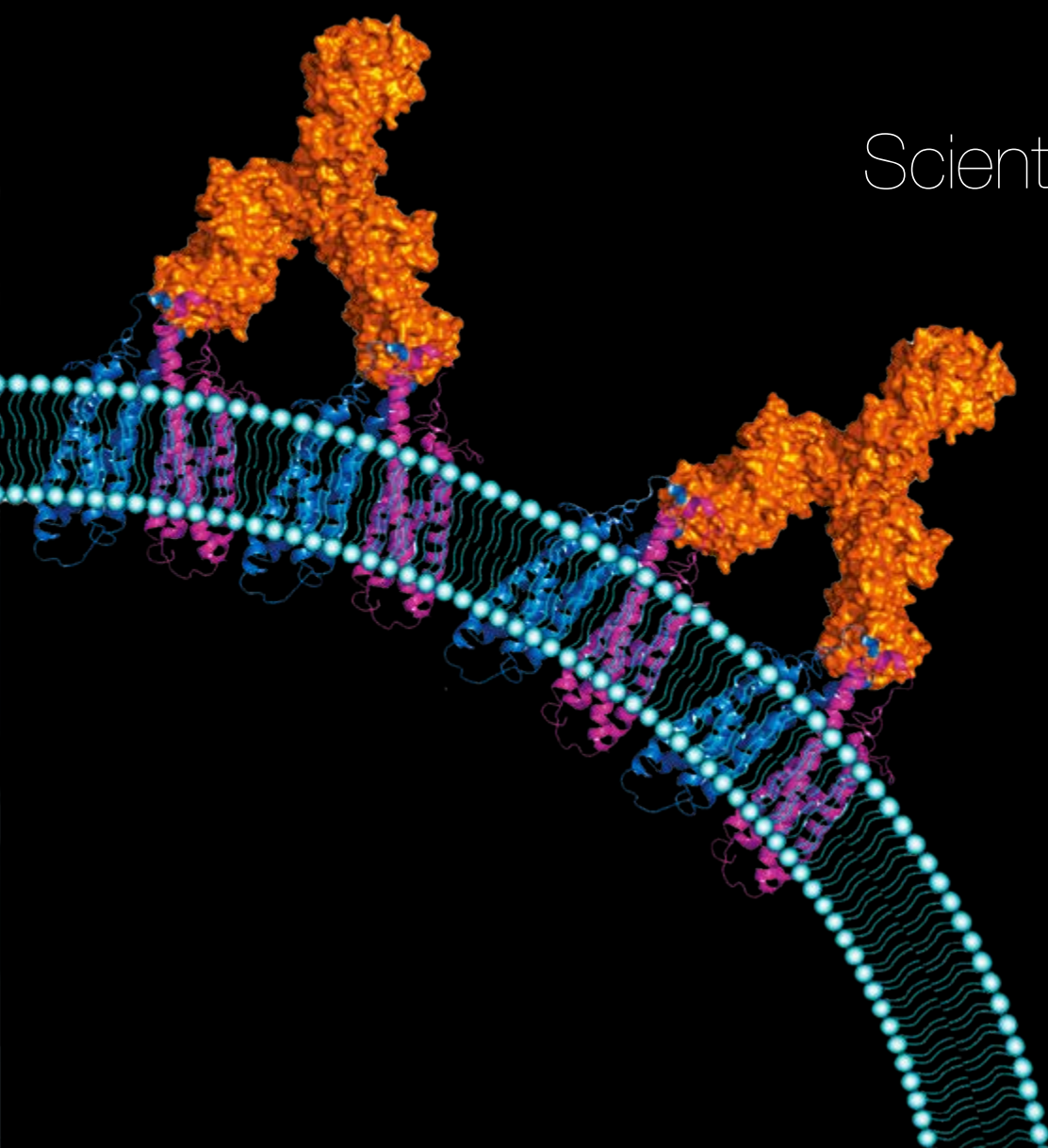




Institut Européen de Chimie et Biologie  
European Institute of Chemistry and Biology



# Scientific Report 2020

On March 5-6, the IECB organized for the first time a 2-day scientific retreat with present and past group leaders. We enjoyed the mild weather, excellent food and stimulating Science in Bazas (Gironde). We were also extremely lucky to have been able to hold this meeting ten days before the general lockdown. For many of the group leaders the Bazas retreat was one of the very few physical scientific meetings that could be held in 2020.



