIMA-1 beamline

11:20 – 11:40 Dominique Housset, IBS, Grenoble
Structural studies of proteins and peptide by cryo-electron diffraction of 3D nano-crystals

11:40 – 12:00 Léonard Chavas, SOLEIL, Gif-sur-Yvette
New access modes for integrated biology at synchrotron SOLEIL

12:00 – 13:30 **Lunch**

Invited Speaker

Chairperson: Joanna Timmins (IBS, Grenoble)

13:30 – 14:00 Malene E. Jensen, IBS, Grenoble
REVEALING the mechanism of action of a temperate bacteriophage by an integrated structural biology approach

Session VI: Multi-scale dynamics in biology

Chairpersons: Carine Van Heijenoort (ICSN, Gif-sur-Yvette), Guy Lippens (TBI, Toulouse)

14:00 – 14:20 Elena Andreeva, IBS, Grenoble
Towards a better understanding of the Orange Carotenoid Protein (OCP) photoactivation mechanism

14:20 – 14:40 Annalisa Pierro, BIP, Marseille
Probing NarJ structural dynamics inside *E. coli* cells by EPR spectroscopy

14:40 – 15:00 Emmanuel Margeat, CBS, Montpellier
Structural dynamics of single metabotropic glutamate receptors

15:00 – 15:20 Kyprianos Hadjidemetriou, IBS, Grenoble
Time-resolved serial femtosecond crystallography on photosensitive proteins

15:20 – 16:15 **Coffee break/Posters**

16:15 – 16:30 Sponsor: Yusuke Nishiyama, Jeol
Electron and NMR nano-crystallography

Session VII: Cellular regulatory mechanisms

Chairpersons: Pascale Marchot (AFMB, Marseille), Marc Delarue (Institut Pasteur, Paris)

16:30 – 16:50 Bouchra Attia, LISM, Marseille
Structural characterization of the GtlJ protein and its interactions with the cytoplasmic platform during the adventurous motility of *Myxococcus xanthus*

16:50 – 17:10 Laurence Blanchard, BIAM/CEA, Saint Paul-lez-Durance
Novel structure of a XRE family transcriptional regulator: insight into the metalloprotease/repressor-controlled radiation response in *Deinococcus*

17:10 – 17:30 Yves Bourne, AFMB, Marseille
Structural insights into substrate binding and catalytic mechanism of heparan sulfate D-glucuronyl C5 epimerase

17:30 – 17:50 Julien Marcoux, IPBS, Toulouse
Hydrogen-deuterium eXchange coupled to Mass Spectrometry highlights a reciprocal crosstalk between the inner and outer rings of the 20S proteasome

Keynote conference

Chairperson: Célia Plisson-Chastang (CBI-LBME, Toulouse)

17:50 – 18:35 Helen Saibil, Birkbeck College, London, UK
Protein aggregation in cells and machinery for disaggregation

Thursday 10 October

Invited Speaker

Chairperson: Eva Pebay-Peyroula (IBS, Grenoble)

09:15 – 09:45 Nathalie Reuter, University of Bergen, Norway
How do soluble proteins bind to lipid membranes? Computational approaches for mapping and predicting protein-membrane interfaces

09:45 – 10:40 **Coffee break/Posters**

Session VIII: Computational methods and modeling

Chairpersons: Marc Bergdoll (IBMP, Strasbourg), Matthieu Chavent (IPBS, Toulouse)

10:40 – 11:00 Julie Cornet, LPT, Toulouse
Monte Carlo and molecular dynamics simulations to explain biomembrane meso-patterning by a composition-curvature coupling mechanism

11:00 – 11:20 Florence Tama, Department of Physics and ITbM, Nagoya University, Japan
Integrative modeling to characterize structure and dynamics of biomolecules from single molecule experiments

11:20 – 11:40 Jérémie Topin, Institut de Chimie de Nice, Nice
Numerical modeling of odorant receptors activation dynamics

11:40 – 12:00 Jérôme de Ruyck, UGSF, Lille
How can oncoprotein Ets-1 interact with DNA repair enzyme PARP-1? A molecular modelling approach to design cancer progression inhibitors

12:00 – 13:30 **Lunch**

Invited Speaker

Chairperson: Eva Pebay-Peyroula (IBS, Grenoble)

13:30 – 14:00 Anna Moroni, University of Milan, Milano, Italy
Relating structure to function in HCN channels

Session IX: Structural organization in membrane and in cellular context

Chairpersons: Coralie Bompard (UGSF, Lille), Alain Dautant (IBGC, Bordeaux)

14:00 – 14:20 Vincent Chaptal, MMSB, Lyon
Cryo-EM and X-ray structures of the multidrug transporter BmrA: revisiting the transport mechanism

14:20 – 14:40 Isabelle Broutin, CiTCom, Paris
New insights into the assembly and opening mechanism of the OprM-MexAB efflux pump from *Pseudomonas aeruginosa*

14:40 – 15:00 Julie Bouckaert, UGSF, Lille
Glycoprotein binding enhances bacterial entry at epithelial membranes

15:00 – 15:20 Arpita Tawani, IECB, Pessac
Nanodomain targeting during bacterial division investigated by solid-state NMR

15:20 – 16:15 **Coffee break/Posters**

16:15 – 16:30 Sponsor: Daisy Paiva, Dynamic Biosensors
Conformational screening of proteins using chip-based friction measurements

French Infrastructure in Structural Biology

Chairperson: Julie Ménétrey (I2BC, Gif-sur-Yvette)

16:30 – 16:55 Members of the FRISBI executive committee
Present and future perspectives

16:55 – 17h15 Round table

17:15 – 18:35 **General assemblies of AFC and SFB**

20:00 – 24:00 **Gala dinner**

Friday 11 October

09:45 – 10:40 **Coffee break/Posters**

Talks selected from posters

Chairpersons: Philippe Dumas (IGBMC, Illkirch), Alain Milon (IPBS, Toulouse)

10:40 – 10:50 Jérémie Buratto, CBMN, Pessac
P3 - Structural insights into hDM2 protein recognition by oligourea foldamers

10:50 – 11:00 Amin Sagar, CBS, Montpellier
P60 - Chemometric decomposition of multiple SAXS datasets to study transient protein-protein complexes

11:00 – 11:10 Paulo Esperito Santo, IBET, Lisboa, Portugal
P28 - Structural insights into the R2TP complex

11:10 – 11:20 Anna Sartori-Rupp, Institut Pasteur, Paris
P81 - 3D ultrastructural studies of tunneling nanotubes by correlative cryo-microscopy

11:20 – 11:35 **Poster prizes**

11:35 – 12:00 **Conclusions**

12:00 – 13:30 **Lunch**

Structural Biology at SOLEIL today and tomorrow (or visit)

Chairperson: Andrew Thompson, SOLEIL, Gif-sur-Yvette

13h30 – 16h00 Presentations and round table

Alternatively: **Visit**

Sponsors


dynamic
BIOSENSORS


Fluidic
Analytics


JEOL
Solutions for Innovation


xenocs
Exploring the very small


Eurisotop
A CAMBRIDGE ISOTOPE LABORATORIES COMPANY

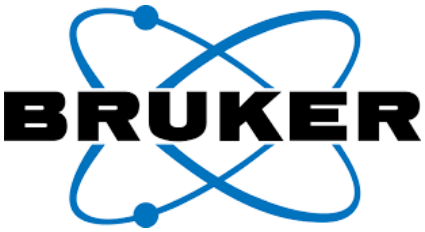

MSVision


Malvern
Panalytical
a spectris company


nano
TEMPER


Advion


Waters
THE SCIENCE OF
WHAT'S POSSIBLE.™


BRUKER

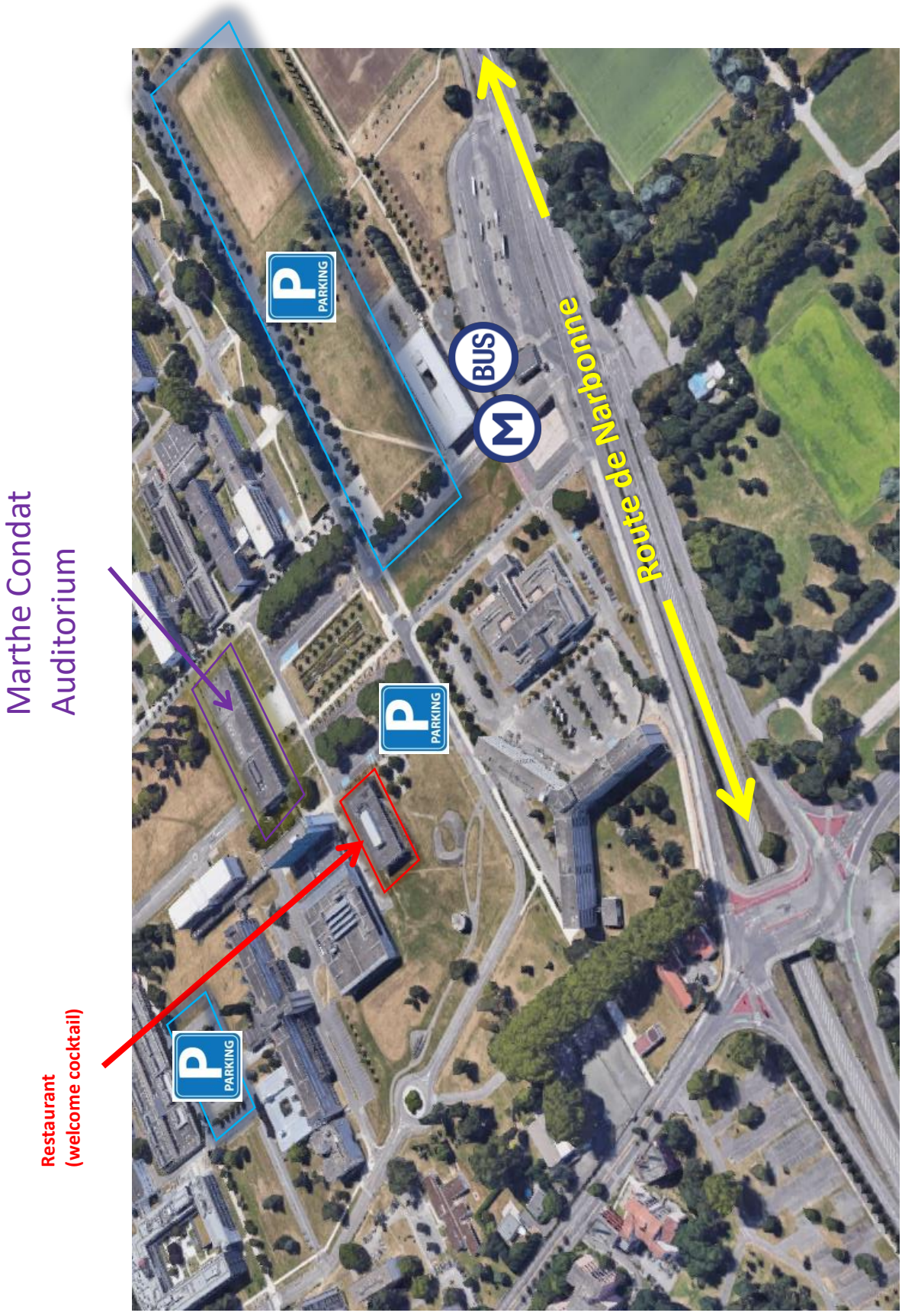

evotec


WYATT
TECHNOLOGY


ThermoFisher
SCIENTIFIC

Institutional partners

Site map


Marthe Condat
Auditorium
Restaurant
(welcome cocktail)
P PARKING
P PARKING
P PARKING
BUS
M
Route de Narbonne

Invited Speakers

Justin Benesch, Oxford University, UK: Justin's research has garnered an international reputation for innovative biophysical chemistry approaches based on combining mass measurement with other experimental methods, simulations, and quantitative thermodynamic and kinetic analyses. This has allowed him and his group to change our thinking as to how proteins assemble, interact, and even evolve.
His group's research impacts broadly the interface between chemistry and the life sciences. Their insights have been important to understanding molecular chaperone (mal)function in humans, and the stress tolerance of plants; and their innovations in mass measurement approaches have provided new means for researchers to quantify biomolecules and their interactions

Chairperson: J. Marcoux (IPBS, Toulouse)

Weighing the evidence for molecular chaperone function

Justin Benesch
Oxford University, UK
Email: justin.benesch@chem.ox.ac.uk

We have been developing and applying mass-measurement approaches to interrogate directly the structure and dynamics of proteins. Here I will focus on the insights this has enabled in studying the evolution of specificity in assembly of molecular chaperone proteins [1], and how they interact with target proteins under the application of force. I also present mass photometry, a new method we have developed that allows the quantitative, label-free interrogation of proteins in solution [2].

[1] Hochberg G.K.A., et al. Structural principles that enable oligomeric small heat-shock protein paralogs to evolve distinct functions (2018) **Science**, 359:930-935
[2] Young, G., et al. Quantitative mass imaging of single biological macromolecules **Science**, 360:423-427 (2018)

Marcelo E. Guerin, CIC Biogune, Derio, Spain: Prof. Marcelo Guerin is the head of the Structural Glycobiology Lab at the Technological Park of Bizkaia, the Basque Country, Spain. He is particularly interested in investigating structural and mechanistic aspects of carbohydrate modifying enzymes. To this end, the group is using a multidisciplinary approach including X-ray crystallography, X-ray free electron laser and Cryo-electron microscopy.

Chairperson: J.D. Pedelacq (IPBS, Toulouse)

Back to Table of Content

Structural basis of glycosyl transfer reactions

Marcelo E. Guerin
Structural Biology Unit, CIC bioGUNE, Spain
Email: mrcguerin@cicbiogune.es

Marcelo will discuss the contribution of his group in investigating the structural and mechanistic properties of enzymes involved in glycosyl transfer reactions. First, he will be focused in the ADP-Glc pyrophosphorylase (AGPase), the enzyme that catalyzes the biosynthesis of the nucleotide sugar ADP-Glc. AGPase, is considered the main regulatory step in glycogen and starch production in bacteria and plants. He will discuss structures of the homotetrameric AGPase from *Escherichia coli* in complex with its physiological positive and negative allosteric regulators, fructose-1,6-bisphosphate (FBP) and AMP. They propose a model in which the energy reporters regulate AGPase catalytic activity by intraprotomer interactions and inter- protomer crosstalk, with a sensory motif and two regulatory loops playing a prominent role. Secondly, they provided for the first time the atomic coordinates of a native Michaelis complex for a glycosyltransferase in the absence of any substrate derivative or protein mutant. Moreover, through a series of unique structural snapshots of the glycosyltransferase GpgS, they visualized how the enzyme guides the substrates into the reaction center and unveiled the mechanism of product release, involving multiple conformational changes not only in the substrates/products but also in the enzyme.

Malene E. Jensen, IBS, Grenoble: Malene R. Jensen is CNRS research director at the Institut de Biologie Structurale in Grenoble. She uses nuclear magnetic resonance (NMR) spectroscopy to study functional protein dynamics. She is mainly interested in understanding the role of large intrinsically disordered proteins in cell signalling.

Chairperson: J. Timmins, (IBS, Grenoble)

Back to Table of Content

Revealing the mechanism of action of a temperate bacteriophage by an integrated structural biology approach

K. K. Rasmussen[a], A. Palencia[b], A. K. Varming[a], H. El-Wali[c], E. Boeri Erba[d], M. Blackledge[d], K. Hammer[c], T. Herrmann[d], M. Kilstrup[c], L.L. Lo Leggio[a], M. R. Jensen[d]

[a]*Department of Chemistry, University of Copenhagen, Denmark*.
[b]*Institute for Advanced Biosciences, INSERM, CNRS, Université Grenoble Alpes, Grenoble, France.*
[c]*Department of Systems Biology, Technical University of Denmark, Lyngby, Denmark.*
[d]*Institut de Biologie Structurale, Université Grenoble Alpes, CEA, CNRS, 38044 Grenoble, France*.
E-mail: malene.ringkjobing-jensen@ibs.fr

Bacteriophages are bacterial viruses. Temperate bacteriophages can enter two different stages of growth, the lytic and the lysogenic life cycle which is mediated by a genetic switch. We have studied the temperate bacteriophage TP901-1 from *Lactococcus lactis*. In TP901-1, the genetic switch is controlled by the anti-repressor MOR and the clear I protein (CI). Here, we employ an integrated structural biology approach, combining NMR spectroscopy, X-ray crystallography, small angle X-ray scattering and native mass spectrometry, to describe the structure and dynamics of CI and its interaction with the DNA operator sites on the phage genome [1,2,3]. In addition, we determine the solution structure of MOR using NMR, and we solve the crystal structure of MOR in complex with the N-terminal domain of CI (CI-NTD). The structure reveals how MOR prevents binding of CI to DNA. ^{15}N NMR relaxation measurements demonstrate that MOR displays molecular recognition dynamics on two time scales involving a repacking of aromatic residues at the interface with CI-NTD. Using sequence alignments and evolutionary couplings, we show that the structural and dynamic features of the MOR:CI-NTD complex are conserved among a larger family of bacteriophages from human pathogens including *Staphylococcus aureus* and *Streptococcus pneumoniae*. Our results, also supported by *in vivo* experiments, provide new insight into the mechanism of action of a larger family of temperate bacteriophages.

[1] K.H. Frandsen, K.K. Rasmussen, M.R. Jensen, K. Hammer, M. Pedersen, J.C. Poulsen, L. Arleth, L. Lo Leggio (2013). Binding of the N-terminal domain of the lactococcal bacteriophage TP901-1 CI repressor to its target DNA: a crystallography, small angle scattering, and nuclear magnetic resonance study. **Biochemistry** 52:6892-6904.

[2] K.K. Rasmussen, K.H. Frandsen, E. Boeri Erba, M. Pedersen, A. K. Varming, M. Kilstrup, P.W. Thulstrup, M. Blackledge, M.R. Jensen, L. Lo Leggio (2016). Structural and dynamics studies of a truncated variant of CI repressor from bacteriophage TP901-1. **Sci. Rep.** 6:29574.

[3] K.K. Rasmussen, A. K. Varming, S. N. Schmidt, K.H. Frandsen, P.W. Thulstrup, M.R. Jensen, L. Lo Leggio (2018). Structural basis of the bacteriophage TP901-1 CI repressor dimzerization and interaction with DNA. **FEBS Lett.** 592:1738-1750.

Cameron Mackereth, IECB, Bordeaux: Cameron Mackereth is an Inserm research director, whose group is located at the Institut Européen de Chimie et Biologie at the University of Bordeaux. He uses NMR spectroscopy coupled with a variety of biophysical, in vitro and cell biology techniques to study the interaction between proteins and RNA. A major theme in the group is the molecular study of alternative splicing related to development and disease.

Chairperson: B. Kieffer, (IGBMC, Illkirch)

Back to Table of Content

Protein recognition of RNA by sequence and shape

Cameron Mackereth
Inserm U1212, Univ. Bordeaux, Institut Européen de Chimie et Biologie,
33076, Pessac, France.
Email: cameron.mackereth@inserm.fr

RNA binding proteins employ a variety of recognition strategies in order to interact with a specific set of targets. We have used complementary in vitro and in vivo tools to explore these interactions, and we also create mutants to strategically perturb the binding in order to gain insight into the biological role of the protein-RNA complexes, or to mimic disease. One goal in the group is to understand how development in multicellular organisms relies on the regulation of splicing factor expression in order to create protein isoforms unique to a given tissue and at a specific time in development. In this context, we are currently investigating the atomic details of RNA recognition by the human splicing factor RBM20 essential for heart development, and the splicing factor MEC-8 required for neural function in C. elegans. For RBM20, we have used NMR spectroscopy and isothermal titration calorimetry to show that RNA recognition involves a coupled folding/binding mechanism with a C-terminal helix required for full affinity interaction with a UCUU motif. For MEC-8, we have discovered that despite low similarity in protein sequence, both the N-terminal and C-terminal RRM domains interact with the same GCAC motif sequence. The additional dimerization of the N-terminal domain [1] imparts further complexity to the interaction of MEC-8 with target RNA. Finally, we have also found and characterized a new double-stranded RNA (dsRNA) binding domain present on the human prolyl isomerase FKBP25 [2]. In this case, the conformation of the RNA rather than sequence features appears to govern RNA recognition. Similar to our other studies, characterization of the interaction between FKBP25 and dsRNA uses a combination of NMR spectroscopy, isothermal titration calorimetry, X-ray crystallography, and cellular assays.

[1] H. Soufari, C.D. Mackereth. (2017). Conserved binding of GCAC motifs by MEC-8, couch potato and the RBPMS protein family. **RNA** 23:308-316
[2] D. Dilworth, S.K. Upadhyay, P. Bonnafous, A.B. Edoo, S. Bourbigot, F. Pesek-Jardim, G. Gudavicius, J. Serpa, E. Petrotchenko, C. Borchers, C.J. Nelson, C.D. Mackereth. (2017). The basic tilted helix bundle domain of the prolyl isomerase FKBP25 is a novel double-stranded RNA binding module. **Nucleic Acids Res.** 45:11989-12004.

Anna Moroni, University of Milan, Italy: Anna Moroni works at the Department of Biosciences of the U. of Milan, Italy. She investigates a class of membrane proteins, ion channels, with the aim of understanding the conformational changes leading to pore gating. To this end, she applies a combination of structural, functional and protein engineering approaches.

Chairperson: E. Pebay-Peyroula (IBS, Grenoble)

Back to Table of Content

Relating structure to function in HCN channels

Anna Moroni
Dept. of Biosciences, Milano, Italy
Email: anna.moroni@unimi.it

Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are the molecular determinants of the If/Ih current, a mixed Na+/K+ current that controls pacemaking in cardiac and neuronal cells. HCN channels are activated by membrane voltage hyperpolarization, and conduct an inwardly directed cation current, which slowly depolarizes pacemaker cells to the threshold for action potential firing. In addition to voltage, HCN channels are modulated by the second messenger cAMP, which enhances channel open probability. How the two signals are integrated by the protein contribute to pore opening is still an open question. We have recently solved the structures of HCN4, the cardiac isoform, with and without cAMP bound by single particle cryoEM. From their comparison we could identified conformational transitions occurring upon cAMP binding and functionally validated them by patch clamp and computational methods. Our data indicate that HCN4 channels undergo a complex mechanism of gating, experiencing multiple closed states before the voltage-operated opening step. Precise structural knowledge of this channel will allow screening for isoform-specific and state-dependent inhibitors.

Jean-Paul Renaud, Urania Therapeutics, Strasbourg: Jean-Paul Renaud had been a CNRS research director at IGBMC in Illkirch, where he worked on nuclear receptors using X-ray crystallography. In 2002 he co-founded NovAliX, an innovative CRO offering an extensive structural biology and biophysics platform for early-stage drug discovery. In 2015, he co-founded Urania Therapeutics (formerly RiboStruct) to leverage the know-how on eukaryotic ribosome crystallography built up by Marat Yusupov and Gulnara Yusupova at IGBMC to develop drug candidates targeting the human ribosome following a structure-based drug design approach.

Chairperson: L. Mourey (IPBS, Toulouse)

The evolving role of structural biology and biophysics in drug discovery

Jean-Paul Renaud
Urania Therapeutics, Ostwald, France
Email: jprenaud@uraniatx.com

The use of structural biology and biophysics has become pervasive in drug discovery, allowing not only to accelerate the hit-to-lead process as in the beginning, but also to discover new biology, to identify low-affinity binders at the screening stage and to obtain detailed information on target / compound interactions [1]. This is perfectly illustrated by the success of FBDD (fragment-based drug design), where the progress of biophysical techniques in terms of sensitivity, speed, and automation has been instrumental [2,3]. Indeed, FBDD is now widely adopted in both academia and the pharmaceutical industry and has led to 2 approved drugs and around 40 compounds being evaluated in clinical trials. Recent progress in cryo-EM has been spectacular and has led to the determination of numerous high-resolution structures of highly interesting macromolecular assemblies, including large dynamical complexes and membrane proteins [4]. Certainly, many of these structures would have been out of reach using X-ray crystallography. Several reviews have recently focused on the potential role of cryo-EM in drug discovery and in particular structure-based drug design (see for instance [5,6]). Even though it has been predicted that "the revolution will not be crystallized" [7], crystallography is still alive and constantly reinventing itself [8] and will certainly remain at least for a couple of years the main technique for high-throughput structure determination in structure-based drug design. X-ray crystallography and cryo-EM as well as NMR should rather be considered complementary techniques [9] and drug discovery scientists should choose in each situation the one better suited to the question to address or, even better, use them in combination. The recent rebirth of chemical biology is the perfect opportunity for structural biologists / biophysicists to work hand in hand with medicinal chemists to exploit the wealth of new modalities and potential new therapeutic targets [1,10] to address challenging unmet medical needs, as illustrated by the development of PROTACs to target previously undruggable proteins.

[1] Renaud J.P. (2019) The evolving role of structural biology in drug discovery in *Structural Biology in Drug Discovery*, J.P. Renaud Ed., Chapter 1, Wiley, in press
[2] Erlanson D. et al. (2016) Twenty years on: the impact of fragments on drug discovery. **Nat. Rev. Drug Discov.** 15:605–619
[3] Renaud J.P. et al. (2016) Biophysics in drug discovery: impact, challenges and opportunities. **Nat. Rev. Drug Discov.** 15:679–698
[4] Cheng Y. (2018) Single-particle cryo-EM – How did it get here and where will it go **Science** 361:876–880
[5] Renaud J.P. et al. (2018) Cryo-EM in drug discovery: achievements, limitations and prospects **Nat. Rev. Drug Discov**. 17:471–492
[6] Ceska T. et al. (2019) Cryo-EM in drug discovery **Biochem. Soc. Trans**., in press
[7] Callaway E. (2015) The revolution will not be crystallized **Nature** 525:172–174
[8] Bricogne G. (2019) High-throughput macromolecular crystallography in drug discovery: Evolving in the midst of revolutions in *Structural Biology in Drug Discovery*, J.P. Renaud Ed., Chapter 10, Wiley, in press
[9] Vénien-Bryan C. et al. (2017) Cryo-electron microscopy and X-ray crystallography: complementary approaches to structural biology and drug discovery **Acta Cryst. F** 73:174–183
[10] Valeur E. and Jimonet P. (2018) New Modalities, Technologies, and Partnerships in Probe and Lead Generation: Enabling a Mode-of-Action Centric Paradigm **J. Med. Chem.** 61:9004−9029

Nathalie Reuter, University of Bergen, Norway: The Reuter group uses protein bioinformatics and molecular simulations to investigate protein dynamics. In particular we are interested in peripheral protein-lipid interactions at membrane interfaces and in conservation of protein flexibility within and across protein families. The Reuter group is at the University of Bergen (Norway) and affiliated to both the Chemistry Department and the Computational Biology Unit.

Chairperson: E. Pebay-Peyroula (IBS, Grenoble)

Back to Table of Content

How do soluble proteins bind to lipid membranes? Computational approaches for mapping and predicting protein-membrane interfaces

Nathalie Reuter
Department of Chemistry & Computational Biology Unit,
University of Bergen, Norway
Email: nathalie.reuter@uib.no

Unlike transmembrane proteins, peripheral proteins bind at the surface of cell or organelle membranes transiently. They can be enzymes such as e.g. phospholipases or membrane-targeting domains such as Pleckstrin Homology or C2 domains. The prototypical peripheral membrane binding site is described as a combination of clusters of basic and hydrophobic amino acids but we know that this model does not hold for all peripheral proteins. In fact, and unlike protein-protein or protein-DNA interfaces, interfacial binding site of peripheral proteins are still poorly characterized both in terms of amino acid composition and structural patterns.

We aim at updating the text-book model for peripheral interfacial binding site and use a combination of computational tools and experimental data. We use molecular simulations on a hand-full of proteins and enzymes, and bioinformatics analyses on larger datasets of protein structures. I will present a summary of our progresses so far, including (i) the role and energetic contributions of aromatic amino acids, (ii) the overestimated role of unspecific electrostatics and (iii) the idea that protruding hydrophobes are a structural pattern distinguishing membrane-binding from non-membrane binding protein surfaces.

Helen Saibil, Birkbeck College, London, UK: Helen Saibil is a structural biologist at the Institute of Structural and Molecular Biology, Birkbeck College London. Her main interests are in analyzing conformational changes in macromolecular machines, mainly using cryo-electron microscopy and image processing. This work has focused on the actions of molecular chaperones in protein misfolding, aggregation and disaggregation in vitro and in cells, and on mechanisms of membrane pore formation in host-pathogen interactions.

Chairperson: C. Plisson-Chastang (CBI-LBME, Toulouse)

Back to Table of Content

Protein aggregation in cells and machinery for disaggregation

Helen Saibil
ISMB, Birkbeck College London
Email: h.saibil@mail.cryst.bbk.ac.uk

Protein misfolding and aggregation cause late onset, degenerative diseases, in which the misfolded protein or peptide can assemble into amyloid fibrils whose formation is associated with cell death. We use correlative fluorescence and 3D electron microscopy to study protein aggregation in models of Huntington's disease. Two different cytosolic disaggregase systems work in cooperation with the abundant and ubiquitous Hsp70 chaperone. In bacteria, fungi and plants, the Hsp100 AAA+ ATPases, including bacterial ClpB and yeast Hsp104, disassemble aggregates and prion fibrils. We have studied the threading action of ClpB by single particle cryo EM. In 30pprox.30, disaggregation activity is provided by the combination of Hsp70 with an Hsp40 co factor and the nucleotide exchange factor Hsp110. We are examining the mammalian Hsp70 chaperone machinery, as it disassembles model amyloid fibrils, by atomic force microscopy and cryo EM.

Claus A. M. Seidel, Heinrich Heine University, Dusseldorf, Germany: Pr. Claus Seidel heads the Chair for Molecular Physical Chemistry at the Heinrich-Heine-University Düsseldorf, Germany. He uses fluorescence spectroscopy with single-molecule detection and super-resolution microscopy combined with Förster Resonance Energy Transfer (FRET) measurements to study the dynamics and function of biomolecules and their assemblies in vitro and in live cells. In an integrative approach, the fluorescence studies are combined with other experimental biophysical techniques (NMR- and EPR-spectroscopy and SAXS) as well as with computer simulations to reach a more complete perspective on the dynamic personality and molecular function of proteins and nucleic acids. This dynamic view in a time range from picoseconds to minutes complements the traditional structure determination showing many detailed static snapshots of biomolecular structures.

Chairperson: E. Margeat (CBS, Montpellier)

Back to Table of Content

Integrative dynamic structural biology with multi-parameter fluorescence spectroscopy and super-resolution FRET microscopy

Claus A.M. Seidel
Chair for Molecular Physical Chemistry, Heinrich Heine University,
Universitätsstraße 1, 40225 Düsseldorf, Germany.
Email: cseidel@hhu.de

We combine super-resolution microscopy via stimulated emission depletion (STED) and Multi-parameter Fluorescence Image Spectroscopy (MFIS) [1,2] to reach molecular resolution with sub-nanometer precision for studying biomolecules and their complexes. While STED-MFIS captures the spatial and temporal information of the cellular context with a resolution down to 20 nm, the concurrent measurement of Förster resonance energy transfer (FRET) between an excited donor and acceptor provides a zoom with Ångström precision. Thus, super-resolution FRET microscopy exploits these synergies to reach molecular resolution. I will present the complete workflow from (I) super-resolution FRET microscopy over (II) FRET-specific data analysis to (III) integrative FRET-restrained structural models. MFIS uses multi- parameter fluorescence detection (MFD) [1] to capture the complete fluorescence information on the biomolecules by registering all eight characteristic fluorescence parameters in a single measurement. This allows us to study the formation of homo- and hetero-complexes by homo- and hetero-FRET in live cells simultaneously.
Using FRET restraints and computer simulations, we established an automated workflow to generate integrative structural models of biomolecular assemblies that can be deposited in the new protein data bank, PDB-dev [3, 4]. We studied Guanylate binding proteins (GBPs) that undergo a conformational transition for GTP-controlled oligomerization to exert their function as part of the innate immune system of mammalian cells – attacking intra-cellular parasites by disrupting their membranes. We identified GBP's intrinsic flexibility, a GTP-triggered association of the GTPase-domains and an assembly-dependent GTP-hydrolysis as functional design principles that control their reversible oligomerization in polar assemblies and the subsequent formation of condensates [5]. Further examples for dynamic structural biology via integrative FRET studies is the mapping of the dynamic exchange network in chromatin fibers by studying a 12-mer nucleosome array (32pprox.. 2,5 Mda) [6] and the detection of a so far hidden functionally important conformational state in the enzyme T4 Lysozyme [7].

[1] Widengren et al. (2006) **Anal. Chem***.* 78:2039-2050.
[2] Weidtkamp-Peters et al. (2009) **Photochem. Photobiol. Sci***.* 8:470-480
[3] Kalinin et al. (2012) **Nat. Methods** 9:1218-1225
[4] Dimura et al.(2016) **Curr. Opin. Struct. Biol***.* 40:163–185
[5] Kravets et. Al.(2016) **eLife** 5:e11479
[6] Kilic et al. (2018) **Nat. Commun***.* 9:235
[7] Sanabria et. Al. **Nat Commun**. (In revision). https://arxiv.org/abs/1812.06937v1

Charlotte Uetrecht, University of Hambug, Germany: Charlotte Uetrecht studied biochemistry at the University of Potsdam, Germany. She performed her doctoral work in the group of Albert Heck at Utrecht University, the Netherlands. An EMBO longterm fellowship funded her postdoc in Uppsala, Sweden, and at European XFEL, Germany, where she is still working as guest scientist. Since 2014, Charlotte runs her own group at Heinrich Pette Institute, Leibnz Institute for Experimental Virology in Hamburg, Germany. She is well-known for structural, especially native, mass spectrometry of viruses and developing native mass spectrometry as delivery system for X-ray based structure determination.

Chairperson: C. Sauter (IBMC, Strasbourg)

Back to Table of Content

Flying viruses – from biophysical to structural characterization

Charlotte Uetrecht
Heinrich Pette Institute, Leibniz Institute for Experimental Virology and European XFEL GmbH, Schenefeld, Germany
Email: charlotte.uetrecht@xfel.eu

Viruses affect basically all organisms on earth. Some are detrimental to human development, whereas those targeting pathogenic bacteria or crop pathogens can be beneficial for us. An integral part of icosahedral viruses is the capsid protein shell protecting the genome. Many copies of the capsid protein often self-assemble into shells of defined size. Low binding affinity of individual subunits allows efficient assembly and gives rise to highly stable particles. These capsids can be studied by native and hydrogen/deuterium exchange mass spectrometry (MS) in terms of stoichiometry, dynamics, assembly pathways and stability. The focus will be on isolate dependent capsid stability and size as well as glycan binding induced dynamics of noroviruses, the main cause of viral gastroenteritis.
Despite the remarkable sensitivity, the structural resolution is limited in native MS. Of special interest to biology is the structural transition upon nucleation of capsid assembly. However, such transient states cannot be purified and are inaccessible for crystallography. Hard X-ray free-electron-lasers (XFELs) offer an opportunity to obtain high resolution structures of single particles. How native MS benefits single particle imaging of transient intermediates at XFELs will be illustrated. Preliminary data and implications for other applications combining native MS and X-rays will be shown, especially how soft X-rays can aid native top-down experiments.

Oral Communications

Session I: Drug design

Chairpersons: V. Delfosse (CBS, Montpellier), L. Maveyraud (IPBS, Toulouse)

Marc Ruff, IGBMC, Illkirch
HIV-1 pre-integration complexes. Structures, functions and drug design

Elizabeth Ramos Morales, IGBMC, Illkirch
The challenge of inhibiting selectively histone deacetylases (HDAC) from eukaryotic pathogens

Gilles Labesse, CBS, Montpellier
Fragment linking yields the first NAD kinase inhibitor active against *Staphylococcus aureus* in vivo

S. Veyron, McGill Center for Structural Biology and Department of Pharmacology and Therapeutics, Montréal, Canada
Structure-based design of small-molecule activators of parkin

Table of Content

HIV-1 pre-integration complexes. Structures, functions and drug design

J. Batisse[a], E. Bruch[a], N. Levy[a], S. Eiler[a], S. Duclaud[a], P. Schultz[a], P. Gouet[b], S. Bouaziz[c], O. Delelis[d], V. Parissi[e] and M. Ruff[a]

[a] IGBMC, Department of Integrated Structural Biology, UMR7104 (CNRS, INSERM, UDS), 1 rue Laurent Fries, 67404, Illkirch, France, [b] MMSB-IBCP, UMR 5086 CNRS Université Lyon 1, 7 passage du Vercors, 69367 Lyon, France, [c] Université Paris Descartes, Sorbonne Paris Cité, Laboratoire de Cristallographie et RMN Biologiques, UMR 8015 CNRS, 4 av. de l'Observatoire, Paris, France. [d] LBPA, ENS Cachan, CNRS UMR8113, Université Paris-Saclay, F-94235 Cachan, France. [e] Fundamental Microbiology and Pathogenicity Laboratory, UMR 5234 CNRS-University of Bordeaux, 146 rue Léo Saignat, Bordeaux Cedex, France.
E-mail: ruff@igbmc.fr

After retroviral infection of a target cell, during the early phase of replication, the HIV-1 genomic RNA is reverse transcribed by the viral reverse transcriptase to generate the double-stranded viral DNA that interacts with viral and cellular proteins to form the pre-integration complex (PIC). Viral integrase (IN) is the key component of the PIC and is involved in several steps of replication notably in reverse transcription, nuclear import, chromatin targeting and integration. Viral components such as IN cannot perform these functions on their own and need to recruit host cell proteins to efficiently carry out the different processes. IN is a flexible protein with intrinsically disordered regions, property allowing its interaction with multiple partners and enabling its multiple functions in viral replication. To study the molecular mechanisms of viral integration we use a bottom – up strategy by assembling *in vitro* and/*or in cellulo* multiprotein complexes around the integrase protein (core protein of the PIC) and DNA. Several complexes have been characterized in our team (IN/LEDGF, IN/LEDGF/INI1-IBD, IN/LEDGF/CA, IN/LEDGF/TNPO3, IN/VBP1/TNPO3, IN/LEDGF/Nucleosome). To reveal the structure – function relationships of PIC complexes, we combine X-ray, NMR and Cryo-EM structures with biochemical and biological data. Two cryo-EM structures of the IN/LEDGF/DNA and IN/LEDGF/INI1-IBD/DNA complexes have been solved at low resolution [1, 2]. With the recent progress of the cryo-EM techniques and our improvement in the complex preparations [3, 4] new cryo-EM datasets are collected (IN/LEDGF/DNA-pal, IN/LEDGF/Nucleosome) which will enable us to increase the structure quality to near atomic resolution. In addition, the use of molecular structures together with in vitro assays allows us to develop a screening platform for interaction/allosteric inhibitors with the pre-integration complexes as targets.

[1] Michel, F., Crucifix, C., Granger, F., Eiler, S., Mouscadet, J.F., Korolev, S., Agapkina, J., Ziganshin, R., Gottikh, M., Nazabal, A., Emiliani, S., Benarous, R., Moras, D., Schultz, P. and Ruff, M., (2009). Structural basis for HIV-1 DNA integration in the human genome, role of the LEDGF/P75 cofactor. **EMBO J.** 28:980-991

[2] Maillot, B., Lévy, N., Eiler, S., Crucifix, C. , Granger, F., Richert, L., Didier, P., Godet, J., Pradeau-Aubreton, K., Emiliani, S., Nazabal, A., Lesbats, P., Parissi, V., Mely, Y., Moras, D., Schultz, P. and Ruff, M., (2013), Structural and functional role of INI1 and LEDGF in the HIV-1 preintegration complex, **PloS ONE** 8(4):e60734.

[3] Levy, N., Eiler, S., Pradeau-Aubreton, K., Maillot, B., Stricher, F., Ruff, M., (2016) Production of unstable proteins through the formation of stable core complexes. **Nature Communications**, 7 : 10932.

[4] Eiler, S., Levy, N., Maillot, B., Batisse, J., Pradeau Aubreton, K., Oladosu, O., Ruff, M., (2018). Unstable protein purification through the formation of stable complexes**. Methods in Molecular Biology** 1764:315-328.

Table of Content

The challenge of inhibiting selectively histone deacetylases (HDAC) from eukaryotic pathogens

E. Ramos Morales, M. Marek, C. Romier

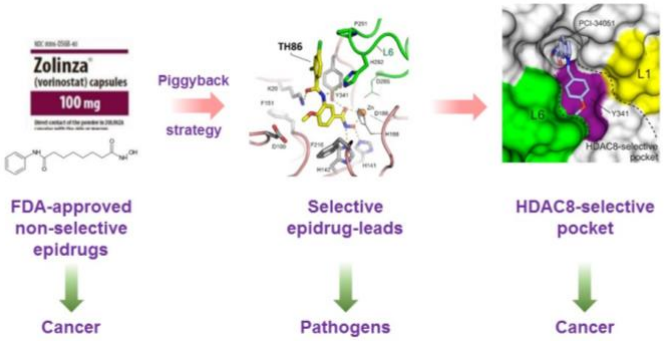
Institut de Génétique et Biologie Moléculaire et Cellulaire, IGBMC, Département de Biologie Structurale Intégrative, Université de Strasbourg, CNRS, UMR7104, INSERM, U1258, Illkirch, France.
E-mail: ramosmoe@igbmc.fr

Epigenetic mechanisms regulate many biological processes, their deregulation causing numerous diseases. Thus, small molecules inhibitors (epidrugs) are currently developed to target human epigenetic enzymes, four of them being already FDA-approved for the treatment of cancers. These epidrugs could also be used for the treatment of human infectious diseases since epigenetic enzymes play crucial roles in eukaryotic pathogens. This raises a selectivity issue where drugs should target the pathogen but not human enzymes.

To address this issue, we applied a "piggyback" strategy where FDA-approved anti-cancer Epidrugs scaffolds were modified to target selectively the pathogen epigenetic enzymes through a structure-based approach. This strategy was applied to target zinc-dependent histone deacetylases (HDAC) from the parasites *Schistosoma mansoni* and *Trypanosoma cruzi*, causing schistosomiasis and the Chagas disease, respectively.

The structure of *S. mansoni* HDAC8 revealed active site differences with its human counterpart that lead to the design of selective small molecules inhibitors targeting selectively the pathogen enzyme, some of these small molecules causing mortality of schistosomes in a dose dependent manner [1, 2]. This work further enabled us to characterize the mechanism of HDAC8-selective inhibition, with implications in anti-cancer therapies [3].

We currently apply the same strategy to target HDAC2 from *T. cruzi* (tcDAC2). By combining protein engineering with biophysical techniques, we could solve the crystal structure of tcDAC2 in complex with non-selective inhibitors. This structure reveals specific structural determinants that pave the way for the design of selective inhibitors.



[1] Marek, M. et al. (2013). Structural basis for the inhibition of histone deacetylase 8 (HDAC8), a key epigenetic player in the blood fluke Schistosoma mansoni. **PLOS Path**. 9:e1003645.
[2] Heimburg, T. et al. (2016). Structure-based design and synthesis of novel inhibitors targeting HDAC8 from Schistosoma mansoni for the treatment of schistosomiasis. **J. Med. Chem**. 59:2423-2435.
[3] Marek, M. et al. (2018). Characterization of histone deacetylase 8 (HDAC8) selective inhibition reveals specific active site structural and functional determinants. **J. Med. Chem**. 61:10000-10016.

Table of Content

Fragment linking yields the first NAD kinase inhibitor active against *Staphylococcus aureus* in vivo

M. Gelin[a], J. Paoletti[b], M.A. Nahori[c], V. Huteau[b], C. Leseigneur[d,e], G. Jouvion[f,g], L. Dugué[b], D. Clément[b], J.L. Pons[a], L. Assairi[h], S. Pochet[b], O. Dussurget[d,e] and G. Labesse[a]

[a] *Centre de Biochimie Structurale, CNRS UMR 5048, INSERM U1054, Université Montpellier, F-34090 Montpellier, France.*
[b] *Unité de Chimie et Biocatalyse, Institut Pasteur, CNRS UMR3523, F-75015 Paris, France.*
[c] *Unité des Toxines Bactériennes, Institut Pasteur, F-75015 Paris, France.*
[d] *Unité de Recherche Yersinia, Institut Pasteur, F-75015 Paris, France.*
[e] *Université de Paris, F-75013 Paris, France.*
[f] *Unité de Neuropathologie Expérimentale, Institut Pasteur, F-75015 Paris, France.*
[g] *Sorbonne Université, INSERM UMR S933, Unité de Génétique Médicale, Hôpital Armand Trousseau, APHP, F-75012 Paris, France.*
[h] *INSERM U759, Institut Curie, 91405 Orsay, France.*
E-mail: gilles.labesse@cbs.cnrs.fr

Antibiotic resistance is a worldwide threat in times of decline in the supply of new antimicrobials. Original targets and innovative strategies are urgently needed to generate pathbreaking druggable compounds. NAD kinase (NADK) is essential for growth in most bacteria as it supports critical metabolic pathways. Here, we present the discovery of a class of antibacterials that targets bacterial NADK.

We generated a series of small synthetic adenine derivatives to screen those harboring promising substituents in order to guide efficient fragment linking. This led to NKI1, a new lead compound inhibiting NADK showing in vitro bactericidal activity against *Staphylococcus aureus*. In a murine model of infection, NKI1 restricted survival of the bacteria, including methicillin-resistant *S. aureus*. Collectively, these findings identify NADK as a potential drug target and NKI1 as a lead compound in the treatment of staphylococcal infections.

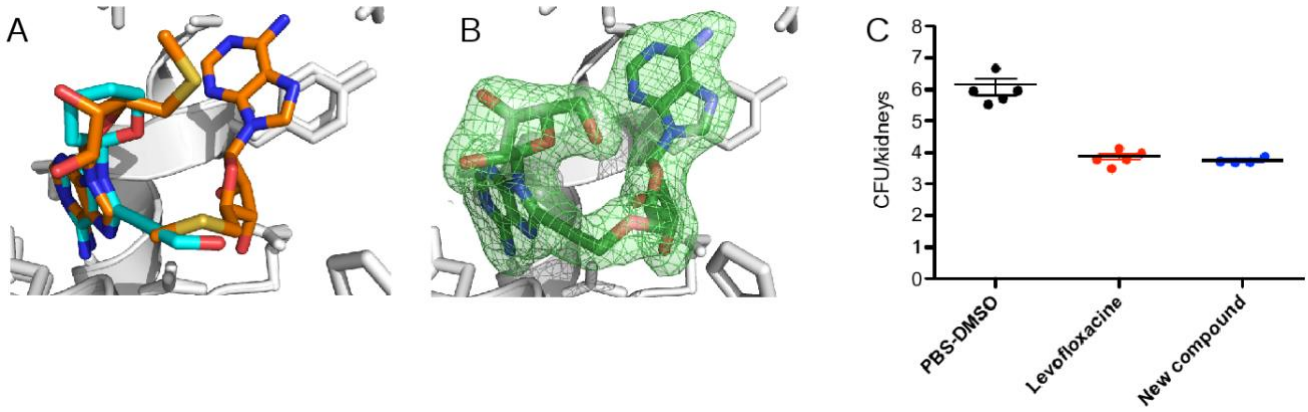


Fig. 1: Fragment linking strategy: (A) Superposed crystal structures of a bacterial NADK with two fragments MTA and 8-propargyl-adenine. (B) Crystal structures of our lead compound NKI1 bound to the active site. (C) In vivo activity of this new compound (and the reference compound levofloxacine) in a murine model of infection.

Table of Content

Structure-based design of small-molecule activators of parkin

S. Veyron [a], A. Bayne [a], M. Vargas [a], N. Croteau [a], D. Drewry [b], J-F. Trempe [a]

[a] *Parkinson's disease and PINK1, McGill Center for Structural Biology and Department of Pharmacology and Therapeutics, McGill University, Montréal, Québec, Canada*
[b] *Medicinal Chemistry, SGC-University of North Carolina, Chapel Hill, North-Carolina, USA*
E-mail: simon.veyron@mcgill.ca

Parkinson's disease (PD) is a common neurodegenerative disease characterized by motor symptoms that are largely caused by the loss of dopaminergic neurons in the substantia nigra. Most cases of PD occur sporadically in older people; however, approximately 5–10% of PD cases are caused by autosomal mutations that usually lead to symptoms in young adults [1]. Recessive mutations in the PARK2 (Parkin) and PINK1 genes are responsible for an early-onset form of PD. Parkin and PINK1 constitute a mitochondrial quality control system that controls autophagy (mitophagy) of depolarized and defective mitochondria. Parkin is a cytosolic E3 ubiquitin ligase basally autoinhibited but that becomes activated by phosphorylation by PINK1, a Ser-Thr protein kinase to induce mitophagy [2, 3]. Molecular mechanism of this activation has been recently described [4,5] and auto-inhibitory element have been identified. In particular, crystallographic studies revealed that tryptophan 403 (W403) is inhibiting Parkin activation binding a pocket and locking the protein [3] (Fig.1a). The importance of this residue in inhibition makes it a very good therapeutic target.
Based on structural data, we synthetized indole-containing compounds that would bind in the W403 binding pocket. We used *in silico*, biophysical and biochemical technics such as Thermal Shift Assay and Saturation-Transfer Difference NMR (STD-NMR) and crystallography to screen the library and test the best compounds (Fig.1b) to develop the first pharmacological activator of Parkin.

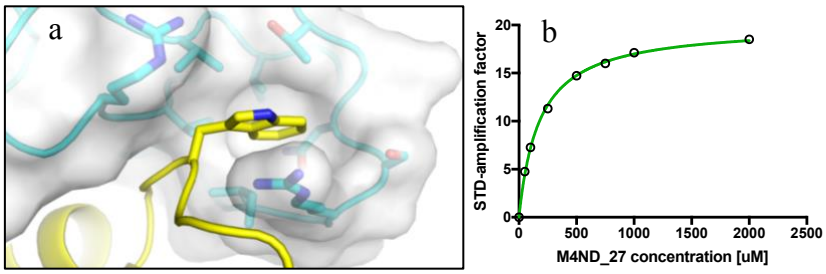


Fig. 1: a: Close-up of W403 (yellow) binding pocket in RING1 domain (cyan) (PDB: 4K7D). b: Compound M4ND_27 bind the protein using STD-NMR.

[1] Martin I, Dawson VL & Dawson TM. (2011). Recent advances in the genetics of Parkinson's disease. **Annu. Rev. Genom. Hum. Genet** 12:301–325.
[2] Bayne AN, Trempe JF. Mechanisms of PINK1, ubiquitin and Parkin interactions in mitochondrial quality control and beyond. **Cellular and Molecular Life Sciences** In revision.
[3] Trempe JF, Sauvé V, Grenier K, Seirafi M, Tang MY, Menade M, Al-Abdul-Wahid S, Krett J, Wong K, Kozlov G, Nagar B, Fon EA, Gehring K (2013). Structure of parkin reveals mechanisms for ubiquitin ligase activation. **Science** 340:1451–1455.
[4] Sauvé V, Sung G, Soya N, Kozlov G, Blaimschein N, Miotto L, Trempe JF, Lukacs GL, Gehring K (2018) Mechanism of parkin activation by phosphorylation. **Nat Struct Mol Biol** 25:623- 630.
[5] Gladkova C, Maslen SL, Skehel JM, Komander D (2018) Mechanism of parkin activation by PINK1. **Nature** 559:410-414.

Session II: DNA & RNA biology

Chairpersons: V. Gervais (IPBS, Toulouse), J.B. Charbonnier (I2BC, Gif-sur-Yvette)

Joanna Timmins, IBS, Grenoble
HU: A key player for nucleoid organization and compaction in *Deinococcus radiodurans*

Clément Charenton, MRC-LMB, Cambridge, UK
Mechanism of 5' splice site transfer for human spliceosome activation

Carine Tisné, IBPC, Paris
m1A modification in tRNAs

Pierre Damien Coureux, École Polytechnique, Palaiseau
Cryo-EM structure of an archaeal translation initiation complex at 3.1 Å resolution

Table of Content

HU: a key player for nucleoid organization and compaction in *Deinococcus radiodurans*

K. Floc'h[a], A-S. Banneville[a], F. Lacroix[a], P. Servant[b], L. Blanchard[c], A. Monari[d], F. Dehez[d], J-P. Kleman[a], J-L. Pellequer[a], D. Bourgeois[a] and J. Timmins[a]

[a] *Institut de Biologie Structurale, CEA-CNRS-Université Grenoble Alpes, Grenoble, France.*
[b] *Institut de Biologie Intégrative de la Cellule, CEA-CNRS-Université Paris-Sud, Orsay, France.*
[c] *Institut de Biosciences et Biotechnologies d'Aix-Marseille, CEA-CNRS-Université Aix-Marseille, Cadarache, France.*
[d] *Laboratoire de Physique et Chimie Théoriques, CNRS-Université Lorraine, Nancy, France.*
E-mail: joanna.timmins@ibs.fr

In all organisms, genomic DNA is compacted several orders of magnitude and yet must remain accessible for essential DNA-related processes including DNA replication, repair and transcription. Most of our knowledge of the organization and dynamics of nucleoids originates from studies of rod- or crescent-shaped bacteria. We have used *Deinococcus radiodurans*, a relatively large, spherical bacterium, well-known for its exceptional radioresistance, to study the structure and dynamics of the nucleoid. Using conventional and super-resolution microscopy approaches, we have revealed that its nucleoid is highly compact at all times, but also surprisingly dynamic, adopting six distinct configurations, including the previously described toroid, as it progresses through its cell cycle [1]. A major player in the organization of bacterial nucleoids is the highly abundant histone-like HU protein that largely coats the genomic DNA. Using a combination of biochemistry, biophysics, microscopy and molecular dynamics simulations, we have investigated HU's mode of DNA binding and its ability to condense the genomic DNA. Taken together, these findings demonstrate that bacterial nucleoids are highly organized and dynamic structures, which are tightly regulated by cell shape and cell cycle progression, and suggest that the HU protein plays a key role in this process.