**Publication director:** Valérie Gabelica & Rémi Fronzes **Graphic design:** A to B communication & Delphine Fleury  
**Photo credits:** Anand Kumar (front cover), Hugette Vanlierde, Claire-Hélène Biard (photo event), Yves Théobald (building, portraits), Marc Grémillon (portraits), Lionel Lizet (IECB-CGFB technology platform).



Institut Européen de Chimie et Biologie  
European Institute of Chemistry and Biology

# Scientific Report 2020

# Director's Foreword



Dr. Rémi Fronzes

Research Director (DR2), CNRS

## 2020: a year like no other

2020 will be remembered as the year of the COVID-19 pandemic. It was a year of profound disruption for everyone. A year that led us to question our role in science and in society. This year everyone learned to value the work of all the people at the service of our society, whatever their grade or role, from the surface cleaners to the research scientist. The response to the pandemic also demonstrated the importance of fundamental research to respond to the grand challenges of humanity.

Three group leaders had the opportunity to install their lab and start their research groups before the beginning of the pandemic. *Emmanuelle Thinon* started her group in November 2019, and works on the chemical biology of transmembrane proteins. In 2020 she was awarded an ANR Young Researchers grant to study the S-palmitoylation of a host factor in Influenza A virus infection. *Petya V. Krasteva* joined the IECB in October 2019, and her research, funded by an ERC Starting Grant, focuses on elucidating bacterial biofilm formation and their role in bacterial pathogenesis. *Nicolas Reyes* moved his group from Institut Pasteur to the IECB in January 2020, and studies membrane solute transporters and membrane receptors involved in human diseases.. His research is also funded by the ERC (Consolidator Grant) and by the American National Institute of Health.

These exemplary research themes illustrate the type of research promoted by IECB: fundamental research questions with deep implications for human health, and an interdisciplinary approach at the crossroads between chemistry and biology. To achieve this, IECB builds on talented researchers at all career stages, with the particularity that early-career researchers are given the opportunity to lead their project with full scientific and financial independence. IECB also gives its researchers time to develop their project, by hosting the groups at the institute for 10 years, with recruitment and periodic evaluations under the auspices of an International Scientific Advisory Board.

The process continued this year with a SAB meeting held in October, and we are extremely grateful to Prof. Helma Wennemers (ETH Zürich), Dr. Anne Houdusse-Juille (Institut Curie) and Prof. Stephen Cusack (EMBL Grenoble) for joining the Scientific Advisory Board. We also express our gratitude to Prof. Dinshaw Patel (Memorial Sloan Kettering Institute, NY), who participated to his Xth and last SAB meeting last October, for his years of service in the SAB of IECB. Five candidates were interviewed, and we will be presenting the new group leaders next year.

As evoked in the preamble, 2020 has been a disruptive year. Although the prime importance of Science for society is clear, fundamental research requires great minds, but also time and investments. The first motivation of Science must remain curiosity. Science is generally profitable for society, but most often in unpredictable ways. Our economic context (the raising costs of high-end, reproducible science, the ageing of infrastructure) is unfortunately making it ever more challenging to maintain international competitiveness. After a couple of years as director of the IECB, I have decided to devote more of my time to research with my team, and to hand over the reins of IECB to our adjunct director Valérie Gabelica. Stewardship of the IECB is no doubt a tough endeavor, but I am fully confident that, with the daily help of Antoine Loquet as future adjunct director, Brice Kauffmann as technical director and Sylvie Djian as administrative director, with the advice of the steering committee and of the SAB, and hand in hand with our trustees, she will lead IECB towards even greater successes for the years to come!

Rémi Fronzes

A handwritten signature in black ink, appearing to be 'R. Fronzes', written in a cursive style.

**The Institut européen de chimie et biologie (IECB)** is a research team incubator placed under the joint authority of the CNRS, the Inserm and the Univ. Bordeaux. It was created in 1998 with the support of the Aquitaine Regional Council to provide promising European chemists and biologists with an environment designed to facilitate the development of first-class interdisciplinary research programs, in collaboration with international public and private research centres.

IECB's International Scientific Advisory Board guides the selection and periodic evaluation of the team leaders. After a probative period of two years, research teams are then hosted for a maximum of 10 years. During their stay at IECB, teams enjoy full financial and managerial autonomy and benefit from state-of-the-art facilities and dedicated technical expertise through IECB's technology platforms in structural biology and preparative and analytical techniques.

The IECB is the largest research team incubator in France, with 15 research teams accounting for 180 researchers and expert technicians.

The company Ureka (Immupharma Group) is hosted at the institute.



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■	Technology platforms	61
■	Technology transfer & start-ups	65

The IECB International Scientific Advisory Board, chaired by Dr Moshe YANIV, interviewed candidates from all over the world for group leader positions.



**Dr. Moshe YANIV**  
Chairman, Institut Pasteur,  
Paris, France



**Dr. Stephen CUSACK**  
EMBL, Grenoble, France



**Dr. Witold FILIPOWICZ**  
Friedrich Miescher Institute for  
Biomedical Research, Basel, Switzerland



**Dr. Bernd GIESE**  
University of Fribourg, Switzerland



**Dr. Anne HOUDUSSE JUILLE**  
Institut Curie, Paris, France



**Pr. Roeland NOLTE**  
Radboud University, Nijmegen,  
Netherlands



**Pr. Dinshaw PATEL**  
Sloan-Kettering Cancer Institute,  
New York, USA



**Pr. Yves POMMIER**  
National Cancer Institute,  
Bethesda, USA



**Dr. Claude SARDET**  
Institut de Recherche en Cancérologie  
de Montpellier, France



**Pr. Helma WENNEMERS**  
ETH, Zurich, Switzerland

# Organisational Structure

# Board Members

## International scientific advisory board (ISAB)

**Dr. Moshe YANIV** President  
Institut Pasteur, Paris, France

**Dr. Stephen CUSACK**  
EMBL, Grenoble, France

**Dr. Witold FILIPOWICZ**  
Institut Friedrich Miescher, Basel, Switzerland

**Dr. Bernd GIESE**  
Departement of Chemistry, University of Basel, Switzerland

**Dr. Anne HOUDUSSE JUILLE**  
Institut Curie, Paris, France

**Pr. Roeland NOLTE**  
Radboud University Nijmegen, Netherlands

**Pr. Yves POMMIER**  
National Cancer Research, NIH, Bethesda, USA

**Dr. Claude SARDET**  
Institut de Recherche en Cancérologie de Montpellier (IRCM), France

**Pr. Helma WENNEMERS**  
ETH Zurich, Suisse

## Former ISAB members

**Dr Herbert WALDMANN**  
Max Planck Institute of Molecular Physiology, Dortmund, Germany

**Dr. Daniel SCHIRLIN**  
Sanofi Aventis, Paris, France

**Prof. Dinshaw PATEL**  
Memorial Sloan-Kettering Cancer Center, New York, USA (2009–2016)

**Dr. Daniel LOUVARD**  
Institut Curie, Paris, France (1999–2014)

**Pr. Iain D. CAMPBELL**  
Departement of Biochemistry, University of Oxford, UK (1999–2013)

**Dr. Simon CAMPBELL**  
Royal Society of Chemistry, London, UK

**Pr. Claude HÉLÈNE**  
Muséum National d'Histoire Naturelle, Paris, France (1999–2003)

**Pr. Georges HUEZ**  
Université Libre de Bruxelles, Brussels, Belgium (2000–2005)

**Pr. Steven LEY**  
Departement de Chemistry, University of Cambridge, UK (1999–2005)