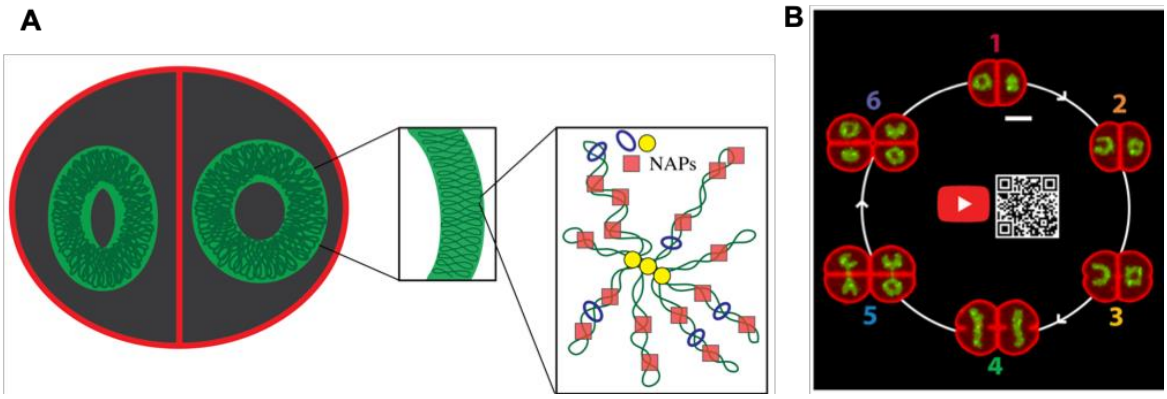


Fig. 1: (A) Schematic representation of a bacterial nucleoid (green). Nucleoid associated proteins (NAPs) and in particular the abundant HU protein, are largely responsible for the high degree of compaction of the nucleoid. (B) Schematic representation of the various shapes and structures adopted by the nucleoid of *D. radiodurans* as it progresses through its division cycle (Phase 1 to 6). Scale bar: 1µm. The QR code provides a link to a simulated movie of this dynamic process.

[1] K. Floc'h, co-authors. (2019). Cell morphology and nucleoid dynamics in dividing *D. radiodurans*. bioRXiv. DOI: 10.1101/582304

Mechanism of 5’ splice site transfer for human spliceosome activation

C. Charenton†, M. E. Wilkinson† & K. Nagai

MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH, United Kingdom.
†These authors contributed equally.
Email: ccharent@mrc-lmb.cam.ac.uk

Pre-mRNA splicing consists of removal of non-coding introns and ligation of coding exons by two transesterification reactions catalyzed by an immense ribonucleoprotein particle called the spliceosome. First, the pre-mRNA 5’ splice site (5’SS) is attacked by the conserved branch-point (BP) adenosine upstream of the 3’SS, yielding the cleaved 5’-exon and lariat-intron/3’-exon intermediate. Then, the freed 3’ hydroxyl of the 5’-exon attacks the 3’SS, ligating the exons and releasing the lariat intron. The spliceosome assembles on every intron in a stepwise manner from five snRNPs, each containing an snRNA (U1, U2, U4, U5 or U6) and then undergoes an extensive remodeling process to form its active site.

The prespliceosome, comprising U1 and U2 snRNPs bound to the pre-mRNA 5’ splice site (5’SS) and branch point sequence, associates with the U4/U6.U5 tri-snRNP to form the fully-assembled precatalytic pre-B spliceosome [1]. In this pre-catalytic spliceosome, U6 snRNA, which will ultimately fold to form the active site, is chaperoned in an inactive conformation through pairing with U4 snRNA, and the 5’SS is still paired with U1 snRNA [2]. Spliceosome activation is initiated by disruption of the 5’SS-U1 snRNP interaction by the DEAD-box helicase Prp28 to transfer the 5’SS to the invariant ACAGAGA box of U6 snRNA and the 5’ exon to U5 snRNA loop 1 [3]. We determined the cryo-EM structures of the human pre-B complex captured before U1 snRNP dissociation at 3.3 Å core resolution, and the human tri-snRNP at 2.9 Å resolution.

Our structures reveal the mechanism of 5’SS delivery to the spliceosome. U1 snRNP presents the 5’SS/U1 snRNA helix between the two RecA domains of the Prp28 DEAD-box helicase. ATP-dependent closure of the Prp28 RecA domains around U1 snRNA releases the 5’SS to pair with the nearby U6 ACAGAGA-box sequence presented as a mobile loop. These structures further suggest that formation of the 5’SS-ACAGAGA helix triggers remodeling of an intricate protein-RNA network to induce Brr2 helicase relocation to its loading sequence in U4 snRNA, enabling Brr2 to unwind the U4/U6 snRNA duplex to allow U6 snRNA to form the catalytic center of the spliceosome. Overall, our study shows how dramatic compositional and conformational changes in the spliceosome are coupled to crucial checkpoints in splice site recognition through the action of RNA helicases [4].

[1] Plaschka, Newman & Nagai. (2018) Structural basis of nuclear pre-mRNA splicing: lessons from yeast. Cold Spring Harb Perspect Biol

[2] Boesler et al. (2015) A spliceosome intermediate with loosely associated tri-snRNP accumulates in the absence of Prp28 ATPase activity. **Nat. Commun**,7:11997

[3] J. Staley & Guthrie. (1999) An RNA switch at the 5’ splice site requires ATP and the DEAD box protein Prp28p. **Mol. Cell**, 3:55-64

[4] Charenton, Wilkinson & Nagai. (2019) Mechanism of 5’ splice site transfer for human spliceosome activation. **Science**, 364:362-367

Table of Content

m1A modification in tRNAs

C. Tisné

Institut de Biologie Physico-Chimique, IBPC, Laboratoire d'Epression génétique microbienne, CNRS, Université de Paris,13 rue Pierre et Marie Curie, 75005 Paris, France.
E-mail: carine.tisne@ibpc.fr

1-methyladenosine (m1A) is a modified nucleoside resulting from the transfer of a methyl group on atom N1 of adenosine [1]. It can be found at positions 9, 14, 22 and 58 of tRNAs. Two different outlines of our works on m1A modifications of tRNAs will be presented:
1) The yqfN gene of Bacillus subtilis encodes the enzyme TrmK responsible for the formation of m1A22 in tRNA. TrmK orthologs are found in a number of Bacteria, including pathogenic species. We showed that TrmK displays a broad substrate specificity, methylating various tRNAs either with large or small variable loop. We also demonstrated that the presence of other modifications decreases the efficiency of TrmK-catalysed methyl transfer. We solved the crystal structure of B. subtilis TrmK and, using NMR chemical shift mapping, we showed that SAM cofactor binding to TrmK is a prerequisite for complex formation with the tRNA [2]. This mapping allowed us to drive a docking model of the TrmK/tRNA complex that is further validated by an extensive analysis of TrmK mutant activity. Our data support a model in which TrmK recognizes tRNA with no to very little deformation of both partners, the G13-A22 base-pair allowing placement of the A22 N1 atom in close contact to the SAM-methyl group in the active site of TrmK.
2) Using an original methodology, we showed that NMR is a powerful tool to monitor tRNA maturation events in a non-disruptive and continuous fashion. Time-resolved NMR measurements of different modification events in complex environments such as yeast cell extracts revealed a defined chronology in the incorporation of tRNA modifications and notably we showed that he m1A58 modification is the latest modification to be incorporated and is favored by the presence of other modifications in the T-arm. We believe that the NMR-based methodology presented here can be adapted to investigate many aspects of tRNA maturation and RNA modifications in general [3].

[1] P. Barraud & C. Tisné. (2019). To be or not to be modified: miscellaneous aspects influencing nucleotide modifications in tRNAs, **IUBMB life** 71: 1126-1140.
[2] C. Dégut[#], M. Roovers[#], P. Barraud, F. Brachet, A. Feller, V. Larue, A. Al Refaii, J. Caillet, L. Droogmans, C. Tisné. (2019). Structural characterisation of *B. subtilis* m^1A_{22} tRNA methyltransferase TrmK: Insights into tRNA recognition, **Nucleic Acids Res.** 47: 4736-4750.
[3] P. Barraud, A. Gato, M. Heiss, M. Catala, S. Kellner & C. Tisné. (2019). Time-resolved NMR monitoring of RNA maturation in cellular extracts revealed complex circuits of modifications in tRNAs, **Nature Communications** 10: 3373-3387.

Table of Content

Cryo-EM structure of an archaeal translation initiation complex at 3.1 Å resolution

P.-D. Coureux, A. Monestier, C. Lazennec-Schurdevin, R. Kazan, G. Bourgeois, Y. Mechulam & E. Schmitt

Laboratoire de Biochimie, École polytechnique, CNRS, 91128 Palaiseau cedex, France
E-mail: pierre-damien.coureux@polytechnique.edu

Universally, protein biosynthesis begins with accurate selection of the start codon on mRNA and consequently the definition of the open reading frame. In eukaryotes, this process involves many initiation factors and it is therefore the target of many regulations. The most frequent mechanism involves a pre-initiation complex (PIC) made up of the small ribosomal subunit bound to the ternary complex eIF2-GTP-Met-tRNAiMet (TC), the two small initiation factors eIF1 and eIF1A, as well as two proteins, eIF5 and eIF3, that have a more regulatory function in the process. In the presence of factors belonging to the eIF4 family, the PIC is recruited near the 5'-capped end and scans the mRNA until an AUG codon in a correct context is found. AUG recognition stops scanning, provokes factor release and the assembly of an elongation-proficient 80S complex through large subunit joining, with the help of eIF5B and eIF1A.

In archaea, there is no long-range scanning because mRNAs have Shine-Dalgarno sequences or very short 5' UTR. However, genomic analyses have shown that three initiation factors homologous to their eukaryotic counterparts, aIF1, aIF1A and aIF2 are found. Thus, even if obvious differences between eukaryotes and archaea exist, in particular for the recruitment of the PIC on the mRNA, start codon selection is achieved within a common structural core made up of the small ribosomal subunit, the mRNA, the methionine initiator tRNA (Met-tRNAiMet) and the three initiation factors e/aIF1, e/aIF1A and e/aIF2.

Previous cryo-electron microscopy structures of the full *Pyrococcus abyssi* PIC led us to propose a spring force model showing how aIF2 controls initiator tRNA pairing to the AUG codon [1]. Then, we used a combination of biochemical experiments and cryo-EM analyses to decipher the molecular mechanisms of start codon selection. In particular the key role of aIF1 was studied [2]. Here, we present the cryo-EM structure of a translation initiation complex obtained in the absence of aIF1 at 3.1 Å resolution. This structure reveals details of full initiator tRNA accommodation in the P site after aIF1 departure. In particular, 30S conformational adjustments and the role of universal ribosomal proteins will be discussed. Finally, we observe for the first time a large set of acetylcytidines along the rRNA sequence likely involved in ribosomal thermostability. This first high-resolution model of the archaeal small ribosomal subunit gives new insights in the molecular evolution of eukaryotic and archaeal translation initiation mechanisms.

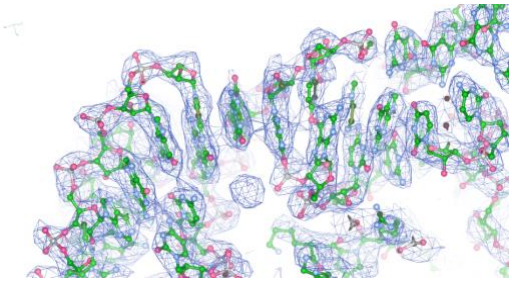


Fig. 1: Electron density map at 3.1 Å resolution of the archaeal translation initiation complex IC2.

[1] Coureux PD, Lazennec-Schurdevin C, Monestier A, Larquet E, Cladière L, Klaholz BP, Schmitt E and Mechulam Y. (2016). Cryo-EM study of start codon selection during archaeal translation initiation. **Nat Commun**, 7:13366.

[2] Monestier A, Lazennec-Schurdevin C, Coureux PD, Mechulam Y, Schmitt E. (2018). Role of aIF1 in Pyrococcus abyssi translation initiation. **Nucleic Acids Res**., 46:11061.

Session IIIa: Combining methods for the study of supramolecular assemblies

Chairpersons: A. Albertini (I2BC, Gif-sur-Yvette), W. Burmeister (IBS, Grenoble)

Stéphanie Petrella, Institut Pasteur, Paris
Overall structures of *Mycobacterium tuberculosis* DNA gyrase reveal the role of a Corynebacteriales GyrB specific insert in ATPase activity

Adrien Favier, IBS, Grenoble
Integrated NMR and cryo-EM atomic-resolution structure determination of a half-megadalton enzyme complex

Benjamin Bardiaux, Structural Bioinformatics Unit, Institut Pasteur, Paris
Integrative structural biology of bacterial type IV pili and pseudopili

Guillaume Tétreau, IBS, Grenoble
An integrative approach to unravel the in vivo crystallization pathway and mechanism of toxicity of Cyt1Aa – a naturally crystalline mosquitocidal toxin

Overall structures of *Mycobacterium tuberculosis* DNA gyrase reveal the role of a Corynebacteriales GyrB specific insert in ATPase activity

S. Petrella [a,b], E. Capton [c], B. Raynal [d], C. Giffard [a,b], A. Thureau [e], F. Bonneté [f], P. M. Alzari [a,b], A. Aubry [c], C. Mayer [a,b]

[a] *Unité de Microbiologie Structurale, Institut Pasteur, CNRS UMR 3528, 25 rue du Docteur Roux, 75724 Paris*
[b] *Université Paris Diderot, Sorbonne Paris Cité, 75724 Paris Cedex 15, France*
[c] *Sorbonne Université, Centre d'immunologie et des maladies infectieuses-Paris, Cimi-Paris, INSERM U1135, Centre National de Référence des Mycobactéries, Laboratoire de Bactériologie-Hygiène, AP-HP, Hôpitaux Universitaires Pitié-Salpêtrière – Charles Foix, F-75013 Paris, France*
[d] *Plateforme de biophysique moléculaire, Institut Pasteur, Centre d'innovation et de recherche technologique, 25 rue du Docteur Roux, 75724 Paris Cedex 15, France ;*
[e] *Synchrotron SOLEIL, l'Orme des Merisiers, F-91410 Saint Aubin, France*
[f] *Institut de Biologie Physico-Chimique (IBPC) – LBPCPM UMR7099, 13 rue Pierre et Marie Curie, 75005 Paris*
E-mail: stephanie.petrella@pasteur.fr

Despite sharing common features, previous studies have shown that gyrases from different species have been modified throughout evolution to modulate their properties. Here, we report two crystal structures of Mycobacterium tuberculosis DNA gyrase, an apo and AMPPNP-bound forms at 2.6-Å and 3.3-Å resolution, respectively. These structures provide high-resolution structural data on the quaternary organization and inter-domain connections of a gyrase (full-length GyrB-GyrA57)2 thus providing crucial inputs on this essential drug target. Together with SAXS studies, they revealed an "extremely open" N-gate state, which persists even in the DNA-free gyrase-AMPPNP complex and an unexpected connection between the ATPase and cleavage-core domains mediated by two Corynebacteriales-specific motifs, respectively the C- and DEEE-loops. We show that the C-loop participates to the stabilization of this open conformation explaining why this gyrase has a lower ATPase activity. Our results image a conformational state which might be targeted for drug discovery.

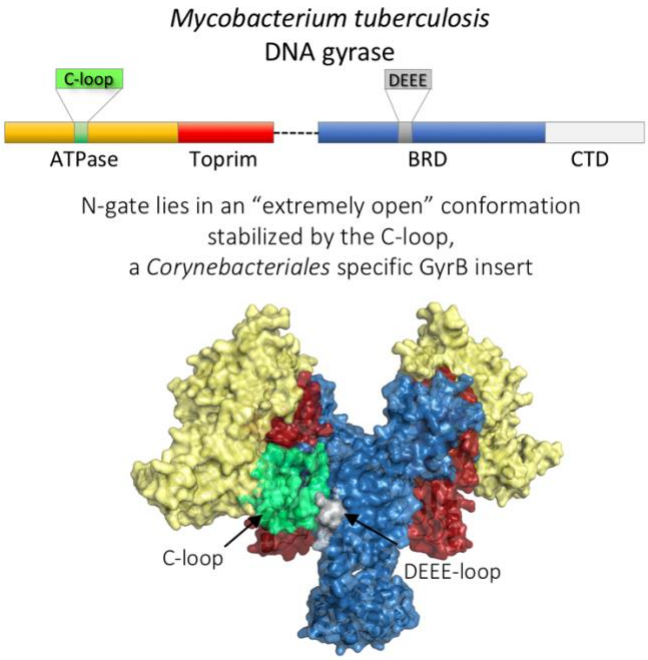


Table of Content

Integrated NMR and cryo-EM atomic-resolution structure determination of a half-megadalton enzyme complex

A. Favier [a], D. F. Gauto [a], L. F. Estrozi [a], C. D. Schwieters [b], G. Effantin [a], P. Macek [a,f], R. Sounier [a,g], A. C. Sivertsen [a], E. Schmidt [c], R. Kerfah [a,f], G. Mas [a,h], J.-P. Colletier [a], P. Guntert [c,d,e], G. Schoehn [a], J. Boisbouvier [a] & P. Schanda [a]

[a] *Univ. Grenoble Alpes, CEA, CNRS, Institut de Biologie Structurale (IBS), 71, avenue des martyrs, F-38044 Grenoble, France.*
[b] *Center for Information Technology, National Institutes of Health, Bethesda, USA.*
[c] *Institute of Biophysical Chemistry, Goethe University Frankfurt am Main, Germany.*
[d] *Laboratory of Physical Chemistry, ETH Z˙urich, Z˙urich, Switzerland.*
[e] *Graduate School of Science, Tokyo Metropolitan University, Tokyo, Japan.*
[f] *NMR-Bio, 5 place Robert Schumann, F-38025 Grenoble, France.*
[g] *Institut de Génomique Fonctionnelle, CNRS UMR-5203 INSERM U1191, University of Montpellier, F-34000 Montpellier, France*
[h] *Present address: Biozentrum University of Basel, Klingelbergstrasse 70, 4056 Basel, Switzerland*
E-mail: adrien.favier@ibs.fr

Atomic-resolution structure determination is the key requirement for understanding protein function. Cryo-EM and NMR spectroscopy both provide structural information, but currently cryo-EM does not routinely give access to atomic-level structural data, and, generally, NMR structure determination is restricted to small (<30 kDa) proteins. We introduce an integrated structure determination approach1 that simultaneously uses NMR and EM data to overcome the limits of each of these methods. The approach enabled determination of the high-resolution structure of the 468 kDa large dodecameric aminopeptidase TET2 to a precision and accuracy below 1 Angstrom by combining secondary-structure information obtained from near-complete magic-angle-spinning NMR assignments of the 39 kDa-large subunits, distance restraints from backbone amides and specifically labelled methyl groups, and a 4.1 Angstrom resolution EM map. The resulting structure exceeds current standards of NMR and EM structure determination in terms of molecular weight and precision. Importantly, the approach is successful even in cases where only medium-resolution (up to 8 Angstrom) cryo-EM data are available, thus paving avenues for the structure determination of challenging biological assemblies.

[1] Gauto D, Estrozi L, Schwieters C, Effantin G, Macek P, Sounier R, Sivertsen AC, Schmidt E, Kerfah R, Mas G, Colletier J-P, Guntert P, Favier A, Schoehn G, Boisbouvier J, Schanda P. (2019) Integrated NMR and cryo-EM atomic-resolution structure determination of a half-megadalton enzyme complex. **Nat. Com**. in press, DOI : 10.1038/s41467-019-10490-9

Table of Content

Integrative structural biology of bacterial type IV pili and pseudopili

B. Bardiaux [a,b], G.C. de Amorim [d,b], A. Lopez-Castilla [a,b], A. Luna-Rico [c,b], J.L. Thomassin [c,b], W. Zheng [e], F. Cordier [a,b], S. Brier, E.H. Egelman [e], M. Nilges [a,b], N. Izadi-Pruneyre [a,b], O. Francetic [c,b]

[a] *Structural Bioinformatics Unit, Institut Pasteur, 75015, Paris, France*
[b] *CNRS UMR 3528, 75015, Paris, France*
[c] *Biochemistry of Macromolecular Interactions Unit, Institut Pasteur, 75015, Paris, France*
[d] *NMR of Biomolecules Unit, Institut Pasteur, 75015, Paris, France*
[e] *Department of Biochemistry and Molecular Genetics, University of Virginia, Charlottesville, USA*
E-mail: bardiaux@pasteur.fr

To interact with their environment, bacteria have acquired a number of proteinaceous appendages that decorate their surface. These appendages, such as pili, secretion systems or flagella, are large macromolecular assemblies that participate in adhesion, auto-aggregation, DNA exchange, motility and virulence factor transport. Type IV pili (T4P) are present in numerous pathogenic bacteria and are crucial for host colonization and virulence of many Gram-negative bacteria. T4P assembly system is very closely related to type II protein secretion systems (T2SS) that are involved in the transport of folded proteins from the periplasm across the outer membrane. The common architecture of T4P and T2SS is based on the assembly of helical fibers in the plasma membrane with membrane protein subunits called pilins. However, their molecular complexity, their dynamics and membrane localization seriously hamper structural characterization of these fibers at atomic resolution with traditional methods. It has become clear that a combination of structural information from many sources is now the key to success. In this context, we will present our integrative strategy to determine the structure of a T4P from enterohemorrhagic E. coli and the first high-resolution structure of a T2SS pseudopili by combining NMR and cryo-Electron Microscopy. data with bioinformatics predictions [1,2]. This integrative modeling approach is completed and validated by site-directed mutagenesis and functional assays, allowing us to define inter-pilins contacts and the key role of calcium in the pseudopilus assembly and stability. Additionally, we will present a comparative analysis of dynamics and solvent accessibility of monomeric and assembled pilins using molecular modeling, NMR and Hydrogen/Deuterium exchange Mass Spectrometry [3].

[1] A. López-Castilla, J.L. Thomassin, B. Bardiaux, W. Zheng, M. Nivaskumar, X. Yu, M. Nilges, E.H. Egelman, N. Izadi-Pruneyre, O. Francetic O. (2017). Structure of the calcium-dependent type 2 secretion pseudopilus. **Nature Microbiology** 2:1686-1695.
[2] B. Bardiaux, G.C. de Amorim, A. Luna Rico, W. Zheng, I. Guilvout, C. Jollivet, M. Nilges, E.H. Egelman, N. Izadi-Pruneyre, O. Francetic (2019). Structure and assembly of the enterohemorrhagic Escherichia coli type 4 pilus. **Structure** 27:1082-1093
[3] B. Bardiaux, F. Cordier, S. Brier, A. López-Castilla, N. Izadi-Pruneyre, M. Nilges. (2019). Dynamics of a type 2 secretion system pseudopilus unraveled by complementary approaches.
Journal of Biomolecular NMR 73:293-303

An integrative approach to unravel the in vivo crystallization pathway and mechanism of toxicity of Cyt1Aa – a naturally crystalline mosquitocidal toxin

G. Tetreau [a], A.S. Banneville [a], A. Brewster [b], E. Andreeva [a], M.S. Hunter [c], I. Snigireva [d], J. Beaudoin [a], A. Burt [a], M. Bacia [a], R.G. Sierra [c], N. Zala [a], N. Burke [a], J.A. Bafna [e], F. Laporte [f], D. Fenel [a], H.W. Park[g,h], J.M. Teulon [a], E. Boeiri-Erba [a], J.A. Rodriguez [i], L. Després [f], M. Vivaudou [a], M. Weik [a], J.L. Pellequer [a], S. Boutet [c], D. Cascio [i], L. Signor [a], M. Winterhalter [e], I. Gutsche [a], N. Coquelle[a,j], N.K. Sauter [b], M.R. Sawaya [i], B. Federici [g], J.P. Colletier [a]

[a] Univ. Grenoble Alpes, CNRS, CEA, IBS, F-38000 Grenoble, France; [b] Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720; [c] Linac Coherent Light Source, SLAC National Accelerator Laboratory, Menlo Park, CA 94025; [d] European Synchrotron Radiation Facility (ESRF), BP 220, 38043 Grenoble, France; [e] Department of Life Sciences & Chemistry, Jacobs University, Bremen, Germany; [f] Univ. Grenoble Alpes, CNRS, LECA, F-38000, Grenoble, France; [g] Department of Entomology and Graduate Program in Cell, Molecular and Developmental Biology, University of California, Riverside, CA 92521; [h] Department of Biological Sciences, California Baptist University, Riverside, CA 92504; [i] UCLA-DOE Institute for Genomics and Proteomics, Department of Biological Chemistry, University of California, Los Angeles, CA 90095-1570; [j] Large-Scale Structures Group, Institut Laue-Langevin, F- 38000 Grenoble, France.

E-mail: guillaume.tetreau@ibs.fr

Mosquitoes are arguably the most detrimental organisms to human health, spreading devastating diseases such as Malaria, Dengue fever and Filiarasis. One of the safest approaches to control mosquito vector populations is the use of *Bacillus thuringiensis* subsp. *israelensis* (Bti), a bacterium that evolved to specifically target mosquito larvae. Bti produces a cocktail of protoxin proteins in the form of naturally occurring nanocrystals during its sporulation. Upon ingestion by mosquito larvae, the crystals dissolve in their gut, characterized by an alkaline pH and by the presence of proteases required for activation of the protoxins into fully functional toxins. One of these, coined Cyt1Aa, is considered a key element of Bti toxicity and the reason for which resistance to Bti was not observed in the field so far. Despite this central role, it remains unclear what structural elements drive the formation and dissolution of Cyt1Aa crystal, and how the toxin exerts its cytotoxicity. This knowledge gap largely originates from the absence of a structure of the Cyt1A protoxin as it naturally occurs in the cell. Indeed, the natural crystals are too small for the structure to be solved by synchrotron methods.

We used Serial Femtosecond Crystallography (SFX) at a X-ray free electron laser to solve the structure of the Cyt1Aa protoxin, from recombinant nanocrystals produced in vivo in an acrystalliferous strain of Bti [1,2]. The 1.8 Å resolution structure suggested residues key to crystal formation, dissolution and toxic action. Recombinant point mutants were expressed as crystals, and characterized by a combination of SFX, solubilization and toxicity assays (FACS), proteomics methods (electrophoresis and mass spectrometry), electrophysiology (black lipid membranes) and microscopy analyses (scanning, transmission, cryo-electron microscopy and atomic force microscopy). Our data shed light into the mechanism of cytotoxicity of Cyt1Aa, from its membrane insertion to its oligomerization leading to membrane perforation and disruption. They furthermore reveal which structural features in the toxin drive in vivo crystal formation and control pH-dependent solubilization, opening new avenues to rationally design mosquitocidal toxins with improved properties.

[1] Colletier JP, et al. (2016) De novo phasing with X-ray laser reveals mosquito larvicide BinAB structure. **Nature** 539:43-47.

[2] Sawaya MR, et al. (2014) Protein crystal structure obtained at 2.9 Å resolution from injecting bacterial cells into an X-ray free-electron laser beam. **Proceedings of the National Academy of Sciences** 111:2769-12774.

Session IIIb: Combining methods for the study of supramolecular assemblies

Chairpersons: I. Billas (IGBMC, Illkirch), A. Roussel (AFMB, Marseille)

Julie Ménétrey, I2BC, Gif-sur-Yvette
Structural characterization of the RH1-LZI tandem of JIP3/4 highlights RH1 domains as a cytoskeletal motor-binding motif

Maxime Bourguet, LSMBO, Strasbourg
Benefits of MS-based approaches for the structural characterisation of the specific association between the coactivator Med1 with the VDR-RXR heterodimer

Julien Henri, Laboratoire de Biologie Moléculaire et Cellulaire des Eucaryotes, Paris
Supramolecular organization of the Calvin-Benson cycle

Antoine Loquet, CBMN, Pessac
Solid-state NMR-based structural biology of functional amyloid fibrils

Structural characterization of the RH1-LZI tandem of JIP3/4 highlights RH1 domains as a cytoskeletal motor-binding motif

F. Vilela[a], C. Velours[a], M. Chenon[a], M. Aumont-Nicaise[a], V. Campanacci[a], A. Thureau[b], O. Pylypenko[c], J. Andreani[a], P. Llinas[a] & J. Ménétrey[a]

[a]Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris-Sud, Université Paris-Saclay, 91198, Gif-sur-Yvette cedex, France. [b] Synchrotron SOLEIL, l'Orme des Merisiers, F-91410 Saint Aubin, France. [c] Structural Motility Team, Institut Curie, PSL Research University, CNRS, UMR 144, Paris, France.
E-mail: julie.menetrey@i2bc.paris-saclay.fr

JIP3 and JIP4 (JNK-interacting proteins 3 and 4) are adaptors for cargo recruitment by two microtubule-based motors, the dynein/dynactin complex and the kinesin1. These adaptor molecules are dimers thanks to two distinct coil-coiled regions, namely Leucine Zippers I and II (LZI and LZII). The LZII is the binding site for dynactin-p150glued and the kinesin1-KLC, whereas the N-terminal part of JIP3/4 which encompasses the LZI, comprises binding sites for the dynein-DLIC and kinesin1-KHC. In contrast to the LZII region, no structural data is available for the N-terminal part of the JIP3/4 adaptors, although both are important microtubule-based motor interaction modules. Here, we characterize the N-terminal part of JIP3/4 using bioinformatical, biophysical and structural approaches. It adopts an elongated all alpha-helical fold, encompassing a RH1 (RILP homology 1) domain followed by the LZ1 region. A 3D model of the RH1-LZI tandem of JIP3 reveals that the binding site of kinesin1-KHC mainly overlaps with the RH1 domain. A sequence comparison search indicates that only RILP (Rab-interacting lysosomal protein) family members (RILP, RILPL1 and RILPL2) exhibit RH1 domains similar to those of JIP3/4. Interestingly, the RH1 domain of RILPL2 is the binding site for an actin-based motor, the myosin5a. Here, we show that JIP3 interacts with myosin5A in vitro and that its RH1 domain is part of the binding site. Overall, these reveal that JIP3/4 are critical “hubs” allowing the coordination of cargo transport between the microtubule and actin cytoskeletons. Further, our results highlight the role of the RH1 domain, as a versatile cytoskeletal motor-binding motif. Finally, we propose that JIP3/4 and RILP family proteins define a unique RH1/RH2-architecture adaptor superfamily which link multiple cytoskeletal motors to Rab GTPases.

Benefits of MS-based approaches for the structural characterisation of the specific association between the coactivator Med1 with the VDR-RXR heterodimer

M. Bourguet [a], A. Belorusova [b], S. Hessmann [a], N. Rochel [b] and S. Cianférani[a]

[a] *Laboratoire de Spectrométrie de Masse BioOrganique, LSMBO, IPHC, Université de Strasbourg, CNRS, Strasbourg, France.*

[b] *Centre de Biologie Structurale, CBS, Institue de Biologie Moléculaire et Cellulaire, IGBMC, Université de Strasbourg, CNRS, Illkirch, France*

E-mail: maxime.bourguet@etu.unistra.fr

Mediator complex subunit 1 (Med1), a core subunit of the Mediator complex, directly binds the AF2 domain of Vitamin D nuclear receptor (VDR) through its second LXXLL signature motif [1], anchoring the Mediator complex required for transactivation of VDR in keratinocytes [2]. To provide structural insights into the mechanism involved in the specific association of Med1 and the heterodimer VDR-RXR (Retinoic X Receptor), we combined structural mass spectrometry (MS) - based approaches, including hydrogen-deuterium exchange coupled to mass spectrometry (HDX-MS) and crosslinking mass spectrometry (XL-MS), to classical biophysical techniques.

In presence of VDR-RXR heterodimer, SEC-MALS and analytical ultracentrifugation data highlighted a binding stoichiometry of 1:1 between Med1 and VDR-RXR with a Kd of the interaction in the micromolar range. HDX-MS was next employed to gain insight into the binding mode of Med1 to VDR-RXR. VDR-RXR and Med1 were analysed either as individual partners and in complex by HDX-MS to highlight conformational changes induced upon Med1 binding. HDX results pointed out several regions of VDR protected upon Med1 binding, comprising the AF2 domain and spatially closed regions due to the interaction with the second NR motif of Med1. More interestingly, several protected regions of RXR were identified upon Med1 binding, encompassing spatially closed regions of the ligand binding domain of RXR. In addition, a deprotected region of RXR LBD corresponding to helix H10 was identified. On Med1, several protected regions were identified upon VDR-RXR binding, namely the second NR motif involved in the interaction with VDR. Finally, to provide spatial constrains to refine the SAXS model, we next performed XL-MS experiments using MS cleavable crosslinkers DSBU and C2-arm [3]. Interestingly, an inter-crosslinked peptide involving Thr 236 of Med1 and Lys 321 of RXR was identified, suggesting a spatial proximity between these regions, which confirmed HDX-MS result. Then, VDR-RXR and Med1 were analysed alone and in complex by SAXS. Thus, SAXS data combined to structure prediction and structural MS data allowed to highlight that both VDR and RXR create an interaction surface on top of the heterodimer to accommodate Med1 binding. These results suggest that other regions of VDR-RXR outside the AF2 VDR may be interacting with Med1 and notably RXR, highlighting MS structural potential in this type of integrative approach.

[1] Heery, D.M., et al. (1997). A signature motif in transcriptional co-activators mediates binding to nuclear receptors. **Nature**. 387:733-6.

[2] Oda, Y., et al. (2003). Two distinct coactivators, DRIP/mediator and SRC/p160, are differentially involved in vitamin D receptor transactivation during keratinocyte differentiation. **Mol Endocrinol** 17:2329-39.

[3] Muller, M.Q., et al. (2010). Cleavable cross-linker for protein structure analysis: reliable identification of cross-linking products by tandem MS. **Anal Chem** 82:6958-68.

Table of Content

Supramolecular organization of the Calvin-Benson cycle

T. Le Moigne[a], M. Zaffagnini[b], P. Crozet[a], D. Tedesco[b], C. Marchand[a], F. Sparla[b], S. Fermani[b], S. Lemaire[a] and J. Henri[a]

[a] *Laboratoire de Biologie Moléculaire et Cellulaire des Eucaryotes, UMR 8226 CNRS Sorbonne Université, Institut de Biologie Physico-Chimique 13 rue Pierre et Marie Curie 75005 Paris*

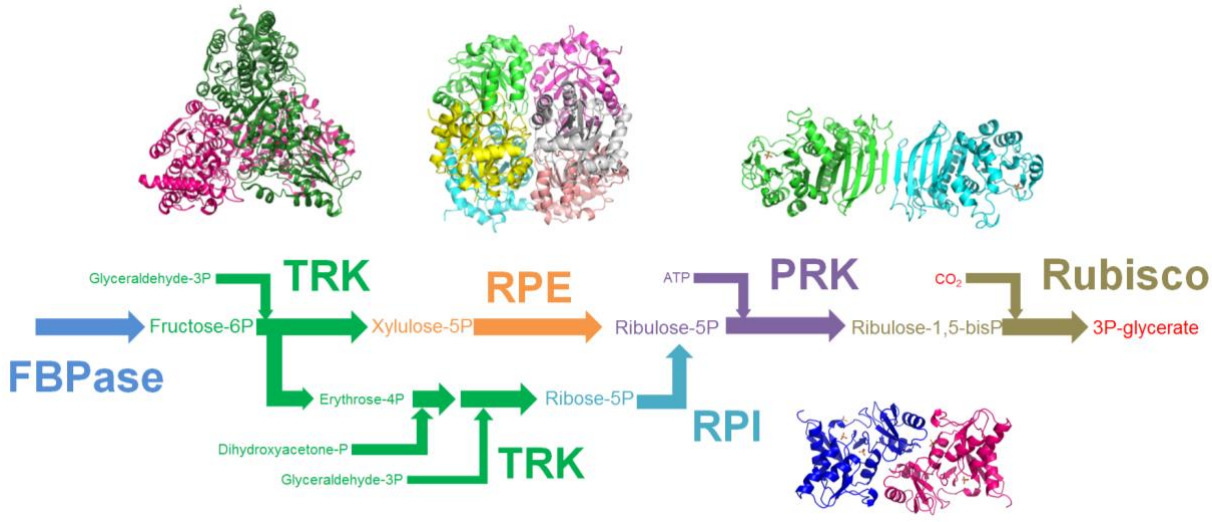
[b] *Université de Bologne, Dipartimento di Chimica Ciamician & Department of Pharmacy and Biotechnology, Bologne Italie*

Email: julien.henri@sorbonne-universite.fr

Photosynthesis is the main energy and carbon source for the biosphere. The Calvin-Benson cycle fixes atmospheric CO2 into organic trioses in the stroma of chloroplasts. While the eleven enzymes individually catalyzing the chemical steps of the cycle are known, little has been reported about their supramolecular organization. Hence, our current research ambitions to redefine the molecular basis for Calvin-Benson photosynthetic carbon fixation, in the light of the regulatory interactions that emerged between its protein components.

We determined the high-resolution crystal structures of four individual enzymes and three redox regulators. Crystal packing analysis confirmed by in vitro small angle X rays scattering revealed homo oligomeric interactions suggesting allosteric cooperativity. Mapping of phospho- and redox-post translational modifications sites supports regulation modes for general or photosynthetic-specific regulations. Surface comparison of the various thioredoxin redox regulators reveals a recognition code towards their enzyme targets. In vitro activity assays complete and validate these structural insights and serve as an integrated model of the final steps of the Calvin-Benson system in the unicellular alga *Chlamydomonas reinhardtii*.

These structure-function study lay the basis to test and determine the supramolecular organization of these enzymes from reconstituted or extracted complexes, as repeatedly hypothesized in the literature. The design of artificial interactions is planned to force the channeling of photosynthetic metabolites in vivo.



Solid-state NMR-based structural biology of functional amyloid fibrils

B. Habenstein, D. Martinez, JK Shenoy, N. El Mammeri, G. Lamon, M. Berbon, M Bertoni, A. Grélard, E. Morvan, A. Saad, A. Lends, A. Daskalov, A. Loquet

CBMN (UMR5248), Univ. Bordeaux - CNRS - IPB, Institut Européen de Chimie et Biologie, 2 rue Escarpit, Pessac, France.
E-mail: a.loquet@iecb.u-bordeaux.fr

Bad reputations are hard to shake off. This applies to individuals but also to certain protein structures. Amyloids have had a very bad one and in part they disserve it. This type of ordered protein aggregate is responsible for the development of more than 30 different devastating human protein-deposition diseases ranging from Alzheimer's disease to type 2 diabetes. But there is nothing intrinsically good or bad about this particular protein polymer architecture. It has now been clarified that alongside disease causing amyloids arising through misfolding, amyloids with functional roles exist in a variety of organisms. Here we will present the structural investigation of various functional amyloids related to cellular processes:

(i) the formation of bacterial biofilms in *Bacillus subtilis* and *Bacillus cereus*
(ii) the execution of programmed cell death reactions
(iii) the architecture of fungal hydrophobins

Using integrative approaches driven by solid-state NMR methods in combination with electron microscopy, FT-IR, mass-per-length measurements and X-ray fiber diffraction, we will show that the structure of functional amyloids emerges as a highly versatile fold that can fulfill a plethora of activities both inside and outside the cells.

Session IV: Molecular interactions and diseases

Chairpersons: S. Fieulaine (I2BC, Gif-sur-Yvette), L. Terradot (IBCP, Lyon)

Paloma Varela, I2BC, Gif-sur-Yvette
Macromolecules interactions in vitro, comparing classical and innovative approaches

Valérie Guillet, IPBS, Toulouse
Structural basis of the addiction of toxin-antitoxin systems to molecular chaperones

Florian Malard, ICSN, Gif-sur-Yvette
The molecular mechanism of the interaction between TCTP and the Bcl-2 proteins (Bcl-xL, Mcl-1) in the context of tumor reversion

Stefan Arold, StruBE/KAUST, Thuwal, Arabie Saoudite
HX repeat motifs and their role in a novel neurocognitive syndrome

Macromolecules interactions in vitro, comparing classical and innovative approaches

P. Fernández Varela, A. Gontier, C. Velours, M. Aumont-Niçaise,
M. Desmadril, P. Minard, J. B. Charbonnier

Institute for Integrative Biology of the Cell, CNRS, Gif sur Yvette, France.
Email: paloma.fernandez-varela@i2bc.paris-saclay.fr

To understand the molecular basis of macromolecules interactions we need to be aware of the advantages and limitations of the available methods. Here we will analyse the principles, strengths and limitations of different methods, classical as analytical ultracentrifugation with or without fluorescence (AUC-FDS), isothermal titration calorimetry (ITC) or size exclusion chromatography coupled to Multi-Angle Light Diffusion (SEC-MALS); and new approaches as bio-layer interferometry (BLI), Microscale Thermophoresis (MST) or switchSENSE (DRX2), for measuring in vitro interactions. We will focus in the study protein-protein and protein-DNA interactions that are studied in our laboratories. We will compare results obtained using six approaches in parallel on two projects (Fig.1). Firstly, a protein-protein interaction between an artificial binder, an alpha-Rep with its target [1]. Secondly, a double-strand break repair factors, Ku70/Ku80 with its DNA substrates [2].

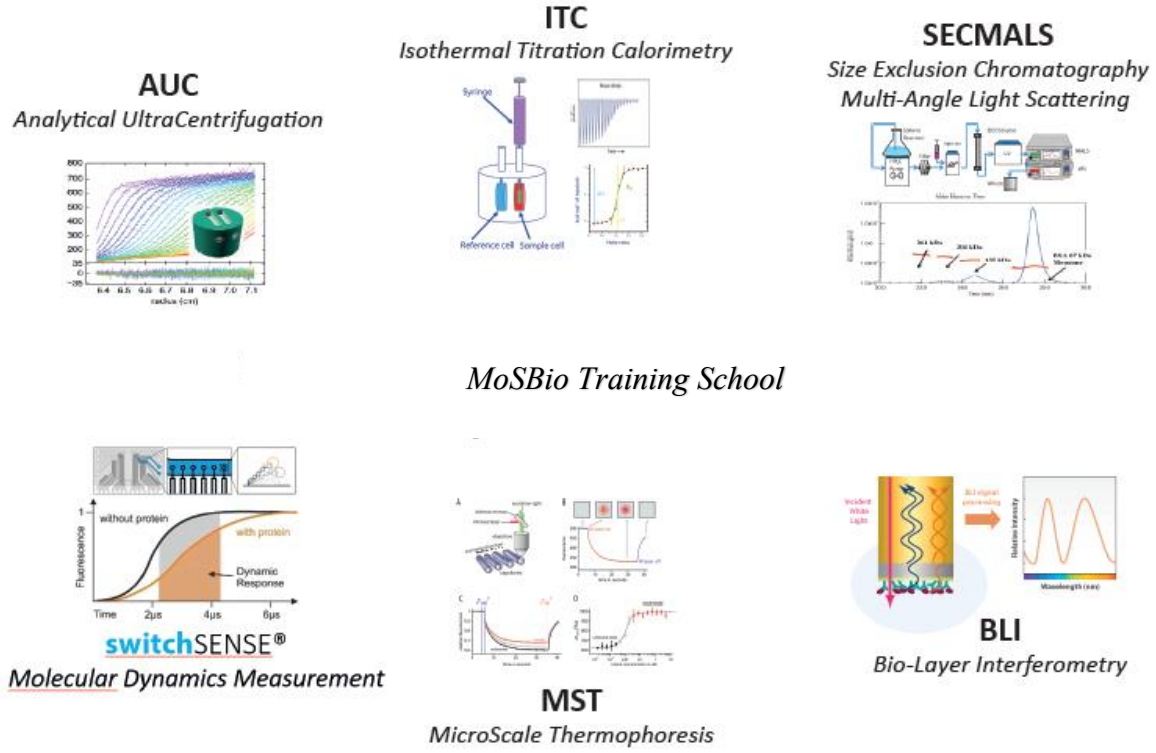


Fig. 1. Classical to innovative approaches to study macromolecules interactions

[1] Guellouz A, Valerio-Lepiniec M, Urvoas A, Chevrel A, Graille M, Fourati-Kammoun Z, Desmadril M, van Tilbeurgh, Minard P. (2013) Selection of Specific Protein Binders for Pre-Defined Targets from an Optimized Library of Artificial Helicoidal Repeat Proteins (alphaRep). **PLoS One**. 8:e71512
[2] Nemoz C, Ropars V, Frit P, Gontier A, Drevet P, Yu J, Guerois R, Pitois A, Comte A, Delteil C, Barboule N, Legrand P, Baconnais S, Yin Y, Tadi S, Barbet-Massin E, Berger I, Le Cam E, Modesti M, Rothenberg E, Calsou P, Charbonnier JB. (2018). XLF and APLF bind Ku80 at two remote sites to ensure DNA repair by non-homologous end joining. **Nat Struct Mol Biol.** 25:971-980

Structural basis of the addiction of toxin-antitoxin systems to molecular chaperones

V. Guillet [a], P. Bordes b, Cécile Bon [a], J. Marcoux [a], V. Gervais [a], A. J. Sala [b], S. Dos Reis [a], N. Slama [b], I. Mares-Mejía [a], A.-M. Cirinesi [b], L. Maveyraud [a], P. Genevaux [b] & L. Mourey [a]

[a] *Institut de Pharmacologie et de Biologie Structurale, IPBS, Université de Toulouse, CNRS, UPS, 31077, Toulouse, France.*
[b] *Laboratoire de Microbiologie et Génétique Moléculaires (LMGM), Centre de Biologie Intégrative (CBI), Université de Toulouse, CNRS, UPS, 31062 Toulouse, France.*
E-mail: valerie.guillet@ipbs.fr

Toxin-antitoxin systems are genetic elements that can control bacterial growth in response to certain stresses. They are usually composed of a toxin and an antitoxin that inhibits the deleterious effect of the toxin. Under certain stress conditions, the less stable antitoxin is degraded and the toxin released can control bacterial growth by targeting, for example, replication or de novo synthesis of proteins. The involvement of some of these systems in bacterial persistence and in the virulence of certain pathogens makes them pertinent therapeutic targets.
Mycobacterium tuberculosis, the bacterium responsible for tuberculosis, causes close to two million deaths worldwide each year and still remains a major public health problem. *M. tuberculosis* possesses a very high number of toxin-antitoxin systems (more than 80) with unknown functions and it has been proposed that these systems could be involved in establishing the persistence phase in this bacterium. Among these toxin-antitoxin systems, the TAC (Toxin-Antitoxin-Chaperone) system is atypical in that it is controlled by a third obligatory partner: a molecular chaperone that assists folding and prevents the degradation of the antitoxin. Remarkably, the dependence of this toxin-antitoxin system on the chaperone is determined by a small region of the antitoxin, called ChAD (for chaperone addiction), which specifically interacts with the chaperone and induces aggregation of the antitoxin in the absence of the latter [1].
Using TAC of *M. tuberculosis* as model system, we solved the crystal structure of the chaperone in complex with the ChAD sequence of the antitoxin. This structure and the exhaustive biochemical and biophysical characterization of the complex help revealing the molecular bases of the phenomenon of addiction of toxin-antitoxin systems to a chaperone [2]. In addition, we showed that the interaction between the chaperone and the antitoxin can be destabilized in vivo and in vitro, inducing controlled activation of the toxin.

[1] P Bordes, AJ Sala, S Ayala, P Texier, N Slama, AM Cirinesi, V Guillet, L Mourey, P Genevaux. (2016). Chaperone addiction of toxin-antitoxin systems. **Nat Commun** 7:13339
[2] V Guillet, P Bordes, C Bon, J Marcoux, V Gervais, AJ Sala, S Dos Reis, N Slama, I Mares-Mejía, AM Cirinesi, L Maveyraud, P Genevaux & L Mourey. (2019). Structural insights into chaperone addiction of toxin-antitoxin systems. **Nat Commun** 10:782

The molecular mechanism of the interaction between TCTP and the Bcl-2 proteins (Bcl-xL, Mcl-1) in the context of tumor reversion

F. Malard [a], C. Sizun [a], E. Jacquet [a], A. Thureau [b], L. Carlier [c] and E. Lescop [a]

[a] *Institut de Chimie des Substances Naturelles, CNRS UPR 2301, Université Paris-Sud, Université Paris-Saclay, 1 avenue de la Terrasse, 91190 Gif-sur-Yvette, FRANCE*
[b] *Synchrotron SOLEIL, l'Orme des Merisiers, F-91410 Saint Aubin, France*
[c] *UPMC Université Paris 06, Laboratoire des BioMolécules UMR 7203, 4 place Jussieu, 75005 Paris, France*
E-mail: florian.malard@cnrs.fr

The role of TCTP in cancer is mainly described in the context of the tumoral reversion program [1]. Cells that undergo such program spontaneously lose their malignant phenotype, leading to the recovery of characteristics associated to benign cells, such as susceptibility to apoptosis. Apoptosis is mediated by the interplay between pro/anti-apoptotic Bcl-2 family proteins and the BH3-only proteins. TCTP is a small (20~kDa) globular protein [2] which exposes a disordered loop [3] and which contains, importantly, a BH3-like motif embedded in the globular part of the protein, making it not readily accessible for interaction. However, it was reported that TCTP directly interacts with the anti-apoptotic proteins Bcl-xL and Mcl-1, reinforcing their pro-survival properties and therefore reducing apoptosis [4,5]. Thus, we investigated the molecular mechanism by which TCTP associates with Mcl-1 and Bcl-xL by using a panel of biophysical methods (NMR, SAXS, CD, SEC, DSF...). So far, we have demonstrated that full length TCTP binds to Bcl-xL and Mcl-1 in their BH3-binding groove. TCTP anchors both proteins by using its BH3-like motif and undergoes a major structural and dynamic change upon complex formation. Importantly, we have shown that only a minor, pre-existing form of TCTP, namely TCTP*, is competent for interactions with the Bcl-2 protein partners. Indeed, TCTP exists in equilibrium between a major state and this minor state, TCTP*, in which both dynamics and tertiary structure are severely affected. Finally, we have demonstrated that the BH3-like motif is unstructured in TCTP*, making it readily accessible for Bcl-2 protein partners. In consequence, current efforts in drug-design targeting TCTP should take into account TCTP* in aim to inhibit TCTP/Bcl-2 proteins interactions. Additionally, this work improves our understanding of BH3-only/Bcl-2 proteins interactions and brings original insights in the molecular basis of the tumoral reversion program.

[1] Telerman, A. & Amson, R. (2009). The molecular programme of tumour reversion: the steps beyond malignant transformation. **Nature Review Cancer** 9:206–216
[2] N. Assrir, F. Malard, and E. Lescop (2017). Structural Insights into TCTP and Its Interactions with Ligands and Proteins, **Cell Death and Differenciation** 64:9-46
[3] F. Malard, N. Assrir, M. Alami, S. Messaoudi, E. Lescop, and T. Ha-Duong. (2018). Conformational Ensemble and Biological Role of the TCTP Intrinsically Disordered Region: Influence of Calcium and Phosphorylation. **Journal of Molecular Biology** 430:1621–1639
[4] Thébault, S. et al. (2016). TCTP contains a BH3-like domain, which instead of inhibiting, activates Bcl-xL. Scientific Reports 6:19725
[5] Liu, H., Peng, H.-W., Cheng, Y.-S., Yuan, H. S. & Yang-Yen, H.-F. (2005). Stabilization and Enhancement of the Antiapoptotic Activity of Mcl-1 by TCTP. **Molecular and Cellular Biology** 25:3117–3126

HX repeat motifs and their role in a novel neurocognitive syndrom

E.E. Palmer [a], S. Hong [b], F. AlZahrani [c], M.O. Hashem, F.A. Aleisa [b], H.M. Jalal Ahmed [b], T. Kandula, R., Merzaban [b], Ł. Jaremko [b], M. Jaremko [b], R.K. Sachdev [a], F.S. Alkuraya [c], S.T. Arold [b]

[a] Sydney Children's Hospital & Randwick, School of Women's and Children's Health, NSW 2031, Australia;
[b] Department of Genetics, King Faisal Specialist Hospital and Research Center, Riyadh 11211, Saudi Arabia;
[c] King Abdullah University of Science and Technology, Division of Biological and Environmental Sciences and Engineering, Thuwal 23955-6900, Saudi Arabia.
E-mail: stefan.arold@kaust.edu.sa

Through an international and integrative effort between clinicians, cellular and structural biologists, we have discovered a novel genetic disease presented by several children across the world. As common origin for this condition, which we term CHEDDA (congenital hypotonia, epilepsy, developmental delay, digit abnormalities), we have identified various mutations that target an unusual 'histidine-X' (HX) repeat motif, present in several human proteins [1]. Combining structural, computational and cellular analyses we observe that this HX repeat produces unusual structural and functional features, that are disrupted by patient mutations [1,2]. Our collective effort allowed to link atomic features with patient phenotypes, and helps informing therapeutic intervention.

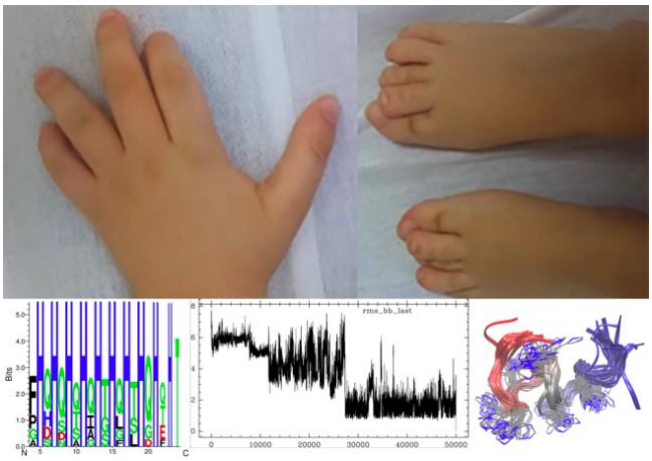


Fig. 1: Top: Hands and feet of a CHEDDA patient showing characteristic features. Bottom (left to right): Sequence features of the HX motif; representative molecular dynamics simulation; superhelical HX structure.