**Pr. Helmut RINGSDORF**  
Institut für Organische Chemie, Johannes Gutenberg Universität, Mainz, Germany (1999–2006)

**Pr. Fritz ECKSTEIN**  
Max Planck Institute for Experimental Medicine, Göttingen, Germany (2003–2006)

**Pr. Jack BALDWIN**  
Departement of Chemistry, University of Oxford, UK (2005 – 2007)

**Pr. Wilfred van GUNSTEREN**  
Laboratory of Physical Chemistry, ETH, Zürich, Switzerland (1999–2007)

**Pr. François DIEDERICH**  
Department of Chemistry and Applied Biosciences, ETH, Zürich, Switzerland (2006–2008)

**Pr. Jean-Yves LALLEMAND**  
Institut de Chimie des Substances Naturelles, CNRS Gif-sur-Yvette, France (1999–2010)

## Board of directors

**Dr. Rémi FRONZES** Executive Scientific Director Research Director, team leader UMR5234 (CNRS, Univ. Bordeaux)

**Mrs. Sylvie DJIAN** Administrative Director (CNRS)

**Dr. Valérie GABELICA** Deputy Scientific Director Research Director, team leader U1212 (Inserm)

## Former directors

**Dr. Jean-Louis MERGNY** Former Executive Scientific Director (2015–2018)

**Dr. Jean-Jacques TOULMÉ** Former Executive Scientific Director (2001–2014)

**Pr. Jean-Yves LALLEMAND** Former Executive Scientific Director (1998–1999)

**Pr. Léon GHOSEZ** Former Deputy Scientific Director (1998–2008)

## Steering committee

**Mrs. Sylvie DJIAN** Administrative Director (CNRS)

**Dr. Rémi FRONZES** Team leader Research Director (CNRS), UMR5234

**Dr. Valérie GABELICA**, Team leader Research Director (Inserm), U1212

**Dr. Gilles GUICHARD** Team leader Research Director (CNRS), UMR5248

**Dr. Axel INNIS**, Team leader Research Director (Inserm), U1212

**Dr. Brice KAUFFMANN** Head of IECB's technology platforms Engineer (CNRS) UMS3033

**Dr. Antoine LOQUET** Team leader Research Director (CNRS), UMR5248

**Dr. Cameron MACKERETH** Team leader Research Director (Inserm), U1212

**Dr. Anne ROYOU** Team leader Research Director (CNRS), UMR5095

## Board of trustees

**Centre National de la Recherche Scientifique**  
3 rue Michel-Ange, 75794 Paris CEDEX 16

**Institut National de la Santé et de la Recherche Médicale**  
101 rue de Tolbiac, 75654 Paris CEDEX 13

**Univ. Bordeaux**  
35 Place Pey Berland, 33000 Bordeaux

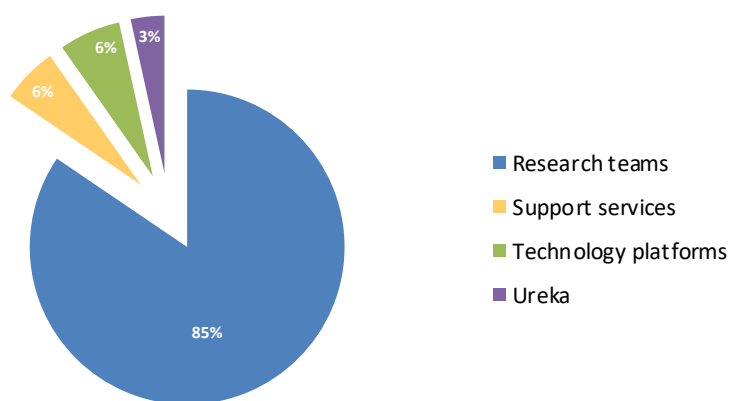
# Organisational Chart



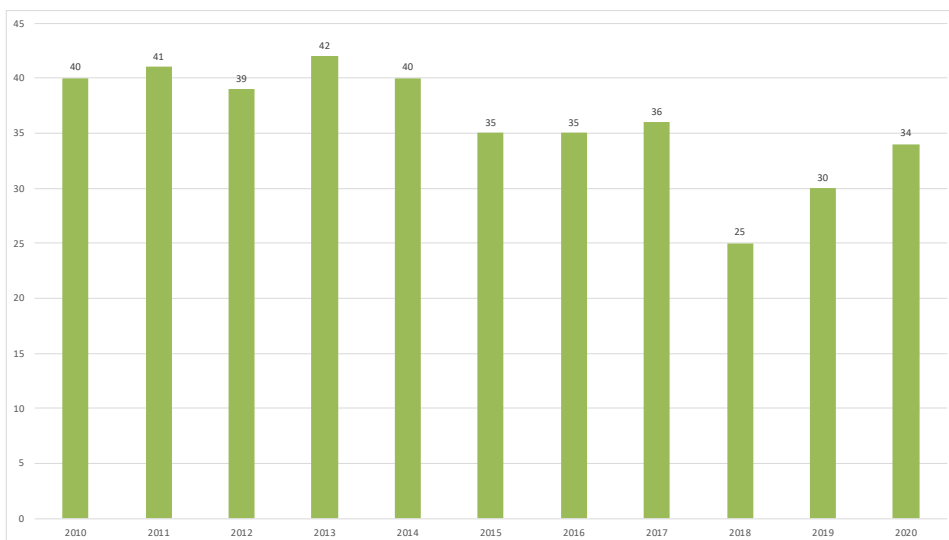
# 2020 Key Figures

In 2020, 174 people were part of the IECB: 147 research staff, 21 employees within the IECB's support services unit and 6 employees of the company Ureka. Young researchers (Master and PhD students, postdoctoral researchers) represent 52% the IECB research staff. This population largely contributes to gender equality and internationalization at IECB. It also testifies to the attractiveness of the institute.