[1] E.E. Palmer, S. Hong, F. AlZahrani, M.O. Hashem, F.A. Aleisa, H.M. Jalal Ahmed, T. Kandula, R. Macintosh, A.E. Minoche, C. Puttick, V. Gayevskiy, A.P. Drew, M.J. Cowley, M. Dinger, J.A. Rosenfeld, R. Xiao, M.T. Cho, S.F. Yakubu, L.B. Henderson, M.J. Guillen Sacoto, A. Begtrup, M. Hamad, M. Shinawi, M.V. Andrews, M.C. Jones, K. Lindstrom, R.E. Bristol, S. Kayani, M. Snyder, M. Mercedes Villanueval, A. Schteinschnaider, L. Faivre, C. Thauvin, A. Vitobello, T. Roscioli, E. P. Kirk, A. Bye, J. Merzaban, Ł. Jaremko, M. Jaremko, R.K. Sachdev, F.S. Alkuraya, S.T. Arold (2019) De novo variants disrupting the HX repeat motif of ATN1 cause a non-progressive neurocognitive disorder with recognisable facial features and congenital malformations. **Am J. Hum. Gen**. 104:778
[2] A.A. Momin, S. Hong, R. Grünberg, F.A. Aleisa, H.M. Jalal Ahmed, Merzaban, Ł. Jaremko, M. Jaremko, R.K. Sachdev, E.E. Palmer, F.S. Alkuraya, S.T. Arold, unpublished

Session V: Technological/methodological innovations

Chairpersons: N. Colloc'h (ISTCT, Caen), E. Ennifar (IBMC, Strasbourg)

Dominique Bourgeois, IBS, Grenoble
Mechanistic investigation of a fluorescent protein reveals a strategy to reduce track interruptions in single particle tracking PALM

Igor Chaussavoine, SOLEIL, Gif-sur-Yvette
Microfluidic positioning of biological macromolecular crystals for serial X-ray diffraction on the PROXIMA-1 beamline

Dominique Housset, IBS, Grenoble
Structural studies of proteins and peptide by cryo-electron diffraction of 3D nano-crystals

Léonard Chavas, SOLEIL, Gif-sur-Yvette
New access modes for integrated biology at synchrotron SOLEIL

Mechanistic investigation of a fluorescent protein reveals a strategy to reduce track interruptions in single particle tracking PALM

E. De Zitter[a], D. Thédié[b], V. Mönkemöller[a], S. Hugelier[a], J. Beaudouin[b], V. Adam[b], M. Byrdin[b], L. Van Meervelt[a], P. Dedecker[a], D. Bourgeois[b]

[a]*Department of Chemistry, KU Leuven, Heverlee, Belgium;*
[b]*Univ. Grenoble Alpes, CEA, CNRS, IBS, Grenoble, France*
E-mail: dominique.bourgeois@ibs.fr

Fluorescent proteins (FPs) are widely used as genetically-encoded markers in practically all fluorescence microscopy schemes. Notably, a large toolbox of so-called phototransformable fluorescent proteins (PTFPs) have been engineered in recent years, of use in many advanced modalities such as super-resolution or single-molecule imaging. PTFPs display a variety of photoactivation, photoconversion, photoswitching, photoblinking and photobleaching properties that can be used at advantage in many cases, but that are also at the origin of major complications and artifacts, making these fascinating "smart" labels still far from ideal and quite poorly understood mechanistically. In this work [1] we studied a green-to-red photoconvertible fluorescent protein called mEos4b, which is intensively used in the super-resolution field but is known to repeatedly enter dark states. These dark states cause, amongst other problems, interrupted tracks in single-particle-tracking localization microscopy (sptPALM), significantly compromising the huge potential of this technique for measuring complex diffusion patterns of proteins in live cells. Using a combination of kinetic crystallography under spectroscopic control and single-molecule measurements, we identified a long-lived dark state in photoconverted mEos4b and managed to trap it in cristallo to solve its structure. We found that the dark state results from a light-induced "frustrated" cis-trans isomerization of the chromophore. In the trans configuration, the dark chromophore was observed to efficiently absorb light at 488 nm. This finding suggested that addition of weak 488-nm light could swiftly reverts the dark state to the fluorescent state. This prediction was experimentally verified in the context of sptPALM, opening the door to a simple strategy to largely eliminate slow blinking and enable the recording of significantly longer tracks with this technique.

[1] E. de Zitter, D. Thédié, V. Mönkemöller, S. Hugelier, J. Beaudouin, V. Adam, M. Byrdin, L. Van Meervelt, P. Dedecker & D. Bourgeois "Mechanistic investigation of mEos4b suggests a strategy to reduce track interruptions in sptPALM", (2019) **Nature Meth**., In press, BioRxiv, DOI: 10.1101/475939.

Microfluidic positioning of biological macromolecular crystals for serial X-ray diffraction on the PROXIMA-1 beamline

I. Chaussavoine, V. Ramakrishna, P. Montaville, L.M.G. Chavas

Synchrotron SOLEIL, L'Orme des Merisier, Saint-Aubin, 91192 Gif-sur-Yvette, France
E-mail : igor.chaussavoine@synchrotron-soleil.fr

One of the major evolutions in recent macromolecular crystallography experiments lies in the implementation of serial crystallography, originally developed for hard x-ray Free-Electron Lasers (FELs), and later adapted to synchrotron sources. These new approaches allow to further expand the spectrum and possibilities in crystallography. The techniques validated to date bring successful results, yet suffer from the requirement of extensive amounts of sample crystals and recorded images to start structure analysis; potentially, this also requires long beamtime shifts at X-ray FELs. At various synchrotron sources including SOLEIL, serial crystallography was adapted by using microfluidic chips [1] to handle batches of macromolecular crystals and expose those to the synchrotron beam. These new developments allow acquiring diffraction data over several degrees per crystal, permitting to drop consequently the number of crystals required for a complete data set. In addition of facilitating a better control during serial crystallography experiments, microfluidic chips provide with the possibility of mastering the crystal environment, the injection of ligands, or even opens access to diffraction experiments on biologically hazardous molecules. Moreover, the trapping method employed within the microfluidic chips facilitates bypassing most of the chemical issues currently occurring when handling and preparing macromolecular crystals for diffraction experiments.
The presentation will highlight the results of microfluidic-based macromolecular serial crystallography at Synchrotron SOLEIL. Using specific patterns inspired from an experiment by Lyubimov [2], macromolecular crystals or macromolecular crystal containing cells are automatically placed at known and precise positions within a microfluidic chip. The chip can then be handled and oriented using a 3D-printed frame placed on the goniometer at PROXIMA-1 beamline. The chip is then identified and the crystals localised within, before being exposed to the X-ray beam for automatic serial data collection by an in-house procedure. Our initial results illustrated here were obtained using in house devices; complete data could and had already been acquired with high redundancy yet a limited and small amount of crystals, prior to solving the structure of a reference protein sample.

[1]. Igor Chaussavoine. Realisation of microfluidic chips for macromolecular X-ray crystallography. M2 report Paris Saclay, 2017
[2]. Artem Y. Lyubimov. Capture and x-ray diffraction studies of protein microcrystals in a microfluidic trap array. **Biological crystallography**, 2015. DOI: 10.1107/S1399004715002308

Structural studies of proteins and peptide by cryo-electron diffraction of 3D nano-crystals

D. Housset[a], M. Bacia[b], J. Lopes[c], J.P. Colletier[c], M. Clabbers[d], T. Blum[d], J.P. Abrahams[d], G. Schoehn[a], W. L. Ling[a]

[a,b,c]Institut de Biologie Structurale, Groupes [a]MEM, [b]MICA & [c]DYNAMOP, UGA, CEA, CNRS, F-38000 Grenoble, France [d]Center for Cellular Imaging and NanoAnalytic, Biozentrum, University of Basel, 4058 Basel, Switzerland
E-mail: dominique.housset@ibs.fr

Electron diffraction of 3D nanometer sized (3D-ED) crystals has recently emerged as a new technique to solve the structure of both small organic molecules and a few model proteins (1, 2). Indeed, electrons constitute an ideal probe for structural studies of sub-micrometer samples, as they interact much more strongly with matter than X-rays (~ 105 times more) and as the energy deposited on the sample per diffracting particle is about 1000 times less (3). A few tens of crystals are sufficient to obtain a complete data set at atomic or quasi-atomic resolution, while more than 100 thousand crystals would be required for X-ray serial crystallography studies. Moreover, electrons can “sense” the atomic charge and the charge of chemical groups or metal ions can be estimated from electron diffraction data (4). Together, these factors make 3D-ED a very attractive and promising technique in structural biology, both easy to implement and very complementary to X-ray crystallography and single particle cryo-EM.
We have collected 3D-ED data from 3 different proteins at resolution ranging from 2.7 to 3.25 Å and from one tetrapeptide at 1.05 Å resolution. The data were processed with XDS and the refinement performed with Refmac. Charge analysis of metal ions bound to the protein (Zn2+ in insulin crystals and Zn2+ and Ca2+ in thermolysin crystals) revealed a partial ionic charge that differs from the formal +2 charge. Multiple diffraction does significantly impact measured intensities in 3D-ED and a simple method to take it into account at the refinement steps has been investigated. Radiation damage has been studied on the tetrapeptide crystals and a comparison between X ray and electron damage will be presented. Last, based on these data, we will present the present limitations of the 3D-ED technique and the required developments to make it a routine tool in structural biology.

New access modes for integrated biology at synchrotron SOLEIL

L. Chavas for the HelioBio scientific section at Synchrotron SOLEIL

Synchrotron SOLEIL, Gif sur Yvette, France.
E-mail: leonard.chavas@synchrotron-soleil.fr

Motivated by the need to support rapid changes in innovative science as closely as possible, Synchrotron SOLEIL is planning to enter a phase for a machine upgrade, which should improve the quality of the synchrotron beam, and hence open a window for new technological and methodological developments using the advantages brought by these new radiation sources. The timing appears then suitable to boost the beamline instrumentation and their associated facilities, laboratories, and infrastructure. With the recent explosion of interest in cryo-electron microscopy and integrated methods, the contribution from synchrotron facilities to the biological community needs to be adapted to a quickly evolving field. The biology and health scientific section, also known as HelioBio, at Synchrotron SOLEIL regroups a large set of instruments with specific equipment dedicated to biological applications. To illustrate the foreseen novelties in synchrotron access modes proposed by the HelioBio community, the seminar/poster will present the prevalent technologies favored by the Synchrotron SOLEIL biology users, complemented by the state-of-the-art methodologies envisioned by the expert communities.

Two main aspects will be introduced. First, the HelioBio is willing to provide on-site emerging/pioneering methods that have a major impact in modern biology and will complement existing approaches at Synchrotron SOLEIL, such as the implementation of cryo-electron microscopy/tomography and soft X-ray tomography. These additional methods should be implemented together with the upgrade of the current beamlines to stay at the state-of-the-art of the performed experiments, and improve compatibilities with other beamlines and instruments (sample environment, data format …). Second, the organization around biological experiments will need rethinking, where the HelioBio community proposes new ways of user access that should allow for on site multimodal data acquisition required for efficient correlative structural/functional biology approaches. A non-exhaustive list of items to include here would regroup multi-beamline access, wet lab hostels to optimize sample preparation before data acquisition, multimodal oriented data processing, and software developments for integrated data analysis and extrapolation. These new access modes to the synchrotron will be pushed forward, facilitating a more flexible, reliable and reactive scheduling of experiments on biological samples.

Members of HelioBio are dedicated to pushing these changes forward, and thus promote better experiments targeted towards the biological user community and more specifically towards projects in need of fully integrated approaches.

Session VI: Multi-scale dynamics in biology

Chairpersons: C. Van Heijenoort (ICSN, Gif-sur-Yvette), G. Lippens (TBI, Toulouse)

Elena Andreeva, IBS, Grenoble
Towards a better understanding of the Orange Carotenoid Protein (OCP) photoactivation mechanism

Annalisa Pierro, BIP, Marseille
Probing NarJ structural dynamics inside *E. coli* cells by EPR spectroscopy

Emmanuel Margeat, CBS, Montpellier
Structural dynamics of single metabotropic glutamate

Kyprianos Hadjidemetriou, IBS, Grenoble
Time-resolved serial femtosecond crystallography

Towards a better understanding of the Orange Carotenoid Protein (OCP) photoactivation mechanism

E. Andreeva[a], A. Wilson[b], G. Schirò[a], S. Jan Nazinski[c], M. Grunbein[d], F. Muzzopappa[b], M. Kloos[d], M. Levantino[e], M. Cammarata[f], N. Coquelle[a], G. Tetreau[a], N. Zala[a], C. Roome[d], M. Hilpert[d], S. Bar-Zv[i], A. Gorel[d], M. Weik[a], K. Hadjidemetriou[a], T. Barends[d], L. Foucar[d], G. Burdzinski[c], R. Shoeman[d], B. Doak[d], M. Sliwa[g], I. Schlichting[d], D. Kirilovsky[b] & J.-P. Colletier[a]

[a]*Institut de Biology Structurale (IBS), Grenoble, France.*
[b]*Institut de Biologie Intégrative de la Cellule (I2BC), CEA Saclay, France.*
[c]*Quantum Electronics Laboratory, Adam Mickiewicz University, Poznan, Poland.*
[d]*Max-Planck-Institut for Medical Research, Heidelberg, Germany.*
[e]*European Synchrotron Radiation Facility (ESRF), Grenoble, France.*
[f]*Department of Physics, University of Rennes, Rennes, France.*
[g]*Univ. Lille, CNRS, UMR 8516 - LASIR - Laboratoire de Spectrochimie Infrarouge et Raman*
Email: eandreeva@ibs.fr

To protect themselves from detrimental light energy excess, cyanobacteria synthesize a small dimeric photoactive Orange Carotenoid Protein (OCP) capable of quenching and dissipating into heat the excess of energy arriving to the cyanobacteria light-harvesting centers. Upon photon absorption, this pigment-encasing two domain protein, undergoes a transition from a compact orange “non-active” state (OCP°) to an extended red “photoactive” state (OCPr) associated with a migration of the carotenoid pigment from the interface between the two domains into the effector N-terminal domain (NTD) of the protein. However, it remains elusive how the energy absorbed by the carotenoid is funneled into the protein scaffold and how the protein structure varies during photoactivation. Using time-resolved (TR) X-ray scattering, we studied the µs-ms photo-triggered structural dynamics of dimeric OCP in solution. We found that upon single-pulse illumination, the transition from the OCP° to the OCPr state occurs before 10 µs, yet under such illumination conditions only a single monomer in the OCP dimer undergoes photoactivation, which falls back into the OCPo state on the hundreds of ms time-scale. Contrastingly, static measurements enable accumulation of the OCPr species, revealing that the latter is a monomer. Thus, the regulation of OCP photocycle involves change in oligomerization. We also used Serial Synchrotron Crystallography (SSX) to shed light on the first intermediate leading to the OCPr photoactive state. This was made possible by a two-day illumination of crystals at 480 nm and revealed that red state exists, wherein the carotenoid is still present at the interface between the two domains, but the carotenoid tunnel in the NTD is already opened. Altogether, our results illustrate how initially localized changes on the ps timescale may trigger global protein structural dynamics of the µs-s timescale and how the structure of this protein has evolved to enable photoactivation only in case of very strong irradiance, despite the use of one of nature’s most potent chromophore.

[1] Coquelle N., Sliwa M., Woodhouse J., Schirò G., …, Colletier J-P, Schlichting I, Weik M. (2018) Chromophore twisting in the excited state of a photoswitchable fluorescent protein captured by time-resolved serial femtosecond crystallography. **Nature chemistry**, 10:31.
[2] Kirilovsky, D. & Kerfeld, C. A., (2016). Cyanobacterial photoprotection by the orange carotenoid protein. **Nat. Plants** 2:16180.
[3] Wilson A., Punginelli C., Gall A., Bonetti C., Alexandre M., Routaboul J.M., Kerfeld C. A…. and Kirilovsky D. (2008). A photoactive carotenoid protein acting as light intensity sensor. **PNAS**, 105:12075-12080

Probing NarJ structural dynamics inside *E. coli* cells by EPR spectroscopy

A. Pierro[a], A. Bonucci[b], G. Karthikeyan[c], G. Casano[c], A. Walburger[d], A. Magalon[d], B. Guigliarelli[a], O. Ouari[c], V. Belle[a], E. Mileo[a]

[a]Aix Marseille Univ, CNRS, BIP, Marseille, France, [b]Magnetic Resonance Center – CERM, University of Florence, Sesto Fiorentino, Italy, [c]Aix Marseille Univ, CNRS, ICR, Marseille, France, [d]Aix Marseille Univ, CNRS, LCB, Marseille, France

E-mail: apierro@imm.cnrs.fr

Understanding how the intracellular medium modulates protein structural dynamics and protein-protein interactions is an intriguing topic that scientists are trying to address by studying biomolecules in their native environment. As the cellular environment is seldom reproducible in vitro, investigation of biomolecules directly inside cells has attracted a growing interest in the past decade.

A major goal in the biostructural field is the development of methodologies that merge advantages of high sensitivity, limitless biomolecule size and the ability to perform in-cell studies of structural transitions and interactions.

Site-Directed Spin Labeling (SDSL) coupled to Electron Paramagnetic Resonance (EPR) spectroscopy is one of the tools available which exhibits competitive and advantageous features to capture protein dynamics inside cells. Recently, we have successfully developed a new nitroxide-based spin label M-TETPO that resists bio-reduction in the cellular context and exhibits efficient EPR properties as a spin label [1].

In this talk, we will discuss the results achieved by the improved properties of M-TETPO in the investigation of the structural dynamics of NarJ, a flexible chaperone protein, by SDSL-EPR directly inside its endogenous host cells (*E. coli*).

Specifically, data obtained by monitoring NarJ structural dynamics in physiologically relevant environment will be discussed in light of data obtained in vitro. We will also show the results obtained by exploring the effects of synthetic crowding agents on NarJ protein dynamics.

[1] G. Karthikeyan, A. Bonucci, G. Casano, G. Gerbaud, S. Abel, V. Thomé, L. Kodjabachian, A. Magalon, B. Guigliarelli, V. Belle, O. Ouari, E. Mileo (2018) A Bioresistant Nitroxide Spin Label for In-Cell EPR Spectroscopy: In Vitro and In Oocytes Protein Structural Dynamics Studies. **Angew. Chem. Int. Ed.** 57:1366-1370.

Structural dynamics of single metabotropic glutamate receptors

R. Quast [a], A.M. Cao [a], F. Fatemi [a], P. Rondard [b], J.P. Pin[b], E. Margeat[a]

[a] *Centre de Biochimie Structurale, CBS, CNRS, INSERM, Université de Montpellier, France.*
[b] *Institut de Génomique Fonctionelle, IGF, CNRS, INSERM, Université de Montpellier, France.*
E-mail: margeat@cbs.cnrs.fr

Metabotropic glutamate receptors (mGluRs) are multidomain proteins belonging to class C G-protein coupled receptors (GPCR). There are essential in controlling synaptic transmission, and as such are important drug targets for the treatment of several disorders including pain, Parkinson's disease, schizophrenia etc...
We recently introduced the use of single molecule FRET combined with Multi-parameter Fluorescence Detection (MFD) to investigate the conformational transitions associated with mGluR activation. We demonstrated that the isolated ligand binding (VFT) domain oscillates between a resting and an active state in a time range of 50-100 µs. Here, we extend these investigations to the full-length receptor solubilized in detergent. Our result confirms the general mechanism observed for the VFT domain, decipher the role of the transmembrane domain, that slightly slows down the receptor dynamics, while stabilizing its active state solely in the presence of a full agonist, and explain the activity of partial agonist from a structural point of view.

Time-resolved serial femtosecond crystallography on photosensitive proteins

K. Hadjidemetriou[a], V. Adam[a], E. Andreeva[a], P. Arnoux[b], A-S. Banneville[a], T. Barends[b], F. Beisson[c], S. Blagny[c], D. Bourgeois[a], S. Boutet[d], M. Byrdin[a], M. Cammarata[e], S. Carbajo[d], J-P. Colletier[a], N. Coquelle[f], B. Doak[b], F. Fieschi[a], L. Foucar[b], A. Gorel[b], M. Grünbein[b], M. Hilpert[b], M. Hunter[b], S. Jakobs[g], J. Koglin[d], G. Nass-Kovacs[b], C. Roomeb, A. Royant[a], C. Ruckebusch[h], G. Schirò[a], I. Schlichting[b], M. Seaberg[d], R. Shoeman[b], R. Sierra[d], M. Sliwa[h], D. Sorigué[c], M. Stricker[b], G. Tetreau[a], M. Thepaut[a], L. Uriarte[h], J. Woodhouse[a], N. Zala[a], M. Weik[a]

[a]*Institut de Biologie Structurale, Grenoble, France.*
[b]*Max-Planck-Institut for Medical Research, Heidelberg, Germany.*
[c]*Biosciences and Biotechnologies Institute of Aix-Marseille, Saint-Paul-lez-Durance, France.*
[d]*Linac Coherent Light Source, Menlo Park, USA.*
[e]*Department of Physics, University of Rennes, France.*
[f]*Institut Laue Langevin, Grenoble, France.*
[g]*Max Planck Institute for Biophysical Chemistry, Göttingen, Germany.*
[h]*Laboratoire de Spectrochimie Infrarouge et Raman, Lille, France.*
E-mail: kyprianos.hadjidemetriou@ibs.fr

The recent advent of X-ray free electron lasers (XFELs) now allow studying structural dynamics of crystalline proteins down to the sub-picosecond time scale by time-resolved serial femtosecond crystallography (TR-SFX) at room temperature [1]. We use TR-SFX to study light-induced dynamics in photosensitive proteins, in particular in reversibly photo-switchable fluorescent proteins (RSFPs) and in the photoenzyme fatty acid photodecarboxylase (FAP).

RSFPs are essential tools in advanced in vivo fluorescence nanoscopy (such as RESOLFT) due to their characteristic light-induced and reversible isomerization between a fluorescent (on) and a non-fluorescent (off) state upon light irradiation at two different wavelengths. Our recent work evidenced a twisted chromophore isomerization intermediate in the electronically excited state 1ps after photoexcitation [2]. This initial work has now been extended to rationally designed mutant of RSFPs (unpublished).

FAP is one of the three enzymes discovered so far whose catalytic activity requires a continuous flux of light [3]. FAP is involved in the lipid metabolism in microalgae by catalyzing the decarboxylation of free fatty acids to alkanes or alkenes. We solved a radiation damage free structure of the resting state of FAP at XFELs and carried out a TR-SFX study on the picosecond to microsecond timescale (unpublished).

[1] Colletier J-P, Schirò G, Weik M. (2018). Time-Resolved Serial Femtosecond Crystallography, Towards Molecular Movies of Biomolecules in Action in X-ray Free Electron Lasers: A Revolution in Structural Biology. Fromme P, Boutet S, Hunter M Eds. Springer International Publishing, 11:331-356

[2] Coquelle N, Sliwa M, Woodhouse J, Schirò G, Adam V, Aquila, A., ..., Colletier J-P, Schlichting I, Weik M. (2018). Chromophore twisting in the excited state of a photoswitchable fluorescent protein captured by time-resolved serial femtosecond crystallography. **Nature chemistry**, 10:31.

[3] Sorigué D, Légeret B, Cuiné S, Blangy S, …, Beisson F. (2017). An algal photoenzyme converts fatty acids to hydrocarbons. **Science** 357:903-907.

Session VII: Cellular regulatory mechanisms

Chairpersons: P. Marchot (AFMB, Marseille), M. Delarue (Institut Pasteur, Paris)

Bouchra Attia, LISM, Marseille
Structural characterization of the GtlJ protein and its interactions with the cytoplasmic platform during the adventurous motility of *Myxococcus xanthus*

Laurence Blanchard, BIAM/CEA, Saint Paul-lez-Durance
Novel structure of a XRE family transcriptional regulator: insight into the metalloprotease/repressor-controlled radiation response in *Deinococcus*

Yves Bourne, AFMB, Marseille
Structural insights into substrate binding and catalytic mechanism of heparan sulfate D-glucuronyl C5 epimerase

Julien Marcoux, IPBS, Toulouse
Hydrogen-deuterium eXchange coupled to Mass Spectrometry highlights a reciprocal crosstalk between the inner and outer rings of the 20S proteasome

Structural characterization of the GtlJ protein and its interactions with the cytoplasmic platform during the adventurous motility of *Myxococcus xanthus*

B. Attia[a], L. My[b], O. Bornet[c], B. Serrano[a], F. Guerlesquin[a], T. Mignot[b] and L. Elantak[a]

[a] *Laboratoire d'Ingénierie des Systèmes Macromoléculaires, CNRS-Aix Marseille University UMR7255, Marseille, France.*
[b] *Laboratoire de Chimie Bactérienne, CNRS-Aix Marseille University UMR 7283, Marseille, France.*
[c] *Institut de Microbiologie de la Méditerranée, CNRS FR3479, Marseille, France.*
Email: battia@imm.cnrs.fr

The motility of bacterial cells promotes a range of physiological phenomena such as nutrient detection, predation, biofilm formation and pathogenesis. *Myxococcus xanthus* uses the so-called adventurous motility (A-motility) to explore new places, change direction and leave the cell swarms. All the studies available revealed that this motility is controlled by cytoplasmic proteins [1-3], MglA and AglZ, coupled to the bacterial cytoskeleton MreB and a molecular motor Agl, as well as a multiprotein complex (Glt) crossing the entire bacterial membrane [4]. This assembly forms focal adhesion complexes (FACs), which propel the cell in a certain direction beforehand defined by the cytoplasmic protein, MglA. Within the Glt multiprotein complex, GltJ is a protein who plays a critical role in FACs assembly and regulation. GltJ is located in the inner membrane with a cytoplasmic N-terminus composed of a "Zinc Ribbon" (ZnR) domain and a "Glycine-Tyrosine-Phenylalanine " (GYF) domain.
In order to understand at the molecular and structural level how GltJ domains regulate the assembly of the FACs, NMR spectroscopy, ITC and genetic experiments as well as phenotypic assays were performed. We solved the NMR solution structures of the ZnR and GYF domains and revealed their specific structural features. We also identified a key interaction of the GYF domain with one of the cytoplasmic proteins and characterized this interaction using NMR and ITC. Also, *in vivo* experiments confirmed and showed that the regulation of the A-motility process is dependent of this interaction. Further fluorescence microscopy analysis are in progress to visualize the fate of these FACs in genetic variants. Our results not only represent the first high resolution structural data concerning the Glt complex but also highlight a direct contact with a cytoplasmic effector of the A-motility machinery. Altogether, we propose that the ZnR and GYF domains of GltJ represent a new type of regulators that are required for the assembly and disassembly of FACs during A-motility of *M. xanthus*.

[1] S. Leonardy, *et al.* (2010) Regulation of dynamic polarity switching in bacteria by a Ras-like G-protein and its cognate GAP. **EMBO J***.* 29:2276–2289.
[2] R. Yang, *et al.* (2004) AglZ Is a Filament-Forming Coiled-Coil Protein Required for Adventurous Gliding Motility of Myxococcus xanthus. **J. Bacteriol***.* 186:6168–6178.
[3] A. Treuner-Lange, *et al.* (2015) The small G-protein MglA connects to the MreB actin cytoskeleton at bacterial focal adhesions. **J. Cell Biol***.* 210:243–256.
[4] S. Islam, T. Mignot. (2015) The mysterious nature of bacterial surface (gliding) motility: A focal adhesion-based mechanism in Myxococcus xanthus. **Semin. Cell Dev. Biol***.* 46:143–154.

Novel structure of a XRE family transcriptional regulator: insight into the metalloprotease/repressor-controlled radiation response in *Deinococcus*

A. de Groot[a], M.I. Siponen[a], R. Magerand[a], R. Martin-Arevalillo[b], J. Doloy[a], D. Lemaire[c], G. Brandelet[a], R. Dumas[b], P. Arnoux[a], D. Pignol[a] and L. Blanchard[a]

[a] *Aix Marseille Univ, CEA, CNRS, BIAM, Molecular and Environmental Microbiology Team, Saint Paul-Lez-Durance, 13108, France*
[b] *Univ. Grenoble Alpes, CNRS, CEA, INRA, IRIG-DBSCI-LPCV, Grenoble, 38000, France*
[c] *Aix Marseille Univ, CEA, CNRS, BIAM, Interaction Protein Metal Team, St Paul-Lez-Durance, France*
E-mail: laurence.blanchard@cea.fr

Exposure to harmful conditions such as radiation and desiccation induce oxidative stress and DNA damage [1]. In radiation-resistant *Deinococcus* bacteria, the radiation/desiccation response is controlled by two proteins, the COG2856 metalloprotease IrrE that cleaves and inactivates the XRE family transcriptional repressor DdrO [2, 3]. Here, we report the biochemical characterization and crystal structure of DdrO, which is the first structure of a XRE protein targeted by a COG2856 protein. DdrO is composed of two domains that fold independently and are separated by a flexible linker. The N-terminal domain corresponds to the DNA binding domain. The C-terminal domain, containing three alpha helices arranged in a novel fold, is required for DdrO dimerization. Cleavage by IrrE occurs in the loop between the last two helices of DdrO and abolishes dimerization and DNA binding. The cleavage site is hidden in the DdrO dimer structure, indicating that IrrE cleaves DdrO monomers or that the interaction with IrrE induces a structural change rendering accessible the cleavage site. Predicted COG2856/XRE regulatory protein pairs are widespread, we confirm here their interaction for two such pairs from other bacteria. Taken together, the data indicate that COG2856/XRE protein pairs have co-evolved into at least two different regulatory mechanisms in which the COG2856 proteins inhibits the function of the XRE repressor, either involving proteolytic cleavage or disruption of oligomerization through competitive binding without proteolytic cleavage. The results open the way to further characterize the stress-induced responses mediated by COG2856/XRE proteins in diverse bacteria including pathogens.

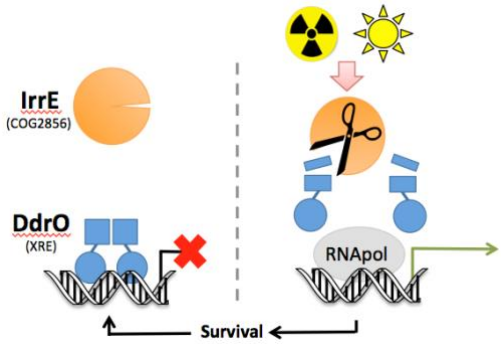


Fig. 1: ***Model for the radiation /desiccation response (RDR) mechanism in* Deinococcus**.
Induction of the RDR regulon after cleavage of a repressor (DdrO) by a separate and site-specific metalloprotease (IrrE) (PDB ID 3DTI).

[1] Lim S, Jung JH, Blanchard L, de Groot A. (2019). Conservation and diversity of radiation and oxidative stress resistance mechanisms in *Deinococcus* species**. FEMS Microbiol Rev**. 43:19-52.
[2] Ludanyi M, Blanchard L, Dulermo R, Brandelet G, Bellanger L, Pignol D, Lemaire D, de Groot A. (2014). Radiation response in *Deinococcus deserti*: IrrE is a metalloprotease that cleaves repressor protein DdrO. **Mol Microbiol**. 94:434-49.
[3] Blanchard L, Guérin P, Roche D, Cruveiller S, Pignol D, Vallenet D, Armengaud J, de Groot A. (2017). Conservation and diversity of the IrrE/DdrO-controlled radiation response in radiation-resistant *Deinococcus* bacteria. **MicrobiologyOpen** 6:e477.

Structural insights into substrate binding and catalytic mechanism of heparan sulfate D-glucuronyl C5 epimerase

C. Debarnot[a], Y. R Monneau[b], V. Roig-Zamboni[a], V. Delauzun[a], E. Richard[c], C. Le Narvor[d], A. Goulet[a], F. Fadel[a], R. R. Vivès[b], B. Priem[c], D. Bonnaffé[d], H. Lortat-Jacob[b], Y. Bourne[a]

[a] *CNRS, Aix Marseille Univ, AFMB, Marseille, France*.
[b] *Univ. Grenoble Alpes, CNRS, CEA, IBS, Grenoble, France*
[c] Univ. Grenoble Alpes, CNRS, CERMAV, Grenoble, France
[d] *ICMMO, LabEx LERMIT, Univ Paris Sud, CNRS, Université Paris-Saclay, Orsay, France*
E-mail: yves.bourne@afmb.univ-mrs.fr

Heparan sulfate (HS) belongs to the family of glycosaminoglycans anchored to core proteins and regulates numerous cell signaling pathways. The large structural variability of HS, orchestrated by sequential enzymatic reactions at the polymer level, greatly affects HS binding activities. These HS biosynthetic enzymes could associate to form different and specific functional "molecular machines" committed to the assembly of specific HS motifs. Glucuronyl C5-epimerase (Glce) is one of these modification enzymes involved in heparan sulfate (HS) biosynthesis where it catalyses epimerisation of D-glucuronic acid (GlcA) into L-iduronic acid (IdoA) at the polymer level. The activity of Glce is key to determine the level and distribution of IdoA residues along the polymer leading to differential sulfation patterns and control the activity of HS proteoglycans. We used a combination of glycoengineering, glycochemistry, real-time NMR spectroscopy and x-ray crystallography to decipher the mode of substrate binding and catalytic mechanism of Glce [1]. The structural characteristics of Glce unveil selective conformational distortions of the HS precursor chain to permit C5-epimerization and provide molecular frameworks for the chemoenzymatic synthesis of heparin or HS analogs.

Work supported by grants from the CNRS-Aix-Marseille University, the French Infrastructure for Integrated Structural Biology (FRISBI), the GDR GAG, the LabEx GRAL, the “Investissements d’avenir” program Glyco@Alps (ANR-15-IDEX-02) and the LabEx LERMIT.

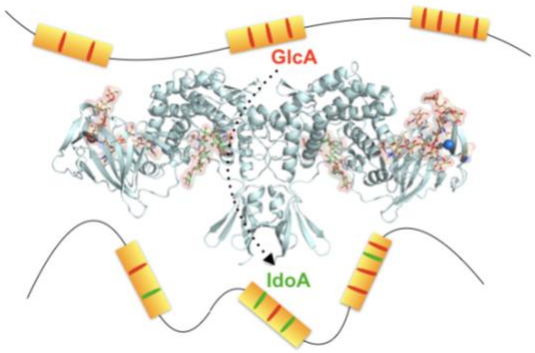


Fig. 1: Schematic view of the structure of the Glce-substrate complex highlighting its role in increasing polysaccharide flexibility in heterogenous HS domains (yellow).

[1] C. Debarnot, Y.R. Monneau, V. Roig-Zamboni, V. Delauzun, C. Le Narvor, E. Richard E, J. Hénault J, A. Goulet, F. Fadel, R.R. Vivès, B. Priem, D. Bonnaffé, H. Lortat-Jacob, Y. Bourne. (2019). Substrate binding mode and catalytic mechanism of human heparan sulfate d-glucuronyl C5 epimerase**. Proc Natl Acad Sci U S A** 116: 6760-6765

Table of Content

Hydrogen-deuterium eXchange coupled to Mass Spectrometry highlights a reciprocal crosstalk between the inner and outer rings of the 20S proteasome

J. Lesne[a], J. Parra[a]; D. Zivkovic[a]; T. Menneteau[a]; M. Chavent[a], M. Locard-Paulet[a], M.P. Bousquet-Dubouch[a]; O. Burlet-Schiltz[a]; J. Marcoux[a]

[a] *Institut de Pharmacologie et de Biologie Structurale, IPBS, Université de Toulouse, CNRS, UPS, 31077, Toulouse, France*.
E-mail: julien.marcoux@ipbs.fr

The 20S proteasome is a multi-catalytic protease composed of 4 heptameric rings. It degrades proteins in a controlled fashion, thereby directly regulating intracellular concentration of cytokines and hub proteins. Alteration of its activity can lead to pathologies including cancers, heart and auto-inflammatory diseases. Its activity can be regulated by replacing its constitutive catalytic subunits and/or by interacting with different activators. However, whether its catalytic subunit composition favors the interaction with a particular regulator is still unclear.
Here, we utilized Hydrogen-Deuterium eXchange coupled to Mass Spectrometry (HDX-MS) to investigate the impact of the catalytic subunit composition of the 20S proteasome on its structure and association to specific activators. Human standard (std20S) and immuno (i20S) proteasomes were deuterated alone of after incubation with the PA28αβ or PA28γ activators.
We successfully optimized the classical HDX-MS workflow in terms of sample preparation, chromatography and MS acquisition to work on both poorly concentrated and very heterogeneous protein complexes. We developed a web application called HDX-Viewer [1] to instantly visualize the raw data of these large complexes, directly from DynamX outputs. Deuteration rates of the three PA28 monomers (forming the PA28αβ or PA28γ heptamers) indicated both common features (Fig.1A), suggesting a similar mode of activation but also local discrepancies. The std20S and i20S clearly showed a faster deuteration on the solvent-exposed surface of the α-ring compared any other ring-interface (Fig1B). Furthermore, we identified flexible regions that are available for interaction with the ~200 Proteasome-Interacting-Proteins described so far. Comparison of the std20S Vs. i20S deuteration highlight subtle but meaningful discrepancies. Similarly, binding of the PA28 regulators influences the deuteration of the 20S proteasomes. Deuteration of the std20S Vs. i20S suggests a first “inner to outer ring allosteric change”. Changes in the inner β-rings upon regulator binding were interpreted as an “outer to inner ring allosteric change”. Altogether, the α-ring region that was more dynamic in the std20S Vs. i20S was also the most protected by activators. Our results thus highlight how the incorporation of different catalytic subunits can alter the proteasome affinity to different regulators.

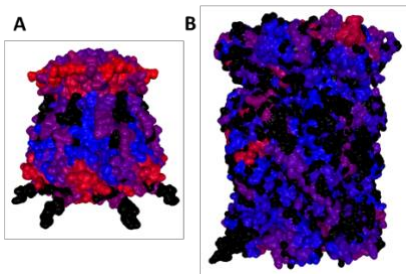


Fig.1: Deuteration of **(A)** PA28γ and **(B)** the full standard 20S proteasome from 0% (blue) to 60% (red).

[1] D. Bouyssié, J. Lesne, M. Locard-Paulet, R. Albigot, O. Burlet-Schiltz, J. Marcoux (2019) HDX-Viewer: interactive 3D visualization of Hydrogen-Deuterium eXchange data” *In Revision*

Session VIII: Computational methods and modeling

Chairpersons: M. Bergdoll (IBMP, Strasbourg), M. Chavent (IPBS, Toulouse)

Julie Cornet, Laboratoire de Physique Théorique, Toulouse
Monte Carlo and molecular dynamics simulations to explain biomembrane meso-patterning by a composition-curvature coupling mechanism

Florence Tama, Computational Structural Biology & Biophysics, Nagoya, Japan
Integrative modeling to characterize structure and dynamics of biomolecules from single molecule experiments

Jérémie Topin, Institut de Chimie de Nice, Nice
Numerical modeling of odorant receptors activation dynamics

Jérôme de Ruyck, UGSF, Lille
How can oncoprotein Ets-1 interact with DNA repair enzyme PARP-1? A molecular modelling approach to design cancer progression inhibitors

Monte Carlo and molecular dynamics simulations to explain biomembrane meso-patterning by a composition-curvature coupling mechanism

J. Cornet[1], M. Chavent[2], M. Manghi[1], N. Destainville[1]

[1]*Laboratoire de Physique Théorique - IRSAMC, UPS, CNRS, 31077, Toulouse, France*
[2] *Institut de Pharmacologie et de Biologie Structurale, IPBS, UPS, CNRS, 31077, Toulouse, France*.
E-mail: julie.cornet@irsamc.ups-tlse.fr

Plasma membrane plays a crucial role in biological functions such as endo and exocytosis, cell communication or adhesion. It is now widely agreed that membrane lipid and protein spatial repartition is not homogeneous but that these components are organized into nanodomains [1] that have proven to be key players in the above-mentioned biological functions. Combining statistical physics analytical tools and numerical simulations, we propose a physical mechanism for this membrane organization in a simple model vesicle. At the meso-scale, we describe the membrane with a composition-curvature coupling mechanism [2]. We perform Monte Carlo simulations for different membrane parameters (temperature, composition, spontaneous curvature, surface tension), determine the range of parameters leading to meso-patterning and quantitatively characterize the emerging membrane domains. In order to propose a valid rationale for membrane structuring at a lower scale, we also perform coarse-grained molecular dynamics simulations (MARTINI) of lipid bilayers including curvature-generating components [4]. To assess the validity of our results, we compare them to available analytical predictions and experimental data.

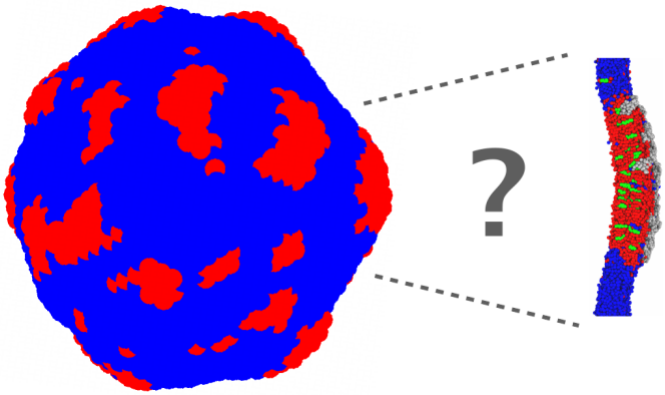


Fig. 1: Meso-scale Monte Carlo simulation of a lipid vesicle (left) and coarse-grained MARTINI simulation of a lipid bilayer (right) featuring curved nanodomains.

[1] F. Schmid. (2017). Physical mechanisms of micro- and nanodomain formation in multicomponent lipid membranes. **Biochimica et Biophysica Acta (BBA) - Biomembranes** 1859:509-528
[2] G. Gueguen, N. Destainville, and M. Manghi. (2014). Mixed lipid bilayers with locally varying spontaneous curvature and bending. **The European Physical Journal E** 37:76-89
[3] M. Chavent, A.L. Duncan, and M. S. P. Sansom. (2016). Molecular dynamics simulations of membrane proteins and their interactions: from nanoscale to mesoscale. **Current Opinion in Structural Biology** 40:8-16

Integrative modeling to characterize structure and dynamics of biomolecules from single molecule experiments

F. Tama

RIKEN-Center for Computational Science, Kobe 650-0047, Japan
Institute of Transformative Bio-Molecules (WPI), Nagoya University, Nagoya 464-8601, Japan
Department of Physics, Graduate School of Science, Nagoya University, Nagoya 464-8602, Japan
E-mail: florence.tama@nagoya-u.jp

Hybrid and integrative modeling methods, that combine computational molecular mechanics simulations with experimental data, have been shown to be powerful to describe the structure and dynamics of large biomolecules. In particular, flexible fitting is a powerful technique to build the 3D structures of biomolecules from cryo-electron microscopy (cryo-EM) density maps. While flexible fitting methods work nicely with very high resolutions maps, there are limitations for medium resolution maps (~5-10 angstrom) in case of complex conformational transitions. To overcome such issues, we proposed a refinement based on conformational ensemble, i.e. performing multiple fittings trials using a variety of parameters. To improve the success rate, an automatic adjustment of the biasing force constants during the fitting process was introduced via a replica-exchange scheme. From such multiple fittings, clustering analysis of the models obtained can be an effective approach to avoid over-fitting [1,2]. Application of such flexible fitting methods into cryo-EM data in conjunction with other experimental methods (Atomic Force Microscopy and NMR) revealed a crucial open-close dynamic of the Oligosaccharyltransferase enzyme, a membrane-bound enzyme that catalyzes the transfer of oligosaccharide chains from lipid-linked oligosaccharides to asparagine residues in a polypeptide chain (Fig 1). These works were performed in collaborations with Professor Kohda, Professor Ando, Dr. Sugita and their group members.

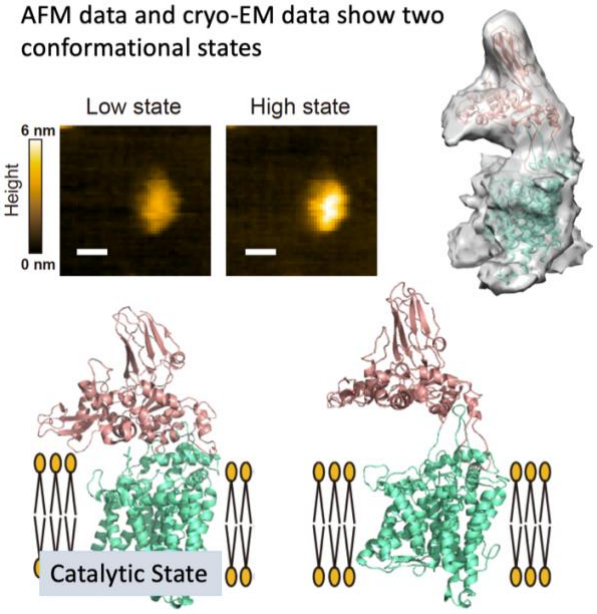


Fig. 1: Integrative modeling the conformational change of the Oligosaccharyltransferase enzyme

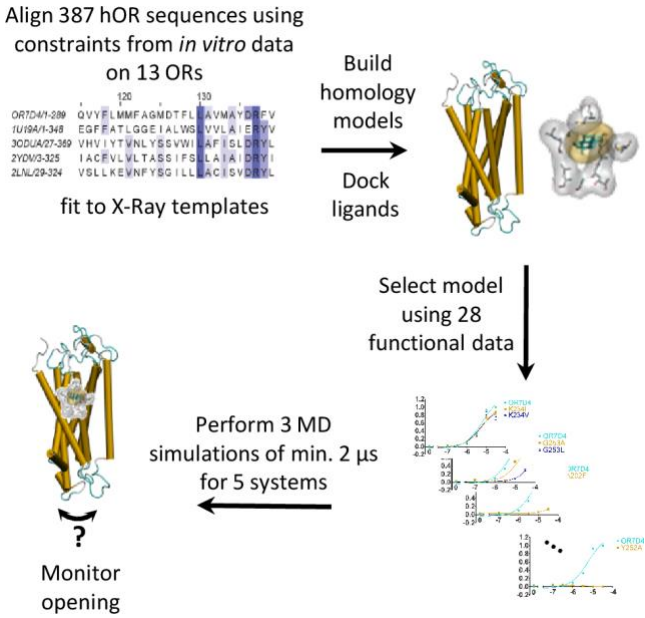
[1]. T. Mori, M. Kulik, O. Miyashita, J. Jung, F. Tama and Y. Sugita. Acceleration of cryo-EM flexible fitting by efficient space partitioning for large biomolecular systems. (2019) **Structure** 27:161-174
[2] O. Miyashita, C. Kobayashi, T. Mori, Y. Sugita, F. Tama. Flexible Fitting to Cryo-EM Density Map using Ensemble Molecular Dynamics Simulations. (2017) **J. Comp. Chem**. 38:1447-1461

Numerical modeling of odorant receptors activation dynamics

J. Topin[a], C. A. de March[a,b], J. Golebiowski[a,c]

[a] *Institute of Chemistry - Nice, UMR 7272 CNRS – Université Côte d'Azur, 06108 Nice cedex, France*
[b] *Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC 27710, USA*
E-mail: jeremie.topin@univ-cotedazur.fr

Our sense of smell is triggered by the activation of odorant receptors (ORs) expressed at the surface of olfactory sensory neurons. ORs are transmembrane proteins which belong to the family of class A G protein-coupled receptors and as such, their activation mechanism can be split into two main events: the binding of the odorant to the OR and then, in the case of an agonist, the activation of the odorant-OR complex. Predicting computationally the activation of ORs represent the first step towards the building of a virtual biomimetic nose.[1]
By combining site-directed mutagenesis and functional assays with computational modeling our work reveals that agonist-induced activation of odorant receptors can be predicted. It involves a dynamic network that spreads over highly conserved sequence motifs which control the downstream signaling from the binding cavity to the G-protein coupling site. Mutant receptors at these positions consistently show differential response upon agonist stimulation.[2]
Our protocol paves the way to deciphering the combinatorial code associated with the perception of smell.



Protocol of activation monitoring and conservation analysis of human odorant receptors.

[1] J. Topin, C. A. de March, L. Charlier, C. Ronin, S. Antonczak, J. Golebiowski (2014). **Chemistry a European Journal**, 20:10227-10230.
[2] C. A. de March, J. Topin, E. Bruguera, G. Novikov, K. Ikegami, H. Matsunami, J. Golebiowski (2018). **Angewandte Chemie**, 57:4554-4558.

How can oncoprotein Ets-1 interact with DNA repair enzyme PARP-1? A molecular modelling approach to design cancer progression inhibitors

J. de Ruyck, G. Brysbaert, M. Aumercier and M. F. Lensink

Univ. Lille, CNRS UMR8576 UGSF – Unité de Glycobiologie Structurale et Fonctionelle, F-59000 Lille, France
E-mail: jerome.de-ruyck@univ-lille.fr

The Ets-1 oncoprotein is a transcription factor that promotes target gene expression in specific biological processes. Most of the time Ets-1 activity is low in healthy cells, but elevated levels of expression have been found in cancerous cells, specifically related to tumor invasion. Given the important role of Ets-1 in major human diseases, it is crucial to identify its partners in a relevant model and characterize their interactions. In this context, we recently identified and validated new interaction partners of Ets-1, which include PARP-1, a DNA repair enzyme and guardian of the genome integrity. It has been shown that these interactions are important for cancer cell survival, but nothing is known about the molecular details.
In the first part of this study, we obtained, via protein-protein docking, a suitable model of interaction for Ets-1 (ETS) and the interacting domain on PARP-1 (BRCT). These models highlight the α-helix 1 (H1) hydrophobic area as a putative interacting surface, including Trp338 and Trp361 which were identified as hot spots (Fig. 1). In the second part, we investigate how to obstruct the interaction between Ets-1 and its partners, including BRCT. Based on the docking results, we built a pharmacophore, screened a large database of available compounds and simulated the complexes between these hits and the ETS domain. In this poster, we present results that open the way to the design of new molecules with the potential to prevent cancer progression.

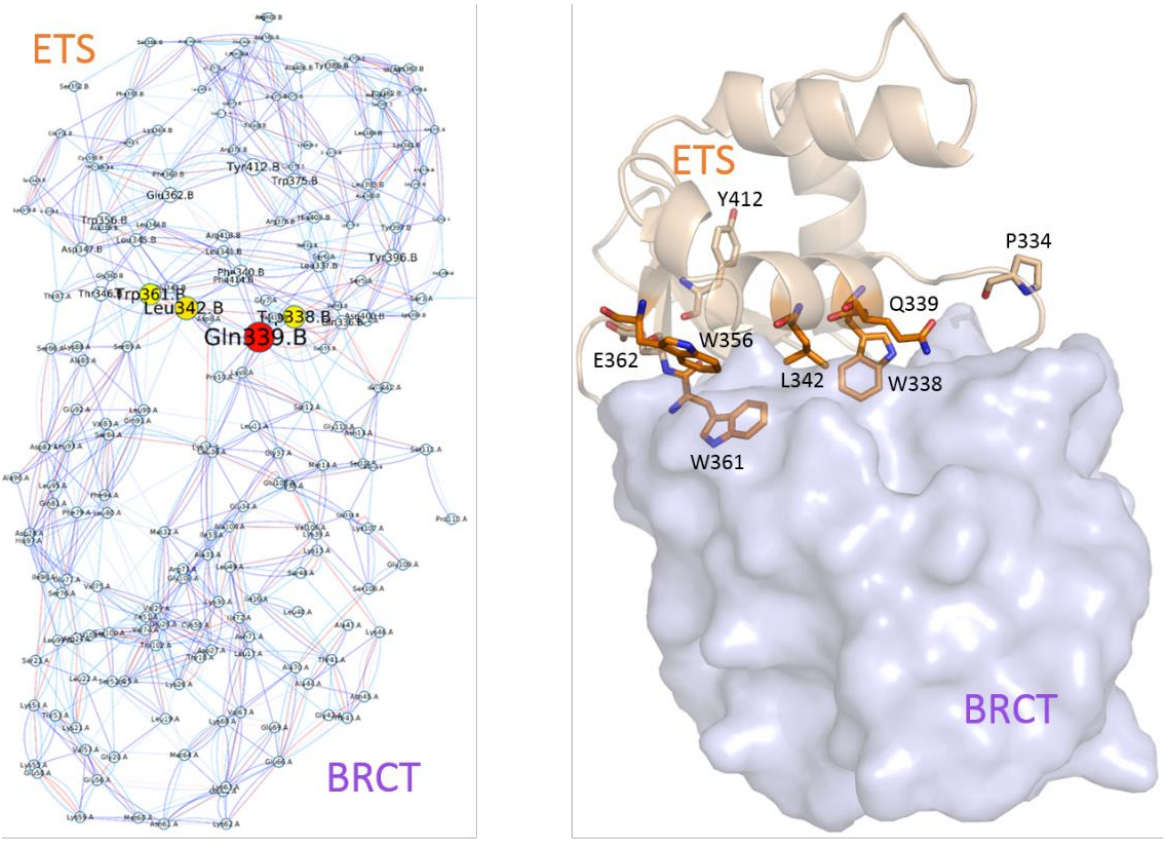


Fig. 1: interaction model for Ets-1 (ETS) and the interacting domain on PARP-1 (BRCT)

Session IX: Structural organization in membrane and in cellular context

Chairpersons: C. Bompard (UGSF, Lille), A. Dautant (IBGC, Bordeaux)

Vincent Chaptal, MMSB, Lyon
Cryo-EM and X-ray structures of the multidrug transporter BMRA: revisiting the transport mechanism

Isabelle Broutin, CiTCoM, Paris
New insights into the assembly and opening mechanism of the OprM-MexAB efflux pump from *Pseudomonas aeruginosa*

Julie Bouckaert, UGSF, Lille
Glycoprotein binding enhances bacterial entry at epithelial membranes

Arpita Tawani, IECB, Pessac
Nanodomain targeting during bacterial division investigated by solid-state NMR

Cryo-EM and X-ray structures of the multidrug transporter BmrA: revisiting the transport mechanism

V. Chaptal[a,f], V. Zampieri[a,f], B. Wiseman[a,b,f], K. N'Guyen[c], S. Magnard[a], A. Kilburg[a], M. Peuchmaur[c], J. Martin[d], C. Orelle[e], J.M. Jault[e], L. Monticelli[d], M. Högbom[b], A. Boumendjel[c] and P. Falson[a]

[a]Drug Resistance & Membrane Proteins group, Molecular Microbiology and Structural Biochemistry Laboratory (CNRS UMR 5086), University of Lyon, IBCP, 7, passage du Vercors, 69367 Lyon, France. [b]Dept. of Biochemistry and Biophysics, Arrhenius Lab. for Natural Sciences, Stockholm University, Stockholm, Sweden. [c]Department of Molecular Pharmacology (CNRS UMR 5063), University of Grenoble-Alpes, 38041 Grenoble, France [d]Modeling Biological Macromolecules group, Molecular Microbiology and Structural Biochemistry Laboratory (CNRS UMR 5086), University of Lyon, IBCP, 7, passage du Vercors, 69367 Lyon, France [e]Bacterial nucleotide-binding proteins group, Molecular Microbiology and Structural Biochemistry Laboratory (CNRS UMR 5086), University of Lyon, IBCP, 7, passage du Vercors, 69367 Lyon, France [f]These authors contributed equally

E-mail: vincent.chaptal@ibcp.fr

Drug resistance is a major health concern, with strong clinical implications in anti-cancer, anti-bacterial and anti-fungal therapies. Several molecular mechanisms will result in drug resistance, mostly sharing a common simple idea; the therapeutic molecule needs to reach its cellular target to be efficient. Cells have devised several strategies to evade the lethal effects of the therapeutic molecules, for example by modifying the target, removing the target, or preventing the molecule to penetrate inside the cell, etc... ABC transporters are a large family of membrane proteins that belong to the later case, being in charge of detoxifying the cell by expelling toxic compounds through the plasma membrane; it thereby decreases the molecule concentration bellow cytotoxic levels, which prevents its activity. It is described that they follow a transport cycle, obeying the alternating access mechanism, utilizing the energy of ATP binding and hydrolysis to transport their substrates. We have solved the X-ray and Cryo-EM structures of the *B. subtilis* multidrug transporter BmrA in two related, yet different, transient outward-facing states (Figure 1). These structures are a missing link between many structures of homologs available today, and provide a detailed molecular movie of drug release. We conducted molecular dynamics simulations that confirm predictions from the structures, and reveal conformational changes post-substrate translocation. Together, these results call for a change of view on how ABC transporters transport their substrates, not through a transport cycle.