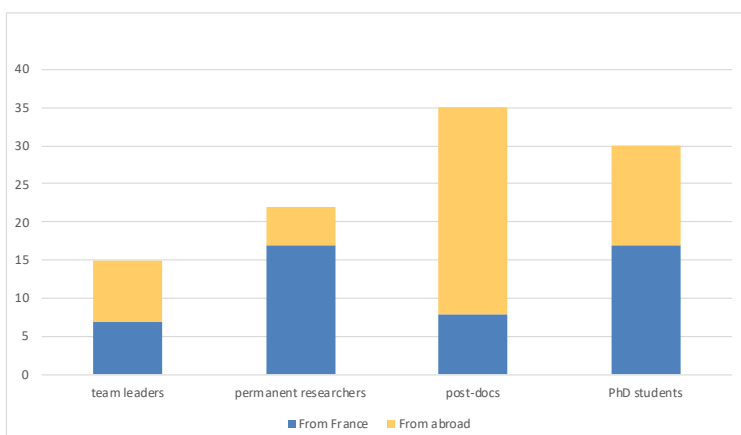
IECB staff by professional category



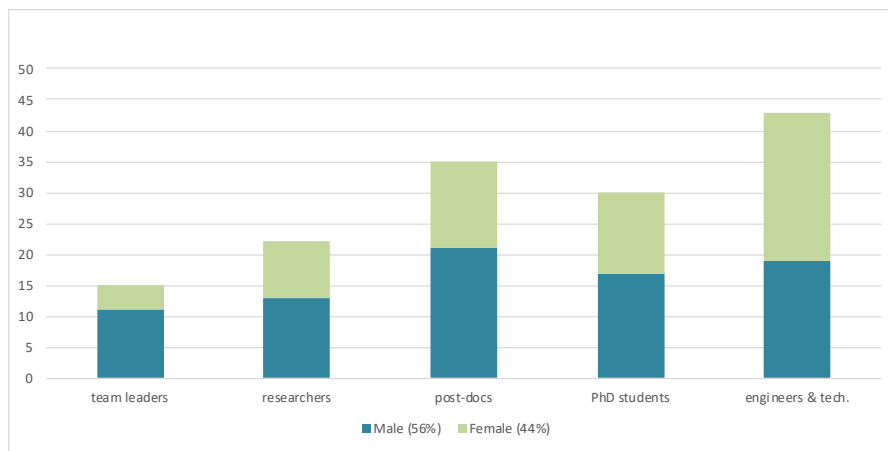
Number of postdoctoral researchers over the past 10 years



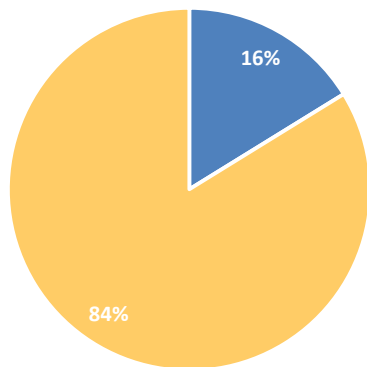
IECB researchers and students by nationality & professional category