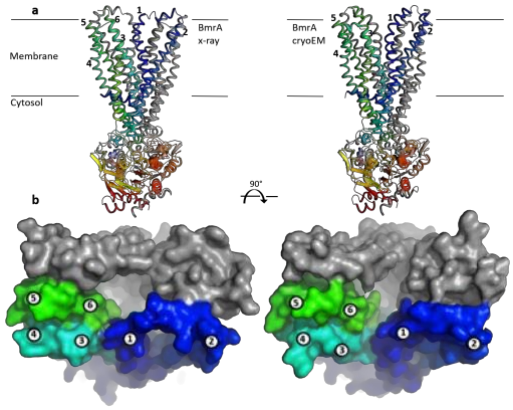


Fig. 1: Xray (left) and Cryo-EM (right) structures of BmrA in transient outward-facing states.

New insights into the assembly and opening mechanism of the OprM-MexAB efflux pump from *Pseudomonas aeruginosa*

Y. Ntsogo [a,d], L. Monlezun [a,c], M. Ma [a,d], M. Glavier [b], L. Daury [b], O. Lambert [b], G. Phan [a], I. Broutin [a]

[a] *Laboratoire Cibles Thérapeutiques et Conception de Médicament, CiTCoM, Université Paris Descartes, CNRS, 75006, Paris, France;* [b] *CBMN, UMR 5248, CNRS/Université de Bordeaux 2, Bordeaux;* [c] *present address: Division of Molecular Microbiology, School of Life Sciences, University of Dundee, UK;* [d] *present address: Department of Biochemistry, University of Cambridge, UK*

E-mail: isabelle.broutin@parisdescartes.fr

Bacterial infections remain a major public concern due to the accelerated increase in the appearance of antibiotic resistance. Among the different mechanisms used by bacteria to resist to antibiotics, the active efflux plays a major role. In Gram-negative bacteria, this is achieved by tripartite efflux pumps that form a macromolecular assembly spanning both membranes of the cellular wall. In *Pseudomonas aeruginosa*, an opportunistic pathogen highly involved in nosocomial diseases, the constitutive pump is OprM-MexAB. Along with functional studies, many crystal structures were solved of the individual components of this pump and homologous ones [1-3]. Nevertheless, a lot of questions concerning the assembly, the mechanism of efflux, and the opening of the whole pump are still a matter of active research, as the blockage of these pumps could restore the utility of the actual therapeutic arsenal.
In order to apprehend the efflux mechanism, a combine approach was used (see figure). New information will be presented on the functional mechanism of the efflux pump MexAB-OprM clarifying the role of the different protein actors.

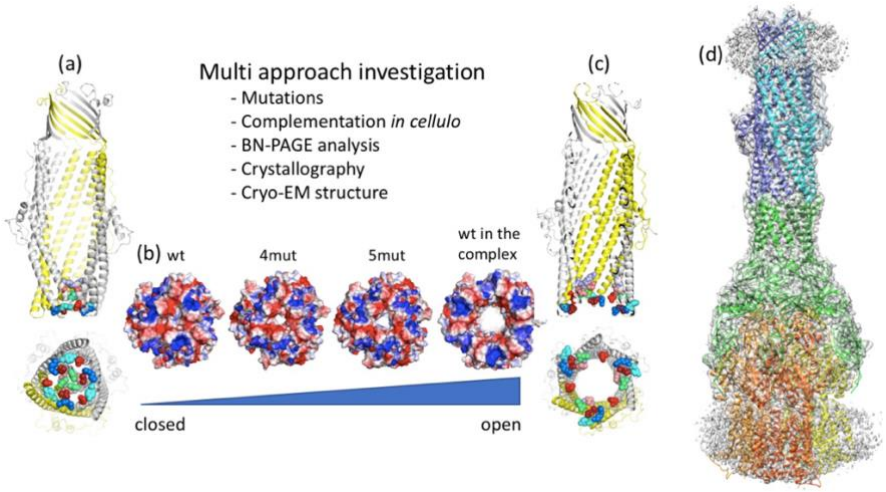


Fig. 1: OprM structures. (a) wt; (b) mutants; (c & d) in the tripartite pump complex

[1] K. Tsutsumi, R. Yonehara et al. (2019). Structures of the wild-type MexAB-OprM tripartite pump reveal its complex formation and drug efflux mechanism. **Nat Commun** 10**:**1520-1529.
[2] Y.V. Ntsogo Enguene, G. Phan, C. Garnier, A. Ducruix, T. Prange & I. Broutin (2017). Xenon for tunnelling analysis of the efflux pump component OprN. **PLoS One** 12**:** e0184045.
[3] L. Daury, F. Orange, J.C. Taveau, A. Verchere, L. Monlezun, C. Gounou, et al. (2016). Tripartite assembly of RND multidrug efflux pumps. **Nat Commun** 7**:**10731-10738.

Table of Content

Glycoprotein binding enhances bacterial entry at epithelial membranes

J. Bouckaert

Unité de Glycobiologie Structurale et Fonctionnelle, UMR 8576 of the CNRS and the University of Lille, Campus CNRS Haute Borne, 50 Avenue de Halley, 59658 Villeneuve d'Ascq, France.
E-mail: julie.bouckaert@univ-lille.fr

A mechanism is revealed in which clinical isolates of adherent-invasive *Escherichia coli* (AIEC) penetrate into the intestinal epithelial cell layer, replicate, and establish biofilms in Crohn's disease [1]. AIEC uses the fimbrial adhesin FimH to bind to oligomannose glycans exposed on the surface of human host cells. Oligomannose glycans exposed on early apoptotic cells serve as the preferred binding targets for AIEC. Upon entry into the epithelial cell layer, the AIEC bacteria propagate into colonies inside the (caco-2) cells and transfer laterally through desmosome-like structures between cells [1]. *N*-glycosylation analyses, using LC-MS/MS, of a membrane glycoprotein, carcinoembryonic antigen related cell adhesion molecule 6 (CEACAM6 or CD66c), as a receptor for the FimH adhesin of AIEC showed high-mannose glycan structures at two distinct sites (Asn197 and Asn224) on the middle Ig-fold domain of CEACAM6. Using confocal laser and structured illumination superresolution microscopy, it is observed that AIEC bacteria engender the clustering of CEACAM6. Crystal structures of FimH in complex with mannose-based ligands and antibacterial antiadhesives have tremendously contributed to the generation of glycomimetics, as alternatives for antibiotics to cure from *E. coli* infections. Integrative structural biology holds the tools to improve future findings.

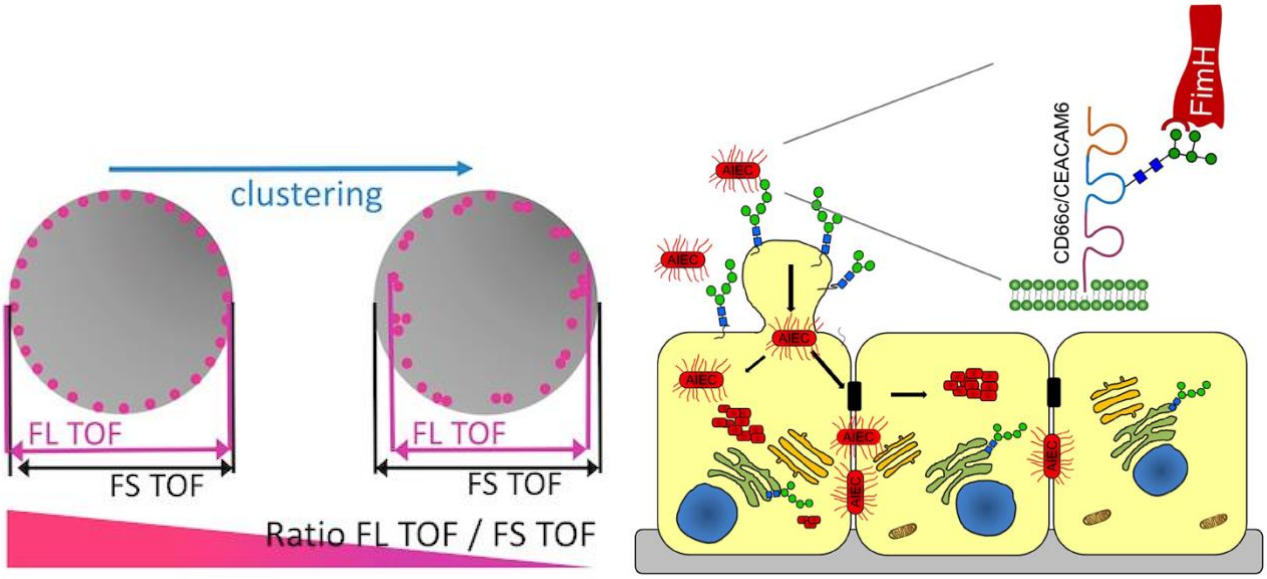


Fig. 1: The CEACAM6 glycoprotein receptor is clustering (*left*) near apoptotic blebs on intestinal epithelial cells when adherent invasive *E. coli* adhere specifically to high-mannose glycans by means of the FimH fimbrial lectin (*right*). Internalization and spreading of the bacteria through lateral membranes allows them to persist.

[1] T Dumych, N Yamakawa, A Sivignon, E Garenaux, S Robakiewicz, B Coddeville, A Bongiovanni, F Bray, N Barnich, S Szunerits, C Slomianny, M Herrmann, S Gouin, AD Lutsyk, L Munoz, F Lafont, C Rolando, R Bilyy, JMJ Bouckaert. (2018) Oligomannose-rich membranes of dying intestinal epithelial cells promote host colonization by adherent invasive *E. coli*. **Frontiers in Microbiology**, 9, art. 742.

Nanodomain targeting during bacterial division investigated by solid-state NMR

A. Tawani[a], A. Saad[a], M. Berbon[a], D. Martinez[a], G. Rivas[b], A. Loquet[a], M. Velez[c], B. Habenstein[a]

[a] *Institute of Chemistry and Biology of Membranes and Nanoobjects, Institut Européen de Chimie et Biologie (CNRS UMR 5248, Université de Bordeaux), 33600 Pessac, France*
[b] *Institute Centro de Investigaciones Biológicas, CSIC, 28040-Madrid, Spain*
[c] *Instituto de Catálisis y Petroleoquímica CSIC, c/Marie Curie, 2, Cantoblanco, 28049 Madrid, Spain*
E-mail: arpita.tawani@u-bordeaux.fr

In bacteria the formation of the division plane is regulated by a membrane-associated ring-shaped assembly, the Z-ring, at the mid-cell [1]. It involves the polymerization of cytoplasmic protein FtsZ, associated to the membrane by the membrane anchors FtsA or ZipA. ZipA contains a small groove in the C-terminal globular domain that tethers FtsZ, while the N-terminal is predicted to form an α-helical transmembrane domain [1]. Membrane lipids and protein-lipid interactions play a significant role in guiding cellular proteins towards their functional sites by heterogeneously distributing over the cell membrane forming lipid nanodomains [2]. Nanodomain organization and the role of lipids during bacterial division remain mainly unexplored because of the complexity of the membrane-protein system. We use solid-state NMR (ssNMR) to address the membrane-protein interplay. ssNMR is emerging as a technology that provides insights into structure and dynamics of membrane-associated proteins and membrane lipids on the atomic level.
We successfully implemented a purification strategy to produce ZipA in sizeable amounts to perform ssNMR. Applying ^{2}H and ^{31}P ssNMR we elucidate the interactions between ZipA with membranes of different putative nanodomain compositions, shedding light on the ZipA-lipid organization during cell division. We examine the phase behavior, structure and dynamics of the membranes in the presence or absence of ZipA. Moreover, in order to discriminate the effect of lipid association mediated by the membrane-binding or by the globular domain, we have employed two constructs encompassing the intact ZipA (wt-ZipA) or only the globular domain (lacking the putative N-terminal helix). We here shed first lights on molecular protein-lipid interactions and potential lipid domain formation governing the bacterial cell division. Furthermore, we employ Magic-Angle Spinning ssNMR to visualize for the first time the membrane anchor ZipA at the atomic level. We find a well-organized structural core of ZipA that shows partial association to the membrane, entailing a rigidified structural regime.

[1] H.P. Erickson. (2001). The FtsZ protofilament and attachment of ZipA--structural constraints on the FtsZ power stroke. **Current Opinion in Cell Biology** 13:55-60.
[2] E. Mileykovskaya, W. Dowhan. (2005). Role of membrane lipids in bacterial division-site selection. **Current Opinion in Microbiology** 8:135-42.

Posters

Session I: Drug Design

Stefan Arold, StruBE/KAUST, Thuwal, Saoudi Arabia
P1 - Structural basis for specific inhibition of the parasitic *striga*

Khaoula Ben Yaala, CRCM, Marseille
P2 - CovaDOTS: a mixed strategy for covalent drug design, combining molecular modeling with medicinal chemistry

Jérémie Buratto, CBMN, Pessac
P3 - Structural insights into hDM2 protein recognition by oligourea foldamers

Coralie Carivenc, IPBS, Toulouse
P4 - Finding inhibitors of targeted enzymes by biophysics and crystallographic approaches for the treatment of infectious diseases

Kendall Carrasco, CRCM, Marseille
P5 - Development of protein-protein interactions inhibitors: application at the Bromodomains

Julien Cros, CBM, Orléans
P6 - Screening, characterization and structural studies of small molecules inhibitors of the DNA glycosylase hNEIL1 and hOGG1, potential therapeutic targets in cancer and neurodegenerative disease

Virginie Gervais, IPBS, Toulouse
P7 - Small molecule–based targeting of TTD-A dimerization to control TFIIH transcriptional activity represents a potential strategy for anticancer therapy

Bruno Kieffer, IGBMC, Illkirch
P8 - Self-organization and interaction properties of a perfluorinated GCPR binding peptide with a perfluorinated tail: a ^{19F} NMR study

Corinne Lionne, CBS, Montpellier
P9 - Structure-based drug design of specific inhibitors of
antibiotic modifying enzymes

Laurent Maveyraud, IPBS, Toulouse
P10 - Fragment based ligand discovery in an academic setup

P1 - Structural basis for specific inhibition of the parasitic *striga*

U.F. Shahul Hameed, I. Haider, M. Jamil, B.A. Kountche, R.A. Zarban, D. Kim, S. Al-Babili, S.T. Arold

Division of Biological and Environmental Sciences and Engineering, Abdullah University of Science and Technology, Thuwal 23955-6900, Saudi Arabia,[c]
E-mail: stefan.arold@kaust.edu.sa

Striga, commonly known as 'witchweed', is a genus of root parasitic plants. Some *Striga* forms infest cereals, causing more than 35 billion USD of losses per year in the Americas and in Africa (where *Striga* affects up to 70% of arable land), while other forms (*Orobanche*, also known as broomrape) target sunflowers in Europe. Currently, there is no effective herbicide to stop the persistence and spreading of *Striga*. Combining structural, biochemical and *in planta* studies, and a good portion of luck, we have identified a first and unexpected lead compound that inhibits *Striga hermonthica* seed germination without affecting host plants. We are currently second-generation compounds to increase efficiency, and are producing structure-based specific herbicides for several other *Striga* species. This study is financed by the Bill & Melinda Gates foundation, and has resulted in a patent.

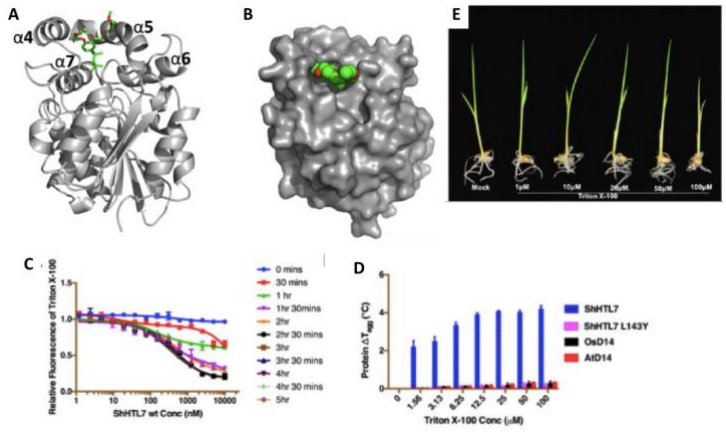


Fig. 1: (A-B). Crystal structures of the *S. hermonthica* sensor for strigolactones (ShHTL7), bound to the specific inhibitor (green). (C-D) The inhibitor binds to ShHTL7 with high affinity and prevents enzymatic activity. (E) The inhibitor does not affect highly similar sensors in rice.

[1] U.F. Shahul Hameed, I. Haider, M. Jamil, B.A. Kountche, R.A. Zarban, D. Kim, S. Al-Babili, S.T. Arold (2018) Structural basis for specific inhibition of Striga hermonthica Seed Germination. **EMBO Reports**, 19: e45619
[2] U.F. Shahul Hameed, I. Haider, M. Jamil, B.A. Kountche, R.A. Zarban, D. Kim, S. Al-Babili, S.T. Arold, **unpublished** results

P2 - CovaDOTS: a mixed strategy for covalent drug design, combining molecular modeling with medicinal chemistry

K. Ben Yaala, L. Hoffer, M. Saez-Ayala, S. Betzi, P. Bremond, S. Combes, P. Dubreuil & X. Morelli

CRCM, CNRS UMR 7258, Inserm UMR 1068, Institut Paoli-Calmettes, Aix-Marseille University, 13009 Marseille, France

E-mail: Khaoula.ben-yaala@inserm.fr

Our team has developed 'DOTS' (Diversity Oriented Target-focused Synthesis), an integrated approach that combines virtual screening with semi-robotized organic synthesis, to accelerate hit-to- lead optimization [1]. The use of a similar approach, dedicated to the design of covalent inhibitors, CovaDOTS, has also been recently proposed [1,2].

We aim here to establish the experimental proof of concept for CovaDOTS by establishing the reactivity profile of the side chains of different nucleophilic residues (Lys, Cys, Met ...) for different protein targets. Our methodology is organized in 5 consecutive steps:

1. Selection of 3'targets'(proteins)bearing different type of nucleophilic residues for compound anchoring (i.e Cysteine, Lysine and Methionine)
2. For each nucleophilic residue, selection a list of potential warheadswith different electrophilic potential [3-5].
3. Evaluation (proteomic approach) of the reactivity and accessibility of all the nucleophilic amino acids in situ, using the selected warheads.
4. Transfer this knowledge to transform reversible drugs / probes into covalent inhibitors, for each of our targets.
5. Evaluate the newly designed and synthesized compounds in biochemical assays.

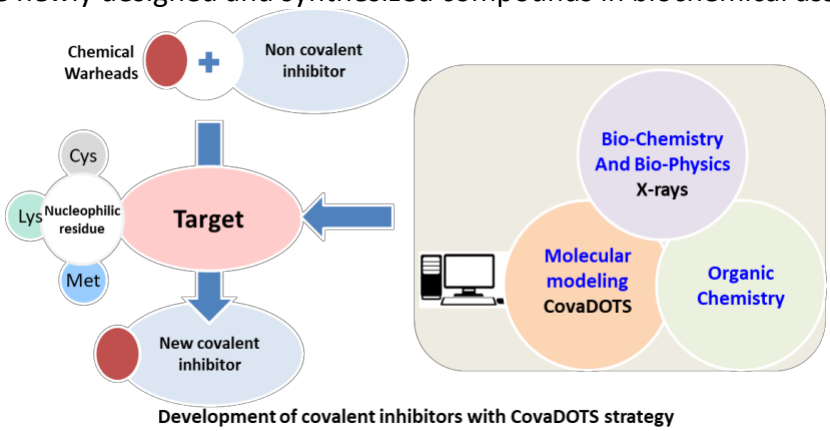


Fig 1. The aim of the strategy is to transform a hit that inhibit the protein target with a non-covalent mode of action into a covalent inhibitor by identifying chemical moieties or warheads that react with a nucleophilic residue in the target protein [1].

[1] Hoffer et al. (2018). Integrated Strategy for Lead Optimization Based on Fragment Growing: The Diversity-Oriented- Target-Focused-Synthesis Approach, **J Med Chem.** 61:5719-5732

[2] Hoffer et al. (2019). CovaDOTS : In Silico Chemistry-Driven Tool to Design Covalent Inhibitors Using a Linking Strategy. **J Am Chem.**, 59:1472-1485

[3] Gambini et al. (2019). Covalent Inhibitors of Protein–Protein Interactions Targeting Lysine, Tyrosine, or Histidine Residues. **J Med Chem**., 62:5616-5627

[4] Dahal et al. (2016). Intrinsic reactivity profile of electrophilic moieties to guide covalent drug design: N-α-acetyl-L-lysine as an amine nucleophile. **Med Chem Commun**, 7:864-872.

[5] Narayanan et al. (2015). Sulfonyl fluorides as privileged warheads in chemical biology. **Chem. Sci**., 6:2650-2659.

P3 - Structural insights into hDM2 protein recognition by oligourea foldamers

J. Buratto [a], L. Mauran [b], S. Fribourg [c], S. Goudreau [b], G. Guichard [a]

[a] *Université de Bordeaux, CNRS, UMR 5248, CBMN, IECB, Pessac (France),*
[b] *UREkA Sarl, Pessac (France),* [c] *Université de Bordeaux, INSERM U 1212 ARNA, Bordeaux (France)*
E-mail: j.buratto@iecb.u-bordeaux.fr

The p53 protein is a transcription factor that regulates the expression of many genes coding for proteins involved in various biological functions. This protein is the guarantor of cellular integrity. The level of p53 expression is highly controlled and kept low by a negative regulatory mechanism driven by the hDM2 protein.[1] The overexpression of the hDM2 protein has been observed in many cases of cancer. Thus, inhibiting the interaction between these proteins, in order to restore the activity of the wild-type p53 is a strategy to fight against cancer.[2] The interaction between these two proteins is mediated by a short α-helix located at the N-terminal extremity of the p53 protein.[3] However, short, isolated peptide helices are generally only weakly populated in aqueous environment and are susceptible to proteolytic degradation, thus limiting their therapeutic potential. A variety of chemical strategies have been proposed to increase the helix folding propensity and stability of α-peptides among which foldamer-based approaches have recently emerged.[4]
In this context, we became interested by the possibility to combine peptide and foldamer helical backbones in a single strand to generate new generations of α-helix mimics. We have shown that oligourea foldamer/peptide chimeras form well-defined helical structures in polar organic solvents with the propagation of a continuous intramolecular H-bond network spanning the entire sequence.[5]
In this presentation, we describe the design of peptide/oligourea hybrid compounds with the ability to modulate the p53/hDM2 interaction, and report our efforts to structurally characterize the interactions between the most potent foldamers in this series and the target protein as a mean to improve design principles and generalize the discovery of foldamer-based inhibitors of protein-protein interactions.

[1] A. Hock and K. H. Vousden. (2010). **Int. J. Biochem. Cell. Biol.** 42:1618–21
[2] P. Chène. (2003). **Nat. Rev. Cancer** 3:102–9
[3] P. H. Kussie et al. (1996). **Science** 274:948–53
[4] M. Pasco et al. (2017). **Comprehensive Supramolecular Chemistry II** 5:89-125
[5] J. Fremaux, et al. (2015). **Angew. Chem Int. Ed.** 54:9816–20

P4 - Finding inhibitors of targeted enzymes by biophysics and crystallographic approaches for the treatment of infectious diseases

C. Carivenc [a], J.D. Pedelacq [a], L. Maveyraud [a,b], V. Nahoum [a,b], G. Prévot [a], C. Chalut [a], C. Guilhot [a], and L. Mourey [a,b]

[a]*Institut de Pharmacologie et de Biologie Structurale, IPBS, Université de Toulouse, CNRS, UPS, 31077, Toulouse, France*.
[b]*Plateforme Intégrée de Criblage de Toulouse, PICT, IPBS, Université de Toulouse, CNRS, UPS, 31077, Toulouse, France*
Email: coralie.carivenc@ipbs.fr

Fatty acid synthases (FAS) (primary metabolism), polyketide synthases (PKS) and non-ribosomal peptide synthetases (NRPS) (secondary metabolism) contain carrier protein domains. These proteins become functionally active after the transfer of the 4'-phosphopantetheinyl (P-Pant) moiety of coenzyme A onto a conserved serine residue in these domains and this reaction is catalyzed by phosphopantetheinyl transferases (PPTases) [1]. PPTases thus activate functionally important classes of megasynthases involved in the biosynthesis of a broad range of complex organic compounds, including specific lipids involved in bacterial virulence and pathogenicity, some of which are essential for the viability of bacteria [2]. Thus, owing to their central role in both primary and secondary bacterial metabolisms, PPTases represent new and attractive therapeutic targets. The aim of this project is to fully characterize such enzymes from different organisms and to identify molecules that display inhibitory activity for the development of novel antibacterial agents. Biochemical, biophysical and structural approaches were used to characterize a set of PPTases from various pathogenic bacteria *(Mycobacterium tuberculosis and M. abscessus, Pseudomonas aeruginosa, and Extra-intestinal Pathogenic Escherichia coli*). In addition, we screened 1000 fragment-like compounds from the Integrated Screening Platform of Toulouse (PICT, IBiSA) against the target enzymes using different approaches, including differential scanning fluorimetry (DSF) and several hits were identified. Besides, we implemented 'dry' co-crystallization for ligand screening using X-ray crystallography [3]. Finally, whole-cell based and *in vitro* assays have been designed that will allow the high-throughput screening of large chemical libraries and validating hits, respectively.

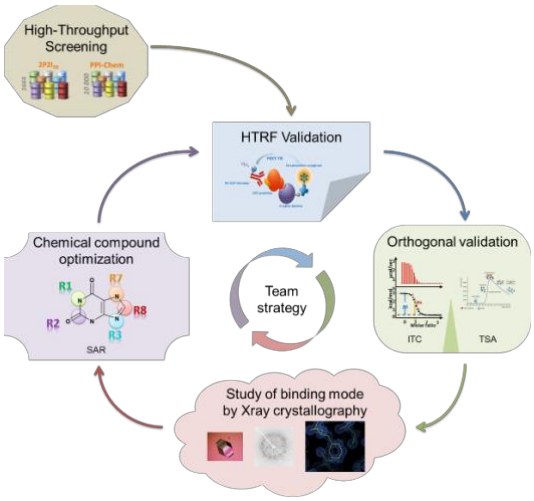
[1] C.T. Walsh et al. (1997). Post-translational modification of polyketide and nonribosomal peptide synthases. **Curr Opin Chem Biol** 1:309
[2] J. Beld et al. (2014). The phosphopantetheinyl transferases: catalysis of a post-translational modification crucial for life. **Nat Prod Rep** 31:61
[3] M. Gelin et al. (2015). Combining 'dry' co-crystallization and *in situ* diffraction to facilitate ligand screening by X-ray crystallography. **Acta Crystallogr D Biol Crystallogr** 71:1890

P5 - Development of protein-protein interactions inhibitors: application at the Bromodomains

K. Carrasco, L. Hoffer, Yuliia V. Voitovich, B. Raux, C. Muller, C. Derviaux, S. Betzi, Y. Collette, S. Combes, P. Roche, et X. Morelli

[a] Centre de Recherche en Cancérologie de Marseille, U1068 INSERM, UMR7258 CNRS, Institut Paoli Calmettes, UM105 Université Aix-Marseille, Laboratoire de Chimie Biologie et Structurale Intégrative
E-mail: kendall.carrasco@inserm.fr

Bromodomains (BRD) are conserved protein-protein interaction modules which can recognize and bind to acetylated lysine residues and particularly in the histone's tails [1]. In the Human, there are 61 bromodomains which are divided in 8 families [1]. In my thesis project and this poster, I focused on the family II, the Bromo and Extra-Terminal domain or BET family. This family is composed of 4 proteins called BRD4, BRD3, BRD2 and BRDT, each protein is composed of two BRDs called BD1 and BD2.
BETs proteins are very interesting therapeutic targets. In fact, there are involved in the regulation of gene transcriptions and implicated in many diseases such cancers or inflammations [2]. BET proteins are widely studied all over the world and it already exists many inhibitors of BET proteins, some of them are engaged in clinical trials.
In my thesis project, I have searched to develop a BET inhibitor with a particular profile, called selective BD1. This type of inhibitors inhibits only the BD1 of BETs bromodomains. For this purpose, we use an identification method, called the Homogeneous Times-Resolved Fluorescence (HTRF), which permits us to screen a data base of 1600 molecules. Molecules which are identified in this primary screen are validated by two experiments, the Thermal Shift Assay (TSA) and the Isothermal Titration Calorimetry (ITC). The binding mode of the compound in the BRD is evaluated by crystallography. The crystallographic structure of complex BRD/compound permits me to drive the compound optimization. These optimizations are essentials to increase the affinity and the selectivity with the goal to obtain a BD1 selective biological probes.



[1] Filippakopoulos, P.; Knapp, S. (2012) The bromodomain interaction module. **FEBS Lett**., 586:2692−2704
[2] Belkina, A. C.; Denis, G. V. (2012) BET domain co-regulators in obesity, inflammation and cancer. **Nat**. **Rev**. **Cancer**, 12:465−477.

P6 - Screening, characterization and structural studies of small molecules inhibitors of the DNA glycosylase hNEIL1 and hOGG1, potential therapeutic targets in cancer and neurodegenerative disease

J. Cros[a], F. Coste[a], S. Goffinont[a], V. Gaudon[a], V. Roy[b], N. Garnier[a], M. Guerin[a], S. Berteina-Rabouin[b], S. Routier[b], L. Agrofoglio[b], B. Castaing[a]

[a]*Centre de Biophysique Moléculaire (CBM), UPR4301 CNRS, rue Charles Sadron, 45071 Orléans*
[b] *Institut de Chimie Organique et Analytique (ICOA), UMR7311 Université d'Orléans-CNRS, Pôle de chimie, Rue de Chartres, 45100 Orléans*
Email : julien.cros@cnrs-orleans.fr

DNA is continuously damaged by physical or chemical agents from endogenous (cell metabolism, replication mistake …) and exogenous (UV, chemicals, ionizing radiations) sources. These damages by interfering with DNA transaction induce mutation or cell death and thus must be eliminated [1]. To avoid these adverse effects, living organisms have various DNA repair strategies that contribute to the maintenance of genetic stability, many DNA repair mechanisms are required to maintain genetic stability. The major pathway to repair DNA base lesions is the Base Excision Repair system (BER) [2]. BER is initiated by DNA Glycosylases such as hNEIL1 and hOGG1, which recognize and remove oxidized bases such as the abundant mutagenic 8-oxoGuanine (8-oxoG) and the replication blocking Thymine glycol (Tg) [3]. Many conventional cancer treatments use genotoxic drugs or physical agents (ionizing radiation…) to induce such damages in DNA of cancer cells, hoping to selectively inducing their death. However, DNA repair machineries can lead to therapeutic resistance. Although involved in genetic stability, DNA repair enzymes can be considered as relevant therapeutic targets in some pathological situations [4-6]. Behind this apparent paradox, the idea of targeting a DNA repair system for anti-cancer therapies is based on the synthetic lethality concept. If we consider that every cancer cells result from at least one gene mutation, we can target the gene, or the product of this gene, that these tumorous cells are depending on, while healthy cells are not. As an example, the synthetic lethality discovered between BRCA1/2 and PARP1 (poly[ADP-ribose] polymerase 1) is currently investigated for the treatment of BRCA-patients [7]. Similarly, to PARP1, several observations suggest that selective inhibition of hNEIL1 and hOGG1 could be relevant in some pathologic contexts (cancer, inflammation, neurodegenerative diseases) [5,6,8].
Here, we will present the screening of small molecule libraries for inhibition of hOGG1 and hNEIL1. Best hits action mode preliminary characterization and structural studies will be presented and discussed.

[1] Lindahl, T. (1993) Instability and decay of the primary structure of DNA. **Nature** 362: 709-715.
[2] Dalhus B. *et al.* (2009) DNA base repair--recognition and initiation of catalysis. **FEMS Microbiol Review** 33: 1044-1078.
[3] Boiteux *et al.* (2017) Repair of 8-oxo-7,8-dihydroguanine in prokaryotic and eukaryotic cells: Properties and biological roles of the Fpg and OGG1 DNA N-glycosylases. **Free Rad Bio Med** 107: 179-201
[4] Kovtun *et al.* (2017) OGG1 initiates age-dependent CAG trinucleotide expansion in somatic cells. **Nature** 447: 447-452.
[5] Ramdzan e*t al.* (2015) The function of CUX1 in oxidative DNA damage repair is needed to prevent premature senescence of mouse embryo fibroblasts. **Oncotarget** 6: 3613-3626.
[6] Visnes *et al*. (2018) Small-molecule inhibitor of OGG1 suppresses proinflammatory gene expression and inflammation. **Science** 362: 834-839.
[7] Ashworth & Lord. (2018) Synthetic lethal therapies for cancer: what's next after PARP inhibitors? **Nat Rev Clin Oncol**. 15: 564-576.
[8] Visnes *et al.* (2018) Targeting BER enzymes in cancer therapy. **DNA Repair (Amst)** 71: 118-126.

P7 - Small molecule–based targeting of TTD-A dimerization to control TFIIH transcriptional activity represents a potential strategy for anticancer therapy

V. Gervais[a], I. Muller[a], P.-O. Mari[a], A. Mourcet[b], K. Tekwani Movellan[a,d], P. Ramos[a], J. Marcoux[a], V. Guillet[a], S. Javaid[c], O. Burlet-Schiltz[a], G. Czaplicki[a], G. Giglia-Mari[b] and A. Milon[a]

[a]*Institut de Pharmacologie et de Biologie Structurale, IPBS, Université de Toulouse, CNRS, Université Paul Sabatier, BP-64182, F-31077 Toulouse, France*
[b]*Université Claude Bernard Lyon 1, INSERM U1217, Institut NeuroMyoGene, CNRS UMR 5310, F-69008 Lyon, France*
[c]*Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center of Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan*
[d] *Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany*
Email: virginie.gervais@ipbs.fr

The TFIIH transcription factor is a huge complex composed of 10 subunits that form an intricate network of protein-protein interactions that are critical for regulating transcriptional and DNA repair activities of TFIIH. The smallest subunit (TTD-A/p8) displays dimerization properties allowing to shift from a homodimeric state in the absence of a functional partner, to a heterodimeric structure with the p52 TFIIH subunit, enabling dynamic binding to TFIIH. Recruitment of p8 at TFIIH stabilizes the overall architecture of the complex, whereas p8's absence reduces its cellular steady-state concentration and consequently decreases basal transcription, highlighting that p8 dimerization may be an attractive target for down-regulating transcription in cancer cells

We considered the dimerization interface of p8 as a "druggable" target. With the aim to design molecules that destabilize the homodimer structure of p8 and alter its recruitment into TFIIH, we applied a fragment-screening strategy based on virtual, biophysical, and NMR screening. We have identified two compounds that bind to and may react with the dimerization motif of p8, provoking destabilization of its protein–protein interface and its precipitation. Using quantitative imaging in living cells, we found that these two compounds have a noticeable effect on concentration and transcription activity of TFIIH.

In conclusion, our study [1] offers p8 as an alternative pharmacological target that could be considered for an effective modulation of transcription. In a more general context where tumor cells require high levels of transcription for proliferation and survival, our work demonstrates the potential of small molecules in targeting protein–protein interactions that are critical for basal transcription machinery, opening new perspectives to design modulators of TFIIH-associated transcription.

[1] Gervais, V., Muller, I., Mari, P. O., Mourcet, A., Movellan, K. T., Ramos, P., Marcoux, J., Guillet, V., Javaid, S., Burlet-Schiltz, O., Czaplicki, G., Milon, A., and Giglia-Mari, G. (2018) Small molecule-based targeting of TTD-A dimerization to control TFIIH transcriptional activity represents a potential strategy for anticancer therapy. **J Biol Chem** 28:14974-14988

P8 - Self-organization and interaction properties of a perfluorinated GCPR binding peptide with a perfluorinated tail: a ^{19}F NMR study

B. Kieffer[a], C. Jourdain de Muizon[a,b], C. Ling[a], D. Bonnet[c], M.A. Delsuc[a,b]

[a] *Institut de Génétique et de Biologie Moléculaire et Cellulaire, IGBMC, Université de Strasbourg, INSERM U596, CNRS UMR7104, Illkirch, France*.
[b] *CASC4DE Le Lodge, 20, Avenue du Neuhof, 67100 Strasbourg, France*
[c] *Faculté de pharmacie, IGBMC, Université de Strasbourg, Illkirch, France*.
E-mail: Kieffer@igbmc.fr

Fluorocarbon-peptide conjugation (FPC) approach provides represents a promising way to enhance the plasma stability of bioactive peptides by enhancing its reversible binding to albumin. This approach has been applied to apelin, a neuro-vasoactive GPCR peptide with potential therapeutic applications for the treatment of cardiovascular diseases and resulted into a sharp increase of *in vivo* efficacy. In order to investigate the underlying molecular mechanisms, fluorine NMR was preformed to study the self-organization properties of the perfluoro-peptide as well as its interaction with serum albumin. The high intrinsic sensitivity provided by the ^{19}F nucleus enabled the measurement of fluorine spectra in a wide range of peptide concentrations enabling the study of the phase transition between micelar and free forms of the apelin. The exchange kinetics between the two forms of the peptide was determined using magnetization transfer experiments. The binding of the fluoro-peptide to the bovine and human serum albumins was studied by fluorine NMR and competition experiments. This work highlights the high potential of using fluorine NMR to study the dynamics of biomolecule's interactions.

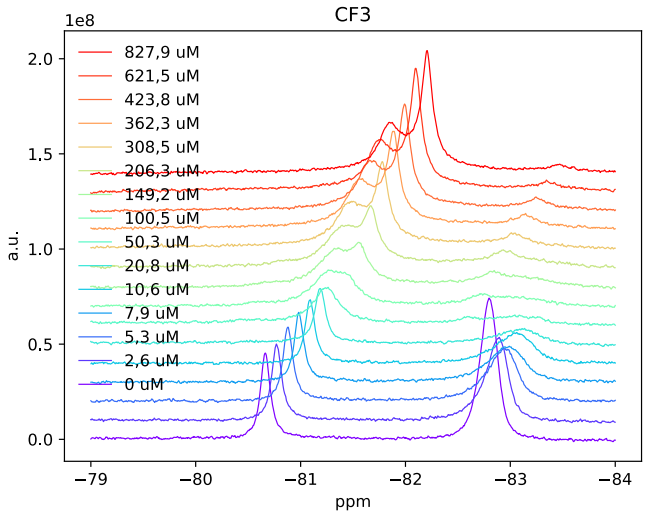


Fig. 1: Titration experiment of a constant concentration of fluoro-apelin (420 µM) by increasing amounts of Bovine Serum Albumin. The two peaks are the signal of the fluoro-methyl group of the fluoro-peptide in the free state (left) and in the micelar state (right).

Table of Content

P9 - Structure-based drug design of specific inhibitors of antibiotic modifying enzymes

E. Chotin[a], E. Kaplan[b], J.F. Guichou[a], M. Gelin[a], G. Labesse[a], C. Lionne[a]

[a] *Centre de Biochimie Structurale CBS, Université de Montpellier, CNRS UMR 5048, INSERM U 1054 34090 Montpellier, France.*
[b] *Department of Pathology, University of Cambridge, Cambridge, United Kingdom.*
Email : lionne@cbs.cnrs.fr

Multidrug resistance (MDR) is a major public health problem requiring urgent development of new antibiotics, as pointed out by the World Health Organization [1]. Modification of aminoglycosides by bacterial enzymes is the major source of resistance against this class of antibiotics [2]. Protein kinase inhibitors, that also inhibit one class of these bacterial enzymes (aminoglycoside phosphotransferases), have been shown to be efficient to restore the sensitivity to aminoglycosides of MDR bacteria [3-5]. However, because these protein kinase inhibitors primarily target human kinases they might have undesirable side effects preventing their clinical use as drug to restore sensitivity to antibiotics. Based on our knowledge of the structures and specificities of aminoglycoside modifying enzymes, our aim is to engineer specific inhibitors of aminoglycoside modifying enzymes in order to restore the bacterial sensitivity to aminoglycosides without affecting human kinases.

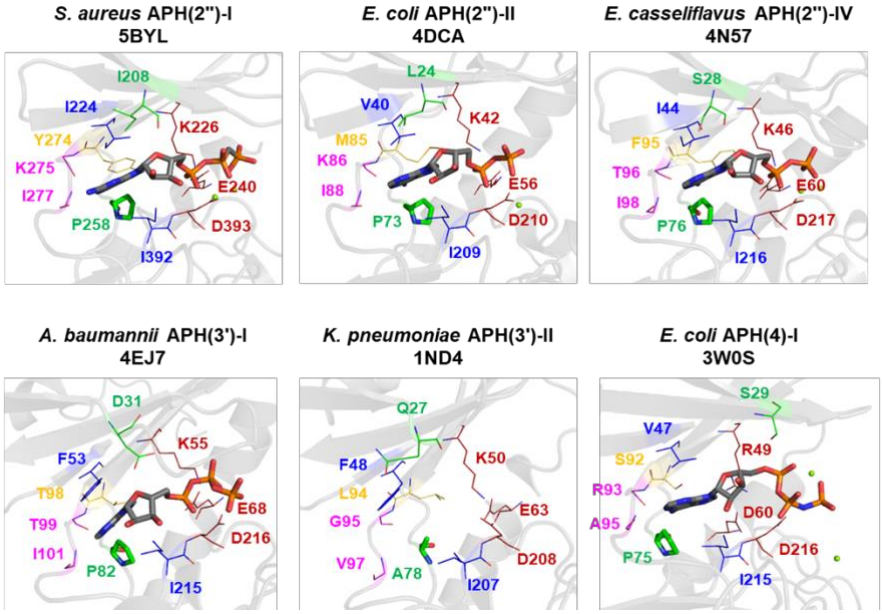


Fig. 1: Structural comparison of the nucleotide binding sites of different APH sub-classes. Nucleotides are shown as sticks, metal ions as small spheres and interacting residues as lines.

[1] https://www.who.int/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed, accession 22th January 2019.
[2] S.B. Vakulenko, S. Mobashery (2003) Versatility of aminoglycosides and prospects for their future. **Clin. Microbiol. Rev.** 16: 430–50.
[3] P.J. Stogios *et al.* (2013) Structure-guided optimization of protein kinase inhibitors reverses aminoglycoside antibiotic resistance. **Biochem. J.** 454: 191–200.
[4] A. Kohl *et al.* (2005) Allosteric inhibition of aminoglycoside phosphotransferase by a designed ankyrin repeat protein. **Structure** 13: 1131–40.
[5] E.D. Scheef, P.E. Bourne (2005) Structural evolution of the protein kinase-like superfamily. **PLoS Comput. Biol.** 1: e49.

P10 - Fragment based ligand discovery in an academic setup

L. Maveyraud [a,b], C. Carivenc [a], J.D. Pédelacq [a], and L. Mourey [a,b]

[a]*Institut de Pharmacologie et de Biologie Structurale, IPBS, Université de Toulouse, CNRS, UPS, 31077, Toulouse, France*.
[b]*Plateforme Intégrée de Criblage de Toulouse, PICT, IPBS, Université de Toulouse, CNRS, UPS, 31077, Toulouse, France*
E-mail: Laurent.Maveyraud@ipbs.fr

To support their drug research programs, pharmaceutical companies have set up huge chemical libraries, with hundreds of thousands or even millions of chemical compounds, that are routinely screened using sophisticated, and expensive, robotic equipment. For an academic laboratory, it is not conceivable to be competitive in this field. Although efficient platforms for high throughput screening (HTS) do exist, chemical libraries are often restricted to a few tens of thousands of compounds. As an example, the French National Chemical Libraries accounts for nearly 90,000 compounds, with close to 55,000 appropriately formatted in 96- or 384-well plates for HTS.
The Fragment-Based Ligand Discovery (FBLD) technology emerged 20 years ago, and contributed 45 drugs in clinical evaluation and 3 approved drugs. FBLD relies on the idea that random small chemical compounds (150-200 Da) are more likely to bind to a macromolecular target than the large drug like compounds (450-550 Da) usually found in corporate libraries. However, binding of such small chemical compounds is usually weak and will not translate into detectable inhibition in classical enzymatic or biological assay; they will therefore not be identified in classical HTS campaign. Detection of such low affinity binders – i.e. expected binding affinity are in the mM range – requires the use of biophysical methods such as ^{1}H-NMR, thermal shift assay, biolayer interferometry. With the advent of crystallization automates, which require small protein volumes, automatic beamlines at synchrotron sites and appropriate data processing and refinement pipelines, it has also become possible to use X-ray crystallography as the primary screening technique.
Here, we will describe the procedure that we used to screen a 939-compounds fragment library using X-ray crystallography. The careful optimization of crystallization conditions is of paramount importance. Crystallization was performed in pre-coated 96-well plates, with individual fragment being deposited in each well prior to protein dispense [1]. About 858 assays yielded crystals, allowing for the collection of 839 datasets, mostly at ESRF and ALBA. 684 structures were determined resulting in 71 structures where a bound ligand could be identified. The use of dedicated ligand-detection procedures, such as PanDDa [2], was instrumental for the detection a weak binders.

[1] M. Gelin et al. (2015). Combining 'dry' co-crystallization and in situ diffraction to facilitate ligand screening by X-ray crystallography. **Acta Crystallogr. D Biol. Crystallogr**. 71:1890
[2] N.M. Pearce et al. (2017). A multi-crystal method for extracting obscured crystallographic states from conventionally uninterpretable electron density. **Nature Com.** 8:15123.

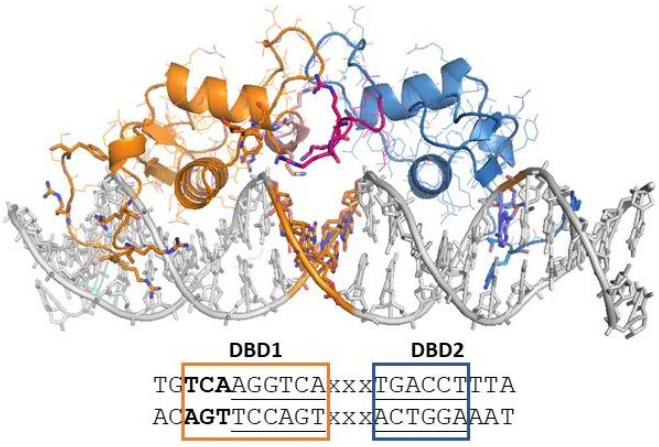
Session II: DNA & RNA Biology

P11 - DNA is the allosteric driver for asymmetric binding of the estrogen-related receptor alpha to its cognate binding sites

K. Mohideen-Abdul, P. Vilela, A. McEwen, I. Hazemann, Y. Chebaro,
B. P. Klaholz, D. Moras and I.M.L. Billas

Centre for Integrative Biology, Department of Integrated Structural Biology, IGBMC, Université de Strasbourg, CNRS UMR 7104, INSERM U1258, 67400, Illkirch, France
E-mail: billas@igbmc.fr

Nuclear receptors (NRs) are transcription factors essential for the proper regulation of the expression of genes involved in major physiological and metabolic pathways. NRs are characterized by two conserved domains, a DNA-binding domain (DBD) composed of two C4 type zinc fingers, and a ligand-binding domain (LBD), which also contains a dimerization interface. Most NRs bind DNA as dimers, either as hetero- or as homodimers on DNA sequences organized as two half-sites with specific orientation and spacing, called response elements (REs), which are present in the promoter-proximal locations or in enhancer regions.
The estrogen-related receptor (ERR) belongs to the steroid hormone nuclear receptor (SHR) family and its DBD shares strong similarity with that of the estrogen receptor (ER). Like ER, ERR binds *in vitro* with high affinity to inverted repeat REs with a 3 bps spacing (IR3), but *in vivo*, it preferentially binds to single half-site REs extended at the 5' end by three base pairs (ERREs), thus explaining why ERR was often inferred as a purely monomeric receptor. Since its C-terminal ligand-binding domain is known to homodimerize with a strong dimer interface, we investigated the binding behavior of the isolated DBD to different response elements using biophysical methods and solved the first crystal structure of homodimeric ERR DBD bound to a natural DNA sequence composed of an extended half-site (ERRE, TCAAGGTCA) embedded into an IR3 RE. Our biophysical and structural results demonstrate that the sequence-driven shape of DNA is essential for the cooperative binding of two ERRα DBD subunits to DNA. Despite a rather symmetrical binding site, the ERRα DBD homodimer features an asymmetrical dimerization interface which is allosterically induced by the DNA conformation and which necessary for proper dimer stabilization. Our results may apply not only to ERR, but also to other members of the SHR family, such as AR or GR, for which a strong well conserved half-site is followed by a weaker one featuring a degenerated sequence.



Overall structure of the asymmetric ERR DBD homodimer bound to a natural ERRE embedded into an IR3 response element at 2.54 Å resolution

P12 - Structural basis of tRNA recognition by cyclodipeptide synthases

G. Bourgeois [a], J. Seguin [b], M. Babin [b], M. Gondry [b], Y. Mechulam [a], E. Schmitt [a]

[a] *Laboratoire de Biochimie, Ecole Polytechnique, Institut Polytechnique, CNRS, 91128 Palaiseau cedex, France*.
[b] *Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris-Sud, Université Paris-Saclay, 91198 Gif-sur-Yvette cedex, France*.
E-mail: gabrielle.bourgeois@polytechnique.edu

CycloDipeptide Synthases (CDPS) catalyze the synthesis of cyclodipeptides, precursors of secondary metabolites with important biological activities. Today the number of putative CDPSs has reached around 800 and about 100 have been experimentally characterized [1]. Phylogenetic studies showed that CDPSs fall into two subfamilies XYP and NYH, characterized by the presence of specific sequence signatures [2]. A recent work showed that the NYH/XYP distinction is indeed observed at the level of the structure of their Rossmann fold [3]. The NYH/XYP subfamilies represent two structural solutions that allow similar positioning of the catalytic residues.
CDPSs use successively two aminoacyl-tRNAs (aa-tRNA) to catalyze the formation of two peptide bonds. The catalytic mechanism begins with the binding of the first aa-tRNA followed by the transfer of the aminoacyl moiety onto the catalytic serine. This aminoacyl-enzyme interacts with the second aa-tRNA to form the dipeptidyl intermediate that undergoes intramolecular cyclisation leading to the final cyclodipeptide [4]. Despite all progress in understanding CDPSs, how they hijack aa-tRNAs from their canonical function in ribosomal protein synthesis remains unknown.
Here, we present the first crystallographic structure of a CDPS:tRNA complex. The crystal structure was solved by molecular replacement using the XYP *Rgry*-CDPS (5MLP) and Phe-tRNA^{Phe} structures as searching models and refined to 5.0 Å resolution. The structure reveals the tRNA binding mode to the enzyme. In particular, we observe that the N and C terminal strands, characteristic of the XYP CDPSs, contact the tRNA acceptor stem. The results will also be discussed in light of the structures of other protein:tRNA complexes involved in translation.

[1] Gondry *et al.*, 2018. A Comprehensive Overview of the Cyclodipeptide Synthase Family Enriched with the Characterization of 32 New Enzymes. **Front Microbiol**. 9:46.
[2] Jacques *et al.*, 2015. Analysis of 51 cyclodipeptide synthases reveals the basis for substrate specificity. **Nat. Chem. Bio** 11:721-732
[3] Bourgeois *et al.*, 2018. Structural basis for partition of the cyclodipeptide synthases into two subfamilies. **JBS**. 203 :17-26.
[4] Moutiez *et al.*, 2014. Specificity determinants for the two tRNA substrates of the cyclodipeptide synthase AlbC from *Streptomyces noursei*. **Nucleic Acids Research**. 42:7247–7258

P13 - Control of PABP1 multifunctionality by lysine acetylation or methylation: are cytoplasmic RNA-binding proteins regulated by 'histone code-like' switches?

E. Dubiez, T. Blee, A. Cook, S. Virdee, N. K. Gray and M. Brook

MRC Centre for Reproductive Health, University of Edinburgh,
E-mail: etienne.dubiez@ed.ac.uk

Poly(A)-binding proteins are multifunctional central regulators of gene expression. The best characterised family member, PABP1, acts as a primary determinant of translation efficiency and stability, regulates specific mRNA fate, and participates in microRNA (miRNA)-mediated regulation and nonsense-mediated mRNA decay (NMD). These disparate, and sometimes antagonistic, functions require PABP1 to interact with numerous, functionally diverse, protein partners. However, many PABP1 partners bind overlapping sites, e.g. up to 16 different PABP-interacting motif 2 (PAM2)-containing proteins compete to bind with similar affinity the same C-terminal PABC domain surface. Thus, it is unclear how these interactions, and therefore different PABP1-mediated functions, are co-ordinated.

Interestingly, PABP1 is subject to extensive, dynamically-regulated, post-translational modification, including mutually-exclusive lysine acetylation and methylation residues that may work as "switches", in a manner reminiscent of the histone code. Specifically, molecular modelling of acetylation or di-methylation of Lysine-606, suggested that modification of PABP1 Lysine-606, which is critical in PABC-PAM2 interactions, may differentially affect these interactions.

Using an orthogonal synthetic biology approach with evolved tRNA-synthetase/tRNACUA pairs, in combination with chemoselective reactions, we have optimised methods for the quantitative, site-specific installation of acetyl-lysine or dimethyl-lysine in recombinant PABP1, allowing comparative kinetic and structural studies using X-ray crystallography. Here we show first pivotal insights into the molecular consequences of PABP1 modification on PABP1-PAM2 interactions that will inform on PABP1 multifunctionality in post-transcriptional control of gene expression.

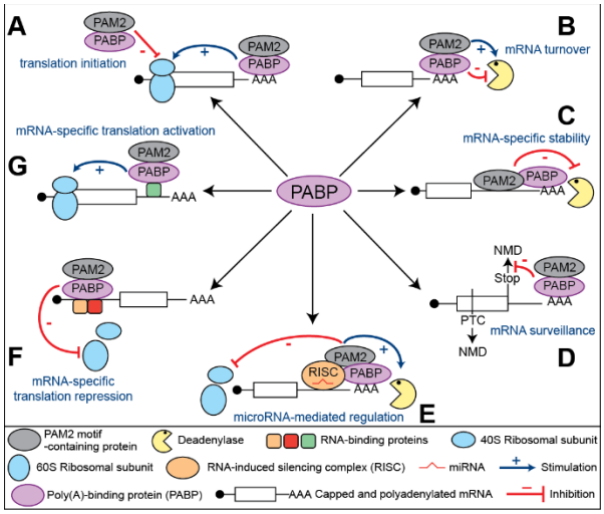


Fig. 1: PAM2 proteins confer PABP multifunctionality

P14 - Structural and functional characterization of human pre-40S ribosome assembly

L. Plassart[a], R. Shayan[a], D. Rinaldi[a], M-F. O'donohue[a], S. Lebaron[a], C. Montellese[b], U. Kutay[b], P-E. Gleizes[a], C. Plisson-Chastang[a]

[a]*Laboratoire de Biologie Moléculaire Eucaryote, Centre de Biologie Intégrative (CBI), Université de Toulouse, CNRS, UPS, 31000 Toulouse, France,*
[b]*Institut für Biochemie, ETH Zurich, Zurich CH-8093, Switzerland*
E-mail: laura.plassart@ibcg.biotoul.fr

The production of ribosomes, the only molecular machine able to synthesize proteins, is one of the most important activity of any living cells. In eukaryotes, ribosome biogenesis is a very complex, multi-step process, which starts in the nucleolus and finishes in the cytoplasm. The correct assembly of both ribosomal subunits requires more than 200 assembly factors for rRNA maturation, ribosomal protein production and subunits assembly [1]. However, the precise activity of most of these assembly factors, as well as the exact chronology of their intervention remain to be determined.
Our project aims at understanding the last cytoplasmic maturation steps of the small ribosomal subunit (also called 40S), in human cells. To reach this goal, we have used both structural and functional approaches. First, using cryo-electron microscopy and single particle analysis methods, we determined atomic 3D structures of pre-40S particles, trapped in very late cytoplasmic maturation steps. Then, using different biochemical strategies, we were able to link and chronologically order these different 3D structures. This work allowed us to better characterize the roles of various proteins involved in the very last stages of maturation of the human small ribosomal subunit, and revealed a new maturation step of the 40S subunit.

[1] Cerezo, E., Plisson-Chastang, C., Henras, A., Lebaron, S., Gleizes, P., & O'Donohue, M. et al. (2018). Maturation of pre-40S particles in yeast and humans. **Wiley Interdisciplinary Reviews: RNA**, 10:e1516. doi: 10.1002/wrna.1516

Session III: Combining methods for the study of supramolecular assemblies

P15 - Blocking antibody targeting the CD73 immunosuppressive pathway

B. Amigues[a], V.Delauzun[a], A.Gaubert[a], S.Spinelli[a], L.Gauthier[b], I.Perrot[b] and A.Roussel[a]

[1]CNRS, Aix Marseille Universite´, AFMB, Architecture et Fonction des Macromole´ cules Biologiques, 13009 Marseille, France
[2]Innate Pharma, 117 Avenue de Luminy, 13009 Marseille, France
E-mail: beatrice.amigues@afmb.univ-mrs.fr

Immune checkpoint inhibitors have revolutionized cancer treatment. However, many cancers are resistant to ICIs, and the targeting of additional inhibitory signals is crucial for limiting tumor evasion. The production of adenosine via the sequential activity of CD39 and CD73 ectoenzymes participates to the generation of an immunosuppressive tumor microenvironment.
In order to disrupt the adenosine pathway, we studied one antibody, IPH5301, targeting human membrane-associated and soluble forms of CD73, and efficiently blocking the hydrolysis of immunogenic AMP into immunosuppressive adenosine.
To get more insight into the mechanism of action of the IPH5301 blocking mAb, we dissected its association with CD73 using different structural approaches. First, the negative staining of the CD73-IPH5301 complex analyzed by electron microscopy revealed that the intact IPH5301 mAb interacts with the N-terminal domains of the CD73 dimers mainly in a 1:1 stoichiometry. Second, we determined the crystal structure of IPH5301 Fab in complex with the ectodomain of CD73 to 2.78A° resolution (PDB: 6HXW). Our data thus support a model for the mode of action of IPH5301, as the intact mAb constrains CD73 in an intermediate state in which AMP could not be hydrolyzed. This model is further supported by the fact that monovalent IPH5301 Fab failed to block membrane associated CD73 enzymatic activity.
To confirm our theory, we produced membrane-associated form of CD73 on extracellular vesicles. We observed the presence of CD73 on vesicles by negative staining. We observed the interaction between IPH5301 mAb and CD73.To go further, a cryoEM study would be necessary. Cryo-tomography would confirm our model of interaction [1].

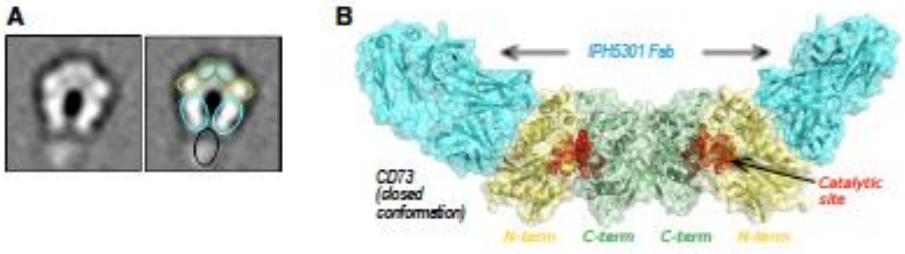


Fig. 1: (A) Negative staining of the CD73-IPH5301 complex. (B) Crysta structure of CD73 and IPH5301Fab complex

[1] I. Perrot *et al*.(2019). Blocking antibodies targeting the CD39/CD73immunosuppressive pathway unleash immune responses in combination cancer therapies. **Cell Reports** 27:2411-2425

P84 - Inactivating mutations of PPARɣ associated with basal bladder tumors

S. Beji, Q. Pippo, C. Peluso-Iltis, N. Rochel

Institut de Génétique et de Biologie Moléculaire et Cellulaire, 1 rue Laurent Fries, BP 10142, 67404 Illkirch
Email: bejis@igbmc.fr

PPARɣ (Peroxisome Proliferator-Activated Receptor Gamma) encodes a transcription factor of the nuclear receptor family that acts as a heterodimer with RXRα. PPARɣ is a key regulator of glucose homeostasis and adipogenesis [1]. In addition to its well-established role in adipocyte differentiation, it has also been shown to be involved in differentiation in several tissues including the urothelium [2]. Several mutations in PPARɣ have been identified in bladder tumors [3]. In this study, inactivating mutations are localized in the important functional ligand binding domain (LBD). Biophysics experiments by nanoDSF (nanoDifferential Scanning Fluorimetry) and MST (Microscale Thermophoresis) revealed that the studied mutants are less stable than the wild-type protein and a decrease of the affinity between the coregulator PGC1-α and the mutants. Cristal structures of these mutants were obtained. We can notice the flexibility around helices 11 and 12, explaining the loss of stability and affinity with coregulators.

[1] Ahmadian, M., Suh, J.M., Hah, N., Liddle, C., Atkins, A.R., Downes, M., Evans, R.M. (2013). PPARγ signaling and metabolism: the good, the bad and the future. **Nat. Med**. 19:557–566.
[2] Varley, C.L. (2004). Role of PPAR and EGFR signalling in the urothelial terminal differentiation programme. **J. Cell Sci**. 117:2029–2036.
[3] Rochel, N., Krucker, C., Coutos-Thévenot, L., Osz, J., Zhang, R., Guyon, E., Zita, W., Vanthong, S., Hernandez, O.A., Bourguet, M., Badawy, K.A., Dufour, F., Peluso-Iltis, C., Heckler-Beji, S., Dejaegere, A., Kamoun, A., de Reyniès, A., Neuzillet, Y., Rebouissou, S., Béraud, C., Lang, H., Massfelder, T., Allory, Y., Cianférani, S., Stote, R.H., Radvanyi, F., Bernard-Pierrot, I., (2019). Recurrent activating mutations of PPARγ associated with luminal bladder tumors. **Nat. Commun**. 10.

P16 - Structural Basis of Membrane Protein Chaperoning within the Mitochondrial Intermembrane Space

K. Weinhäupl[a], I. Sucec[a], O. Dakhlaoui[a], C. Lindau[b], Y. Wang[c], C. Schütze[b], A. Hessel[a], T. Jores[d], H. Kalbacher[d], M. Brennich[e], B. Bersch[a], D. Rapaport[d], K. Lindorff-Larsen[c], N. Wiedemann[b], Paul Schanda[a]

[a] *Institut de Biologie Structurale (IBS), UGA-CNRS-CEA, 38044 Grenoble, France*.
[b]*Institut für Biochemie und Molekularbiologie, Universität Freiburg, Germany.*
[c]*The Linderstrøm-Lang Centre for Protein Science, University of Copenhagen, Denmark.*
[d]*Interfaculty Institute of Biochemistry, University of Tübingen, Germany*
[e]*European Molecular Biology Laboratory, Grenoble, France*
E-mail: beate.bersch@ibs.fr

Mitochondria are ubiquitous eukaryotic cell organelles that are surrounded by two membranes. 99% of all mitochondrial proteins are not synthesized in the mitochondrion, but in the cytosol. Mitochondria therefore possess different protein translocases that shuttle mitochondrial protein precursors to their particular destinations, depending on specific import signals. In this context, the transport of membrane proteins is particularly critical, due to their high propensity to precipitate in aqueous environments. After translocation through the TOM complex, the precursors are taken over by ATP-independent complexes of small Tim chaperones in the mitochondrial intermembrane space. Two hexameric complexes comprising TIM9/10 or TIM8/13 have been identified in yeast. Chaperoned by these Tim complexes, the precursors are guided to their respective insertion machinery in the outer or inner mitochondrial membrane.
We have developed an experimental approach for the reproducible formation of complexes between the aggregation-prone membrane protein precursors and the recombinant, hexameric TIM9/10 or TIM8/13 chaperones. We first employed an integrated structural biology approach, combining NMR, SAXS, *in-vivo* experiments and various biophysical methods with data-guided molecular dynamics simulations to determine the structural ensemble and dynamics of a chaperone-preprotein complex [1]. Our findings decipher how the small TIM hexameric complexes protect both α-helical and β-barrel proteins within the mitochondrial intermembrane space. The precursor protein binds to a conserved cleft on the chaperone in a highly dynamic manner, ensuring high overall affinity through avidity of multiple binding sites. We also found that the stoichiometry of TIM9/10 or TIM8/13 in complex with different preproteins depends on the substrate-protein length. Recently, we addressed the relative affinity of TIM9/10 and TIM8/13 chaperones for different substrate proteins and further characterized the complex formed by TIM8/13 and Tim23 in which the IDP-like N-terminal part of Tim23 was found to interact with the hexameric chaperone.

[1] Weinhäupl, K., Lindau, C., Hessel, A., Wang, Y., Schütze, C., Jores, T., Melchionda, L., Schönfisch, B., Kalbacher, H., Bersch, B., Rapaport, D., Brennich, M., Lindorff-Larsen, K., Wiedemann, N., Schanda, P. (2018) Structural basis of membrane protein chaperoning through the mitochondrial intermembrane space. **Cell** 175 :1365-1379

P17 - Nucleosomes as a target for HIV-1 pre-integration complexes

E. Bruch[a], J. Batisse[a], K. Mohideen Abdul[a], N. Levy[a], S. Eiler[a], Y. Mely[b], V. Parissi[c], M. Lavigne[d], S. Bouaziz, O. Delelis[e], B. Klaholz[a], M. Ruff[a]

[a] *IGBMC, Department of Integrative Structural Biology, UDS, U596 INSERM, UMR7104 CNRS Illkirch, France.*
[b]*LBP, Faculté de Pharmacie, UDS, UMR7213 CNRS, Illkirch, France.*
[c]*Fundamental Microbiology and Pathogenicity UMR5234 CNRS-Bordeaux university*
[d]*Institut Cochin, Inserm U1016 - CNRS UMR8104 - Paris Descartes university*
[e]*LBPA UMR 8113 CNRS - ENS Cachan*
Email: bruche@igbmc.fr

Viral integrase (IN) is involved in several steps of the viral life cycle including reverse transcription, nuclear import, chromatin targeting and DNA integration. The diversity of roles for IN requires the interaction with several cellular and viral partners. Following reverse transcription, HIV double stranded DNA interacts with cellular and viral components, such as IN or LEDGF, to form the pre-integration complex (PIC). The location of retroviral integration into the human genome is thought to play a role in the clonal expansion of infected cells and HIV persistence. The chromatin status and the presence of IN partners can affect DNA integration. For example, LEDGF modulates the DNA integration site specificity by preferentially targeting transcriptionally active sites. A better understanding of the IN molecular partners in the context of the DNA integration and the dynamics of the integration event are key to fully understand HIV infection. We decided to focus in the interaction of the IN containing complexes and nucleosomes. The lab master methodologies that enable the production of stable complexes for structural and functional studies [1] as well as system to produce multi-protein complexes from mammalian cells enabling assembly of entire complexes within cells. Previous results showed the role of the C-terminal domain of IN in the recognition of N-terminal Histone tails [2, 3]. Currently we are characterizing the interaction of IN/LEDGF and intact human nucleosomes. We succeeded in the generation of IN/LEDGF/nucleosome complexes that were characterized using pull-down, gel filtration and native gels, among other techniques. Currently we are using electron microscopy (both using negative stained and in cryo samples) to characterize structurally the complexes. Our results will help us to understand the structure-function relationships and the molecular mechanisms of the integration reaction as well as to make drug design studies for the definition of integrase allosteric inhibitors.

[1] Levy N, *et al*. (2016) **Nature communications** 7: 10932
[2] Mauro E, *et al*. (2019) **Nucleic Acids Research**, doi: 10.1093/nar/gkz091, in press.
[3] Benleulmi, MS, *et al.* (2017) **Retrovirology** 14:54.

P18 - Intermediate resolution crystal structure of the adenovirus AD3 fibre knob in complex with the EC2-EC3 fragment of desmoglein 2

W. P. Burmeister, E. Vassal-Stermann, S. Hutin and P. Fender

Institut de Biologie Structurale, CEA, CNRS, Université Grenoble Alpes,
71 avenue des Martyrs, F-38042 Grenoble, France.
E-mail: wim.burmeister@ibs.fr

Adenoviruses are dsDNA viruses with an icosahedral capsid where a trimeric fibre protein terminated by a receptor binding knob is inserted into the vertices. Adenoviruses are wide-spread pathogens with potential applications in oncology and gene therapy, which use different cell-surface proteins as receptor. The cryo-electron microscopy (cryo-EM) of the complex between the human adenovirus Ad3 trimeric fibre knob and human desmoglein-2 fragments containing cadherin domains EC2 and EC3 has been published recently showing 3:1 and 3:2 complexes [1]. Here we present the crystal structure determined at 4.5 Å resolution with one EC2-EC3 desmoglein fragment bound per fibre knob monomer in the asymmetric unit leading to an apparent 3:3 stoichiometry. However, in solution the 3:3 stoechiometry has never been observed, only 3:1 and 3:2 complexes are present. A majority of 3:2 complex is present in small-angle X-ray scattering (SAXS) experiments, while the cryo-EM structure using lower sample concentrations showed a majority of 3:1 complex. It is likely that more or less tight binding sites are distributed randomly around the crystallographic 3-fold axis limiting the resolution of the diffraction experiments. Substitution of the calcium ions bound to the desmoglein domains by terbium ions allowed confirmation of the X-ray structure using their anomalous scattering and shows that at least one binding site per cluster of calcium ions is intact and exchangeable and, combined with SAXS data, that the cadherin domains are folded even in the distal part invisible in the cryo-EM reconstruction.

[1] Vassal-Stermann, E., Effantin, G., Zubieta, C., Burmeister, W., Iseni, F., Wang, H., Lieber, A., Schoehn, G. & Fender, P. (2019). CryoEM structure of adenovirus type 3 fibre with desmoglein 2 shows an unusual mode of receptor engagement. **Nat. Commun**. 10:1181

P19 - Structural study of proteins from an integrative and conjugative element involved in horizontal gene transfer in *Streptococcus thermophilus*

J. Cappèle [1], B. Douzi [2], A. Mohammad Ali [2], N. Leblond [2], P. Tsan [1], C. Didierjean [1], F. Favier [1]

[1] *Laboratoire de Cristallographie, Résonance Magnétique et Modélisations, CRM², UMR CNRS-UL 7036, Université de Lorraine, Vandoeuvre-Lès-Nancy, France.*
[2] *Dynamique des Génomes et Adaptation Microbienne, DynAMic, UMR CNRS-INRA 1128, Université de Lorraine, Vandoeuvre-Lès-Nancy, France.*
E-mail : julien.cappele@univ-lorraine.fr

Antimicrobial resistance has emerged as a major threat to public health since the 2000s [1]. In Gram-positive bacteria, integrative and conjugative elements (ICE) are one of the most common means for the transfer of virulence and antibiotic resistance genes [2]. These genome-integrated sequences are excised from the donor cell, duplicated and sent to the recipient cell through a conjugation pore. Then they both reintegrate their respective cell genome. If the conditions are met, the recipient cell is able to repeat the same process [2]. It is proposed that all the genes necessary for the mechanism are included in the transferred and integrated sequence [2]. One of the large protein complexes responsible for this cell-to-cell transfer is called T4SS (Type IV Secretion Systems). When compared to similar transfer systems in Gram-negative bacteria, these elements all reveal proteins that support 1) the replication and transfer of the conjugative element, 2) the assembly of a transfer transmembrane macro-complex, 3) various enzymatic activities such as ATPases or peptidoglycan hydrolase [3]. By NMR and X-Ray crystallography, our group studies proteins of the integrative and conjugative element ICESt3 of *Streptococcus thermophilus*. Here, we will present preliminary results for two of them. The study of these isolated proteins will help in understanding the molecular mechanism of ICE gene transfer, and in building a model of the Gram-positive type-IV secretion system.

[1] Ventola, C. L. (2015). The Antibiotic Resistance Crisis: Part 1: Causes and Threats. **P. T.** 40(4): 277.
[2] Johnson, C. M. and A. D. Grossman (2015). Integrative and Conjugative Elements (ICEs): What They Do and How They Work. **Annu Rev Genet** 49: 577-601.
[3] Goessweiner-Mohr, N., K. Arends, W. Keller and E. Grohmann (2014). Conjugation in Gram-Positive Bacteria. **Microbiol. Spectr.** 2: 0004--2013.

Bellanger, X., A. P. Roberts, C. Morel, F. Choulet, G. Pavlovic, P. Mullany, B. Decaris and G. Guedon (2009). Conjugative transfer of the integrative conjugative elements ICESt1 and ICESt3 from Streptococcus thermophilus. **J. Bacteriol.** 191:2764-2775.
Carraro, N. and V. Burrus (2014). Biology of Three ICE Families: SXT/R391, ICEBs1, and ICESt1/ICESt3. **Microbiol. Spectr.** 2(6).
Grohmann, E., P. J. Christie, G. Waksman and S. Backert (2018). Type IV secretion in Gram-negative and Gram-positive bacteria. **Mol. Microbiol.** 107:455-471.

P20 - Biophysical characterization of Vitamin D receptor activities modulated by RXR ligands

S. Chalhoub [a], D. Rovito [b], A. I. Rerra [b], G. Laverny [b], R. Natacha [a]

[a]*Integrative Structural Biology Department, Institute of Genetics and Molecular and Cellular Biology (IGBMC), Illkirch, France* [b]*Functional Genomics and cancer Department, Institute of Genetics and Molecular and Cellular Biology (IGBMC), Illkirch, France / Centre National de Recherche Scientifique (CNRS) UMR 7104 / University of Strasbourg*.

E-mail: chalhous@igbmc.fr

The active form of vitamin D, calcitriol, is a key hormone for the regulation of calcium homeostasis [1]. Calcitriol controls many biological functions, including the proliferation and differentiation of many cells, and has anti-inflammatory and immune activities modulatory [2]. It regulates the expression of many target genes by binding to the nuclear receptor Vitamin D (VDR). VDR is thus an important therapeutic target for various pathologies (osteoporosis, neurodegenerative diseases, autoimmune diseases and cancers) [3].

VDR acts as a heterodimer with the retinoid X nuclear receptors (RXRs) [4], to control the transcription of target genes. Contradictory data in the literature about the role of RXR ligands Transcriptional Activity mediated by VDR. To study the effects of RXR ligands on the VDR interactions and transcriptional activities induced by various VDR ligands and to determine if RXR ligands can synergize ligand activities of VDR, we combined biophysical methods. Biacore Surface Plasmon Resonance and Micro Scale Thermophoresis have been used to quantify the effect of RXR ligand on DNAs binding and hetero-dimerization. For the DNA binding we choose different genes response elements characterized to be regulated by VDR.

[1] Brown AJ, Dusso A, Slatopolsky E. (1999). Vitamin D. **Am J Physiol**. 277(2):F157-75.

[2] Campbell, M. J. & Adorini, L. (2006). The vitamin D receptor as a therapeutic target. **Expert Opin Ther Targets**. 10:735–48.

[3] Chiang, K.-C. & Chen, T. C. (2013).The anti-cancer actions of vitamin D. **Anticancer Agents Med Chem**. 13:126–39.

[4] Bouillon R[1], Carmeliet G, Verlinden L, van Etten E, Verstuyf A, Luderer HF, Lieben L, Mathieu C, Demay M. (2008). Vitamin D and human health: lessons from vitamin D receptor null mice. **Endocr Rev**. (6):726-76.

P21 - Multidisciplinary approaches for the study of structure-activity relationships of GH70 glucansucrases

G. Cioci,[a] M. Claverie,[b] P. Roblin,[c] M. Guionnet,[1] J. Schörghuber,[d] R. Lichtenecker,[d] C. Moulis,[1] G. Lippens[1] and M. Remaud-Simeon[1]

[a] TBI, INSA-Toulouse, France ; [b] IPREM-University of Pau, France ; [c] LGC, Toulouse, France
[d] Institute for Organic Chemistry, University of Vienna, Austria
E-mail: cioci@insa-toulouse.fr

Glucansucrases belonging to the GH70 Family (CAZY) represent an important class of enzymes that are widely used for biotechnology and industrial applications. Glucansucrases are able to catalyze the polymerization of glucose units forming a wide range of α-glucan polymers using sucrose, an abundant and inexpensive agro resource, as the sole substrate. Therefore, the understanding of structure-activity relationships of GH70s is essential for the development of tailor-made enzymes able to synthesize polysaccharides or oligosaccharides with precise chemical structure and molecular weight. Glucansucrases are difficult-to-study enzymes, given their high molecular weight (100-250 kDa), their multi-domain organization with intrinsic flexibility and the complexity of the products formed.
In the present work, we have applied a combination of multidisciplinary techniques to structurally and functionally characterize the DSR-M enzyme, an efficient dextransucrase that is able to synthetize very linear dextrans with high specificity (99% of α-1,6 linkages).[1] The crystal structure of DSR-M has been solved in complex with substrates and acceptor molecules which revealed the important residues and the structural features that play a role in the catalysis. Small angle X-ray scattering confirmed the *horseshoe* structure of DSR-M which shows a unique glucan binding domain (GBD) that plays a role into dextran binding and elongation. [2]
Nuclear magnetic resonance on the ^{15}N labelled enzyme has been used to get insights into the active site dynamics of DSR-M and to characterize one interesting mutant (Trp624Ala) which shows a product distribution shifted to the formation of shorter dextran chains. Finally, using a Monte Carlo simulation of the elongation process, we shed new lights on the mechanistic behaviour of sugar polymerases, explaining how the probability of enzyme-acceptor encounters directly affects the product distribution length. [3]

[1] D. Passerini, M. Vuillemin, L. Ufarté, S. Morel, V. Loux, C. Fontagné-Faucher, P. Monsan, M. Remaud-Siméon, C. Moulis. (2015). Inventory of the GH70 enzymes encoded by Leuconostoc citreum NRRL B-1299 - identification of three novel α-transglucosylases **FEBS J** 282:2115–2130.
[2] M. Claverie, G. Cioci, M. Vuillemin, N. Monties, P. Roblin, G. Lippens, M. Remaud-Simeon, C. Moulis. (2017). Investigations on the Determinants Responsible for Low Molar Mass Dextran Formation by DSR-M Dextransucrase **ACS Catalysis** *7*:7106–7119.
[3] M. Claverie, G. Cioci, M. Guionnet, J. Schörghuber, R. Lichtenecker, C. Moulis, M. Remaud-Simeon, G. Lippens G. (2019). Futile Encounter Engineering of the DSR-M Dextransucrase Modifies the Resulting Polymer Length **Biochemistry** DOI:10.1021/acs.biochem.9b00373.

P22 - Understanding the recognition mechanism driving to the covalent association of the Jo/In complex

N. Cox[a], C. Y. Montanier[a], T. Vernet[b], A.-M. Di Guilmi[C] & G. Lippens[a].

[a]Toulouse biotechnology institute, TBI, Université de Toulouse, CNRS, INRA, INSA, 31077 Toulouse, France ; [b]1Institut de Biologie Structurale, IBS, Université Grenoble Alpes, CEA, CNRS, 38044 Grenoble, France ; [c]CEA, 18 route du panorama, 92265, Fontenay-aux-roses, France

Email : ncox@insa-toulouse.fr

Spatial arrangement of proteins is a crucial feature in nature, where enzymes are clustered towards so called substrate channelling, where spatial organisation offers kinetic advantages. Such organisation is a challenge to consider *in vitro*, especially for highly complex scaffolds [1].
The Jo-In complex, derived from *streptococcus pneumoniae* pili [2] is composed of two small (~15 kDa) proteins that present the ability to spontaneously covalently bind together. This biotechnological tool is used as expression tags to covalently link proteins together and offers interesting features towards the *in vitro* reconstruction of spatially resolved enzymatic sub complexes [3]. However, the reconstruction of these natural scaffold would gain from the availability of multiple specific pairs, but to date only one Jo-In pair is available.
With the goal of engineering the Jo-In complex to create new specific pairs, the mechanism by which the recognition and interaction of the two proteins occurs is first to be understood. Although the catalytic mechanism of isopeptidic covalent bond formation has been studied [4], the overall recognition and interaction mechanism of the complex is yet to be described. Using NMR based experiments, we highlight a strong flexibility of the proteins in their free state, followed by a strong structuration event upon biding towards a globular complex. Using inactivated mutant proteins, we further characterized and decomposed the interaction and recognition mechanism, highlighting that protein-protein interaction is independent but required for covalent bond formation. Overall, these data allow a better understanding of the recognition mechanism of the Jo-In pair, and highlight promising leads towards interface engineering.

[1] C.M.G.A. Fontes & H.J. Gilbert. (2010). Cellulosomes: highly efficient nanomachines designed to deconstruct plant cell wall complex carbohydrates. **Annual Review of Biochemistry** 9:655–81
[2] J. Bonnet, J. Cartannaz, G. Tourcier, C. Contreras-Martel, J. P. Kleman, C. Morlot, T. Vernet & A. M. Di Guilmi. (2017). Autocatalytic association of proteins by covalent bond formation: a Bio Molecular Welding toolbox derived from a bacterial adhesin. **Scientific reports** 7:43564
[3] C.Y. Montanier, M. Fanuel, H. Rogniaux, D. Ropartz, A.M. Di Guilmi & A. Bouchoux. (2019). Changing surface grafting density has an effect on the activity of immobilized xylanase towards natural polysaccharides. **Scientific reports** 9:5763
[4] R.M. Hagan, R. Björnsson, S.A. McMahon, B. Schomburg, V. Braithwaite, M. Bühl, J.H. Naismith, and U. Schwarz-Linek. (2010). NMR spectroscopic and theoretical analysis of a spontaneously formed Lys–Asp isopeptide Bond. Angewandte Chemie International Edition 49:8421 –8425

P23 - Structure elucidation of helical aromatic foldamer-protein complexes

S. Post [a], B. Langlois d'Estaintot [a], T. Granier [a], C. Mackereth [b], L. Fischer [a], I. Huc [c]

[a] CBMN (UMR5248), Univ. Bordeaux – CNRS – IPB, Institut Européen de Chimie et Biologie, 33600 Pessac, France; [b] ARNA (U1212), Univ. Bordeaux – INSERM – CNRS, Institut Européen de Chimie et Biologie, 33600 Pessac, France; [c] Department Pharmazie and Center for Integrated Protein Science Ludwig-Maximilians-Universität, D-81377 München, Germany.

E-mail: l.fischer@iecb.u-bordeaux.fr

Inspired by naturally folding biomolecules, aromatic foldamers[1] emerge as a new class of folded synthetic oligomers that may be decorated with proteinogenic side chains to interact with proteins and eventually serve as modulators of protein-protein interactions (PPIs)[2].
Our group has developed methodologies to produce highly functionalized aromatic amide quinoline-based foldamers. With their medium size compatible with the large surface areas involved in PPIs, their stability and their well-defined structures, these foldamers are good candidates to recognize protein surfaces.
As first steps towards the design of protein surface ligands based on these scaffolds, we have explored an anchoring approach that consists in confining a foldamer at the surface of a protein to investigate foldamer-protein interactions even in case of weak binding. Following this strategy, we have identified several aromatic foldamers interacting with protein surfaces.
Different crystal structures of the complexes were obtained allowing the analysis of the mode of action of protein recognition by the foldamer ligands which act as supramolecular inducers of protein assemblies[3]. These complexes were also studied in solution by circular dichroism, SPR as well as native mass spectrometry and NMR[4]. These results constitute the basis of modeling studies towards the rational design of selective host foldamer ligands.

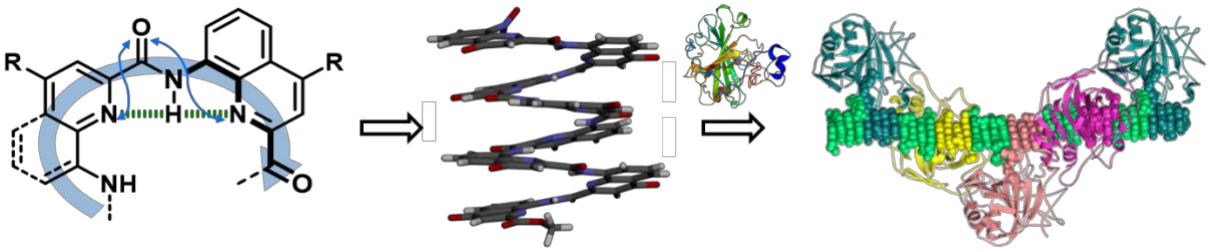


Fig. 1: Scaffold of an aromatic oligoamide foldamer equipped with proteinogenic side chains (R) able to adopt a helical structure and drive protein/foldamer complex supramolecular assemblies.

[1] G. Guichard, I. Huc, (2011), **Chem. Commun.**, 47:5933–5941.
[2] S. van Dun, C. Ottmann, L.-G. Milroy, L. Brunsveld, (2017), **J. Am. Chem. Soc.**, 139:13960–13968.
[3] J. Buratto, et al., (2014), **Angew. Chem. Int. Ed.**, 53:883–887; M. Jewginski, T. Granier, B. Langlois d'Estaintot, L. Fischer, C. D. Mackereth, I. Huc, (2017), **J. Am. Chem. Soc.**, 139: 2928–2931; M. Jewginski, L. Fischer, C. Colombo, I. Huc, C. D. Mackereth, (2016), **ChemBioChem**, 17:727–736.

P24 - Structural dissection of amyloid fibrils of different TDP-43 constructs by solid-state NMR

J. S. Krishnashenoy Padmabai[a], N. El Mammeri[a], A. Dutour[a], M. Berbon[a], B. Habenstein[a], A. Loquet[a], F.-X. Theillet[b], B. Kauffmann[c]

[a] *CBMN, IECB (CNRS UMR 5248), 33600 Pessac, France*
[b] *LSBR, IIBC (CEA, CNRS, University Paris South), Gif-sur-Yvette, France*
[c] *IECB (CNRS INSERM UMS3033, US001), 33600 Pessac, France*
E-mail: jayakrishna-shenoy.krishnashenoy-padmabai@u-bordeaux.fr

The TAR DNA binding protein of 43 kDa (TDP-43) is observed as the main component in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar dementia (FTLD) cytoplasmic inclusions [1]. TDP-43 consists of a well-folded N-terminal domain (NTD), two RNA recognition motif domains (RRM1 and RRM2) and an intrinsically disordered C-terminal domain. The prion-like C-terminal domain possesses most of the pathologically relevant mutations and plays a critical role in the spontaneous aggregation of TDP-43 and associated proteinopathy [2, 3]. For a detailed structural analysis of the amyloid-forming C-terminal region, we have analyzed the full-length TDP-43, two C-terminal fragments (TDP-35 and TDP-16) and a C-terminal truncated fragment (TDP-43 ΔGaroS2) in their fibrillar state. Although the different protein constructs exhibit similar fibril morphology and a typical cross-β signature by X-ray diffraction, solid-state NMR indicates that TDP-43 and TDP-35 share the same polymorphic molecular structure, while TDP-16 encompasses a well-ordered amyloid core. We identified several residues in the so-called C-terminal GaroS2 region that participate in the rigid core of TDP-16 fibrils, underlining its importance during the aggregation process. Our findings demonstrate that C-terminal fragments can adopt a different molecular conformation in isolation or in the context of the full-length assembly, suggesting that the N-terminal domain play a significant role in the TDP-43 amyloid transition [4].

[1] Neumann, M. et al. (2006) **Science** 314:130–3
[2] Johnson, B. S. et al. (2009) **J. Biol. Chem**. 284:20329–20339
[3] Pesiridis, G. et al. (2009) **Hum. Mol. Genet**. 18
[4] Shenoy et al., manuscript submitted

P25 - Thermodynamics of antimicrobial peptides binding to different bacterial ribosomes

A-M. Lobstein[a], C. Silva Da Veiga[a], K. Brillet[a], Q. Vicens[a], A. Simonetti[a], Y. Hashem[b], A. Innis[b], D. Burnouf[a]

[a] *CNRS-UPR 9002, Institut de Biologie Moléculaire et Cellulaire, IBMC, 67084, Strasbourg, France.*
[b] *Institut Européen de Chimie et Biologie, IECB, 33600, Pessac, France.*
E-mail: am.lobstein@ibmc-cnrs.unistra.fr

The current worldwide emergence of bacteria resistant to most commercialized antibiotics is a major health issue [1], exacerbated by diminishing pharmaceutical innovations. Research into antimicrobial peptides, naturally occurring peptides possessing antibacterial activity but with a radically different structure than classic antibiotics, may be one way to find new strategies to address this persistent problem.
We focused our attention on PrAMPs (Proline-rich AntiMicrobial Peptides), derived from insects. PrAMPs block the protein exit tunnel of bacterial ribosomes (70S), thus inhibiting translation. Only Gram-negative bacteria possessing the SbmA transporter (such as *Escherichia coli*) are naturally sensitive to PrAMPs [2].
However, could PrAMPs bind in the same way to ribosomes of naturally resistant bacteria (lacking the SbmA transporter)? Isothermal Titration Calorimetry (ITC) was used to characterize the interaction of a PrAMP, pyrrhocoricin (Pyrr), with ribosomes of bacteria naturally sensitive (*E. coli*) or naturally resistant to PrAMPs: a Gram-negative bacteria, *Pseudomonas aeruginosa*, and two Gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*. Preliminary cryo-EM structures of Pyrr bound to the *P. aeruginosa* ribosome will be analysed to understand the differences in thermodynamic and kinetic data.

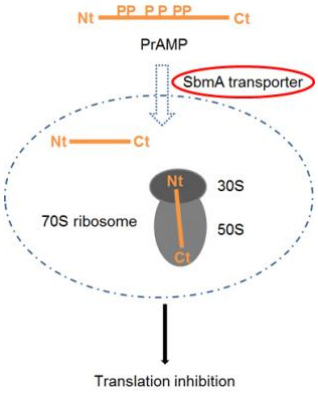


Fig. 1: Gram-negative bacteria possessing the SbmA transporter are sensitive to PrAMPs. (P : proline)

[1] J. O'Neill (2014). Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations. **The Review on Antimicrobial Resistance** p5
[2] A. C. Seefeldt, *et al.* (2015). The proline-rich antimicrobial peptide Onc112 inhibits translation by blocking and destabilizing the initiation complex. **Nature Structural and Molecular Biology** 22:470–475

P26 - Structural study of the CagI/CagL complex from the Cag-type IV secretion system of *Helicobacter pylori*

Losfelt M. [a], Bergé C. [a], Bezault A. [b], Fronzes R. [b,c], Terradot L. [a]

[a] *CNRS- Université Lyon UMR5086, Molecular Microbiology and Structural Biochemistry, Lyon, France.*
[b] *Institut Européen de Chimie et Biologie, University of Bordeaux, Pessac, France.*
[c] *CNRS UMR 5234 Microbiologie Fondamentale et Pathogénicité, Bordeaux, France*
E-mail : marine.losfelt@ibcp.fr

Helicobacter pylori is a Gram-negative bacterium that colonises the human stomach in half of the world population and can cause ulcers and gastric cancers. The most virulent strains of the bacteria produce the oncoprotein CagA, and the "cag" type IV secretion system (*cag*T4SS) used by the bacteria to deliver CagA into the host cell, thereby promoting cancer development. The *cag*T4SS is composed of 27 proteins that assemble into a molecular nanomachine that spans both bacterial membranes (Figure 1). This assembly is composed a cytoplasmic complex, a core complex forming a channel in the periplasm and an external pilus that contact directly host cells. Three proteins CagI, CagL and CagH, produced from a single operon, were found to form a complex *in vivo* and are essential for the function of the *cag*T4SS [1]. *H. pylori* strains deleted of CagI or CagL are unable to produce pilus while strains deleted of CagH present longer and thicker pili. These data suggest that the three proteins are involved in the *cag*T4SS pilus assembly. To understand the link between CagI, CagL and CagH, we have performed protein-protein interaction studies. We have first used the bacterial two-hybrid to determine the interaction network. The individual proteins were then purified and microscale thermophoresis experiments were carried out to characterize these interactions. The results prompted us to investigate the CagI/CagL complex. The two proteins were co-expressed in *E. coli* and the complex purified by affinity chromatography after membrane extraction. This complex was analysed by cryo-electron microscopy. These preliminary studies allowed us to obtain a first low-resolution three-dimensional structure of the complex CagI/CagL.

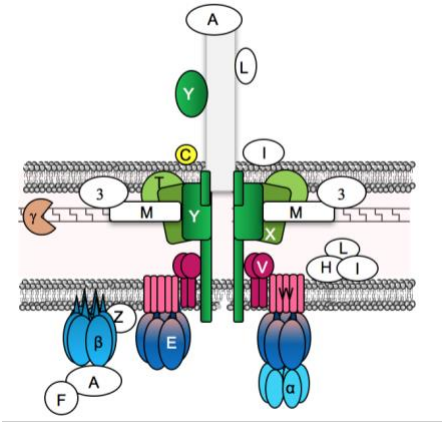


Fig. 1: Schematic representation of the cagT4SS of Helicobacter pylori

[1] Shaffer *et al*. (2011). *Helicobacter pylori* exploits a unique repertoire of type IV secretion system components for pilus assembly at the bacteria-host cell interface. **PLoS Pathog** 7:e1002237

P27 - Packaging of RNAs and polyelectrolytes in viral capsids

L. Marichal[a], R. Leite Rubim[a], S. Bressanelli[b], G. Tresset[a]

[a] Laboratoire de Physique des Solides, CNRS, Univ. Paris-Sud, Université Paris-Saclay, 91405 Orsay Cedex, France. [b]Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris-Sud, Université Paris-Saclay, 91198 Gif-sur-Yvette Cedex, France

E-mail: laurent.marichal@u-psud.fr

Single-stranded (ss)RNA viruses are ubiquitous pathogens that possess a relatively simple structure consisting of genomic RNAs packaged into protein shell called capsid. Despite a great deal of work on the structural and molecular biology of viruses the physicochemical mechanisms underlying the in vivo viral life cycles are still elusive. In particular, how viral RNAs (and not non-viral ones or other molecules) get selectively encapsidated is still a mystery.

One model virus called cowpea chlorotic mottle virus (CCMV) was used for this study. Its capsid possesses an icosahedral shape and is made of 180 identical capsid proteins (CP). Using purified CP, we studied the capsid assembly in the presence of either RNAs or synthetic polyelectrolytes mimicking RNAs. A key step of the virion formation is the intermediate state called nucleoprotein complex (NPC) where CPs and RNA interact with each other but don't form a capsid. We previously showed that under the same physicochemical condition, CPs mixed with RNAs formed NPCs while CPs mixed with polyelectrolytes could form well-structured filled capsids [1]. In order to see more subtle differences, we compared the capsid formation in the presence of either homologous or heterologous RNAs. To do so three complementary approaches have been used: time-resolved small-angle X-ray scattering (TR-SAXS), isothermal titration calorimetry (ITC), and small-angle neutron scattering (SANS, work in progress). SAXS data showed that, under the same conditions, CPs mixed with homologous or heterologous RNAs formed respectively capsids and NPCs (Fig. 1). This may indicate a potential sequence or structural specificity that had not been seen before. However, based on the ITC data (Fig. 2) not significant differences could be found between RNAs both in terms of affinity or thermodynamics.

These results could help us understanding how (ss)RNA viruses manage to package selectively their genome and to assemble their capsid in an error-free manner, within a short time frame.

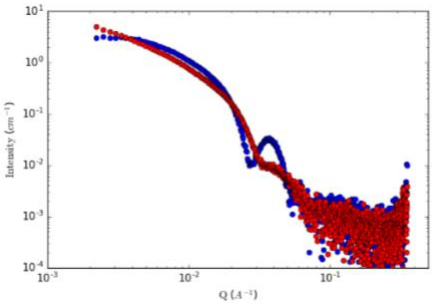


Fig. 1: SAXS curves of CCMV CP mixed with homologous RNA (blue curve) or heterologous RNA (red curve) under NPC forming conditions

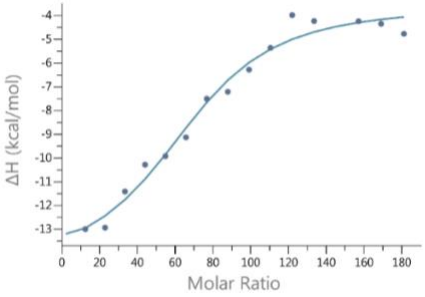


Fig. 2: ITC curve of CCMV CP interacting with homologous RNA under NPC forming conditions. Heterologous RNA gives similar results.

[1] Chevreuil M., Law-Hine D., Chen J., Bressanelli S., Combet S., Constantin D., Degrouard J., Moller J., Zeghal M., and Tresset G. (2018) Nonequilibrium self-assembly dynamics of icosahedral viral capsids packaging genome or polyelectrolyte. **Nat. Commun**. 9:3071.

P28 - Structural insights into the R2TP complex

P. E. Santo [a,b], S.T.N. Silva [a], A.C.F. Paiva [b], P.M.F. Sousa [b], P.M. Matias [a,b], T.M. Bandeiras [a,b]

[a] *Instituto de Tecnologia Química e Biológica, ITQB-UNL*
[b] *Instituto de Biologia Experimental e Tecnológica, iBET*
E-mail: psanto@ibet.pt

The R2TP complex, so named as it contains Ruvb1, Ruvb2, Tah1 and Phi1 proteins, was first discovered in *S. cerevisiae* as a HSP90 interacting partner, and its human counterpart is the R2TP complex, containing RuvBL1, RuvBL2, RPAP3 and PIH1D1.
The R2TP complex plays an important role in a variety of process: in the assembly of RNA polymerase II and of snoRNPs, in PIKK signalling, and in apoptosis [1]. The participation of R2TP in different cellular processes is due to the ability of RuvBL1, RuvBL2, PIH1D1 and RPAP3 to interact with specific components from each complex. RuvBL1 and RuvBL2 are capable of assembling into homo-hexamers, dimers of homo-hexamers (dodecamers) [2,3] and hetero-oligomers with alternating RuvBL1/RuvBL2 subunits [4]. RuvBL's ability to assemble into different oligomeric forms may relate to the different processes R2TP complex is involved.
Since R2TP has been related to hepatocellular, renal and gastric carcinomas [5], understanding the molecular mechanisms of how it assembles its different clients/targets may open avenues for anti-cancer drug discovery programs.
Work in our group has been focused on the R2TP components RuvBL1 and RuvBL2, but we also mapped the protein:protein interactions within the R2TP complex using a unique approach called cellular biotinylation-coupled Surface Plasmon Resonance. Aiming to solve the structure through X-ray Crystallography and Cryo-EM we designed and purified minimal R2TP constructs. These minimal constructs were characterized by SEC-MALS and Native-MS to access complex stoichiometry, while mapping the protein:protein interaction regions were analysed by Cross-Link-MS.

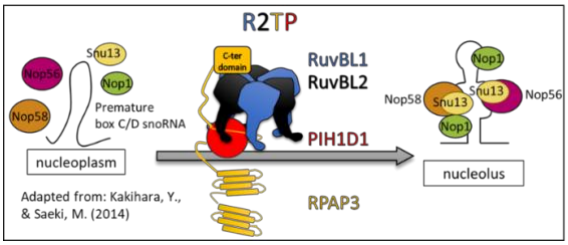


Fig 1: Assembly process of yeast Box CD SnoRNP by human R2TP complex

[1] Y. Kakihara & W.A. Houry, (2012). The R2TP complex: Discovery and functions. **Biochimica et Biophysica Acta**, 1823:101
[2] P. M. Matias et al., (2006). Crystal structure of the human AAA+ protein RuvBL1. **J. Biol. Chem.**, 281: 38918
[3] T.N. Sara et al., (2018). X.ray Structure of full-lentgh human RuvB-Like 2-mechanistic insights into coupling between ATP binding and mechanical action. **Scientific reports**, 8:13726.
[4] S. Gorynia et al., (2011). Structural and functional insights into a dodecameric molecular machine–the RuvBL1/RuvBL2 complex. **J. Struct. Biol.**, 176:279
[5] Y. Kakihara and M. Saeki (2014). The R2TP chaperone complex: its involvement in snoRNP assembly and tumorogenesis. **BioMol Concepts**, 5:513

P29 - Characterization of a multi-modular GH3-CBM48-CE1 enzyme isolated from termite gut microbiota

L. Fermon[a,b], F. Moncassin[a,b], S. Nazef[a,b], R. Fauré[a], C. Montanier[a], G. Cioci[a], L. Mourey[b], M. O'Donohue[a], S. Tranier[b], C. Dumon[a]

[a] *Toulouse Biotechnology Institute, TBI, INSA, INRA, CNRS, Toulouse, France.*
[b] *Institut de Pharmacologie et de Biologie Structurale (IPBS) Université de Toulouse (Université Paul Sabatier - Toulouse III), CNRS 205 route de Narbonne 31077 Toulouse Cedex 04*
E-mail: samuel.tranier@ipbs.fr

Valorization of the lignocellulosic biomass represents a major stake for our society. The living organisms capable of degrading plant wall polysaccharides use different strategies. Bacteria of the genus *Bacteroides* produce enzymes encoded by gene clusters called Polysaccharides Using Loci (PULs) dedicated to the degradation of a given polysaccharide. Multi-modular enzymes consisting of a GH3 glycoside hydrolase domain, a carbohydrate binding module (CBM48) and a carbohydrate esterase CE1 domain have been discovered in a termite gut metagenome [1]. These two similar enzymes are found in a different genetic environment. CBM48 are generally appended to starch degrading enzymes or pullulan, even though recent results showed that CBM48 appended to CE1 are able to bind xylan, here the CBM48 doesn't show any binding on amylose, starch nor xylan polymers. The biochemical characterization of the GH3 and CE1 domains showed that the GH3 domain is a β-glucosidase and the CE1 is active on pNP-acetate.
Crystal structure of the GH3 and the CBM48-CE1 fragment revealed the presence of a glucose molecule in their respective active site. This glucose molecule is at a canonical position in the GH3 domain. In the CBM48-CE1 crystal structure, the glucose moiety is located in the putative active site of the CE1 domain and tightly bound to an aspartic acid residue from de CBM48 domain. This revealed the important role of such a carbohydrate-binding module in the ligand stabilization in the CE1 active site.

[1] G. Bastien, G. Arnal, S. Bozonnet, S. Laguerre, F. Ferreira, R. Faure, B. Henrissat, F. Lefevre, P. Robe, O. Bouchez, *et al.* (2013). Mining for hemicellulases in the fungus-growing termite *Pseudacanthotermes militaris* using functional metagenomics. **Biotechnol Biofuels 6**:78

P30 - Fabrication of X-ray compatible microfluidic devices for biomacromolecular structural studies

R. Vasireddi, I. Chaussavoine and M.G. Chavas Leonard

Synchrotron SOLEIL, L'Orme des Merisier, Saint-Aubin, 91192 Gif-sur-Yvette, France
E-mail: vasireddi.ramakrishna@synchrotron-soleil.fr

Microfluidics enables the precise control of liquid volumes on the nanoliter scale within micron-sized channels. These very well-defined flow conditions make this technology predestined for the study of biomacromolecules both in steady state and time-resolved environments using microfocused X-ray sources with limited and slow sample consumption. Microfluidic devices can be manufactured using a number of different polymers, such as polydimethylsiloxane (PDMS) [1], cyclic olefin polymers (COC) or polyimide films (Kapton®)[2], each having different optical and mechanical properties and varying ease in manufacturing. With the advent of more powerful and brighter X-ray sources, Kapton and COCs have received great interests as materials for X-ray compatible microfluidic devices due to their low-background and stability against X-ray-induced damages. Taking apart sample consumption, another approach for low-background sample environments are microfluidic liquid jet devices, offering a free-flowing sample stream, for experiments at XFELs and highly brilliant synchrotron sources. At the Synchrotron SOLEIL, the microfluidic group, together with the HelioBio scientific section is currently providing expertise in the design, manufacturing and experimental implementation of microfluidic devices at x-ray sources, providing with easer in sample handling and specialized sample environments that address specific experimental conditions at synchrotrons and XFEL facilities. We are also attempting to make standardized microfluidic trapping devices for biomacromolecular structural studies for industrial users at Synchrotron SOLEIL [3].

[1] R. Vasireddi, J. Kruse, M. Trebbin *et al* Solution blow spinning of polymer/nanocomposite micro-/nanofibers with tunable diameters and morphologies using a gas dynamic virtual nozzle **Scientific Reports** (Under Review).
[2] M. Vakili, R. Vasireddi**,** M. Trebbin *et al* 3D-Micromachined Polyimide Mixing Devices for in situ X-ray Imaging of Solution-based Block Copolymer Phase Transitions **Langmuir** (Under Review).
[3] M. Vakili, R. Vasireddi, M. Trebbin *et al* Fully Polyimide-based Liquid Jet Systems for In situ X-ray Studies In preparation

P31 - Structural characterization of assembly intermediates of eukaryotic ribosome biogenesis

M. Vayssières, M. Blaud, N. Leulliot

Laboratoire Cibles Thérapeutiques et Conception des Médicaments, CiTCoM, UMR8038, Faculté de Pharmacie, Université Paris Descartes, Paris, France.
E-mail: marlene.vayssieres@parisdescartes.fr

The ribosome is the cell's huge ribonucleic machinery in charge of synthesizing proteins from the mRNA. In yeast, the ribosome is composed of three ribosomal RNAs (rRNAs), the 25S, 5.8S and 18S, associated to 79 proteins. The different steps of ribosome biogenesis, including rRNA transcription, processing, and ribosomal protein assembly require the coordinated action of over 200 non ribosomal proteins, also named assembly factors. Assembly of the ribosomal proteins onto the rRNA is highly coordinated with rRNA processing. Indeed, the maturation of ribosomes is a multistep and dynamic process in which the protein complexes are remodeled and recruited following specific maturation events, thereby providing several quality control checkpoints. One of the most striking proofreading steps is the last pre-18S rRNA cleavage, mediated by the endonuclease Nob1 [1,2]. The maturation of the pre-rRNA 20S into mature 18S rRNA seems to occur in an 80S-like particle composed of a pre-40S subunit bound to a mature 60S subunit, in a translation-like cycle [3,4]. The assembly factor Rio1 is one of the proteins needed to the 20S pre-rRNA processing, found in the 80S-like particle [5]. We solve the structure by cryo-EM at 3.2Å, of an 80S-like particle with a Rio1 mutant as bait, in order to better understand this translation-like checkpoint.