IECB research staff by gender & professional category

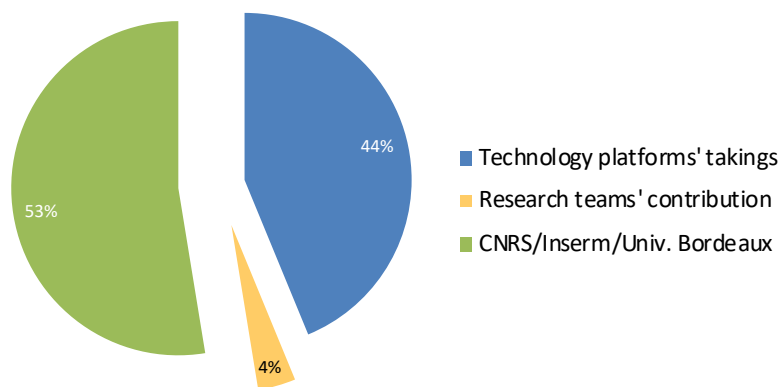


IECB's 2020 budget



■ Support services ■ Research teams' own funding

Support services funding



■ Technology platforms' takings  
 ■ Research teams' contribution  
 ■ CNRS/Inserm/Univ. Bordeaux

The budget of the institute, which amounts to 11,4 million euros including salaries, can be divided into two separate parts: the budget of the support services (UMS3033/US001) and the research teams' own resources.

The first one is mainly granted by the trustees (CNRS, Inserm, Université de Bordeaux), while the other comes from public and private research grants and contracts.

SUPPORT SERVICES (UMS3033 & US001)

Support services at IECB consist of staff in administration and finance, infrastructure and maintenance, as well as 11 engineers and technicians dedicated to IECB's technology platforms. The support services unit UMS3033 & US01 is jointly funded by the CNRS, the Inserm and the Univ. Bordeaux, and receives financial support from the Nouvelle Aquitaine Regional Council. Research teams also contribute to financing those general services.

Administration and finance

- Administrative director**  
Sylvie DJIAN, IR, CNRS
- Executive assistant officer**  
Claire-Hélène BIARD, AI, Inserm
- Accounting and administration officers**  
Laetitia COINTEMENT, Tech, Inserm  
Catherine DUPRAT, Tech, Inserm  
Laurent KUBICKI, Tech, Inserm  
Sandra LAVENANT, Tech, Univ. Bordeaux  
Amélie STOTZINGER, Tech, Inserm
- IT management**  
Gérald CANET, IE, Inserm  
Eric ROUBIN, Tech, Inserm
- Infrastructure officer**  
Patrice DUBEDAT, AJT, Univ. Bordeaux

Structural biology facilities

- Head of structural biology facilities and crystallography engineer**  
Brice KAUFFMANN, IR, CNRS
- Crystallography engineer**  
Stéphane MASSIP, IE, Univ. Bordeaux
- Nuclear magnetic resonance engineer**  
Estelle MORVAN, IE, CNRS
- Mass spectrometry engineer**  
Frédéric ROSU, IR, CNRS
- Surface plasmon resonance engineer**  
Laetitia MINDER, AI, Inserm
- Electron microscopy engineer**  
Armel BEZAULT, IE, CNRS
- Biochemistry and molecular biology engineer**  
Jean-Michel BLANC, IE, Inserm
- Biochemistry and molecular biology technicians**  
Thierry DAKHLI, Tech, Inserm  
Myriam MEDERIC, Tech, Inserm
- Quality approach**  
Loïc KLINGER, AI, CNRS



# Research Teams & Output



**Dr. Rémi Fronzes**  
Research Director (DR2), CNRS

Rémi Fronzes has a long-term research experience in biochemistry and structural biology of macromolecular assemblies. He trained as a membrane protein biochemist during his PhD in Bordeaux (France). In 2005, he moved to Gabriel Waksman's laboratory at the Institute of Structural and Molecular Biology in London (UK) to work as a postdoctoral research associate. In 2009, he was appointed as a junior research scientist at the CNRS and as a group leader at Institut Pasteur, in Paris (France). In 2011, RF was awarded an ERC (European Research Council) starting grant. In 2015, Rémi Fronzes was awarded a "Chaire d'excellence Senior" by the university of Bordeaux and Aquitaine regional Council. He moved his research group to IECB and CNRS unit UMR 5234 « Microbiologie Fondamentale et Pathogénicité » in 2016. In 2017, RF was awarded an ERC consolidator grant. He is the coordinator of the EquipEx+ project NanoCryoCLEM awarded in 2020.