[1] Fatica, A., Tollervey, D., and Dlakić, M. (2004). PIN domain of Nob1p is required for D-site cleavage in 20S pre-rRNA. **RNA N. Y. N** 10:1698–1701.
[2] Pertschy, B., Schneider, C., Gnädig, M., Schäfer, T., Tollervey, D., and Hurt, E. (2009). RNA helicase Prp43 and its co-factor Pfa1 promote 20 to 18 S rRNA processing catalyzed by the endonuclease Nob1. **J. Biol. Chem.** 284:35079–35091.
[3] Lebaron, S., Schneider, C., van Nues, R.W., Swiatkowska, A., Walsh, D., Böttcher, B., Granneman, S., Watkins, N.J., and Tollervey, D. (2012). Proofreading of pre-40S ribosome maturation by a translation initiation factor and 60S subunits. **Nat. Struct. Mol. Biol.** 19:744–753.
[4] Strunk, B.S., Novak, M.N., Young, C.L., and Karbstein, K. (2012). A translation-like cycle is a quality control checkpoint for maturing 40S ribosome subunits. **Cell** 150:111–121.
[5] Ferreira-Cerca, S., Kiburu, I., Thomson, E., LaRonde, N., and Hurt, E. (2014). Dominant Rio1 kinase/ATPase catalytic mutant induces trapping of late pre-40S biogenesis factors in 80S-like ribosomes. **Nucleic Acids Res.** 42:8635–8647.

P32 - GG, an atypical iron-sulfur protein conserved in the Mimiviridae family

A. Villalta [a], A. Lartigue [a], B. Burlat [b], F. Chaspoul [c], B. Guigliarelli [b] and C. Abergel [a]

[a]*Information Génomique et Structurale, CNRS-AMU UMR 7256, 13288 Marseille*
[b]*Bioénergétique et Ingénierie des Protéines, UMR7281, 13402 Marseille*
[c]*Institut Méditerranéen de la Biodiversité et d'Ecologie marine et continentale, UMR 7263, 13288 Marseille, France*
E-mail: villalta@igs.cnrs-mrs.fr

Giant viruses, since discovered 16 years ago, surprise by their unexpected features such as the size of the virions (>0.5µm), their complex genomes (600kb-2.5Mb), containing 650 to 2500 genes, most of them (>60%) encoding proteins without cellular or viral homologs. To date, four families have been identified: Mimiviridae, Pandoraviridae, Pithoviridae and Molliviridae [1] and the number of identified giant viruses is still increasing worldwide [2].
Transcriptional and proteomic analyses of Mimivirus, infecting *Acanthamoeba castellanii*, revealed that an unpredicted gene (*R633b*) was among the most transcribed at the late stage of the infection and encoded one of the most abundant proteins (GG) in the virions [3], showing its importance for the virus. GG is a small protein of ~6kDa conserved in the Mimiviridae, with an atypical sequence composed of ~40% glycine and 12% cysteine, coordinating an uncommon Fe-S cluster. Its function remains unknown.
Aiming to elucidate the structure and function of the GG-FeS family, we characterized the Fe-S cluster combining UV-Vis, EPR analyses of the wild type and mutant proteins. This unusual Fe-S cluster reminds the inactive aconitase's linear 3Fe-4S [4] with the behavior of the IssA atypical thioferrate protein [5]. Our structural studies show that GG can oligomerize to form 20nm structures that can assemble into long chains. The expression in *Acanthamoeba castellanii* of GG fused with GFP reveals its localization at the cellular membrane. I will also present some surprising properties of the protein that could serendipitously provide us the means to elucidate its 3D structure.

[1] C. Abergel, et al. (2015). The rapidly expanding universe of giant viruses: Mimivirus, Pandoravirus, Pithovirus and Mollivirus. **FEMS Microbiology Reviews** 39:779-796
[2] J. Abrahão et, et al. (2018). Tailed giant Tupanvirus possesses the most complete translational apparatus of the known virosphere. **Nature Communication** 9:749-61.
[3] M. Legendre, et al. (2010). mRNA deep sequencing reveals 75 new genes and a complex transcriptional landscape in Mimivirus. **Genome Research** 20:664–74.
[4] M.C. Kennedy, et al. (1984) Evidence for the Formation of a Linear [3Fe-4S] Cluster in Partially Unfolded Aconitase. **Journal of Biological Chemistry** 259:14463-14471.
[5] B.J. Vaccaro, et al. (2017). Biological iron-sulfur storage in a thioferrate-protein nanoparticle. **Nature Communication** 8:1-9

Session IV: Molecular Interactions and Diseases

Fanomezana Moutse Ranaivoson, Institut Curie, Paris
P45 - Structural specificities of the mitotic kinesin MKlp2 and implications to its mechanism and function
`

Carine van Heijenoort, ICSN, Gif-sur-Yvette
P46 - Structural and dynamic characterization of the intrinsically disordered tail of ErbB2, phosphorylation and interaction

Paloma Varela, I2BC, Gif-sur-Yvette
P47 - Macromolecules interactions *in vitro*, comparing classical
and innovative approaches

Armelle Vigouroux, I2BC, Gif-sur-Yvette
P48 - The plant defense signal galactinol is specifically used as a nutrient by the bacterial pathogen agrobacterium fabrum

P33 - Structural basis for the recognition of LDL-receptor family members by VSV glycoprotein

L. Belot[a], J. Nikolic[a], H. Raux[a], P. Legrand[b], Y. Gaudin[a], and A. Albertini[a]

[a] *Institut de Biologie Intégrative de la Cellule (I2BC), CEA, CNRS, Univ. Paris-Sud, Université Paris-Saclay, 91198, Gif-sur-Yvette cedex, France.*
[b] *Synchrotron SOLEIL, 91192, Gif-sur-Yvette cedex, France.*
E-mail: laura.belot@i2bc.paris-saclay.fr

Vesicular stomatitis virus (VSV) is the prototype of the Vesiculovirus genus and the Rhadboviridae family. VSV is an oncolytic virus and its glycoprotein (G) is widely used to pseudotype other viruses for gene therapy.
VSV G is a transmembrane protein that mediates both virus attachment to its cellular receptor and fusion of the viral envelope with the endosomal membrane. Low-density lipoprotein receptor (LDL-R) is implied in regulating cholesterol homeostasis and also serves as a major entry receptor for VSV. To elucidate the complex interplay between the virus and its receptor, we combined several techniques to understand the molecular basis of the interaction between G and the LD-R.
We first performed a structural and functional study of VSV G in complex with the LDL-R.
The LDL-R contains a ligand binding domain which is composed by 7 cysteine-rich domains (*e.g.* CR1 to CR7). We purified all those domains and shown that G can bind two individual CR: CR2 and CR3. We also characterized the interactions between G and the separate CR and solved the crystal structures of VSV G in complex with CR2 and in in complex with CR3. This reveals that the binding sites of CR2 and CR3 on G are identical. G engages the same basic residues in each structure to bind the CR domains. We also performed binding tests and infectiosity tests using mutated G. Those experiments show that although VSV can use alternative receptors, the mutations of basic residues on G, which are keys for interaction with LDL-R CR domains, abolish VSV infectivity. Our data indicate that the only receptors of VSV are members of the LDL-R family and that VSV G has specifically evolved to interact with their CR domains. This provides structural insights on the interaction between VSV G and host cell receptors and basis for the design of recombinant viruses with an altered tropism.
Key words: Vesicular stomatitis virus, LDL-receptor, X-ray structures, viral receptor, viral entry, viral glycoprotein, pseudotype, oncotherapy

Nikolic, J*****., Belot, L*****., Raux, H., Legrand, P., Gaudin, Y., and A Albertini, A. (2018). Structural basis for the recognition of LDL-receptor family members by VSV glycoprotein. **Nat. Commun**. 9:1029. *equal contribution to the work

P34 - Structural study of the inhibition mechanism of the *Mycobacterium tuberculosis* dehydratase HadAB by thiacetazone

Y. Grimoire [a], J. Rima [a], A. Villalta [a], S. Julien [a], S. Tranier [a], J. Nommé [a], J. Marcoux [a], N. Eynard [a], F. Bardou [a], Tercio de Freitas-Paulo [b], V. Bernardes-Genisson [b], A. Quémard [a], L. Mourey [a], C. Bon [a]

[a] *Institut de Pharmacologie et de Biologie Structurale, IPBS, Université de Toulouse, CNRS, UPS, 31077, Toulouse, France*.
[b] *Laboratoire de chimie de coordination, LCC, CNRS, 31077, Toulouse, France*.
E-mail: cecile.bon@ipbs.fr

A new generation of drugs and vaccines effective against the *Mycobacterium tuberculosis* resistant strains is urgently needed [1]. First and second-line anti-TB drugs mainly target the mycolic acid biosynthesis pathway. This pathway involves two fatty acid synthase systems (FAS): FAS-I, which is responsible for the synthesis of C16-C18 and C22-C26 fatty acids (FAs), and FAS-II, which elongates C16-C18 FAs into C42-C62 FAs [2]. HadAB, a dehydratase belonging to the FAS-II system, is targeted by two anti-TB drugs, isoxyl (ISO) and thiacetazone (TAC), but these drugs are no longer used due to their low bioavailability (ISO) and toxicity (TAC). It has been proposed that ISO and TAC could inhibit HadAB by the formation of a covalent adduct with Cys61 of HadA, which could then lead to the formation of a disulfide bridge between Cys61 and Cys105 [3,4], but this mechanism has not been structurally validated. We present here the structural results we obtained concerning the inhibition of HadAB by TAC, as well as another molecule that we have recently shown to inhibit HadAB *in vitro*.

[1] **WHO, Report** 2017.
[2] A. Quémard. (2016) New insights into the mycolate-containing compound biosynthesis and transport in mycobacteria. **Trends Microbiol** 9:725-738.
[3] A.E. Grzegorzewicz, N. Eynard, A. Quémard, E.J. North, A. Margolis, J.J. Lindenberger, V. Jones, J. Korduláková, P.J. Brennan, R.E. Lee, D.R. Ronning, M.R. McNeil, M. Jackson (2015). Covalent modification of the *Mycobacterium tuberculosis* FAS-II dehydratase by Isoxyl and Thiacetazone. **ACS Infect Dis.** 1:91-97.
[4] A.E. Grzegorzewicz, J. Korduláková, V. Jones, S.E. Born, J.M. Belardinelli, A. Vaquié, V.A. Gundi, J. Madacki, N. Slama, F. Laval, J. Vaubourgeix, R.M. Crew, B. Gicquel, M. Daffé, H.R. Morbidoni, P.J. Brennan, A. Quémard, M.R. McNeil, M. Jackson (2012). A common mechanism of inhibition of the *Mycobacterium tuberculosis* mycolic acid biosynthetic pathway by isoxyl and thiacetazone**. J Biol Chem**. 287:38434-41.

P35 - Investigation of the structure and hub protein function of RSV phosphoprotein

C. Cardone [a], N. Pereira [a], C.-A. Richard [b], J. Fix [b], V. Richeval [c], M.-A. Rameix-Welti [c], M. Galloux [b],J.-F. Eléouët [b], C. Sizun [a]

[a] *Institut de Chimie des Substances Naturelles, ICSN, CNRS, Université Paris-Saclay, 91190, Gif-sur-Yvette.*
[b] *Unité de Virologie et Immunologie Moléculaires, VIM, INRA, 78352, Jouy-en-Josas.*
[c] *Unité Infection et Inflammation Chroniques, 2IC, INSERM, Université de Versailles Saint Quentin, 78180 Montigny le Bretonneux.*
E-mail: christophe.cardone@cnrs.fr

Worldwide, the human Respiratory Syncytial Virus (hRSV) causes acute lower respiratory tract infections and is responsible for most of bronchiolitis cases. This virus belongs to the *Pneumoviridae* family of the Mononegavirales order. The hRSV genome consists of a genomic RNA molecule packed into a viral capsid. To replicate and transcribe its genomic RNA, the virus uses its own RNA polymerase machinery. This machinery is a complex, in which the main co-factor, the hRSV phosphoprotein (hRSV P), is essential to position the polymerase complex onto its template, a ribonucleoprotein complex formed of genomic RNA and the bound hRSV nucleoprotein (hRSV N). We have undertaken a structural investigation of hRSV P [1] as well as of its interactions with protein partners by using nuclear magnetic resonance (NMR) spectroscopy. NMR is an atomic scale tool well adapted to study highly dynamic proteins. hRSV P is a tetrameric 4x241-residue protein with a short ~40-residue oligomerization domain (OD), flanked by large intrinsically disordered regions (IDRs) at its N- and C-termini. We have analyzed the helical regions, OD included, by NMR. NMR moreover shows that the IDRs are very heterogeneous, with some regions displaying high and other regions very weak secondary structure propensity. We found long range contacts inside these intrinsically disordered domains of hRSV P as well as contacts between almost stable C-terminal helices formed downstream of the OD and the OD itself. These contact regions can be involved in protein interactions with different protein partners, like hRSV N or the hRSV transcription cofactor M2-1, which is recruited by hRSV P to viral cytoplasmic inclusion bodies, where viral RNA is produced. hRSV M2-1 is dephosphorylated by the cellular phosphatase PP1α. However, it does not bind to PP1α. We showed that hRSV P serves as a platform that binds both hRSV M2-1 and PP1α in nearby regions [2]. Due to the close proximity in the ternary complex, hRSV M2-1 can be dephosphorylated by PP1α. Thus, hRSV P contributes to M2-1 distribution between viral inclusion bodies and the cytoplasm, linked to cycling of hRSV M2-1 between phosphorylated and unphosphorylated states.

[1] N. Pereira, C. Cardone, S. Lassoued, M. Galloux, J. Fix, N. Assrir, E. Lescop, F. Bontems, J.-F. Eléouët, C. Sizun. (2017). New Insights into Structural Disorder in Human Respiratory Syncytial Virus Phosphoprotein and Implications for Binding of Protein Partners. **J Biol Chem**. 292:2120-2131.
[2] C.-A. Richard, V. Rincheval, S. Lassoued, J. Fix, C. Cardone, C. Esneau, S. Nekhai, M. Galloux, M.-A. Rameix-Welti, C. Sizun, J.-F. Eléouët. (2018) RSV hijacks cellular protein phosphatase 1 to regulate M2-1 phosphorylation and viral transcription. **PLoS Pathog** 14:e1006920.

P36 - Structure analysis uncovers a novel effector family related to killer proteins in *Zymoseptoria tritici*

K. de Guillen[a], T. Kroj[b], A. Genissel[c], L. Mammri[a], P. Barthe[a], F. Hoh[a], M. H. Lebrun[c], and A. Padilla[a]

[a]*Centre de Biochimie Structurale, INSERM U1054, CNRS UMR5048, Montpellier University, Montpellier, France.*
[b]*INRA, BGPI, Biology and Genetics of Plant-Pathogen Interactions.*
Campus International de Baillarguet, Montpellier, France.
[c]*INRA, BIOGER, Campus AgroParisTech, Thiverval-Grignon, France*
E-mail: karine.deguillen@cbs.cnrs.fr

Zymoseptoria tritici has a large number of secreted protein effectors coding genes, most of which are expressed during infection of wheat leaves. We determined the 3-dimensional structure of *Z. tritici* effector Mycgr3-91409. Previous structure-based HMM pattern searches suggested that Mycgr3-91409 is the only representative of the MAX (*Magnaporthe* AVRs and ToxB) effector family [1] in *Z. tritici*. MAX effectors have a conserved β-sandwich fold and highly diverse primary protein sequences. *Mycgr3-91409* was expressed in *E. coli* without its signal peptide and the purified protein was analysed for its 3D structure by NMR spectroscopy, and X-ray crystallography. Mycgr3-91409 had a α/β-sandwich structure completely different from MAX effector, but similar to KP6 killer proteins from *Ustilago maydis*. KP6α and KP6β are forming a protein complex toxic to non-producing fungi [2]. Accordingly, *Mycgr3-91409* was named *Zt-KP6-1. Zt-KP6-1* orthologs were identified in *Z tritici* populations and in closely related species *Z. brevis,* but not in other fungi. Survey of *Zt-KP6-1* genetic diversity, indicated that it was subjected to purifying selection, suggesting a functional role for this protein. A paralog *of Zt-KP6-1*, Mycgr3-96389, was identified in *Z. tritici* and in related species *Z. pseudotritici* and *Z. ardabiliae*, but not in other fungi. Structure modeling suggested that Mycgr3-96389 had the same protein fold as KP6α and Zt-KP6-1, and it was named Zt-KP6-2. RNAseq data indicated that *Zt-KP6-1* and *Zt-KP6-2* were not expressed during fungal growth *in vitro,* but they were highly expressed during the asymptomatic phase of wheat leaf infection. Therefore, these two proteins are interesting candidate effectors, expressed specifically during infection. It will be interesting to determine whether these effectors have a toxic activity on fungi and/or plants. These experiments also clearly showed that determining the 3D structure of effectors, is a powerful method to assign these proteins to families.

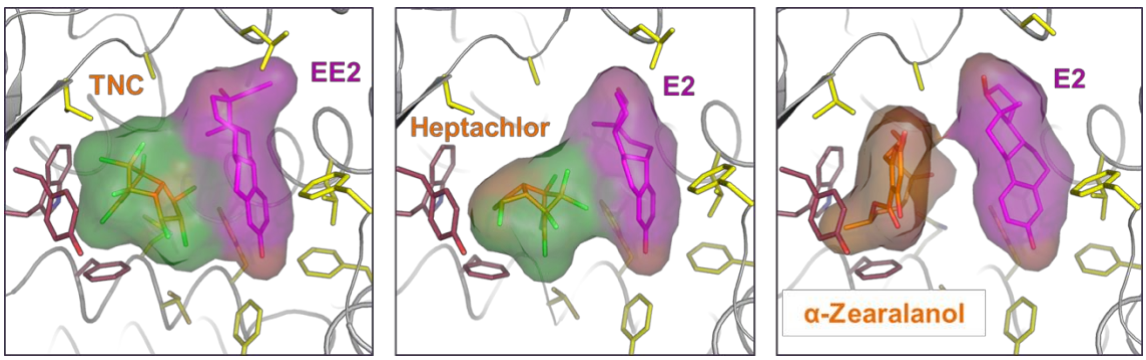
[1] de Guillen, K., Ortiz-Vallejo, D., Gracy, J., Fournier, E., Kroj, T., and Padilla, A. (2015). Structure Analysis Uncovers a Highly Diverse but Structurally Conserved Effector Family in Phytopathogenic Fungi. **PLOS Pathog** 11:e1005228.
[2] Allen, A., Chatt, E., and Smith, T.J. (2013). The Atomic Structure of the Virally Encoded Antifungal Protein, KP6**. J. Mol. Biol**. 425:609–621.

P37 - The synergistic activation of pxr by xenobiotics relies on their cooperative binding in the ligand binding pocket

V. Delfosse [a], M. Granell [a], C. Gardia-Parège [b], M. Grimaldi [b], P. Balaguer [b], W. Bourguet [a]

[a] *Centre de Biochimie Structurale, CNRS UMR5048, Inserm U1054, UM, 34090, Montpellier, France*.
[b] *Institut de Recherche en Cancérologie de Montpellier, Inserm U1194, 34090, Montpellier, France*.
E-mail: delfosse@cbs.cnrs.fr

Humans and wildlife are chronically exposed to cocktails of chemicals referred to as xenobiotics. A vast body of literature now links exposure to these chemicals with an increased incidence of reproductive, developmental, metabolic, neurological disorders, or cancers. Recent experimental data clearly demonstrate that when used in combination chemicals have outcomes that are not easily predicted from their individual behaviour. They could exert additive, synergistic or antagonistic effects that are far from being understood. The nuclear receptor PXR is the principal actor in mediating the mammalian xenobiotic response by regulating the expression of phase I, II and III detoxifying enzymes. Our previous study shed light on the molecular mechanisms involved in the synergistic effects of a binary cocktail of xenobiotics on PXR activation [1]. We have found that the combined use of the pesticide trans-nonachlor (TNC) and the synthetic estrogen ethinylestradiol (EE2) produced a more than additive effect. By solving the structure of PXR in complex with both EE2 and TNC, we observed inter-ligand contacts that generate a mutual stabilization of the compounds in the ligand binding pocket and account for the enhanced affinity of the mixture. To gain further insights into the identification and characterization of a larger number of mixtures, we are using complementary cell-based, biophysical, structural and *in vivo* approaches. We recently identified binary cocktails ranging from “non-active” to “synergistically active” on PXR for which we solved the structures in complex with the receptor. These structures suggest a link between the cooperative binding of molecules in the ligand binding pocket and their synergistic activity observed on PXR.



[1] V. Delfosse, B. Dendele, T. Huet, M. Grimaldi, A. Boulahtouf, S. Gerbal-Chaloin, B. Beucher, D. Roecklin, C. Muller, R. Rahmani, V. Cavaillès, M. Daujat-Chavanieu, V. Vivat, J. M. Pascussi, P. Balaguer and W. Bourguet. (2015). Synergistic activation of the human pregnane X receptor by a binary cocktail of pharmaceutical and environmental compounds. **Nature Communications** 6:8089.

P38 - The crystal structure of TYMV PRO/DUB complexed to ubiquitin reveals how the enzyme distinguishes its substrates

S. Fieulaine [a], M. Witte [b], C. Theile [b], M. Ayach [a], H. Ploegh [b], I. Jupin [c], S. Bressanelli [a]

[a] *Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ Paris Sud, Université Paris-Saclay, 91198, Gif sur Yvette cedex, France*
[b] *Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142, USA*
[c] *Institut Jacques Monod, UMR 7592, CNRS - Univ Paris-Diderot, 15 rue Helene Brion, 75205 Paris Cedex 13, France*
E-mail: sonia.fieulaine@i2bc.paris-saclay.fr

Ubiquitylation and deubiquitylation events are often involved during viral infection. In some cases, host can tag viral proteins with ubiquitin (Ub), likely to target them towards proteasome for degradation, and virus is able to remove or edit these resulting poly-ubiquitin chains. Deubiquitylation of tagged viral proteins is done by deubiquitinases (DUB), which are either host enzymes recruited by the virus or viral encoded enzymes. Interestingly, DUBs encoded by some compact RNA viruses are actually bifunctional enzymes also responsible for the viral polyprotein maturation through a protease activity (PRO). In this case, both PRO and DUB activities rely on a single catalytic site, able to cleave a peptide or an isopeptide bond respectively. The molecular determinants allowing the enzyme to switch from a substrate to another and to an activity to another remain largely unknown.
In order to address the key question of how a viral PRO/DUB can discriminate substrates and act as a PRO or a DUB, we study *Turnip yellow mosaic virus* (TYMV), whose replication cycle appears to involve reversible ubiquitylation events [1]. TYMV PRO/DUB is indeed a PRO involved in proteolytic processing of the viral replication precursor, and also a DUB that is able to rescue the ubiquitylated viral polymerase from degradation. The polyprotein cleavage sites are well described while the type of poly-ubiquitin chain(s) that are attached to the viral polymerase is not yet know.
In order to document how TYMV PRO/DUB recognizes the ubiquitylated viral polymerase, we have solved the crystal structure of TYMV PRO/DUB in complex with one module of ubiquitin. We show that the enzyme uses two remarkable non-conserved loops to contact the two conserved hydrophobic patches of Ub. Strikingly these loops contain polar residues that make labile hydrogen bonds and salt bridges with Ub but mostly van der Waals interactions with the aliphatic part of their side chains. A structure-guided mutagenesis study revealed that hydrophobic contacts make the major contribution of the interaction. Of note, our results also show that DUB activity of the enzyme can be strongly increased by substituting polar residues of its two interacting-loops by hydrophobic residues, highlighting a sub-optimal surface complementarity between the two proteins. These results thus establish one cause of the low DUB activity of TYMV PRO/DUB and further raise the possibility that it may be functionally important, rather than a tradeoff of multi-substrate recognition. In addition, the comparison of this PRO/DUB-Ub structure to that of a PRO-PRO complex that occurs during polyprotein processing [2] reveals largely overlapping binding surfaces, which gives us clues to understand how the bifunctional enzyme can distinguish two of its substrates.

[1] M. Chenon, L. Camborde, S. Cheminant, I. Jupin. (2012) A viral deubiquitylating enzyme targets viral RNA-dependent RNA polymerase and affects viral infectivity. **EMBO J**. 31:741–753.
[2] C. Lombardi, M. Ayach, L. Beaurepaire, M. Chenon, J. Andreani, R. Guerois, I. Jupin, S. Bressanelli. (2013) A compact viral processing proteinase/ubiquitin hydrolase from the OTU family. **PLoS Pathog**. 9:e1003560.

P39 - Because structure means function - Quantifying binding-induced conformational changes of proteins using hydrodynamic protein size measurements

F. M. Moeller [a], H. Daub [a], R. Staffler [a], M. Greber [a]

[a] *Dynamic Biosensors GmbH, Lochhamer Str. 15, 82152 Martinsried/Planegg, Germany*
E-mail: greber@dynamic-biosensors.com

Function, activity, and interactions of proteins crucially depend on their three-dimensional structures and the relative arrangement of individual protein domains. We present a novel method for the measurement of the Stokes radii of proteins and demonstrate how this approach can be used to detect analyte binding-induced conformational changes in proteins to open new perspectives for hit validation through in-depth information on protein conformation, oligomeric state, and stability.
We show two examples of functional biophysical switchSENSE assays which correlate protein structure and function.

Transglutaminase2 (TG2) is a multifunctional enzyme, involved in autoimmune and neurodegenerative diseases. Its activity is allosterically regulated by Ca^{2+} and nucleotides and involves conformational changes. Here, we combine real-time nucleotide binding kinetics with protein size measurements and observe nucleotide and ion induced conformational changes. The quick exchange of analytes provides dynamic information on TG2 conformation and yields new insights in TG2 regulation and the mode-of-action of pharmaceutically interesting peptidic inhibitors, e.g. the reversibility of structural changes.

Tumor necrosis factor α (TNFα) is a homotrimeric cell signaling protein involved in inflammation and the regulation of immune cells. It loses its bioactivity when decaying to a monomer. We succeed in observing the monomerization kinetics of TNFα in real-time by changes in protein friction. By controlled monomerization and evaluation of TNFα's protein size before each experiment, we show the dependence of antibody binding kinetics and affinity on the structural integrity of the homotrimer and the importance of structural quality testing of proteins for binding assays.

P40 - Regulation of the cell division of the bacterium *Streptococcus pneumoniae* by the serine/threonine-kinase StkP

V. Gueguen-Chaignon[b], C. Freton[a], C. Mercy[a], F. Galisson[a], S. Nagarajan[a], P. Gouet[a], C. Grangeasse[a]

[a] Molecular Microbiology and Structural Biochemistry, MMSB, CNRS, Université de Lyon, UMR5086, Lyon, France.
[b] Protein Science Facility, SFR Biosciences, CNRS, INSERM, ENS Lyon, Université de Lyon, UMS3444, Lyon, france.
E-mail: virginie.gueguen-chaignon@ibcp.fr

The eukaryotic-like serine/threonine kinase StkP is a central regulator of the cell division and morphogenesis of the human pathogen *Streptococcus pneumoniae*. StkP is a membrane protein possessing an intracellular kinase domain and an extracellular domain composed of 4 PASTA repeats (Fig. 1) [1]. How StkP is activated remains elusive. The aim of this study is to determine the structural organization of both extra- and intra-cellular domains to decipher the molecular features responsible for the activation of StkP. For that, we first identified the autophosphorylation sites of the catalytic domain of StkP by mass spectrometry and generated a series of mutants with phospho-ablative or -mimetic mutations to access their impact on the structure of StkP. A first structure allowed to determine the main structural features of the catalytic domain. The structure of the other mutants is ongoing. In addition, we determine the structure of the membrane-distal PASTA repeat using evolution- and structural-guided approaches. We showed that surface exposed residues are required for the interaction with the cell division protein LytB, a cell wall hydrolase needed for final cell separation [2]. Altogether, our work allows to draw a working model in which the extracellular domain of StkP would play a dual function in interconnecting StkP activation, and consequently phosphorylation of its endogenous targets, with cell wall remodeling to allow the separation of the two daughter cells (Fig.2).

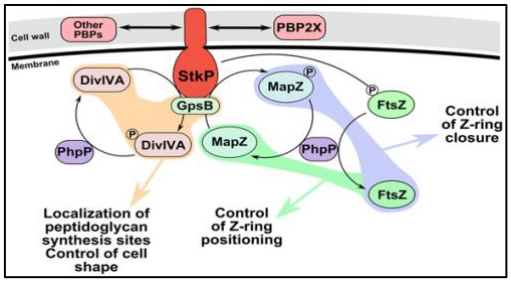


Fig. 1: Role of StkP in pneumococcal cell division [2]

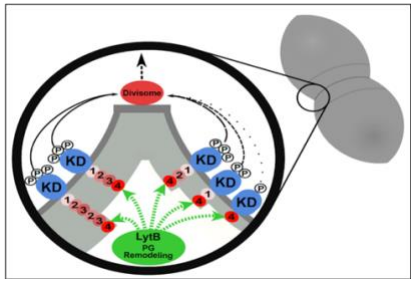


Fig.2 : Model for the coordination of cell constriction and septal cell wall hydrolysis by StkP

[1] S. Manuse, A. Fleurie, L. Zucchini, C. Lesterlin and C. Grangeasse (2016). Role of eukaryotic-like serine/threonine kinases in bacterial cell division and morphogenesis. **FEMS Microbiology Reviews**, 40:41–56.
[2] L. Zucchini, C. Mercy, P.S. Garcia, C. Cluzel, V. Gueguen-Chaignon, F. Galisson, C. Freton, S. Guiral, C. Brochier-Armanet, P. Gouet and C. Grangeasse. (2018). PASTA repeats of the protein-kinase StkP interconnect cell constriction and separation of *Streptococcus pneumoniae*. **Nature Microbiology**, 3:197-209.

P41 - Interaction of histone variant H2A.Z with its chaperones YL1 and ANP32E: link to chromatin remodeling and disease

P. Klein [A], N. Aleksandrova [A], M. Marek [A], C. Romier [A]

[a] *Institut de génétique et de biologie moléculaire et cellulaire, IGBMC, CNRS UMR 7104 - Inserm U 1258, Illkirch, France*.
E-mail: kleinp@igbmc.fr

Modulation of chromatin structure by epigenetic effectors regulates cellular homeostasis by influencing the accessibility to the genetic information. The dysregulation of epigenetic mechanisms is linked to numerous pathologies, including cancers [1]. Histone variant H2A.Z is an important epigenetic player with unique functions ranging from transcription regulation and DNA repair to chromosome segregation, with direct implications in cancers.
Two chaperones of human H2A.Z have been identified: YL1 and ANP32E. Whereas YL1 deposits H2A.Z onto the chromatin, ANP32E removes it from the chromatin [2,3], (Fig.1). Both chaperones belong to large chromatin remodeling complexes, p400/Tip60 and SRCAP, which have key roles in maintaining genome integrity. It is therefore essential to decipher in molecular terms the interplay of these epigenetic effectors and their mode of action to tackle their pharmacological modulation as potential therapeutic targets for cancers.
To address this issue, we have combined biochemical, biophysical and structural approaches to characterize the interaction of YL1 and ANP32E with (i) the H2A.Z-H2B histone pair and (ii) the nucleosome. Using Isothermal Titration Calorimetry (ITC), we have studied the interaction of YL1 and ANP32E H2A.Z-Interacting Domains (ZID) with H2A.Z-H2B (Fig.1). Our data revealed marked differences between the two chaperones, but also suggested that other domains of these chaperones are important for interaction with H2A.Z-H2B.
Using biochemical techniques and X-ray crystallography, we have showed that the N-terminal part of ANP32E could also play a role in H3-H4 chaperoning. We are currently investigating the interaction of YL1 and ANP32E with the nucleosome to further characterize their functional roles in molecular terms.

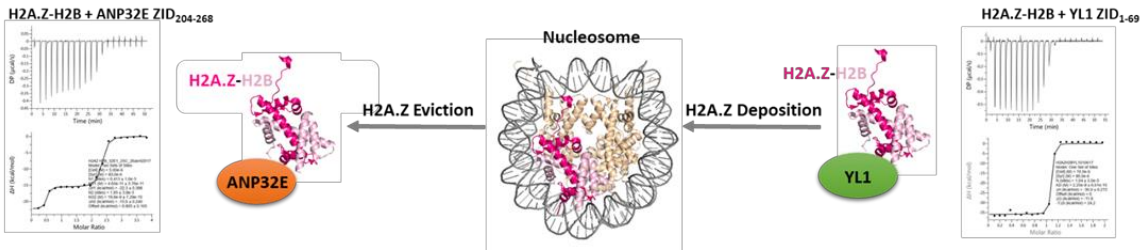


Fig. 1: (Center) Model of H2A.Z deposition and eviction by ANP32E and YL1 chaperones. (Edges) Interaction probing of YL1 and ANP32E with histone pair H2A.Z/H2B by ITC.

[1] T. O. Tollefsbol. (2018). Epigenetics of Human Disease. **Translational epigenetics** 6:3-10
[2] A. Obri *et al*., (2014). ANP32E is a histone chaperone that removes H2A.Z from chromatin. **Nature** 505:648–653
[3] C. M. Latrick et al., (2016). Molecular basis and specificity of H2A.Z–H2B recognition and deposition by the histone chaperone YL1. **Nat. Struct. Mol. Biol.** 2:309–316

P42 - The alpha/beta-hydrolase fold: a structural motif with multiple variations and functions

P. Marchot [a], A. Chatonnet [b], Y. Bourne [a]

[a] *Architecture et Fonction des Macromolécules Biologiques (AFMB), Centre National de la Recherche Scientifique / Aix-Marseille Univ, Marseille, France.*
[b] *Dynamique Musculaire et Métabolisme (DMeM), Institut National de la Recherche Agronomique / Univ Montpellier, Montpellier, France.*
E-mail: pascale.marchot@univ-amu.fr

The superfamily of proteins and protein subdomains displaying an alpha/beta-hydrolase fold comprises many members with a demonstrated enzymatic function and a few others with varying other functions [1]. All members diverged from a common ancestor. The canonical fold consists of a central, highly twisted beta-sheet made of eight to eleven beta-strands and flanked on both sides by alpha helices. Additional motifs of variable sizes, structures and positions often decorate this common core and confer on it specific properties for substrate selectivity or regulation of catalysis (enzyme members) or for heterologous partner recognition or other functions (non-enzyme members). Another level of variability is found for some enzymes that display the promiscuous ability to hydrolyze alternative substrates or are true moonlighting proteins acting also as transporters, receptors, chaperones... [2]. A reverse situation has been encountered for members initially identified as cell-adhesion proteins and later shown to be enzymes.
The hydrolytic enzymes lipases and cholinesterases, the cell-adhesion molecules neuroligins, and the hormonogenic macromolecule thyroglobulin are only a few of the many members of the superfamily. Despite their distinctive modes of action, their canonical subunits share surface binding patches and determinants for forming homodimers and for accommodating structural subunits or protein partners. Several of these surface regions of high functional relevance have been mapped through structural or mutational studies, while others have been proposed based on biochemical or molecular docking data. We will describe these binding interfaces and, by showing how specific or overlapped they are, will emphasize their specificity *versus* potentially multifunctional character, and the diversity of the functional adaptations of the alpha/beta-hydrolase fold [3].

[1] Hotelier T, Renault L, Cousin X, Negre V, Marchot P, Chatonnet A (2004) ESTHER, the database of the alpha/beta-hydrolase fold superfamily of proteins. **Nucleic Acids Res** 32(Database issue):D145-7. (PMID: 14681380)
[2] Marchot & Chatonnet (2012) Enzymatic activity and protein interactions in α/β-hydrolase fold proteins: moonlighting versus promiscuity. **Protein Peptide Lett** 19:132-43. (PMID: 21933125)
[3] Bourne & Marchot (2017) Hot spots for protein partnership at the surface of cholinesterases and related alpha/beta-hydrolase fold proteins or domains - a structural perspective. **Molecules** 23:35. (PMID: 29295471)

P43 - Structural and functional studies of post-translational modification of mycobacterial type I polyketide synthase

M. C. Nguyen, O. Saurel, C. Carivenc, S. Gavalda, S. Saitta, M. Phuong Tran, A. Milon, C. Chalut, C. Guilhot, L. Mourey and J.D. Pedelacq

Institut de Pharmacologie et de Biologie Structurale, IPBS, Université de Toulouse, CNRS, UPS, 31077, Toulouse, France.
Email: Minh-Chau.Nguyen@ipbs.fr

Pathogenic mycobacteria continue to pose major threats to human health. Among them is *Mycobacterium tuberculosis,* the etiologic agent of tuberculosis, one of the most dangerous diseases in human history. *Mycobacterium abscessus* is also gaining attention as it causes pulmonary diseases, especially in vulnerable hosts such as patients with cystic fibrosis. Polyketide synthases (PKSs) participate in the biosynthesis of various complex lipids of the mycobacterial cell envelope, which constitutes the first line of defense against toxic molecules from the host and has been involved in pathogenesis. To be catalytically active, PKSs must be modified post-translationally by 4'-phosphopantetheinyl transferases (PPTases). PPTases transfer the Ppant arm of coenzyme A (CoA) to the conserved serine residue of acyl carrier protein (ACP) domains of PKSs in the presence of metal ions [1]. Once activated, the ACP domain brings intermediate products tethered to the extremity of the Ppant to other catalytic domains of PKSs. Interactions between ACP domain with its partners, including PPTases and other PKS domains, is central to the mechanism of action of this important class of megasynthases. Thus, PPTases represent novel interesting therapeutic targets, which have been scarcely explored [2]. In this study, we characterized both biochemically and structurally PptAb, the PPTase from *M. abscessus*. We found that Mn^{2+} ions have a positive effect on stability of PptAb whereas the *in vitro* transfer reaction completes quicker in the presence of Mg^{2+} ions. We obtained crystals of PptAb in various conditions and we observed an effect of the pH on cofactor binding. In addition, we have determined the crystal structure of PptAb in complex with the ACP domain of the PKS PpsC from *M. tuberculosis*, revealing a snapshot of the Ppant transfer intermediate. NMR experiments performed on the ACP domain revealed flexibility on regions that participate in interactions with PptAb. Our study suggests a new mechanism for the post-translational modification of modular megasynthases. Moreover, our structural data should be beneficial for rational drug design and the development of therapeutic alternatives to treat patients with mycobacterial infections.

[1] Beld J., Sonnenschein E., Vickery C., Noel J., Burkart, Michael D. (2014) The Phosphopantetheinyl Transferases: Catalysis of a Posttranslational Modification Crucial for Life. **Natural Product Reports**, 31:61-108.
[2] Leblanc C., Prudhomme T., Tabouret G., Ray A., Burbaud, S., Mourey, L., Guilhot, C., Chalut C. (2012) 4'-Phosphopantetheinyl Transferase PptT, a New Drug Target Required for *Mycobacterium tuberculosis* Growth and Persistence in vivo. **PLoS Pathogens**, 8:e1003097.

P44 - Structural and biophysical studies on the innate immune NOD2-RIP2 signaling

E. Pellegrini[a], A. Desfosses[b], M. Lethier[a], A. Wallaman[c], W.M. Schulze[a], K. Rehbein[c], L. Signor[d], G. Zenkeviciute[a], S. Gaudon[a], P. Mas[b], M.Hons, H. Malet[b], I. Gutsche[b], C. Sachse[e], Hartmut Oschkinat[c], G. Schoehn[b], E. Boeri Erba[d] and S. Cusack[a]

[a] *European Molecular Biology Laboratory, Grenoble, France*
[b] *Univ. Grenoble Alpes, CNRS, CEA, CNRS, IBS, F-38000 Grenoble, France*
[c] *FMP, Berlin, Germany*
[d] *University Grenoble Alpes, CEA, CNRS, IBS, F-38000 Grenoble, France*
[e] *European Molecular Biology Laboratory, Heidelberg, Germany*
Email: epellegr@embl.fr

NOD2 (Nucleotide Oligomerization Domain 2) is an innate immune pattern recognition receptor that plays a critical role in the defense against bacterial pathogens and in the maintenance of the integrity of the mucosal barrier. Binding of the bacterial peptidoglycan breakdown MDP (Muramyl dipeptide) to NOD2 leads to receptor oligomerization and subsequent recruitment via CARD-CARD interaction of downstream adaptor protein kinase RIP2 (Receptor Interacting Protein 2). RIP2, upon auto-phosphorylation and ubiquitination, becomes a scaffold for downstream effectors. Eventually, the NOD2 signalling pathway triggers an efficient inflammatory response through NF-kB, MAPK activation and autophagy [1].

Aberrant NOD2-RIP2 signalling is implicated in both genetic and non-genetic inflammatory diseases, such as Crohn's disease, Blau syndrome, early-onset Sarcoidosis, inflammatory arthritis and multiple sclerosis [1]. Therefore, the NOD2-RIP2 signaling pathway has become an attractive pharmacological target, with a particular focus on the inhibition of the RIP2 kinase activity. Despite its critical importance, little is known about the detailed molecular mechanism underling the RIP2 kinase activation by NOD2 and the importance of such activation for the recruitment of downstream signalling regulators, including several E3 ligases, such as XIAP (X-chromosome-linked inhibitor of apoptosis).

Here, we present our biophysical and structural data on NOD2-RIP2 signalling, which strongly support the hypothesis that interaction between the two proteins leads to the formation of a polar fibrillary assembly complex analogous to the signalosome already described for other innate immune pathways, e.g. RIG-I, NLRP3 and CARMA1. We report the cryo-electron microscopy (cryo-EM) structure at 4 Å of the core of such a complex, which reveals the molecular details underlying the assembly mechanism of the RIP2CARD domain. By applying structure-driven mutagenesis in both *in-cell assay* and *in vitro*, we proved the relevance of RIP2 polymerisation in the activation of NF-κB signalling by NOD2 [2]. RIP2CARD polymerization likely promotes the subsequent RIP2 kinase (RIP2K) dimerization, which we show to be essential for RIP2 activation [3]. Furthermore, our preliminary data show that such dimerization might be relevant for the recognition of the E3 ligase XIAP.

[1] D.J. Philpott, M.T. Sorbara, S.J. Robertson, K. Croitoru & S.E. Girardin. (2014) NOD proteins: regulators of inflammation in health and disease.**Nature reviews. Immunology.** 14:9-23.

[2] E. Pellegrini, A. Desfosses, A. Wallmann, W.M. Schulze, K. Rehbein, P. Mas, L. Signor, S. Gaudon, G. Zenkeviciute, M. Hons, H. Malet, I. Gutsche, C. Sachse, G. Schoehn, H. Oschkinat & S. Cusack (2018). RIP2 filament formation is required for NOD2 dependent NF-κB signalling. **Nat Commun.** 9:4043.

[3] E. Pellegrini, L. Signor, S. Singh, E. Boeri & S. Cusack (2017) Structures of the inactive and active states of RIP2 kinase inform on the mechanism of activation. **PloS one** 12:e0177161

P45 - Structural specificities of the mitotic kinesin MKlp2 and implications to its mechanism and function

F.M. Ranaivoson[a], A. Abdalla Mohammed Khalid[b], S. Inagaki[c], C. Schmidt[b], S.S. Rosenfeld[c], A. Houdusse[a]

[a]*Institut Curie, Structural Motility team, UMR 144, Paris, France*
[b]*Georg-August-Universität, Fakultät für Physik, Göttingen, Germany*
[c]*Mayo Clinic, Department of Neuro-Oncology, Jacksonville, FL, United States*
Email : fanomezana-moutse.ranaivoson@curie.fr

During cell division, the kinesin-6 MKlp2 plays a critical role from the metaphase-anaphase transition to the cytokinesis. In particular, in late metaphase, MKlp2 localizes at the antiparallel microtubule (MT) bundles that form at the spindle midzone, where it mediates the translocation of the Chromosome Passenger Complex (CPC) from the centromeres to the mitotic spindle and then to the contractile ring at the equatorial cortex [1]. At this stage, the ensemble {Mklp2-CPC} regulates the furrow ingression process and eventually the abscission to split the two daughter cells apart. Due to this important function in cell division and because several cancer cells overexpress MKlp2, a growing interest in 1) investigating the peculiar MKlp2's molecular mechanism and 2) specifically targeting this mitotic kinesin by anti-tumoral drugs arose during the past decade [2]. Like other kinesin-6s, MKlp2 is characterized by a large and poorly conserved insertion in the loop L6 of its motor domain (MD) and an unusually long neck linker (NL), a fragment that connects the C-terminus of one MD to the dimerization (coiled-coil) domain. Our initial cryo-EM and biochemical data highlighted MKlp2's mechanistic specificities when compared to other kinesins. Importantly, we showed that the ADP state of this kinesin displays an unusually high affinity towards MTs, which led to the first structural characterisation of a MT-bound ADP-kinesin [3].

Here we further decipher the structural and functional properties of MKlp2. Using X-ray crystallography, we reached a resolution of 2.1 Å that allowed us to confidently define the conformation of the L6 insert and to establish that its N-terminal part is compactly folded with the core MD, while its C-terminal part is disordered. At the opposite side of the structure, we show that the nucleotide-free state is accompanied by a backward-docked NL, which is a newly observed structural feature of a kinesin motor pre-stroke state. In addition, although deleterious to its velocity, the MKlp2 long NL (~50 residues) would allow one MD of a dimer to explore a wide space while the other binds to a microtubule, presumably reaching another microtubule. Indeed, using motility assays, we demonstrate that dimeric MKlp2 constructs move along microtubule at low rate. Instead, these constructs allow the bundling of MTs and their organisation into a three-dimensional network, a property usually observed with tetrameric kinesins. Altogether, our results provide unprecedented structural and physical exploration into the molecular mechanism of MKlp2 and shed new lights towards a fine understanding of MKlp2 functions at the mitotic spindle.

[1] J. Landino, S.R. Norris, M. Li, E.R. Ballister, M.A. Lampson and R. Ohi, Mol. Biol. (2017). **Cell.** 28:3634
[2] R.D. Baron and F.A. Barr, Kozielski. (2015). FSB F. (eds) Kinesins and Cancer. Chapter 12. Springer, Dordrecht.
[3] J. Atherton, I-M. Yu, A. Cook, J. M. Muretta, A. Joseph, J. Major, Y. Sourigues, J. Clause, M. Topf, S.S. Rosenfeld, A. Houdusse , C.A. Moores. (2017). **eLIFE**. e27793.

P46 - Structural and dynamic characterization of the intrinsically disordered tail of ErbB2, phosphorylation and interaction

L. Pinet [a], Y.H. Wang [a], E. Lescop [a], F. Guerlesquin [b], D. Durand [c], N. Assrir [a], C. van Heijenoort [a]

[a] *Department of Analytical and Structural Chemistry and Biology, ICSN, CNRS UPR 2301, Gif-sur-Yvette*
[b] *Laboratoire d'Ingénierie des Systèmes Macromoléculaires, IMM, UMR 7255, Marseille*
[c] *Biochemistry, Biophysics and Structural Biology Department, I2BC, UMR 9198, Gif-sur-Yvette*
E-mail: carine.van-heijenoort@cnrs.fr

ErbB2 is a member of the ErbB family of receptor tyrosine kinases, which are involved in signaling that control cell proliferation, cell migration and apoptosis. It is the only ErbB for which no ligand is needed for efficient signaling. Its overexpression is observed in many cancers and is especially correlated to poor prognosis in breast cancer. Signal transduction is triggered by dimerization, activating the kinase domain that phosphorylates the C-terminal end of the protein, which we showed to be an intrinsically disordered region (IDR). This tail, called CtErbB2, is the hub for the interactions that will determine cell fate, via its phosphorylated tyrosines. Although several drugs targeting the extracellular and kinase domains are effective, resistance is acquired within a few years of therapy. It is therefore necessary to expend the range of available treatments, and CtErbB2 remains an unexplored, but potentially critical target.
CtErbB2 is a 268-residue, proline-rich IDR. Using high-field NMR, we showed that unphosphorylated CtErbB2 retain very little residual structure [1], and is locally particularly extended. However, a N-to-C terminal contact occurs, potentially modulating the accessibility of certain sites to partners.
In addition to phosphotyrosines that interact with SH2 or PTB domains, CtErbB2 contains PxxP motifs prone to interact with SH3 domains. Grb2, a central protein in Ras-dependent pathways, contains both SH2 and SH3 domains and could therefore be subject to particular interaction mechanisms. We determined the solution organization of Grb2 domains, and studied the interaction between unphosphorylated CtErbB2 and Grb2, as well as between Grb2 and phosphopeptides of ErbB2, showing that the SH3 domains indeed modulate the interaction.
Strategies to obtain phosphorylated CtErbB2 are also tested, to study the interdependence of phosphorylation events, behavior of the modified protein, and role of phosphorylation in regulating interactions.

[1] Y.H. Wang, L. Pinet, N. Assrir, L. Elantak, F. Guerlesquin, A. Badache, E. Lescop and C. van Heijenoort (2017). 1H, 13C and 15N assignments of the C-terminal intrinsically disordered cytosolic fragment of the receptor tyrosine kinase ErbB2, **Biomol NMR Assign** 12:23-26.

P47 - Macromolecules interactions *in vitro*, comparing classical and innovative approaches

P. Fernández Varela, A. Gontier, C. Velours, M. Aumont-Niçaise, M. Desmadril, P. Minard, J.-B. Charbonnier

Institute for Integrative Biology of the Cell, CNRS, Gif sur Yvette, France.
Email: paloma.fernandez-varela@i2bc.paris-saclay.fr

To understand the molecular basis of macromolecules interactions we need to be aware of the advantages and limitations of the available methods. Here we will analyse the principles, strengths and limitations of different methods, classical as analytical ultracentrifugation with or without fluorescence (AUC-FDS), isothermal titration calorimetry (ITC) or size exclusion chromatography coupled to Multi-Angle Light Diffusion (SEC-MALS); and new approaches as bio-layer interferometry (BLI), Microscale Thermophoresis (MST) or switchSENSE (DRX^2), for measuring *in vitro* interactions. We will focus in the study protein-protein and protein-DNA interactions that are studied in our laboratories. We will compare results obtained using six approaches in parallel on two projects (Fig.1). Firstly, a protein-protein interaction between an artificial binder, an alpha-Rep with its target [1]. Secondly, a double-strand break repair factors, Ku70/Ku80 with its DNA substrates [2].

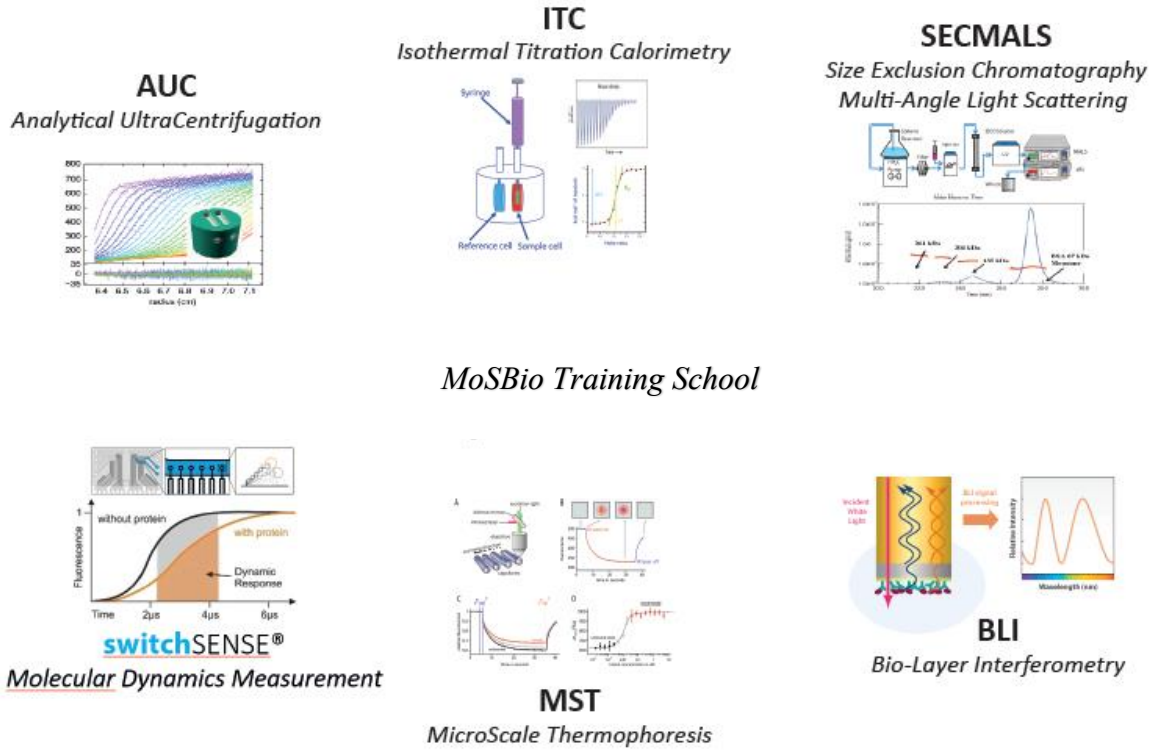


Fig. 1. Classical to innovative approaches to study macromolecules interactions

[1] Guellouz A, Valerio-Lepiniec M, Urvoas A, Chevrel A, Graille M, Fourati-Kammoun Z, Desmadril M, van Tilbeurgh, Minard P. (2013) Selection of Specific Protein Binders for Pre-Defined Targets from an Optimized Library of Artificial Helicoidal Repeat Proteins (alphaRep). **PLoS One**. 8:e71512

[2] Nemoz C, Ropars V, Frit P, Gontier A, Drevet P, Yu J, Guerois R, Pitois A, Comte A, Delteil C, Barboule N, Legrand P, Baconnais S, Yin Y, Tadi S, Barbet-Massin E, Berger I, Le Cam E, Modesti M, Rothenberg E, Calsou P, Charbonnier JB. (2018). XLF and APLF bind Ku80 at two remote sites to ensure DNA repair by non-homologous end joining. **Nat Struct Mol Biol**. 25:971-980

P48 - The plant defense signal galactinol is specifically used as a nutrient by the bacterial pathogen agrobacterium fabrum

A. Vigouroux[a], T. Meyer[b], M. Aumont-Nicaise[a], G. Comte[b], L. Vial[b], C. Lavire[a], and S. Moréra[a]

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

[b] *UMR Ecologie Microbienne, CNRS, INRA, VetAgro Sup, UCBL, Université de Lyon, F-69622, Villeurbanne, Lyon, France*

E-mail: armelle.vigouroux@i2bc.paris-saclay.fr

The bacterial plant pathogen *Agrobacterium fabrum* uses periplasmic-binding proteins (PBPs) along with ABC transporters to import a wide variety of plant molecules as nutrients. Nonetheless, how *A. fabrum* acquires plant metabolites is incompletely understood. Using genetic approaches and affinity measurements, we identified here the PBP MelB and its transporter as being responsible for the uptake of the raffinose family of oligosaccharides (RFO), which are the most widespread d-galactose-containing oligosaccharides in higher plants. We also found that the RFO precursor galactinol, recently described as a plant defense molecule, is imported into *Agrobacterium* via MelB with nanomolar range affinity. Structural analyses and binding mode comparisons of the X-ray structures of MelB in complex with raffinose, stachyose, galactinol, galactose, and melibiose (a raffinose degradation product) revealed how MelB recognizes the nonreducing end galactose common to all these ligands and that MelB has a strong preference for a two-unit sugar ligand. Of note, MelB conferred a competitive advantage to *A. fabrum* in colonizing the rhizosphere of tomato plants. Our integrative work highlights the structural and functional characteristics of melibiose and galactinol assimilation by *A. fabrum*, leading to a competitive advantage for these bacteria in the rhizosphere. We propose that the PBP MelB, which is highly conserved among both symbionts and pathogens from *Rhizobiace* family, is a major trait in these bacteria required for early steps of plant colonization.