## Research team

**Dr. Rémi FRONZES** Research Director DR2, (CNRS)

**Dr. Esther MARZA** Maître de conférence (Univ. Bordeaux)

**Prof. Jean-Paul BOURDINEAUD** Professeur (Univ. Bordeaux)

**Dr Leonardo TALACHIA ROSA** Post-doctoral fellow ERC

**Dr Pauline PONY** Post-doctoral fellow ERC

**Pierre NOTTELET** PhD Student ANR

**Robin ANGER** PhD student ERC

**Nina LOPEZ-LOZANO** PhD Student (Univ. Bordeaux)

**Dr Pierre MAISONNEUVE** Visiting scientist Research scientist, (University of Toronto)

**Laure BATAILLE** Visiting Scientist Engineer, CIRI, (INSERM, Lyon)

This team is part of the unit "Microbiologie fondamentale et pathogénicité" (MFP), CNRS UMR5234/Univ. Bordeaux

# Structure and Function of Bacterial Nanomachines

Bacteria are extremely adaptable and able adjust their lifestyle very quickly when these changes occur. One dramatic illustration of this capacity is the spread of antibiotic resistance among bacterial pathogens. During the last decade, the emergence of multi-resistant bacteria, which are resistant to several treatments, led to increase mortality caused by common infections. The 2014 report on antimicrobial resistance from the World Health Organization warns against the beginning of a "post-antibiotic" era, when most of the bacterial pathogens will become resistant to all treatments available.

In this context, it is crucial to fully understand the molecular mechanism of bacterial adaptability to ultimately target and limit this ability. To survive in a changing environment, bacteria have to resist to stresses induced by these changes and ultimately to adapt their lifestyle if these changes persist. These two processes are almost contradictory since the first aims at maintaining cell integrity while the second allows long term variability through the acquisition of new traits.

For 10 years, the team engaged several lines of research on this topic in the lab, first at Institut Pasteur and from 2016 within the MFP unit and at the Institut Européen de Chimie et Biologie (IECB) in Bordeaux. Over the last 5 years, we focused our research on the main projects listed below. The lab has also been instrumental in setting up a state of the art cryo-electron microscopy (CryoEM) facility at IECB. We have several on-going collaborations related to our expertise in CryoEM. We are also involved in technological development projects such as the implementation of super-resolution correlative microscopy in cryo conditions.

## Project 1: Natural transformation and gene repair in bacterial pathogens (Funded by ERC)

In this project, we want to understand how DNA can be uptaken and recombined in the bacterial genome during bacterial transformation.

Natural genetic transformation, first discovered in *Streptococcus pneumoniae* by F. Griffith in 1928, is observed in many Gram-negative and Gram-positive bacteria. This process promotes genome plasticity and adaptability. In particular, it enables many human pathogens such as *Streptococcus pneumoniae*, *Neisseria gonorrhoeae* or *Vibrio Cholerae* to acquire resistance to antibiotics and/or to escape vaccines through the binding and incorporation of new genetic material. While it is well established that this process requires the binding, internalization of external DNA and its recombination in the bacterial genome, the molecular details of these steps are unknown. In this project, we aim at acquiring a detailed understating of each of these steps. We discovered a new appendage at the surface of *S. pneumoniae* cells and showed that this appendage is similar in morphology and composition to appendages called Type IV pili commonly found in Gram-negative bacteria. We demonstrated that this new pneumococcal pilus is essential for transformation and that it directly binds DNA (PLOS Pathogens 2013 and 2015). We are also actively studying the DNA translocation apparatus. We isolated most of its components and are in the process of determining their structure and studying their function in vitro and in vivo. Finally, we identified a new key ATPase involved in the recombination process. We determined the crystal structure of this protein and identified its function in vitro and in vivo in collaboration with Patrice Polard's team in Toulouse (France) (**Nature communications, 2017**). We also explored the initiation and molecular mechanism of the recombination event. We determined the structure of RecA filaments from *S. pneumoniae* and revealed the structural basis of its interaction with its loader on ssDNA during transformation (called DprA) (Manuscripts in preparation). Finally, we are also exploring the architecture of the transformation apparatus in its native cellular environment using Cryo-tomography and correlative microscopy approaches.