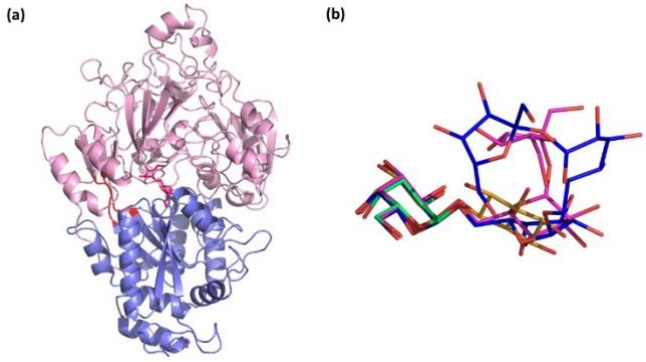


Fig. 1: Ribbon representation of MelB structures and superposition of ligands in binding site (a) Raffinose in magenta is located in the cleft between the lobes 1 and 2 shown in slate and in pink, respectively, and the hinge region is in red. (b) Superposition of the bound galactose, melibiose, galactinol, raffiose and stachyose shown in green, yellow, orange, magenta, blue sticks, respectively, in the binding site of MelB,

Meyer T, Vigouroux A, Aumont-Nicaise M, Comte G, Vial L, Lavire C, Moréra S. (2018). The plant defense signal galactinol is specifically used as a nutrient by the bacterial pathogen Agrobacterium fabrum. **J Biol Chem**.293:7930 -7941.

Session V: Technological/Methodological Innovations

Nathalie Colloc'h, ISTCT, Caen
P49 - Neuroglobin under every kind of pressure

Vincent Delauzun, AFMB, Marseille
P50 - Extracellular vesicles as a new platform to study cell surface membrane protein structure and interaction with ligand

Evolène Deslignière, LSMBO, Strasbourg
P51 - Toward automation of Collision Induced Unfolding experiments through online Size Exclusion Chromatography coupled to native Mass Spectrometry

Philippe Dumas, IGBMC, Strasbourg
P52 - Rigorous equations for Isothermal Titration Calorimetry

Eric Ennifar, IBMC, Strasbourg
P53 - New biophysical approaches to study interactions between ribosome and viral RNA

Jean-Luc Ferrer, IBS, Grenoble
P54 - FIP2-BM07: a post-EBS automated beamline designed for a large range of macromolecular crystallography experiments

Sabine Gavalda, TBI, Toulouse
P55 - Engineering the first highly efficient PET-depolymerizing enzyme

Eric Girard, IBS, Grenoble
P56 - Developments of lanthanide complexes for structural biology

Oscar Hernandez Alba, LSMBO, Strasbourg
P57 - Intact- and middle-level Collision Induced Unfolding experiments to decipher gas-phase unfolding mechanism of hybrid and canonical mAbs

Robin Lener, SOLEIL, Gif-sur-Yvette
P58 - State-of-the-art instrumentation for optimised integrated structural biology experiments – the MX approach at proxima-1

Yuan-Yuan Liao, SOLEIL, Gif-sur-Yvette
P59 - Development of microfluidic mixers for the preparation of time-resolved Cryo-EM biological samples

Amin Sagar, CBS, Montpellier
P60 - Chemometric decomposition of multiple SAXS datasets to study transient protein-protein complexes

Claude Sauter, IBMC, Strabourg
P61 - ChipX: a new microfluidic design for efficient biomolecule crystallization and in situ structural analysis by serial crystallography

P49 - Neuroglobin under every kind of pressure

N. Colloc'h [a], E. Girard [b], P. Carpentier [c], T. Prangé [d], B. Vallone [e]

[a] *ISTCT UMR 6030 CNRS Université de Caen-Normandie CEA, CERVOxy team, centre Cyceron, Caen, France*
[b] *IBS UMR 5075 Université Grenoble Alpes CEA CNRS, Grenoble, France*
[c] *CEA/DRF/BIG/CBM/BioCat LCBM CNRS Université Grenoble Alpes, Grenoble, France*
[d] *CitCoM UMR 8038 CNRS Université Paris Descartes Faculté des Sciences Pharmaceutiques, Paris, France*
[e] *Instituto Pasteur-Fondazione Cenci Bolognetti, Sapienza univesrité di Roma, Italy*
E-mail: colloch@cyceron.fr

Protein dynamics and plasticity are intrinsically correlated to functional efficiency. Internal cavities present within proteins facilitate conformational transitions between conformers and are crucial for conformational flexibility. On one hand, pressure promotes high energy conformers of lower volumes, and thus becomes an ideal tool to study conformational fluctuations and relationships between local rigidity an overall flexibility. On the other hand, noble gas labelling is the best way to map hydrophobic cavities and tunnels, and to identify ligand migration pathways.
Neuroglobin structures has been determined under high hydrostatic pressure for WT and mutants (up to 3000 bar), under moderate xenon pressure for WT and mutants (40 bar), under high noble gas pressure (krypton up to 150 bar, argon up to 2000 bar) and moderate dioxygen pressure (up to 50 bar). These combined approaches allow to determine the most flexible and the most rigid parts of neuroglobin in relation with cavity modifications, and to characterize dioxygen migration pathway and storage binding site [1-3]. In combination with coarse-grained simulations, a mechanical nucleus around which neuroglobin hinges during its conformational transition induced by dioxygen migration has been evidenced, to achieve coordination through the heme sliding mechanism.

[1] N. Colloc'h, S. Sacquin-Mora, G. Avella, A.C. Dhaussy, T. Prangé, B. Vallone, E. Girard. (2017). Determinants of neuroglobin plasticity highlighted by joint coarse-grained simulations and high pressure crystallography. **Sci. rep**. 7:1858
[2] N. Colloc'h, P. Carpentier, L.C. Montemiglio, B. Vallone, T. Prangé. (2017). Mapping hydrophobic tunnels and cavities in neuroglobin with noble gas under pressure. **Biophys. J.** 113:2199
[3] C. Ardiccioni, A. Arcovito, S. Della Longa, P. van der Linden, D. Bourgeois, M. Weik, L.C. Montemiglio, C. Savino, G. Avella, C. Exertier, P. Carpentier, T. Prangé, M. Brunori, N. Colloc'h, N. Vallone. (2019) Ligand pathways in neuroglobin revealed by low temperature photodissociation and docking experiments. **IUCr J**., in press

P50 - Extracellular vesicles as a new platform to study cell surface membrane protein structure and interaction with ligand

V. Delauzun[a], S. Spinelli[a], B. Amigues[a], A. Boubou Coulibaly[a], L. Gauthier[b], A. Roussel[a]

[a]*CNRS, Aix Marseille Université, AFMB, Architecture et Fonction des Macromolécules Biologiques, 13009 Marseille, France*
[b]*Innate Pharma, 117 Avenue de Luminy, 13009 Marseille, France*
E-mail: vincent.delauzun@afmb.univ-mrs.fr

Membrane proteins are a central subclass of the proteome. Producing intact membrane proteins in order to study them is an inherently challenging task due to their requirement for a lipid environment. Most procedures developed involve isolating the protein by detergent solubilization and further reconstitutions into artificial membrane such as liposomes or nanodiscs. These procedures are highly time consuming and suffer from further drawbacks, including low yields and high cost.
Most eukaryotic cells have the physiological ability to secrete extracellular vesicles (EVs). They are made with a lipid bilayer comparable to a cytoplasmic membrane containing membrane receptors. Their size varies from about ten to a few hundred nanometers. EVs constitute true intercellular communication channels and are also involved in cellular waste disposal processes.
Due to their physico-chemical properties, EVs are an advantageous tool for the study of membrane receptors as close as possible to their natural state.
We present here an innovative method for rapidly obtaining EVs derived from cells in culture and coated with a membrane protein of biological interest. These EVs can be easily purified and observed by electron microscopy to confirm the presence on their surface of the protein to be studied. Interactions with ligands such as antibodies can be visualized directly on the surface of these modified EVs by electron microscopy, and can be analyzed by biolayer interferometry as well. In order to validate this new method, two different membrane proteins were studied: the glycoprotein of the Ebola virus envelope and the CD73 membrane enzyme. [1]

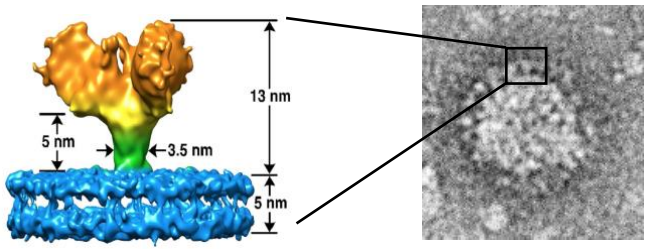


Fig. 1: electron microscopy image in negative staining of recombinant EVs with the Ebola virus envelope glycoprotein

[1] Booth.TF et al, (2017), Structure of the Ebola virus glycoprotein spike within the virion envelope at 11A resolution. **Sci Rep** 7:46374

P51 - Toward automation of Collision Induced Unfolding experiments through online Size Exclusion Chromatography coupled to native Mass Spectrometry

E. Deslignière [a], A. Ehkirch [a], T. Botzanowski [a], O. Hernandez-Alba [a], A. Beck [b], S. Cianférani [a]

[a] *Laboratoire de Spectrométrie de Masse BioOrganique (LSMBO), Université de Strasbourg, IPHC, CNRS - UMR 7178, 67000 Strasbourg, France*
[b] *IRPF - Centre d'Immunologie Pierre-Fabre (CIPF), 74160 Saint-Julien-en-Genevois, France*
E-mail : evolene.desligniere@etu.unistra.fr

Native ion mobility (IM) and collision induced unfolding experiments (CIU) play a key role in the characterization of biotherapeutics [1]. CIU detects subtle structural differences of monoclonal antibodies (mAbs) based on their gas-phase unfolding patterns and stabilities [2], by sequential increase of collision voltages. However, CIU lacks in automation and remains tedious and time-consuming, hampering its routine use. We thus present here an automated CIU data acquisition workflow using size-exclusion chromatography (SEC) coupled to native IM-MS for mAb analysis.
Automated CIU data acquisition using SEC-native IM-MS was evaluated for several intact mAbs of different isotypes. CIU fingerprints obtained with our automated workflow are compared to manually performed experiments. Online automated SEC-CIU experiments present several benefits over manual CIU, among which i) improved and fast desalting efficiency compared to manual buffer exchanges used for classical CIU experiments [3]; ii) drastic reduction of the overall data acquisition time process from 2 hours to 30 minutes along with iii) maintaining CIU key features (number of transitions and CIU50 values) to reach similar CIU fingerprints.
Our results demonstrate the possibility of automating CIU experiments using an online SEC-CIU coupling. Our strategy provides an automated workflow, from sample preparation to CIU data interpretation. This fast isotype screening method proved its efficiency for mAb analysis and could be adapted to the analysis of other systems.

[1] S. M. Dixit, D. A. Polasky, B. T. Ruotolo. (2018). Collision induced unfolding of isolated proteins in the gas phase: past, present, and future. **Curr. Opin. Chem. Biol.** 85:715-736
[2] O. Hernandez-Alba, E. Wagner-Rousset, A. Beck, S. Cianférani. (2018). Native Mass Spectrometry, Ion Mobility, and Collision-Induced Unfolding for Conformational Characterization of igG4 Monoclonal Antibodies. **Anal. Chem.** 91:8865-8872
[3] A. Ehkirch, O. Hernandez-Alba, O. Colas, A. Beck, D. Guillarme, S. Cianférani. (2018). Hyphenation of size exclusion chromatography to native ion mobility mass spectrometry for the analytical characterization of therapeutic antibodies and related products. **J. Chromatogr. *B*** 1086:176-183.

P52 - Rigorous equations for Isothermal Titration Calorimetry

P. Dumas

Institut de Génétique et Biologie Moléculaire et Cellulaire, IGBMC,
Université de Strasbourg, CNRS, UMR 7104, Illkirch, France.
E-mail: dumasp@igbmc.fr

New mathematical methods have been developed for processing titration curves (TC) obtained from Isothermal Titration Calorimetry (ITC). Exact TC equations for the usual multi-injection method (MIM), or for the single-injection method (SIM) with continuous injection, were derived by taking into account rigorously the effect of dilution resulting from the titration process [1]. The only hypothesis being made is that the cell content is always well mixed. Considering the classical one-step association/dissociation mechanism A + B ⇄ C with B initially in the syringe, the commonly used approximate method leads to a heat power per injected mole of B equal to:

$$Q(v) = (\Delta H/B_0)\, dC/dv$$

with B_0 the concentration of B in the syringe and the reduced volume $v = V/V_{cell}$ with V the total injected volume of B at any step of the titration. As intuitive the latter result may be with this direct proportionality between Q(v) and dC/dv, it is wrong. The exact method leads to the non-intuitive equation:

$$Q(v) = (\Delta H/B_0)\, [dC/dv + C(v)]$$

The analysis can be generalized to any complex mechanism. The novelty is that, in all situations where the concentrations at equilibrium of the different species can be obtained at any step of the titration in 'closed form' (*i.e.* with an equation), the method provides an exact equation for the titration curve. The result obtained with the usual one-step mechanism A + B ⇄ C was compared with the results from four currently used programs, which identified a systematic error pervading three of them, NanoAnalyze from TA being much less in error.

The exact TC equation for the one-step mechanism A + B ⇄ C is readily programmed in common tools like Microsoft Excel and allows fitting an experimental TC by using its Solver functionality (an Excel file with an example is available at https://doi.org/10.1101/512780 . NB: take care of uploading the most recent version of the Excel file). The exact methods are also being integrated in the program AFFINImeter [2].

This exact analysis is also applicable for processing data obtained from the single-injection method (SIM). The improvement on existing methods is described in [1] and was assessed with data from [3].

[1] Dumas P. Rigorous equations for isothermal titration calorimetry: theoretical and practical consequences. bioRxiv 512780; doi: https://doi.org/10.1101/512780
[2] https://www.affinimeter.com/site/
https://www.affinimeter.com/site/download/affinimeter-itc-windows/
[3] Markova N., Hallen D. (2004) The development of a continuous isothermal titration calorimetric method for equilibrium studies. **Analytical Biochemistry** 331:77-88. doi: 10.1016/j.ab.2004.03.0

P53 - New biophysical approaches to study interactions between ribosome and viral RNA

E. Schenckbecher [a], .G. Bec [a], L. Traxler [b], T. Sakamoto [c], E. Ennifar [a],

[a] Architecture et Réactivité de l'ARN, IBMC, CNRS/Université de Strasbourg, Strasbourg, France
[b] Dynamic Biosensors GmbH, Planegg, Germany
[c] Department of Life Science, Chiba Institute of Technology, Chiba, Japan
E-mail: e.ennifar@unistra.fr

Translation initiation, in both eukaryotes and bacteria, requires essential elements such as mRNA, ribosome, initiator tRNA and finally initiation factors. For each domain of life, canonical mechanisms and signals are observed to initiate protein synthesis. However, in some cases other ways of initiation can be used, as for viral mRNAs. Viruses hijack cellular machinery to translate some of their mRNAs through a non-canonical initiation pathway using Internal Ribosome Entry Site (IRES), a highly structured RNAs which can directly recruit the ribosome.
Here we took advantage of innovative biophysical approaches to study interactions between the intergenic IRES from the cricket paralysis virus (CrPV) and the eukaryotic yeast ribosome. Isothermal Titration Calorimetry (ITC) and kinetic ITC (kinITC) provided thermodynamic and kinetic data [1]. A comparison is made with data collected on a biosensor using the new switchSENSE technology, based on electroswitchable DNA chips [2].

[1] Burnouf, D.; Ennifar, E.; Guedich, S.; Puffer, B.; Hoffmann, G.; Bec, G.; Disdier, F.; Baltzinger, M.; Dumas, P. (2012) **J Am Chem Soc**, 134:559-65.
[2] Kaiser, W.; Rant, U. (2010) **J Am Chem Soc**, 132:7935-45.

P54 - FIP2-BM07: a post-EBS automated beamline designed for a large range of macromolecular crystallography experiments

J.-L. Ferrer [a], Y. Sallaz-Damaz [a], C. Berzin [a], E. Mathieu [a], P. Jacquet [a], P. Israel-Gouy [a], D. Cobessi [a], F. Borel [a], M. Budayova-Spano [a], X. Vernede [a]

[a] *Univ. Grenoble Alpes, CEA, CNRS, IBS, F-38000 Grenoble, France*
E-mail: jean-luc.ferrer@ibs.fr

In the context of the ESRF-EBS upgrade, a new French CRG beamline dedicated to macromolecular crystallography (MX) is being built on port BM07, in replacement of the FIP-BM30A beamline that ended operations in Nov. 2018 [1]. This new beamline, named FIP2, will be a flexible and highly automated beamline with improved performances with respect to FIP-BM30A, without loosing its specificities. It is designed to host a large variety of MX experiments, including anomalous phasing, neutron and spectroscopy coupled diffraction, *in situ* diffraction, etc. (*i*) Regarding anomalous diffraction, it will be possible to perform faster experiments with improved signal/noise, still with homogeneous exposure of sample, in an extended sample size and energy range (long wavelength S/K-SAD). Limited radiative damages will also make possible the systematic anomalous diffraction experiment for heavy atoms detected by X-ray fluorescence, to identify unambiguously the type and position of these atoms). (ii) The increased intensity will facilitate X-ray sample identification *in situ*. Automated data collection on series of crystals in the same plate will be facilitated. This will open the possibility for high-throughput ligand screening [2], and the analysis at room temperature of structures previously solved at 100 K. (iii) Induced UV/X-ray radiation damage phasing and on-line absorption spectroscopy experiments will also benefit from the beam improvements. Homogeneous exposure to X-ray beam will be possible for a larger extent of sample sizes, with higher flux and therefore faster measurements. And of course, as for FIP, FIP2 will also focus on instrumental and methodology developments. FIP2 is planned to start normal operation by the end of 2020.

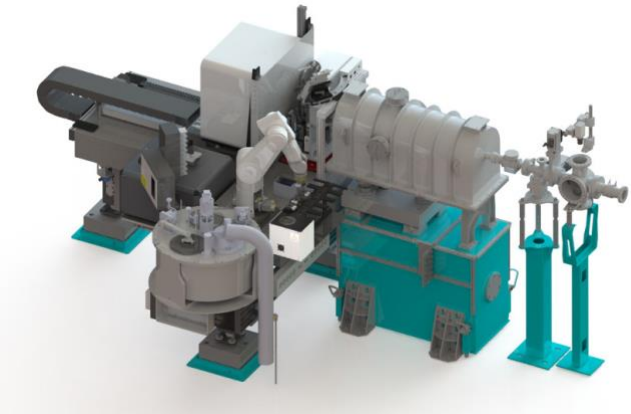


Fig. 1: Experimental setup of FIP2-BM07.

[1] M. Roth, P. Carpentier, O. Kaïkati, J. Joly, P. Charrault, M. Pirocchi, R. Kahn, E. Fanchon, L. Jacquamet, F. Borel, A. Bertoni, P. Israel-Gouy, and J.-L. Ferrer (2002). FIP: a Highly Automated Beamline for Multiwavelength Anomalous Diffraction Experiments. **Acta. Cryst. D** 58:805-814
[2] M. Gelin, V. Delfosse, F. Allemand, F. Hoh, Y. Sallaz-Damaz, M. Pirocchi, W. Bourguet, J.-L. Ferrer, G. Labesse, J.-F. Guichou (2015). "Dry" co-crystallization and in situ diffraction for automatic ligand screening by X-ray crystallography. **Acta Cryst. D** 71:1777-1787

P55 - Engineering the first highly efficient PET-depolymerizing enzyme

V. Tournier[b], C. M. Topham[a], A. Gilles[a], E. Kamionka[a], S. Gavalda[a], J. Nomme[a], G. Cioci[a], I. André[a], S. Duquesne[a], A. Marty[a,b]

[a] *Toulouse Biotechnology Institute, Bio & Chemical Engineering (ex.LISBP)*
Université de Toulouse - CNRS 5504 - INRA 792 – INSA
135 avenue de Rangueil, 31077 Toulouse CEDEX 04.
[b] *Carbios, 3, rue Emile Duclaux; Biopôle Clermont Limagne; 63360 Saint-Beauzire, France.*
E-mail: sgavalda@insa-toulouse.fr

In the last century, plastics became a ubiquitous material with a wide range of applications in every aspect of life. They are commonly used in packaging, automotive, buildings, electronics, clothes, etc. To date, more than 335 million tons per year of plastics are produced worldwide [1], and 150 million tons of plastic wastes are generated every year. Most of them are issued from the chemistry of petroleum such as polyethylene, polyethylene terephthalate (PET)... Sometimes adored, often deplored, plastic is becoming a threat to our planet both on earth and in oceans and its accumulation in the environment is disastrous. The main recycling process for PET, via mechanical means, results in a loss of mechanical properties [2]. Consequently, de novo synthesis is preferred, and PET waste continues to accumulate.
The selected protein is an enzyme that has recently received attention because of its potential application for degrading such plastics. To optimize the depolymerization yields, we sought to improve this protein by enzymatic engineering. For this we obtained the 3D structure of the protein at very high resolution and combining this with modeling approaches we improved the activity of the enzyme and its thermostability (Tm evaluation by differential scanning fluorimetry DSF). Finally, we obtained a PET hydrolase that ultimately achieves a minimum of 90% PET depolymerization into monomers in a timescale of 10 hours compatible with economically viable industrialization.

[1] PlasticsEurope, Plastics - The Facts 2017: An Analysis of European Plastics Production, demand and waste data. **PlasticsEurope** (2017).
[2] Ragaert, K., Delva, L. & Van Geem, K. (2017) Mechanical and chemical recycling of solid plastic waste. **Waste Manag.** 69:24-58.

P56 - Developments of lanthanide complexes for structural biology

Z. Alsalman[a], S. Engilberge[a], T. Wagner[b], C. Sauter[c], G. L. Santoni[d], F. Gabel, F. Riobé[e], O. Maury[e], E. Girard[a]

[a] Institut de Biologie Structurale, IBS, CEA, CNRS, UMR 5075, Grenoble, France, [b] Max Planck Institute for Terrestrial Microbiology, Marburg, Germany, [c] Institut de Biologie Moléculaire et Cellulaire (IBMC), UPR 9002, Strasbourg, France, [d] Structural Biology Group, European Synchrotron Radiation Facility, Grenoble, France, [a]ENS de Lyon, CNRS UMR 5182, Université Claude Bernard, Lyon, France.

E-mail : eric.girard@ibs.fr

X-ray crystallography is considered the most productive method to obtain high-precision structural information about biological macromolecules, with 90% of structures deposited in the Protein Data Bank (PDB) were resolved using this method. However, this method still suffers from two main issues: production of high quality/well-diffracting crystals and solving the phase problem inherent to the method. For that, a new molecule named crystallophore (Xo4) [1] was proposed.

Crystallophore is a cationic lanthanide complex with nucleating properties and with a high-phasing power [1]. Thus, it overcomes the two major bottlenecks of macromolecular crystallography and highly facilitates the crystal structure determination process. We will present examples of successes obtained thanks to Xo4 [2,3] including serial crystallography as well as in-situ experiments [4]. We will also present preliminary results on the second generation of Xo4 developed in order to improve the Xo4 capabilities and to extend the nucleating possibilities to targets of interest such as membrane proteins. Finally, we will explore the possibility of using such molecules for other structural methods such as SAXS.

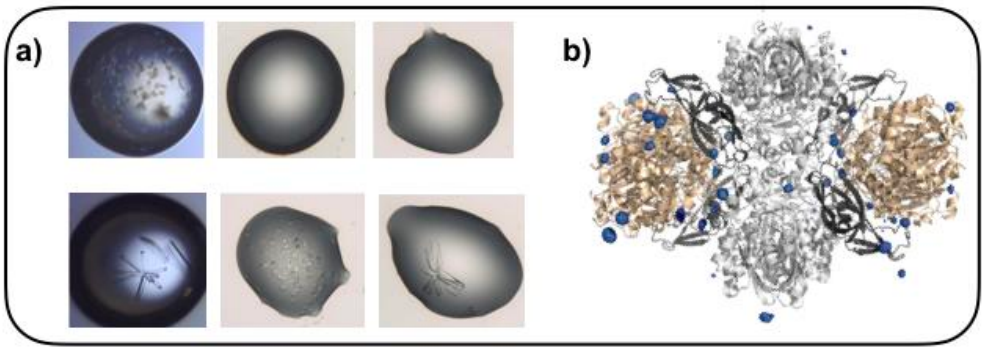


Fig. 1: a) Crystallization drops obtained without (top) and with Xo4 (bottom) for three different proteins. b) Structure of the complex thiolase/HMGCS/DUF35 [2].

[1] Engilberge S, Riobé F, Di Pietro S, Lassalle L, Coquelle N, Arnaud C-A, Pitrat D, Mulatier J-C, Madern D, Breyton C, et al. (2017) Crystallophore: a versatile lanthanide complex for protein crystallography combining nucleating effects, phasing properties, and luminescence. **Chem Sci** 8: 5909–5917.

[2] Vögeli B, Engilberge S, Girard E, Riobé F, Maury O, Erb TJ, Shima S, Wagner T (2018) Archaeal acetoacetyl-CoA thiolase/HMG-CoA synthase complex channels the intermediate via a fused CoA-binding site. **Proceedings of the National Academy of Sciences** 115: 3380–3385.

[3] Bernhardsgrütter I, Vögeli B, Wagner T, Peter DM, Cortina NS, Kahnt J, Bange G, Engilberge S, Girard E, Riobé F, et al. (2018) The multicatalytic compartment of propionyl-CoA synthase sequesters a toxic metabolite. **Nat Chem Biol** 14: 1127–1132.

[4] de Wijn R, Hennig O, Roche J, Engilberge S, Rollet K, Fernandez-Millan P, Brillet K, Betat H, Mörl M, Roussel A, et al. (2019) A simple and versatile microfluidic device for efficient biomacromolecule crystallization and structural analysis by serial crystallography**. IUCrJ** 6: 454–464.

P57 - Intact- and middle-level Collision Induced Unfolding experiments to decipher gas-phase unfolding mechanism of hybrid and canonical mAbs

O. Hernandez Alba[a], T. Botzanowski[a], E. Wagner-Rousset[b], O. Colas[b], A. Beck[b], S. Cianférani[a]

[a] *Laboratoire de Spectrométrie de Masse BioOrganique, Université de Strasbourg, CNRS, IPHC UMR 7178, 67000 Strasbourg, France.*
[b] *IRPF - Centre d'Immunologie Pierre-Fabre (CIPF), 74160 Saint-Julien-en-Genevois, France*
E-mail: ahernandez@unistra.fr

Most currently approved mAbs for clinical treatment of several diseases are selected from three human IgG isotypes (1, 2, and 4) [1]. Since different isotypes also differ in their ability to support secondary immune functions, there is a real interest in developing new strategies to characterize the influence of the inter-chain disulfide bridges in the global mAb structure and stability thus, further understanding their structure-function relationship. Here we aim at developing new CIU-based strategies at intact and middle level to tackle structural differences/similarities on biotherapeutic mAbs not only from different isotypes, but also for hybrid IgG formats.
All the therapeutic mAb-based formats were deglycosylated and buffer desalted against 100 mM ammonium acetate at pH 7.0. Native CIU experiments were acquired on a hybrid SynaptTM G2 HDMS coupled to an automated chip-based nanoelectrospray device. Ions were progressively accelerated by increasing the acceleration voltage in the trap prior to IM separation by 5 V steps from 0 to 200 V. CIUSuite 2 [2] software was used to generate and compare all the CIU fingerprints.
CIU experiments were used to differentiate and characterize the gas-phase stability of several iso-cross-section mAbs isotypes. At the intact level, several differences are distinguished between the three mAb isotypes in terms of unfolding energies and number of unfolding transitions. However, no clear-cut evidences could be obtained to assign the CIU fingerprints to specific mAb isotypes. Subsequently, middle-CIU experiments were performed on the F(ab')2 and Fc subunits, giving rise to a higher number of unfolding transitions, allowing to assign specific unfolding signatures to each individual isotype class. In this case, middle-CIU experiments provided more diagnostic fingerprints to efficiently characterize/categorize mAb isotypes. CIU experiments were also conducted to evaluate the stabilization of the mAb structure upon payload conjugation [3] or to study the differences of the unfolding mechanism of different IgG4 formats after Ser to Pro mutation in the hinge region [4].
Altogether, our study highlights the suitability of CIU experiments, in particular at the middle-level, for the characterization of the gas-phase unfolding dynamics of different therapeutic mAbs formats. We envision that specific signatures characteristic of each individual mAb isotype from different IgG-subunits can be used to characterize/categorize new IgG-based biotherapeutics.

[1] Beck, A.; Wagner-Rousset, E., et al. (2013), **Anal. Chem.,** 85:715-736.
[2] Polasky, D. A.; Dixit, S. M., et al. (2019), **Anal. Chem.,** 91:3147-3155.
[3] Botzanowski, T.; Erb, S., et al. (2017) **mAbs,** 9:801-811.
[4] Hernandez-Alba, O.; Wagner-Rousset, E., et al. (2018), **Anal. Chem.,** 90:8865-8872.

P58 - State-of-the-art instrumentation for optimised integrated structural biology experiments – the MX approach at proxima-1

R. Lener, T. Isabet, P. Legrand, S. Sirigu, L. Chavas

Synchrotron SOLEIL, Gif sur Yvette, France.
E-mail: robin.lener@synchrotron-soleil.fr

Synchrotron machines worldwide are witnessing a revolution while evolving towards brighter sources, opening to methodology developments and better-implemented instrumentation at experiment-oriented beamlines. Following these exciting advances, the field of biology brilliantly succeeds in combining state-of-the-art experiments performed within a plethora of dedicated new approaches. Among these techniques, macromolecular crystallography beamlines are already subject to automation and very high-throughput data collection procedures, yet need to adapt to a quickly evolving field.

At the Synchrotron SOLEIL PROXIMA-1 beamline, the whole sample environment has been designed for optimized diffraction data measurements, to get the most of weakly diffracting crystals as much as the best of better diffracting ones. Similarly, the incoming x-ray beam has been shaped and treated to avoid noisy background generation and recording, again for optimized diffraction measurements. Finally, the control of the experiments at the beamline is interfaced through an adapted version of the collaborative MXCuBE project.

In the current poster, we intend to highlight the principle of the experiments at the beamline PROXIMA-1, and how these experiments are fully integrated within the HelioBiology life-sciences scientific section at Synchrotron SOLEIL. A new model for synchrotron access needs to be put in place in order for most of the biological projects to benefit from the best of each instrument in a common effort towards integrated biology.

P59 - Development of microfluidic mixers for the preparation of time-resolved Cryo-EM biological samples

Y.-Y. Liao[a], R. Kazan[b], P-D. Coureux[b], S. Lefrancois[a], E. Schmitt[b], L. Chavas[a], P. Legrand[a], Y. Mechulam[b], B. Lassalle-Kaiser[a]

[a] *Synchrotron SOLEIL, Laboratoire de microfluidique, L'Orme des Merisier, Saint-Aubin, BP 48, Gif-sur-Yvette Cedex F-91192;* [b]*Laboratoire de Biochimie 7654 CNRS-Ecole Polytechnique, Palaiseau cedex F-91128*
E-mail: yuan-yuan.liao@synchrotron-soleil.fr

We designed a mixing-spraying microfluidic system for time-resolved Cryo-EM sample preparation. The system allows the reactants to mix rapidly following a defined time interval (10 - 100ms), and to be sprayed onto an EM grid as a thin film (< 200nm). The micro-device used in the system is made of one micro-mixer (Figure 1) and one micro-sprayer (Figure2).

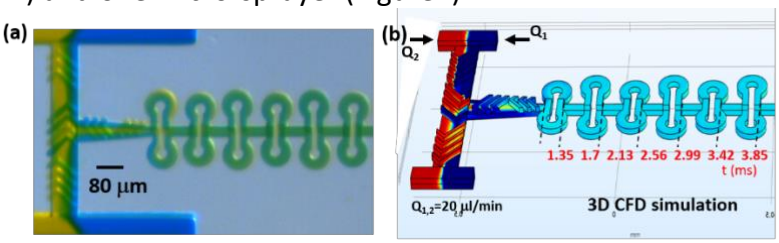


Figure 1: (a) Two-layers micro-mixer with colored solution introduced by syringe pumps (b) Computational fluid dynamics simulation by Comsol®.

A novel 3D-chaotic microfluidic mixing device was designed to achieve fast mixing (*ca.* 10 ms). It is a combination of a double T-type pre-mixer with staggered herring-bone ridges followed by pairs of single-butterfly mixing elements (Fig. 1), to enhance the entropy and achieve fast mixing. The swept volume of the whole mixing region is less than 8nL, which allows minimal consumption of sample. After thorough mixing, the solution is introduced to the micro-sprayer chip. The tested-chip is composed of two identical PDMS layers, with the design inspired by a 3D-microjet [1] and a gas-assisted annular micro-sprayer.[2]

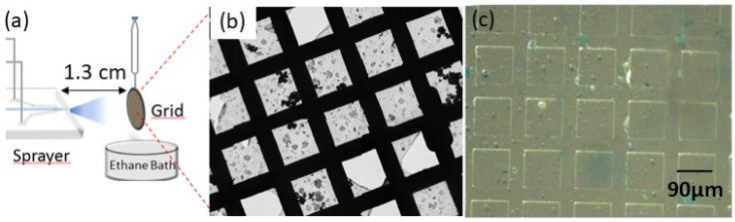


Figure 2: Visualization of the droplets sprayed on the EM grids.

The distribution and the thickness of the droplets that are caught by the grid are both crucial parameters in determining the quality of the cryo-EM image. The high surface tension of the grid is essential to the spreading of the droplets (Figure 2b), which size is about 10µm (Figure 2b-c), with very a good distribution and minimal statistical variation. These preliminary results are very promising and should soon allow the preparation of time-resolved samples for cryo-EM experiments.

[1] Trebbin, M.;Krüger, K.;DePonte, D.;Roth, S. V.;Chapman, H. N.; Förster, S. (2014) Microfluidic liquid jet system with compatibility for atmospheric and high-vacuum conditions. **Lab on a Chip**, 14:1733
[2] Lu, Z.;Barnard, D.;Shaikh, T. R.;Meng, X.;Mannella, C. A.;Yassin, A.;Agrawal, R.;Wagenknecht, T.; Lu, T.-M. (2014) Gas-Assisted Annular Microsprayer for Sample Preparation for Time-Resolved Cryo-Electron Microscopy. **Journal of micromechanics and microengineering : structures, devices, and systems,** 24:115001

P60 - Chemometric decomposition of multiple SAXS datasets to study transient protein-protein complexes

A. Sagar[a], F. Herranz-Trillo[b], R. Tauler [c], B. Vestergaard[b], P. Bernadó[a]

[a] *Centre de Biochimie Structurale(CBS), CNRS, INSERM, Montpellier, France*.
[b] *Department of Pharmacy and Department of Drug Design and Pharmacology, University of Copenhagen, Universitetsparken 2, Copenhagen, Denmark*
[c] *Institute of Environment Assessment and Water Diagnostics (IDAEA-CSIC), Barcelona, Spain*
E-mail: amin.sagar@cbs.cnrs.fr

Many biologically important systems are inherently polydisperse e.g. transient protein-protein complexes, oligomerizing proteins or proteins with different co-existing conformations. Such systems are always in an equilibrium of different species and are therefore heterogenous. Importantly, such systems cannot be meaningfully studied in a holistic sense by physically separating the components of the system as they exist in context of an equilibrium with multiple species. Small Angle X-ray Scattering (SAXS) is an excellent tool to study such systems as SAXS data are additive and it contains structural information of all co-existing species within the sample. With the appropriate tools, in principle SAXS data of these complex mixtures can be decomposed into the scattering profiles of the components. We propose a chemometrics based, model free method to decompose SAXS data into the contributions of the individual components and recover the relative populations at the same time [1]. Our method can use multiple SAXS datasets and multiple representations of the SAXS profiles to prepare augmented data matrices which improves the resolution of the curves and reduces the ambiguity of the solutions.

We have tested our method on the simulated SAXS titrations datasets of transient protein-protein complexes where one of the proteins is titrated with different ratios of the other protein. It must be noted here that as the dissociation constants of the complexes are high, the complex is never enriched in the solutions. However, using our method, we are able to recover the SAXS profile, and therefore the size and shape parameters, of the complex from the SAXS dataset. At the same time, we are also able to accurately extract the relative populations of the complex and the dissociated components through the titration series, giving insight into the thermodynamics of the system.

In addition of the transient complexes, our method can be extremely useful to study many kinds of the evolving SAXS data sets (oligomerization, Time-resolved SAXS, Fibrillation, etc.) and increases the reach of SAXS into the area of complex, heterogenous, evolving biological systems.

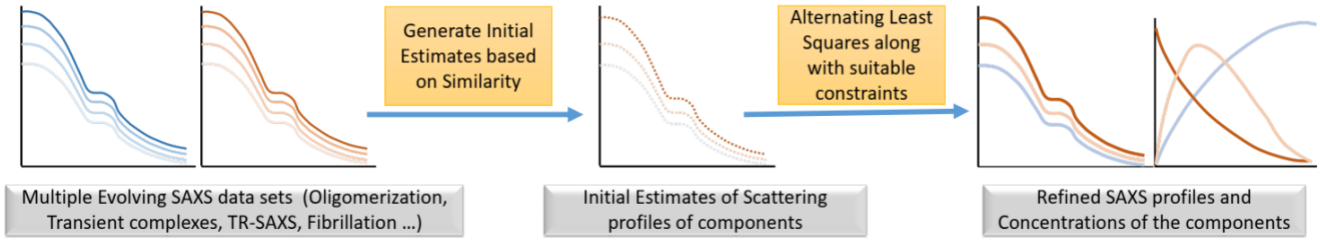


Fig. 1: Steps involved in the decomposition of SAXS profiles

[1] Herranz-Trillo, F. *et al.* (2017) Structural Analysis of Multi-component Amyloid Systems by Chemometric SAXS Data Decomposition. **Structure** 25:5-15

P61 - ChipX: a new microfluidic design for efficient biomolecule crystallization and in situ structural analysis by serial crystallography

R. de Wijn[a], K. Rollet[a], O. Hennig[b], J. Roche[c], S. Engilberge[d], P. Fernandez-Millan[a], K. Brillet[a], H. Betat[b], M. Mörl[b], A. Roussel[c], E. Girard[d], C. Mueller-Dieckmann[e], G.C. Fox[f], V. Oliéric[g] J.A. Gavira[h], B. Lorber[a], C. Sauter[a]

[a]Institut de Biologie Moléculaire et Cellulaire, UPR 9002 CNRS, Strasbourg, France ; [b]Biochemistry and Molecular Biology, Inst. for Biochemistry, Leipzig University, Leipzig, Germany; [c]Architecture et Fonction des Macromolécules Biologiques, UMR 7257 CNRS, Marseille, France ; [d]Institut de Biologie Structurale, UMR 5075 CEA-CNRS-UGA, Grenoble, France ; [e]European Synchrotron Radiation Facility, , Grenoble, France ; [f]Synchrotron SOLEIL, Gif-sur-Yvette, France ; [g]Paul Scherrer Institute, Swiss Light Source, Villigen, Switzerland; [h]Laboratorio de Estudios Cristalográficos, CSIC,Granada, Spain.

E-mail: c.sauter@ibmc-cnrs.unistra.fr

For about fifteen years, microfluidics has opened up new possibilities and brought many benefits for the crystallization of biomolecules. Indeed, microfluidic systems facilitate the manipulation of nanovolumes of sample solutions, as well as extreme miniaturization and parallelization of crystallization assays. In addition, they provide a convection-free environment that favors the growth of high-quality crystals [1].

We present a new multifunctional microchip design [2] combining: 1) the search and optimization of crystallization conditions of biomolecules by the counter diffusion method, 2) crystal identification by fluorescence microscopy, 3) microcrystalline seeding, 4) derivatization of crystals by substrate soaking, and 5) *in situ* crystal analysis at room temperature. The concept was tested on a large panel of biomolecules including RNA and various soluble or membrane proteins. Several crystal structures were solved *in situ* by the serial crystallography approach at room temperature under synchrotron radiation.

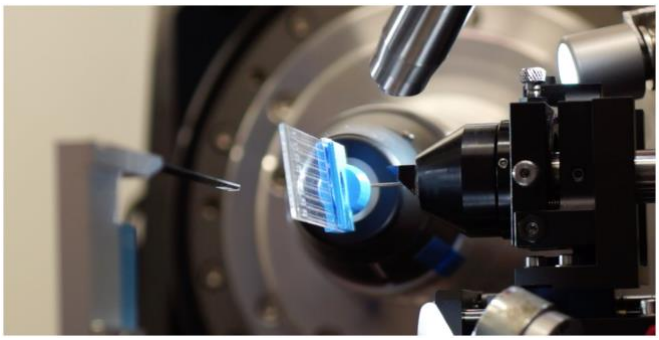


Fig. 1: *On-chip* crystal analysis by serial crystallography under synchrotron radiation.

[1] C. Sauter, K. Dhouib, B. Lorber. (2007). From macrofluidics to microfluidics in the crystallization of biological macromolecules. **Crystal Growth & Design**, 7:2247-50.

[2] R. de Wijn, O. Hennig, J. Roche, S. Engilberge, K. Rollet, P. Fernandez-Millan, K. Brillet, H. Betat, M. Mörl, A. Roussel, E. Girard, C. Mueller-Dieckmann, G.C. Fox, V. Oliéric, J.A. Gavira, B. Lorber, C. Sauter. (2019). A simple and versatile microfluidic device for efficient biomacromolecule crystallization and structural analysis by serial crystallography. **IUCrJ**, 6:454–464.

P62 - Ten years of PROXIMA1

S. Sirigu, L. Chavas, T. Isabet, P. Legrand, R. Lener

Synchrotron SOLEIL, Gif sur Yvette, France
E-mail: serena.sirigu@synchrotron-soleil.fr

The undulator beamline PROXIMA1 at the Synchrotron SOLEIL located in heart of the scientific cluster Paris-Saclay is a hard x-ray beamline dedicated to macromolecular crystallography.
Originally designed to be extremely compact for a very high stability and reproducibility, PROXIMA1 has gone through multiple rounds of technological implementations and evolutions over the past ten years.
We present here the most relevant advances on PROXIMA1 ranging from the optics to the diffraction detection, to the goniometry and automation. The state-of-the-art instrumentation made available at PROXIMA1 allowed to allocate over 3000 8-hours shifts to date and greatly contributed to obtaining high quality data as illustrated by more than 1100 structure depositions to the Protein Data Bank, coupled with high-impact factor scientific publications generated so far, making of PROXIMA1 one of the most productive beamline in Europe.

P63 - Recent improvements in the SWING beamline at synchrotron SOLEIL

A. Thureau [a], O. Roudenko [a], P. Roblin [b], T. Biziens [a], J. Pérez [a]

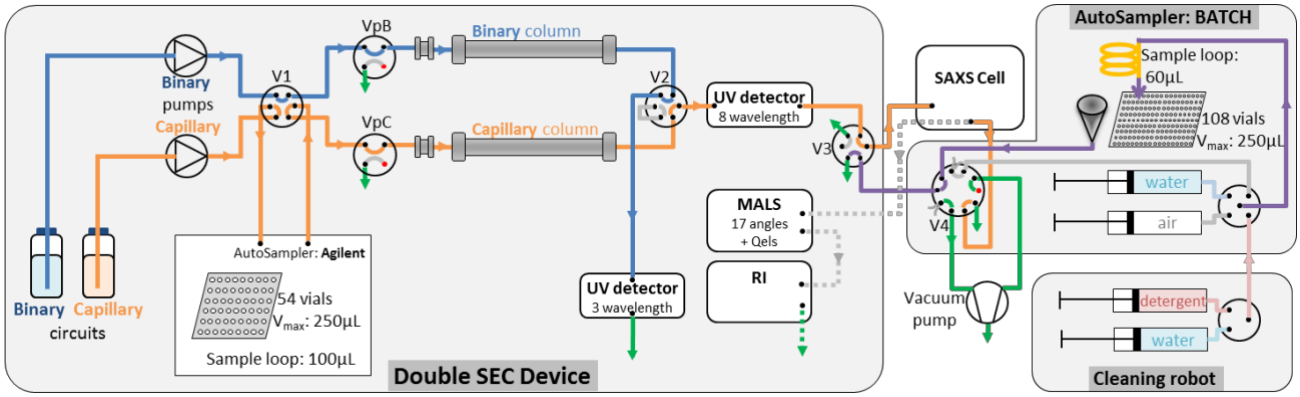
[a] *Synchrotron SOLEIL, L'Orme des Merisiers BP 48 St-Aubin, 91192 Gif sur Yvette*
[b] *Laboratoire de génie chimique, UMR5503, 31 432 Toulouse.*
E-mail: aurelien.thureau@syncrotron-soleil.fr

SWING is the SAXS/WAXS beamline at Synchrotron SOLEIL, dedicated to structural biology and soft condensed matter. SWING has pioneered SEC-SAXS set-up [1], now available at all BioSAXS beamlines in synchrotrons [2]. We present, here, the recent developments in hardware and software [3,4] that can offer the SWING beamline to structural biologists.

The BioSaxs set-up is fully automated for BATCH and SEC-SAXS mode. In Batch mode, a cycle, including the injections, the measurements, the washing and the drying of 2 buffers and 1 sample take 9 min in total. The installation of 2 parallels circuit for the SEC-SAXS makes a change of conditions (buffers, columns) possible without losing equilibration time. The measurement time with SEC corresponds to the column elution time and 3 additional minutes for the injection loop washing and for the sample pipetting/injection. Some Agilent columns can be provided to users with an elution time of 15min (0.3ml/min). MALS and RI devices can also be added in series under user's request.

An Eiger-4M on motorized table is installed in a vacuum chamber. A smaller beamstop allows a default q range comprised between $3.8E^{-3}$ and 0.56 $Å^{-1}$. The q_{min} value can be decreased easily by moving the detector-sample distance to analyse bigger object (up to 300nm). It takes less than 5min to move the detectors.

The acquisition and visualization Gui's are simplified which render the launch easier. The data treatment is done by users on site with Foxtrot, a software developed by a Soleil's IT group. It uses some semi-automatic macros to select the relevant frames and to obtain the final macromolecules saxs curve. In parallel, an automatic workflow is running using the same macros and the ATSAS suite [5]. Then the data are published in the ispyB database [6].To further analyse Saxs data, the Dadimodo webserver is now available to users. The goal of this software is to propose complete atomic models including flexible parts (terminals, linkers and loops) based on an atomic structure and a saxs curve.



[1] David, G.; Perez, J. (2009). **J. Appl. Crystallogr.** 42: 892-900.
[2] Pérez, J. and Vachette, P. (2017) Biological Small Angle Scattering: Techniques, Strategies and Tips, **Advances in Experimental Medicine and Biology** 1009:183-199.
[3] Roudenko O., Thureau A. & Pérez J. (2018). **https://dadimodo.synchrotron-soleil.fr.**
[4] Pérez J. & Koutsioubas, A. (2015). ***Acta Cryst.***, D71:86-93.
[5] Petoukhov, M.V. et al.(2012). **J. Appl. Cryst.** 45:342-350.
[6] Delageniere, S. et al. (2011). **Bioinformatics**. 27(22):3186-3192.

Session VII: Cellular Regulatory Mechanisms

Luisa M Bessa, IBS, Grenoble
P64 - Interplay between the scaffold protein POSH (Plenty of SH3s) and JNK signaling cascade proteins by nuclear magnetic resonance

Célia Deville, IGBMC, Illkirch
P65 - Activation of the ClpB disaggregase initiates sequential substrate threading by the main ATPase motor

Marie M. Grandjean, LISM, Marseille
P66 - A cytoplasmic disulfide oxidase chaperone associated to H1-T6SS effector in *Pseudomonas aeruginosa*

Allegra Mboukou, Expression Génétique Microbienne, Paris
P67 - Molecular recognition of a new class of nuclear localization signals by transportin 1

P64 - Interplay between the scaffold protein POSH (Plenty of SH3s) and JNK signaling cascade proteins by nuclear magnetic resonance

L. M. Bessa, T. Orand, D. Maurin, M. R. Jensen

Univ. Grenoble Alpes, CNRS, CEA, IBS, F-38000 Grenoble France
E-mail: luiza.mamigonian-bessa@ibs.fr

The JNK (c-Jun N-terminal kinase) signaling pathway plays a critical role in apoptosis in eukaryotes. It includes a three-tiered kinase phosphorylation cascade of MAPKs (Mitogen-activated protein kinases). These kinases bind to flexible scaffold proteins that can play a role in proper cellular localization and specificity of the cascade enzymes. Two of the scaffold proteins involved in the JNK signaling pathway are JIP1 (JNK-interacting protein 1) and POSH (Plenty of SH3s). We are interested in the interplay between the scaffold protein POSH and other proteins in the signaling pathway. POSH, as its name suggests, contains four SH3 (SRC homology 3) domains as well as an N-terminal zinc-finger domain (Fig. 1). This protein was originally identified through a yeast two-hybrid screen due to its ability to bind the small GTPase Rac1 [1]. POSH and JIP1 have been shown to interact *in vivo* and *in vitro* and it has been proposed that together they form a single scaffold for all the MAPKs of the pathway [2]. However, little structural information is known about the interaction between the two scaffold proteins. We studied the interaction between the structured POSH SH3 domains and the N-terminal disordered region of JIP1 using nuclear magnetic resonance spectroscopy. The disordered domain of JIP1 contains a number of poly-proline motifs that can potentially be recognized by the SH3 domains of POSH. Furthermore, we obtained a ^{1}H-^{15}N HSQC spectrum of a construct of POSH (amino acids 260-445) containing the Rac1-binding region. This region lacks a canonical CRIB (Cdc42- and Rac-interactive binding) motif, present in most Rac1 binding partners. This work may provide new structural insight on how Rac1 activates the JNK signaling pathway and the role of the POSH-JIP interaction.

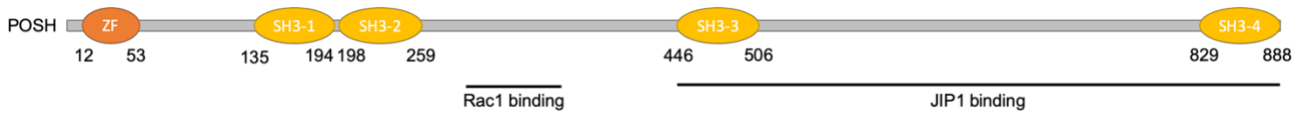


Fig. 1: Organization of the scaffold protein POSH. POSH contains four SH3 domains and an N-terminal zinc-finger domain (ZF). Regions previously identified in the binding of Rac1 and JIP1 are labeled.

[1] N. Tapon, K. Nagata, N. Lamarche, A. Hall. (1998). A new Rac target POSH is an SH3-containing scaffold protein involved in the JNK and NF-κB signaling pathways. **The EMBO Journal** 17: 1395-1404
[2] N. V. Kukekov, Z. Xu, L. A. Greene. (2006). Direct interaction of the molecular scaffolds POSH and JIP is required for apoptotic activation of JNKs. **Journal of Biological Chemistry** 281:15517-15524

P65 - Activation of the ClpB disaggregase initiates sequential substrate threading by the main ATPase motor

C. Deville [a], K. Franke[b], A. Mogk[b], B. Bukau[b] and Helen R. Saibil[c]

[a] *IGBMC, INSERM, U964, CNRS, UMR-7104, Université de Strasbourg, Illkirch-Graffenstaden, France*
[b] *DKFZ-ZMBH Alliance,Im Neuenheimer Feld 282, 69120 Heidelberg, Germany*
[c] *Birkbeck, University of London, Malet Street,London WC1E 7HX, UK*
E-mail: c.deville@unistra.fr

AAA+ proteins form asymmetric hexameric rings that use energy derived from ATP hydrolysis to thread nucleic acids or polypeptides though a central channel. Understanding the interplay between the ATPase activity and substrate translocation steps and its regulation is key to explain their molecular mechanisms. ClpB, a bacterial disaggregase, contains two tandem AAA domains. It is involved in extraction and refolding of aggregated proteins, to fight cellular proteotoxic stress. A belt of coiled-coil regulatory M-domains encircle the first AAA ring (AAA1) and regulates the protein that can shift between low and high ATPase and polypeptide threading activities. We dissected the activation and substrate threading mechanisms of ClpB by comparing the ATPase activities and cryo-EM structures of ClpB wild type and of a constitutively active M-domain mutant, all bound to a polypeptide substrate. The ClpB hexamer adopts a remarkably asymmetric arrangement with a spiral distortion closed by a seam. The substrate polypeptide threads through the entire central channel where it is contacted by a spiral of conserved tyrosine pore loops. Different conformations of activated ClpB reveal a sequential mode of ATP hydrolysis in the AAA2 ring, the main motor, coupled with repositioning of the tyrosine pore loop at the seam from the bottom to the top of the spiral track of interactions. AAA1 and AAA2 rings work in an out of phase manner maintaining a constant level of interactions with the substrate. Altogether, these conformational changes suggest polypeptide translocation via a rope climbing mechanism ensuring a high grip to extract polypeptides from aggregates.

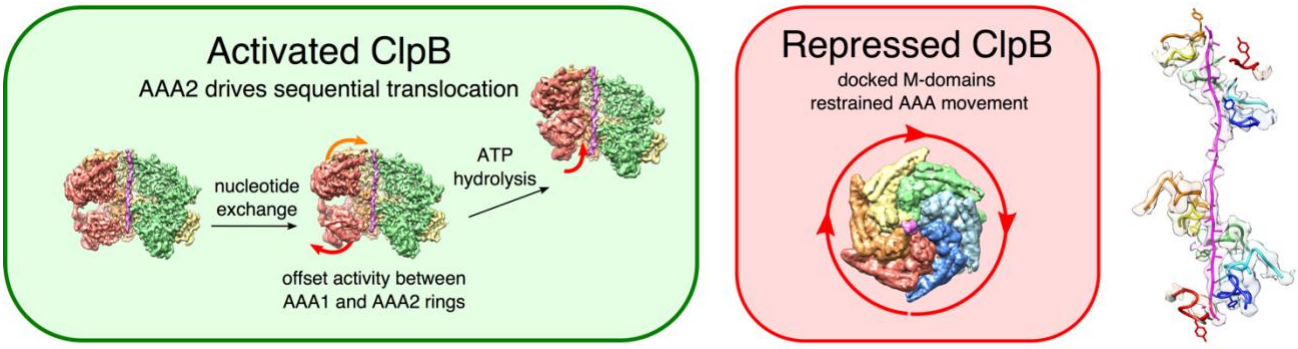


Fig. 1: Activated and repressed modes of ClpB and polypeptide substrate contacted by spirals of pore loops.

[1] C. Deville, M. Carroni, K. Franke, M. Topf, B. Bukau, A. Mogk and H. Saibil (2017). Structural pathway of regulated substrate transfer and threading through an Hsp100 disaggregase. **Science Advances** 3:e1701726.
[2] C. Deville, K. Franke, A. Mogk, B. Bukau and Helen R. Saibil (2019). Two-Step Activation Mechanism of the ClpB Disaggregase for Sequential Substrate Threading by the Main ATPase Motor. **Cell Reports** 27:3433–3446.

P66 - A cytoplasmic disulfide oxidase chaperone associated to H1-T6SS effector in *Pseudomonas aeruginosa*

M. M. Grandjean, E. B. Garcin, L. Houot, L. Elantak, C. Bordi and C. Sebban-Kreuzer

Laboratoire d'Ingénierie des Systèmes Macromoléculaires (LISM), IMM, CNRS – UMR7255– IMM-IFR88, CNRS, 31 chemin Joseph Aiguier, 13402 Marseille cedex 20
E-mail: mgrandjean@imm.cnrs.fr

The cytoplasm of most organisms is a highly reducing environment in which cysteines of proteins are maintained in thiol/thiolate form thus settling the dogma that there is no disulphide bond in the cytoplasm. In that sense, thioredoxin proteins are known to reduce disulphide bonds of abnormally oxidized proteins in the cytoplasm. Remarkably, we found an unusual cytoplasmic thioredoxin (Patrx2) from the bacterium *Pseudomonas aeruginosas*. The active site of Patrx2 does not contain the consensus thioredoxin sequence, instead it presents the active site sequence of the eukaryotic protein disulfide isomerase (PDI) whose role is to oxidize proteins. We have characterized the Patrx2 thioredoxin. Using NMR spectroscopy, we solved the structure of Patrx2, we investigated its catalytic oxidase and reductase activities, and we analysed its redox properties. We observed important differences between Patrx2 and the canonical bacterial thioredoxin and our results are likely indicative of the involvement of Patrx2 in disulfide isomerisation of distinct substrates within the cytoplasm of the bacteria. Using a bacterial two-hybrid genome fragment library of the *P. aeruginosa* PAO1 strain, we identified Tse1, a toxin effector of the type six secretion system (H1-T6SS), as a substrate of Patrx2. Further NMR experiments showed that Patrx2/Tse1 complex formation results in the oxidation of Tse1. Using genetics, we explored *in vivo* the role of Patrx2 and demonstrated that Patrx2 is directly involved in Tse1 secretion. Altogether our data allowed us to propose that Patrx2 is part of a new redox pathway for the regulation of T6SS effector secretion.

P67 - Molecular recognition of a new class of nuclear localization signals by transportin 1

A. Mboukou, M. Catala, C. Tisné & P. Barraud

Laboratoire d'Expression Génétique Microbienne, Institut de Biologie Physico-Chimique, IBPC, CNRS, Université de Paris, 75005, Paris, France.
E-mail: allegra.mboukou@ibpc.fr

A hallmark of eukaryotic cells is the physical separation of the genetic material and its associated proteins in the nucleus from the translational machinery located in the cytoplasm. Trafficking of macromolecules between the nucleus and cytoplasm through nuclear pore complexes (NPCs) is a selective and efficient process orchestrated by transport receptors [1]. Transportin 1 (Trn1), a transport receptor involved in nuclear import, binds directly to its cargos without adaptors in the cytoplasm, targets them to the nucleus through the NPC and releases them in the nucleus. Trn1 recognizes a well-characterized group of nuclear localization signals (NLSs) called PY-NLSs [2]. In addition, Trn1 also mediates the import of multiple proteins lacking a PY-NLS (non PY-NLSs), including some ribosomal proteins, histones and viral proteins [3]. Although it is clear that Trn1 must adapt to recognize different classes of NLSs, the molecular mechanisms controlling the recognition of non PY-NLSs by Trn1 are poorly characterized. With the help of X-ray crystallography and other biophysical methods, we want to characterize the interaction between Trn1 and a set of non PY-NLSs. We also aim at defining a set of rules governing the recognition of non PY-NLSs by Trn1. Our latest results on this topic will be presented.

[1] A. Cook, F. Bono, M. Jinek and E. Conti. (2007). Structural Biology of Nucleocytoplasmic Transport. **Annual Review of Biochemistry** 76:647-671
[2] B. Lee, A. Cansizoglu, K. Suel, T. Louis, Z. Zhang and Chook. (2006). Rules for Nuclear Localization Sequence Recognition by Karyopherin β2. **Cell** 126:543-558
[3] L. Twyffels, C. Gueydan and V. Kruys. (2014). Transportin-1 and Transportin-2: Protein nuclear import and beyond. **FEBS Letters** 588:1857-18

Session VIII: Computational Methods and Modelling

P68 - Structural study of LESV from *Arabidopsis thaliana*, a new protein involved in starch metabolism

E. Rimbaut, X. Roussel, C. D'Hulst, N. Szydlowski, F. Wattebled & C. Bompard

UMR CNRS-8576 Unité de Glycobiologie Structurale et fonctionnelle, Université de Lille, faculté des sciences et technologies, VILLENEUVE D'ASCQ
Email: coralie.bompard@univ-lille.fr

Starch is vital in human nutrition and its unique physical properties are widely used in industrial applications. Starch accumulates in plants as water-insoluble, semi-crystalline granules. Plants produce and consume their starch over time scales from hours to years. Leaf starch is metabolized in chloroplasts during the diel cycle. The synthesis and degradation rates are under photoperiodic control. Starch is comprised of two glucan polymers (amylose and amylopectin) and its physicochemical properties are defined by their organization within the granule. It has been assumed that the polymers self-organize to form the granule matrix. Recently, however, two conserved but uncharacterized proteins Early Starvation 1 (ESV1) and Like Early Starvation 1 (LESV) were proposed to organize the starch polymers. The mechanism by which this occurs is unknown. This work presents the structural and biophysical study of AtLESV by molecular modelling and SAXS. The aim of this project is to characterize the structure LESV and relate the structure/function of this molecules to starch metabolism.

P69 - Development a Computing Methodology to Study Assembly of Molecules: Application to the Norovirus Capsid

Y. Boulard, J. C. Carvaillo, T. Tubiana, S. Bressanelli

Institut de Biologie Intégrative et de la Cellule, I2BC, Gif-sur-Yvette, CNRS-CEA, 91191, Gif sur Yvette, France.
E-mail: yves.boulard@i2bc.paris-saclay.fr

The capsid of noroviruses (viruses responsible for gastroenteritis) is composed of 90 dimers of the same protein, viral protein 1 or VP1. The 3D structure of the capsid was resolved by X-ray in 1999 [1]. From this structure, the authors proposed a model of assembly based on an isotropic growth starting from a pentamer of dimers, which was identified as the most stable substructure. In 2013, Tresset *et al.* studied the kinetic of self-assembly of VP1 dimers into capsid using time-resolved-small-angle X-ray scattering (TR-SAXS) method [2] and identified only one structural intermediate of assembly in solution which corresponded to 10-11 dimers with an elongated shape that could not result from an isotropic growth as proposed by Prasad *et al.* To understand how from a perfect symmetric structure (pentamer of dimers) it is possible to grow in a privileged direction, we developed a computing strategy based on all-atom and coarse-grained models, to perform protein docking and molecular dynamics simulations. This methodology not only allows us to explain the anisotropic growth of the pentamer of dimers structure and to redefine the model obtained from TR-SAXS data but could also be used to study general assembly molecular mechanisms that are mostly crucial in many biological processes.

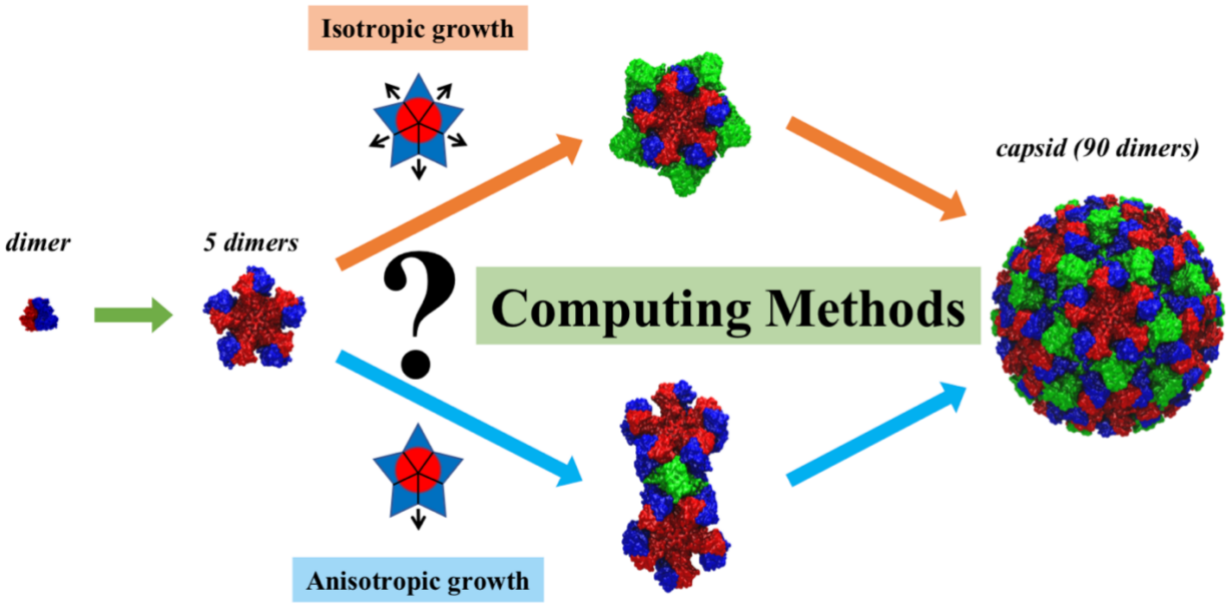


Fig. 1: Illustration of the problematic we want to address with computing methods. The orange arrows show the growth pathway that was proposed from the crystal structure of the norovirus capsid [1]. The blue arrows show a pathway consistent with actual time-resolved SAXS data [2].

[1] Prasad, B. V., Hardy, M. E., Dokland, T., Bella, J., Rossmann, M. G., and Estes, M. K. (1999) X-ray crystallographic structure of the Norwalk virus capsid. **Science**. 286:287–290
[2] Tresset, G., Le Coeur, C., Bryche, J.-F., Tatou, M., Zeghal, M., Charpilienne, A., Poncet, D., Constantin, D., and Bressanelli, S. (2013) Norovirus capsid proteins self-assemble through biphasic kinetics via long-lived stave-like intermediates. **J. Am. Chem. Soc**. 135:15373–15381

P70 - Autoprocessing at Proxima1 & Proxima2a @ synchrotron SOLEIL

T. Isabet , F.E. PIcca, I. Chado, L. Chavas, P. Legrand, W. Shepard, S. Sirigu, M. Savko

Synchrotron SOLEIL, Gif sur Yvette, France.
E-mail: tatiana.isabet@synchrotron-soleil.fr

Synchrotron SOLEIL is the French national synchrotron source located in the Paris suburbs. Two beamlines dedicated to the macromolecular crystallography, PROXIMA1 and PROXIMA2A welcome academic and industrial users from Wednesdays to Sundays.
One of the requirements coming out from our user community lied in the implementation of an automated data processing pipeline, now installed within our local computing infrastructure. The process launches the *XDSME* suite automatically for processing data, and results are then pushed in the ISPyB database for visualization from outside SOLEIL.

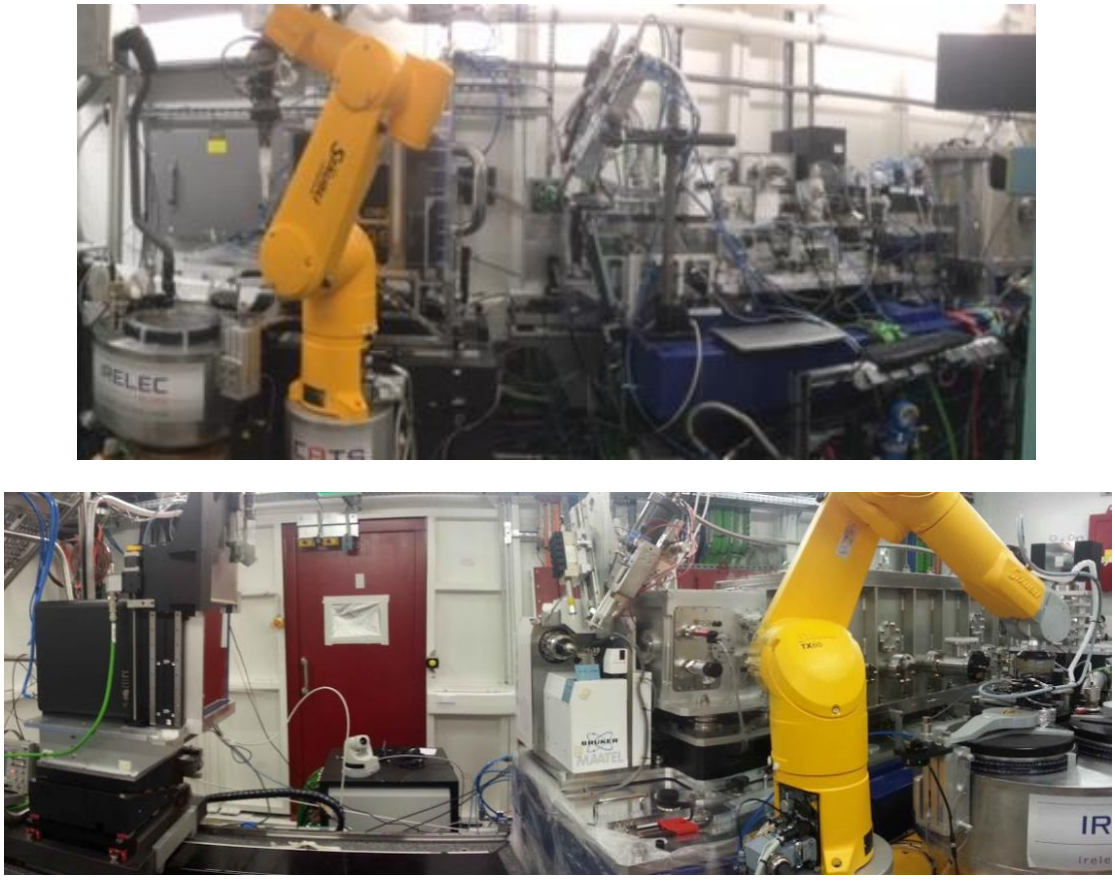


Fig. 1: Panorama of the PROXIMA1 (top) and PROXIMA-2A end stations, respectively.

P71 - CAPRI: The diverse challenges of computational protein-protein docking

M. F. Lensink [a], G. Brysbaert [a], N. Nadzirin [b], S. Velankar [b], S. J. Wodak [c]

[a] *University of Lille, CNRS UMR8576 UGSF, F-59000 Lille, France*
[b] *European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Hinxton, UK*
[c] *VIB Structural Biology Research Center, VUB, B-1050 Brussels, Belgium*
E-mail: marc.lensink@univ-lille.fr

A wealth of genomic information has recently become available, but whereas sequence and structure space are being rapidly filled, the sampling of the interactome remains sparse. Protein-protein interaction (PPI) is omnipresent in life and plays a central role in all biological processes. Many large-scale studies focusing on PPI have emerged in recent years, both experimental as well as computational ones. These studies display cobweb-like networks to represent the protein components interacting with each other. The establishment of such binary interaction provides invaluable information for the understanding of biological function and owing to the sheer massiveness of the underlying data, these representations capture a wealth of information. However, the representation itself remains abstract and incomplete; no information as to time, place or specificity is included, which in short is not enough to adequately guide mutagenesis studies, let alone devise drugs.
The CAPRI protein docking experiment is a community-wide effort, aimed at the improvement of computational docking methods. It does so by working in close collaboration with experimental scientists, who make their data available to this community, in the fullest confidence and prior to publication.
Participants in CAPRI are asked to predict the three-dimensional structure of a protein complex; these predictions are then assessed in a double-blind procedure against an unpublished and confidential high-resolution target structure. Present-day docking scenarios use increasingly integrative approaches, combining literature mining with homology modeling and guided FFT docking.
The targets in CAPRI follow the demand of the experimentalists and as such represent a wide variety of biological processes. The experiment now includes the prediction of multi-component assemblies, protein-nucleic acid and protein-polysaccharide binding, but also the prediction of binding affinities and positions of interfacial water molecules. The CAPRI project gives a fair assessment of the performance of present-day protein docking methods for the more difficult targets and as such it provides an upper limit of what can be expected from docking algorithms.

You can participate!

You are a developer of docking algorithms? You want to test these in a blind and unbiased experiment? Check the web, or send an e-mail for details on how to participate!
You have an interesting crystal structure that could serve as target? You want world-renowned computational structural biologists to work on your structure? You want a gazillion extra citations? Get in touch with us. Rest assured that your structure and any information associated with it will be treated in the greatest confidentiality!

Lensink et al. (2018). The challenge of modeling protein assemblies: the CASP12-CAPRI experiment. **Proteins** 86:257
Lensink and Wodak. (2017). Modeling protein-protein and protein-peptide complexes: CAPRI 6th edition. **Proteins** 85:359

P72 - Deep learning for protein fold recognition

M. Haller[1], A. Buzon[1], L. Moulinier[1], O. Poch[1], A. Jeannin[1], C. Mayer[1,2]

[1] *CSTB, ICube, UMR CNRS 7357, Strasbourg*
[2] *Université Paris Diderot, UMS, Institut Pasteur, Paris*
Email: mayer@pasteur.fr

Knowledge of protein domain folds is crucial for understanding protein function and their determination is mainly based on experimental methods (crystallography, NMR, electron cryomicroscopy). To date, more than 140,000 protein structures are available in the Protein Data Bank (PDB), which are divided into approximately 1,300 different folds. It is estimated that a few thousand different folds may exist in the living world for about 110 million sequences, making the problem of fold prediction a classification problem. Artificial intelligences (AI) are well adapted to this classification problem, particularly by using deep learning. In order to train and test these AIs, different benchmarks have been designed from sequences whose fold is already known and taken from the SCOPe structural classification database. After comparing several automatic learning methods for fold prediction, we have shown that the most effective method is based on processing 54 descriptors applied to the EDD dataset in a set of neural networks. The created classifier has an accuracy of 87%, slightly lower than that obtained in the literature, but with fewer descriptors (50 versus 400) and less computation time. In addition, during a structural analysis of the data used by these different AIs described in the literature, we highlighted various problems inherent in the data that could lead to a bias in the predictive effectiveness of these automatic learning algorithms.

P73 - Polysaccharide-lipid interactions: a theoretical and experimental study using molecular dynamics simulations, quantum chemical ^{13}C NMR spectra computation and solid-state ^{13}C NMR

A. Schahl[a,b], V. Réat[b], F. Jolibois[a]

[a] *Université de Toulouse-INSA-UPS, LPCNO, CNRS UMR 5215, 135 av. Rangueil, F-31077, Toulouse, France*
[b] *IPBS Toulouse France*
Email: Adrien.Schahl@ipbs.fr

Understanding the interactions of polysaccharides with lipids is of major importance for understanding the physicochemical properties of membranes. Among the different experimental approaches used to analyze such molecular complexes, solid-state NMR is a method of choice. However, theoretical approaches are sometimes necessary to understand the NMR spectral signatures obtained experimentally and to reveal the detailed three dimension structure of such complexes.
In this context, amylose-palmitic acid and amylose-POPC complexes were studied theoretically and experimentally. Molecular dynamic simulations using modified GLYCAM force field have been undergone and, based on structures extracted from MD simulations, Density Functional Theory (DFT) B3LYP using GIAO method has been employed in order to simulate ^{13}C NMR spectra of these amylose-lipid complexes and to compare it to experiments. This study revealed the ability of the protocol to decipher the structuration of heterogeneous systems and can safely be extrapolated to other systems (proteins, DNA,…).

Session IX: Structural Organization in Membrane and in Cells

P74 - Cryo-EM structures of heme import complexes from *S. marcescens*

V. Biou [a], R. Adaixo [b], M. Chami [c], P.-D. Coureux [d], Y. Ntsogo [a], P. Delepelaire [a]

[a] *Laboratoire de Biologie Physico-Chimique des Protéines Membranaires, UMR 7099 CNRS/Univ. Paris Diderot, Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie 75005 Paris – France.* [b] *Center for Cellular Imaging and NanoAnalytics, Biozentrum, University of Basel, Mattenstrasse 26, CH-4058 Basel, Switzerland.* [c] *BioEM lab, Biozentrum, University of Basel, Mattenstrasse 26, CH-4058 Basel, Switzerland.* [d] *Laboratoire de Biochimie, Ecole polytechnique, CNRS, Université Paris-Saclay, 91128 Palaiseau CEDEX, France.*
E-mail: valerie.biou@ibpc.fr

Gram-negative bacteria adapt to their environment by responding to external stimuli via signal transduction pathways. This results in gene expression to increase nutrient uptake and/or emission of virulence factors. Heme import in particular uses two-membrane spanning complexes involving TonB-like proteins. The transport is energised via a proton-motive-force driven complex located in the inner membrane composed of ExbB that assembles with two other proteins: a one-transmembrane domain protein with a periplasmic domain called ExbD and TonB analog HasB.
Crystal structures of ExbB with or without ExbD from *E. coli* have been published. One is a quasi-symmetrical pentamer with one ExbD TM helix in the transmembrane pore [1]. The other is a hexamer of ExbB and cryo-EM studies of the ExbB-D complex shows three ExbD per hexamer [2]. In both cases, the assembly (pentamer or hexamer) forms a channel. But neither shows indications of the relative orientation between the two partners and the structure does not explain how the transport is energised.
We present here cryo-EM structures of ExbB and ExbB-D from *Serratia marcescens*, an opportunistic pathogen that takes the heme from its environment via the Has system. ExbB from *S. marcescens* presents 37 additional residues at its N-terminus. Figures 1 and 2 show 2D classes and projection of the cryo-EM map that allows tracing of supplementary residues from *S. marcescens* ExbB, disclosing their mutual arrangement.

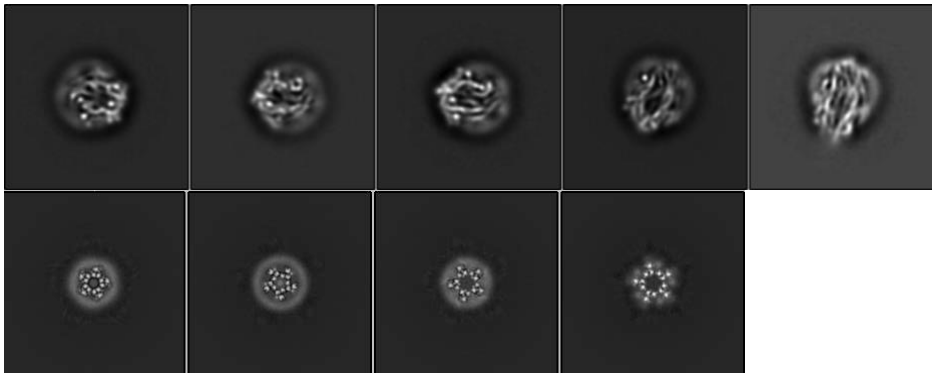


Figure 1: selected 2D classes of ExbB

Figure 2: projections of cryo-EM map of ExbB.

Acknowledgement We thank the ESRF for providing cryo-EM data collection, the Labex Dynamo for funding, Benoist Laurent for software installation and the Laboratoire de Biologie Théorique for providing access to the calculation cluster; the Curie Institute facility for access to their microscope.

[1] Celia, H., Noinaj, N., Zakharov, S.D., Bordignon, E., Botos, I., Santamaria, M., Barnard, T.J., Cramer, W.A., Lloubes, R., and Buchanan, S.K. (2016). Structural insight into the role of the Ton complex in energy transduction. **Nature** 538:60-65.
[2] Maki-Yonekura, S., Matsuoka, R., Yamashita, Y., Shimizu, H., Tanaka, M., Iwabuki, F., and Yonekura, K. (2018). Hexameric and pentameric complexes of the ExbBD energizer in the Ton system. **ELife** *7*:e35419.

P75 - Structural characterization of EPAC1 by HDX-MS

E. Del Nero[a], C. Sartre[b], M. Bourguet[a], Y. Ferrandez[b], J. Cherfils[b], S. Cianférani[a]

[a]*Laboratoire de Spectrométrie de Masse BioOrganique, Université de Strasbourg, CNRS, IPHC UMR 7178, 67000 Strasbourg, France*
[b]*Laboratoire de Biologie et Pharmacologie Appliquée, CNRS and Ecole Normale Supérieure, Paris-Saclay, 94235 Cachan, France*
E-mail: edelnero@unistra.fr

Introduction: EPAC1 (Exchange Protein Activated by cAMP 1) is a transmembrane protein that activates small GTPAses by stimulating exchange of GDP by GTP [1]. EPAC1 is involved in many cell functions is proposed as a therapeutic target for many human diseases, namely cardiovascular diseases. EPAC1 is activated by 3',5-adenosine monophosphate (cAMP). As no complete 3D structure of EPAC1 exists, we used Hydrogen/Deuterium Exchange coupled to Mass Spectrometry (HDX-MS) [2] to investigate EPAC1 conformational changes upon cAMP activation in presence or absence of liposomes as membrane mimics.
Methods: HDX of EPAC1 was carried out with or without liposomes (100-fold excess) and/or cAMP (300-fold excess). Samples were incubated in 95% deuterated buffer. Analyses were realized with a Q-TOF Synapt G2Si HDMS (Waters) coupled to a CTC PAL robot (Leap Technologies) and an Acquity UPLC (Waters). DynamX 3.0 (Waters) and MEMHDX were used to validated peptides.
Results: The comparison of deuterium uptake between EPAC1 and EPAC1/cAMP shows conformational modifications into DEP (Disheveled / Egl-10 / Pleckstrin), CNB (Cyclic Nucleotide Binding) and GEF (Guanidine Nucleotide Exchange) domains. Indeed, solvent accessibility decreases with cAMP in CNB domain, especially at its fixation site. A part of GEF domain is also less accessible with the ligand. With liposomes, all domains are impacted by cAMP. However, the difference of deuterium uptake is very significant for CNB domain (fixation site of ligand): cAMP decreases solvent accessibly. All EPAC1 domains show a significant difference of deuterium uptake with liposomes. Finally, the liposomes cause a difference of deuterium uptake for all domains of EPAC1 with cAMP, but the GEF domain is the most impacted.
Conclusion: HDX-MS was able to highlight regions of EPAC1 affected upon cAMP binding in presence or absence of liposomes to mimic the membrane.

[1] W. G. Robichaux, X. Chen. (2018). Intracellular cAMP Sensor EPAC: Physiology, Pathophysiology, and Therapeutics Development. **Physiol Rev** 98:919-1053
[2] J. R. Engen, T. E. Wales. (2015). Analytical Aspects of Hydrogen Exchange Mass Spectrometry. **Annu Rev Anal Chem (Palo Alto Calif)** 8:127-148

P76 - Regulation and inhibition of the guanine nucleotide exchange factor EPAC1 on membranes.

Y. Ferrandez[a], C. Sartre[a], F. Peurois[a], R. Fischmeister[b], M. Zeghouf[a], G. Peyroche[a], J. Cherfils[a]

[a] *Laboratoire de Biologie et Pharmacologie Appliquée, CNRS et Ecole Normale Supérieure, Paris-Saclay, 94230 Cachan, France.*

[b] *Laboratoire de Signalisation et Physiopathologie Cardiaque, Faculté de Pharmacie, Université Paris Sud, 92290 Chatenay Malabry, France.*

Email: yann.ferrandez@ens-cachan.fr

The guanine nucleotide exchange factor (GEF) Epac1 activates the small GTPase Rap1 in cardiovascular physiology by stimulating exchange of GDP for GTP. It is currently considered a potential therapeutic target, which is motivating intense research in drug discovery. EPAC1 has a complex regulatory regime, in which binding of the second messenger cyclic AMP (cAMP) promotes a large conformational change leading to auto-inhibition release. In this study, we investigated the role of membranes in EPAC1 activity by reconstitution of EPAC1 and Rap1 in artificial membranes combined with fluorescence-based kinetics, structural analysis and inhibition by a small molecule. We found that cAMP and membranes cooperate to potentiate EPAC1 activity by more that 2 orders of magnitude. We identified structural determinants for this effect, and used a small molecule that blocks EPAC1 activity to highlight intermediates between the auto-inhibited and the fully active conformations. These findings demonstrate a major contribution of membranes to EPAC1 structure and activity, which can inspire new routes for the discovery of drugs against cardiovascular diseases

P77 - Structural characterization of *Aspergillus fumigatus* cell wall architecture by solid-state NMR

G. Lamon[a], V. Aimanianda[c], I. Valsecchi[b], J. Tolchard[a], A. Grélard[a], B. Habenstein[a], I. Guijarro[b], A. Loquet[a]

[a]*Institut Européen de Chimie et de Biologie, IECB, Institut de Chimie et de Biologie des Membranes et Nano-objets, CBMN, UMR5248, CNRS, Bordeaux, France.*
[b]*Unité de Bioinformatique Structurale, Institut Pasteur, UMR3528 CNRS, Paris, France.*
[c]*Unité des Aspergillus, Institut Pasteur, UMR3528 CNRS, Paris, France.*
E-mail: g.lamon@iecb.u-bordeaux.fr

The filamentous fungus *Aspergillus fumigatus* is one of the major fungal pathogen of the respiratory system. Aspergillosis displaying both high incidence and mortality rates, is becoming a massive public health issue. The spores of *Aspergillus fumigatus* are surrounded by a cell wall, essential for their growth and allowing them to resist against host defense mechanisms. The cell wall is composed of a set of polysaccharides, covered by melanin and a layer of proteins called hydrophobins [1,2], characterized by their amphipathic properties and their ability to self assemble at hydrophilic/hydrophobic interfaces. Seven hydrophobins were identified in the genome of *Aspergillus fumigatus*. Among them, RodA is the most studied hydrophobin and it forms a layer of amyloid fibers (with a rodlet morphology) at the spores' surface to prevent the cells from the desiccation.
In this study [3], we aim at investigating the structural architecture of *Aspergillus fumigatus* cell wall at atomic resolution using magic-angle spinning solid-state NMR spectroscopy. We use multidimensional solid-state NMR to identify the cell wall components, decipher their rigidity / mobility and understand their interaction with bulk water molecules. By comparing wild type conidia, swollen conidia and germinating conidia we investigate the composition of the cell wall during the germination process. Moreover, using *Δags* conidia (α-glucan deficient), we analyze the compensatory mechanism [4] occurring when α-glucan is deleted.

[1] Beauvais, A., Fontaine, T., Aimanianda, V., Latgé, J. P. (2014). Apergillus cell wall and biofilm. **Mycopathologia** 178:371-377.
[2] Wosten, H. A., Asgeirsdottir, S. A., Krook, J. H., Drenth, J. H. & Wessels, J. G. (1994). The fungal hydrophobin Sc3p self-assembles at the surface of aerial hyphae as a protein membrane constituting the hydrophobic rodlet layer. **European Journal of Cell Biology** 63:122-129.
[3] Supported by the ANR project HYDROFUN to I. G.
[4] Henry, C., Latgé, J. P., Beauvais, A. (2012). α-1,3 Glucans Are Dispensable in *Aspergillus fumigatus*. **Eukaryotic Cell** 11:26-29.

P78 - A structurally conserved effector family in the plant pathogenic fungus *Leptosphaeria maculans* and in related fungal pathogens.

N. Lazar[a], I. Li de la Sierra-Gallay[a]; E. Zélie[a]; K. Blondeau[a]; C. Mesarich[b]; A. Idnurm[c]; I. Fudal[d]; H. van Tilbeurgh[a]

[a]*Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris-Sud, Université Paris-Saclay, 91198, Gif-sur-Yvette cedex, France*
[b]*School of Fundamental Sciences, Massey University, Palmerston North, New Zealand.*
[c]*School of BioSciences, University of Melbourne, Melbourne, Australia*
[d]*UMR1290 BIOGER, INRA / AgroParisTech, Thiverval-Grignon, France*
Email: noureddine.lazar@i2bc.paris-saclay.fr

During plant infection, pathogenic fungi secrete effectors, key elements of pathogenesis which modulate plant immunity and which can also be targeted by resistance (R) proteins from the plant (and then termed avirulence proteins / AVR). Recently, we identified in *Leptosphaeria maculans*, the fungus responsible for oilseed rape stem canker, and in other Dothideomycetes, an effector family (called AvrLm) which is structurally conserved, some members being recognized by the same resistance gene, and others showing antagonistic interactions, suggesting competition for a same plant target. Two of these AVR genes, *AvrLm3* and *AvrLm5-9*, respectively recognized by the oilseed rape R genes *Rlm3*, *Rlm5* and *Rlm9*, share homology at the protein level. Their recognition by cognate R genes is masked by the presence of another AVR gene, *AvrLm4*-7. Orthologues of *AvrLm3* and *AvrLm5-9* have been identified in fungi of the Dothideomycetes family, including an AVR gene of *C. fulvum*, a fungal pathogen of tomato, called *ECP11-1*, recognized by the tomato R gene *CfECP11-1*. In this work, we produced and purified AvrLm5-9 and Ecp11-1, determined their three-dimensional structures and identified a conserved structural pattern.

We produced AvrLm5-9 and Ecp11-1 in *Pichia pastoris* and determined their crystal structures. AvrLm5-9 and Ecp11-1 share structural analogies with AvrLm4-7 crystal structure (Blondeau et al., 2015): they are all have of a four stranded anti parallel sheet and have some disulfide bridges in common. This suggests that the antagonistic interaction between AvrLm5-9 and AvrLm4-7 could be due to a competition for a same plant target. AvrLm4-7, AvrLm5-9 and ECP11-1 (and possibly AvrLm3) may be part of a more extensive structurally conserved family of effectors. The AvrLm effector family is then an exceptional model not only for studying the molecular mechanisms underlying recognition by common cognate R genes, but also for deciphering the mechanisms underlying competition for common plant targets.

P79 - Molecular mechanisms behind remorin nanodomain formation by solid-state NMR

A. Legrand [a], D. Martinez [a], J. Gronnier [b], M. Berbon [a], M. Decossas [c], P. Gouguet [a], V. Germain [b], A. Grélard [a], O. Lambert [c], A. Loquet [a], S. Mongrand [b], B. Habenstein [a]

[a] *Institute of Chemistry & Biology of Membranes & Nanoobjects (UMR5248 CBMN), IECB, CNRS, Université Bordeaux, Institut Polytechnique Bordeaux, All. Geoffroy, Saint-Hilaire, 33600 Pessac, France*
[b] *Laboratoire de Biogènese Membranaire – UMR 5200 – CNRS, Université de Bordeaux, 71 Avenue Edouard Bourlaux, 33883 Villenave d'Ornon Cédex, France*
[c] *Institute of Chemistry & Biology of Membranes & Nanoobjects (UMR5248 CBMN), CNRS, Universite Bordeaux, Institut Polytechnique Bordeaux, 14 All. Geoffroy Saint-Hilaire, 33600 Pessac, France*
E-mail: anthony.legrand@u-bordeaux.fr

Protein and lipid components in biological membranes act as a dynamic network of subtle molecular interactions segregating the membrane into particular regions called nanodomains. Nanodomains act as functional platforms enriched in specific lipids (such as sterols and phosphoinositides) and proteins to perform their diverse activities. Remorins (REMs) are plant proteins and well-established nanodomain markers and, as such, they can be considered as a paradigm to provide a mechanistic description of membrane organisation into functional nanodomains. Using solid-state nuclear magnetic resonance (ssNMR) and building upon our initial knowledge of *St*REM1.3 and its C-terminal membrane anchor, we reveal the delicate balance between hydrophobic and electrostatic effects leading up to the protein's characteristic affinity for negatively charged phospholipids. In a divide-and-conquer approach, we describe the impact of *St*REM1.3's C-terminal anchor, its oligomerisation domain and itsintrinsically disordered region on membrane structure and dynamics. Furthermore, we tackle the structural features of *St*REM1.3 when associated to nanodomain-mimicking membranes. We reveal that *St*REM1.3 drives nanodomain organisation by concerted lipid-protein and protein-protein interactions.

P80 - Multiscale investigation of *in vivo* heterologous protein crystallization

P. Montaville, F. Jamme, I. Chaussavoine, M. Réfrégiers, L. Chavas

Synchrotron SOLEIL, Gif-sur-Yvette, France.
E-mail: pierre.montaville@synchrotron-soleil.fr

In vivo crystallography is a singular phenomenon in which the highly ordered packing of macromolecular molecules occurs within living cells or organisms. Naturally occurring *in vivo* protein crystals have been first described as a highly controlled way to achieve specific functions like protein storage or efficient catalysts, or as accidental uncontrolled consequences of cell dysfunctions or protein mutations in the context of diseases such as cataract, hemoglobin C disease, Charcot Leyden Crystals associated diseases. More recently, several heterogously expressed proteins have been reported to crystallize *in vivo*, allowing even in cellulo high resolution X-ray structure determination. This route for macromolecules X-ray structure determination is appealing for the structural biology community as it passes by both major bottlenecks of the classic in vitro approach, namely protein expression and purification. Moreover, it can be considered as an attractive alternative for unsuccessful classical in vitro crystallization trials. However only a handful of *in vivo* crystallized heterogously expressed protein cases are reported so far and very little is known concerning the molecular mechanisms involved. In order to determine the real potential of *in vivo* crystallization, a study has been initiated at Synchrotron SOLEIL within the Heliobio community. A multimodal/multiscale approach has been chosen to decipher key parameters underlying this counterintuitive process using as a model protein an engineered 26 kDa coral fluorescent protein (XPA) shown to crystallize in HEK293 cells. We combine methods such as fluorescence and SHG microscopies, UV imaging, SXR tomography, X-ray diffraction, and tools such as tailored protein expression and targeting plasmids, semi random mutated protein target bank, and microfluidics to gain insight on *in vivo* crystallization at the cell culture, single cell, subcellular and molecular levels.
The goals here are multiple:

- To observe in situ and understand the principles of protein crystal nucleation, growth, in a highly heterogeneous and complex medium such as the intracellular medium.
- To determine the macromolecules properties amenable for *in vivo* crystallization and explore the potential of this approach.
- To set up a workflow from the cell biology lab to the serial crystallography based data acquisition on the MX beamline.

All the gained knowledge combined to the technical advances will allow the implementation of an *in vivo* based crystallography platform open to users within a Synchrotron facility.

P81 - 3D ultrastructural studies of tunneling nanotubes by correlative cryo-microscopy

A. Sartori-Rupp [a], A. Pepe[b], S. Corroyer-Dumont [a], D. Cordero-Cervantes[b], E. Delage, K. Gousset, C. Schmitt [a], J. Krijnse-Locker [a], C. Zurzolo[b]

[a] *UBI, C2RT, Institut Pasteur, 28 rue du Dr Roux, 75015 Paris, France.*
[b] *Membrane Traffic and Pathogenesis Unit, Institut Pasteur, 28 rue du Dr Roux, 75015 Paris, France.*
E-mail: anna.sartori-rupp@pasteur.fr

The investigation of the structure-function relationship of molecules and of molecular machineries directly in the intracellular environment is one of the ultimate goals of functional studies in biology. 'Zooming in' continuously from the whole cell into its supramolecular architecture on the same sample can currently not be performed using only one instrument but makes the use of different microscopes, techniques and sample preparation necessary.
Techniques based on Fluorescent Light Microscopy (FLM) offer the possibility to image and locate fluorescently labelled molecules in living cells and to follow and record dynamical processes. Transmission Electron Microscopy (EM) and Electron Tomography (ET) allow to visualise all structures (labelled and unlabelled) directly in the cellular environment with greater spatial resolution. The advantages of the two microscopy methods can be combined by a correlative light-electron microscopy approach in which the fluorescently labelled structures are targeted in the light microscope and then relocated and imaged in 2 and 3D in the electron microscope.
In particular the combination of light microscopy techniques with cryo-ET where the 3D structure of the sample can be determined at molecular resolution in a close-to-physiological conditions has proven to be very powerful for the investigation of cellular systems with nanometer resolution.
In this context, we developed correlative light and cryo-EM strategies in order to investigate fragile samples whose structure can be altered by conventional EM sample preparation [1, 2]. We applied our approaches to the study of Tunnelling NanoTubes (TNTs), which are long, actin-rich, fragile membranous cell protrusions that form suspended bridges between distant cells [3, 4]. In recent years these novel structures have emerged as an important mean of cell-to-cell communication, mediating the bi- and unidirectional transfer of various cargoes, including organelles pathogens, ions, proteins and genetic material, both in vitro and in vivo. To better preserve TNTs' structure, we have set-up a workflow for correlative light- and cryo-ET that has allowed us to elucidate at high resolution the ultrastructural organization of TNTs in neuronal cells providing an unprecedented insight into these structure in an unperturbed environment.

[1] A. Sartori, R. Gatz, F. Beck, A. Rigort, W. Baumeister, J.M. Plitzko. (2007). Correlative microscopy: bridging the gap between fluorescence light microscopy and cryo-electron tomography. **J. Struct. Biol.** 160:135.
[2] M. Schorb, L. Gaechter, O. Avinoam, F. Sieckmann, M. Clarke, C. Bebeacua, Y.S. Bykov, A.F. Sonnen, R. Lihl, J.A. Briggs. (2017). New hardware and workflows for semi-automated correlative cryo-fluorescence and cryo-electron microscopy/tomography. **J. Struct. Biol.** 197:83.
[3] A. Rustom, R. Saffrich, I. Markovic, P. Walther, H.H. Gerdes. (2004). Nanotubular highways for intercellular organelle transport. **Science** 303:1007.
[4] A. Sartori-Rupp, D. Cordero Cervantes, A. Pepe, K. Gousset, E. Delage, S. Corroyer-Dulmont, C. Schmitt, J. Krijnse-Locker, C. Zurzolo. (2019). Correlative cryo-electron microscopy reveals the structure of TNTs in neuronal cells. **Nat. Comm.** 21:342.

P82 - Investigating UreG structural dynamics in cell by EPR spectroscopy

K. C. Tamburrini[a], O. Ouari [b], B. Guigliarelli [a], V. Belle[a], B. Zambelli[c], E. Mileo [a]

[a] *Bioénergétique et Ingénierie des Protéines, BIP, Aix Marseille Univ, CNRS, 13009, Marseille, France*.
[b] *Institut de Chimie Radicalaire, ICR, Aix Marseille Univ, CNRS, 13013, Marseille, France*.
[c] *Laboratory of Bioinorganic Chemistry, Department of Pharmacy and Biotechnology, University of Bologna, 40127, Bologna, Italy.*
E-mail: ktamburrini@imm.cnrs.fr

It is now appreciated that the biophysical properties of proteins inside cells are modulated by the "supercrowded", heterogeneous cytoplasm. As the cellular environment is hardly reproducible *in vitro*, investigation of biomolecules directly inside cells have attracted a growing interest in the past decade.
Site-Directed Spin Labeling (SDSL) coupled to Electron Paramagnetic Resonance (EPR) spectroscopy is part of the toolbox available and this technique exhibits competitive and advantageous features to capture protein dynamics inside cells.
The effects of macromolecular crowding and of intracellular viscosity appears particularly pronounced for protein regions with high degrees of structural flexibility, such as Intrinsically Disordered Proteins (IDPs) and chaperones.
Thanks to recent progress in the field of *in-cell* EPR spectroscopy [1], we will present the results of the investigation of the structural dynamics of UreG from *S. pasteurii* directly inside bacterial cells. UreG is a chaperone protein involved in the biosynthesis of the Urease and is responsible for hydrolysis of GTP; it presents a flexible fold that makes this protein the first discovered intrinsically disordered enzyme [2]. This protein likely uses disorder to couple its catalytic function to a non-enzymatic role as a molecular chaperone, undergoing disorder-to-order transition upon interaction with its partners. [3] EPR results obtained by monitoring UreG structural dynamics *in cell* will be discussed in light of the data obtained *in vitro* and in the presence of crowders.

[1] G. Karthikeyan, A. Bonucci, G. Casano, G. Gerbaud, S. Abel,V. Thomé, L. Kodjabachian, A. Magalon, B. Guigliarelli, V. Belle, O. Ouari, and E. Mileo. (2018). A Bioresistant Nitroxide Spin Label for In-Cell EPR Spectroscopy: In Vitro and In Oocytes Protein Structural Dynamics Studies. **Angew. Chem. Int. Ed.,** 57:1366-1370.
[2] B. Zambelli, M. Stola, F. Musiani, K. De Vriendt, B. Samyn, B. Devreese, et al. (2005). UreG, a chaperone in the urease assembly process, is an intrinsically unstructured GTPase that specifically binds Zn^{2+}. **J. Biol. Chem**, 280: 4684–4695
[3] M. Palombo, A. Bonucci, E. Etienne, S. Ciurli, V. N. Uversky, B. Guigliarelli, V. Belle, E. Mileo & B. Zambelli. (2017). The relationship between folding and activity in UreG, an intrinsically disordered enzyme. **Sci. Rep.**, 7:5977.

P83 - Tuning specifically galectin-1/glycoprotein interactions at the cellular level

P. Touarin [a], O. Bornet [b], B. Serrano [a], L.G. Scott [c], S.M. Mancini [d], C. Sebban-Kreuzer [a] and L. Elantak [a]

[a] *Laboratoire d'Ingénierie des Systèmes Macromoléculaires, CNRS UMR 7255, Aix-Marseille Université, Marseille, France*
[b] *Plateforme RMN, IMM FR3479, CNRS, Aix-Marseille Université, Marseille, France*
[c] *Cassia, LLC 3030 Bunker Hill Street, Ste.214San Diego, California 92109, United States*
[d] *CRCM, Institut Paoli-Calmettes, CNRS, INSERM Aix-Marseille université, Marseille, France*
e-mail: ptouarin@imm.cnrs.fr

Galectins are bridging glycosylated molecules by their β-galactoside moieties, forming a network involved in many physiological functions, such as inflammation, immune responses, cell migration, autophagy and signaling. Extracellularly, galectins function has been mainly described through carbohydrate binding activity. The first example of a carbohydrate-independent interaction outside the cell concerns galectin-1 (Gal1) and its ligand, the pre-B cell receptor (pre-BCR)[1]. This interaction has a crucial role in B-cell development through the formation of a Gal1-dependent lattice between pre-BII and stromal cells and thus leading to proper B cell differentiation [1, 2]. Our previous study revealed that Gal1 interacts with a motif of the pre-BCR (λ5-UR) on a surface adjacent to the Gal1 CBS (Carbohydrate Binding Site) and allows Gal1 to undergo selective affinity changes in its carbohydrate-binding activity *in vitro* [3, 4].
Using solution state on cell NMR spectroscopy, we investigated the effect of λ5-UR interactions on Gal1 binding activity for its physiological ligands expressed on pre-BII and stromal cell surfaces. We show that λ5-UR can specifically induce a ligand binding shift of Gal1 when bound to its cell surface ligands. We also identified one cell surface glycan epitope for which Gal1 increases its affinity upon λ5-UR interaction and deciphered the intermolecular contact changes induced by λ5-UR. In addition, glycan arrays screening combined to NMR relaxation data showed that λ5-UR changes the internal dynamics of specific Gal1 CBS residues resulting in the targeting of specific glycan epitopes. This cellular and structural NMR study ever conducted at atomic resolution for a member of the galectins demonstrates that Gal/unglycosylated protein interactions can act as physiological regulators of Gal1 lattice interactions at the cell surface.

[1] L. Gauthier et al. (2002). Galectin-1 is a stromal cell ligand of the pre-B cell receptor (BCR) implicated in synapse formation between pre-B and stromal cells and in pre-BCR triggering. **Proc Natl Acad Sci U S A**, 99(20): 13014-9.
[2] F. Mourcin et al. (2011). Galectin-1 expressing stromal cells constitute a specific niche for pre-BII cell development in mouse bone marrow. **Blood**, 117(24): 6552-61.
[3] L. Elantak et ak. (2012). Structural Basis for Galectin-1 dependent Pre-BCR Activation. **J Biol Chem**, 287(53): 44703-13
[4] J. Bonzi et al. (2015). Pre-B cell receptor binding to galectin-1 modifies galectin-1/carbohydrate affinity to modulate specific galectine-1/glycan lattice interactions. **Nat Commun**, 6: 6194

List of Participants

A

ALBERTINI Aurélie
ALSALMAN Zaynab
AMIGUES Beatrice
ANDREEVA Elena
ARNOUX Pascal
AROLD Stefan
ASSEMAT Olivier
ATTIA Bouchra
AUMONT Magali

B

BARDIAUX Benjamin
BARDOU Fabienne
BEJI Syrine
BELOT Laura
BENESCH Justin
BEN YAALA Khaoula
BERGDOLL Marc
BERGER Sylvie
BERSCH Beate
BILLAS Isabelle
BIOU Valérie
BLANCHARD Laurence
BLAUD Magali
BOMPARD Coralie
BON Cécile
BONNETÉ Françoise
BONTEMS François
BORDES Florence
BORIES Pascaline
BOUCKAERT Julie
BOULARD Yves
BOURGEOIS Dominique
BOURGEOIS Gabrielle
BOURGEOIS Stéphanie
BOURGUET Maxime
BOURGUET William
BOURNE Yves
BOUSQUET Marie-Pierre
BOUTANT Jérôme
BROUTIN Isabelle
BRUCH Eduardo
BRUNETTI Guillaume
BURATTO Jérémie
BURMEISTER Wim
BUSSIERE Dirksen

C

CAPPELE Julien
CARDONE Christophe
CARIVENC Coralie
CARRASCO Kendall
CASTEL Cyril
CASTILLO Sebastian
CAVARELLI Jean
CHALHOUB Sandra
CHAPTAL Vincent
CHARBONNIER Jean-Baptiste
CHARENTON Clément
CHAUSSAVOINE Igor
CHAVAS Leonard
CHAVENT Matthieu
CHERFILS Jacqueline
CIOCI Gianluca
COHEN-GONSAUD Martin
COLLOC'H Nathalie
CORNET Julie
COSTE Franck
COUREUX Pierre-Damien
COX Neil
CROS Julien
CZAPLICKI Georges
CZERNECKI Dariusz

D

DAUTANT Alain
DECLERCK Nathalie
DE GUILLEN Karine
DE RUYCK Jerome
DEL NERO Elise
DELARUE Marc
DELAUZUN Vincent
DELFOSSE Vanessa
DEMANGE Pascal
DESCHANEL Louis
DESLIGNIERE Evolène
DESMADRIL Michel
DEVILLE Celia
DHIFAOUI Ahmed
DOS REIS Suzana
DUBIEZ Etienne
DUCLOS Stéphanie
DUCOUX-PETIT Manuelle
DUMAS Philippe

E

ELANTAK Latifa

QUEMARD Annaïk

R

RAMOS Pascal
RAMOS MORALES Elizabeth
RANAIVOSON Fanomezana
RAVIER Nicolas
REAT Valérie
RENAUD Jean-Paul
REUTER Nathalie
RICHET Nicolas
RIMA Julie
ROUSSEL Alain
RUFF Marc

S

SAGAR Amin
SAIBIL Helen
SARTORI-RUPP Anna
SAUREL Olivier
SAUTER Claude
SAVKO Martin
SCHAEFFER Corinne
SCHAHL Adrien
SCHILTZ Odile
SCHMITT Emmanuelle
SEIDEL Claus A. M.
SENIOR Hazel
SHEPARD William
SIPONEN Marina

T

TAMA Florence
TAMBURRINI Ketty Concetta
TAWANI Arpita
TEBOUL David
TERME FERRARI Stéphanie
TERRADOT Laurent
TETREAU Guillaume
THOMPSON Andrew
THUREAU Aurélien
TIBERTI Marion
TIMMINS Joanna
TISNE Carine
TOPIN Jérémie
TOUARIN Pauline
TRANIER Samuel

U

UETRECHT Charlotte

V

VAN HEIJENOORT Carine
VASIREDDI Ramakrishna
VAYSSIERES Marlène
VEYRON Simon
VIGOUROUX Armelle
VILLALTA CASARES Alejandro
VITORIA GOMES Marina

W

WORTEL Nico

Z

ZERBIB Didier
ZIVKOVIC Dusan