**Project 2: Bacterial competition systems (Type 6 and type 7 secretion systems) (funded by the IDEX/regional Chair).**

The bacterial Type 6 secretion (T6S) system is one of the key players for microbial competition, as well as an important virulence determinant during bacterial infections. It assembles a nano-crossbow-like structure that propels an arrow made of Hcp tube and VgrG spike into the cytoplasm of the attacker cell and punctures the prey's cell wall. The nano-crossbow is stably anchored to the cell envelope of the attacker by a membrane core complex. In collaboration with Eric Cascales' laboratory in Marseille (France), we recently have shown that this membrane complex is assembled by the sequential addition of three proteins –TssJ, TssM and TssL– and presented a structure of the fully assembled complex (**Nature 2015**). Since our arrival at IECB and MFP, we solved the cryoEM structure of this complex (**EMBO J. 2019**). We also solved the cryoEM structure of another key element of the T6S system, the baseplate (**Nature microbiology 2018**) and of the T6SS substrate from pathogenic *Escherichia coli* in complex with the T6SS spike (**EMBO J. 2020**). While at Institut Pasteur, our group started to work on type 7 secretion systems (T7SS). These systems are mostly found in mycobacteria and other Gram-positive bacteria such as *Staphylococcus aureus* or *Bacillus subtilis*. While it is well established that mycobacterial T7SS are directly used in virulence, their exact function in other Gram-positive bacteria was unclear. We recently revealed that the T7SS found in *B. subtilis* is an anti-microbiobal device used by these bacteria to kill other Gram-positive bacteria (**BioRxiv 2020**). We also performed an in-depth biochemical study and solved the crystal structure of a key component of this system (YukC). Overall, our work shows that *B. subtilis* Yuk T7SS is a bona fide and functional T7SS that can be used as a model system to study T7SSs.

**Project 3: Metabolic adaptability of bacterial pathogens (funded by the ERC)**

Acetaldehyde-alcohol dehydrogenase (AdhE) enzymes are a key metabolic enzyme in bacterial physiology and pathogenicity. They convert acetyl-CoA to ethanol via an acetaldehyde intermediate during ethanol fermentation in an anaerobic environment. This two-step reaction is associated to NAD<sup>+</sup> regeneration, essential for glycolysis. The bifunctional AdhE enzyme is conserved in all bacterial kingdoms but also in more phylogenetically distant microorganisms such as green microalgae. It is found as an oligomeric form called spiroosomes, for which the function remains elusive. We used cryo-electron microscopy to obtain structures of *E. coli* spiroosomes in different conformational states. We showed that spiroosomes contain active AdhE monomers, and that AdhE filamentation is essential for its activity *in vitro* and function *in vivo*. The detailed analysis of these structures provides insight showing that AdhE filamentation is essential for substrate channeling within the filament and for the regulation of enzyme activity. This work was published in **Nature communications in 2020**.

**Project 4: Structure and function of a mycoplasma antibody cleavage device (funded by the ANR, in collaboration Yonathan Arfi, INRA, Bordeaux)**

Mycoplasmas cause various chronic diseases in animals and humans. They have evolved strategies to evade the host immune response, including the Mycoplasma Ig Binding (MIB)– Mycoplasma Ig Protease (MIP) antibody degrading system. The Fab domain of many types of immunoglobulins is recognized by MIB. This interaction allows the recruitment of the serine protease MIP, which cleaves the VH domain of the antibody. To understand the molecular basis of this system, we have solved the structure of the ternary complex Fab–MIB–MIP antibody degrading system by cryo-electron microscopy. The structure of the complex between MIB, MIP and the Fab fragment of a goat IgG has been solved to a 3 Å resolution, by single particle cryoEM. Together with biochemical and *in vivo* data, our work reveals very original binding mechanism of the complex to the antibody (**Science Advances, 2020**).

**Project 5: Development of super-resolution cryo-correlative microscopy**

The project 5 started very recently in collaboration with the laboratories of Daniel Choquet (IINS), Brahim Lounis, (LP2N) Gregory Giannone (IINS), Bordeaux imaging center and the UMS of the IECB. The major challenge in cell and structural biology today is to combine two cutting-edge technologies, super-resolution fluorescence microscopy and cryoEM, to enable a new revolution in the determination of atomic structure and the understanding of the function of molecules in their natural context. Multimodal or correlative microscopy approaches combining the power of high-resolution optical and electronic microscopy are at the forefront of technology at the national and international level. This technological development will make it possible to go beyond the limits of existing technologies and will revolutionize our understanding of the molecular mechanisms of living organisms, particularly in the fields of neurobiology, cancerology and the study of pathogens such as parasites, bacteria that are multi-resistant to antibiotics or emerging viruses. Rémi Fronzes is the coordinator of an EquipEx proposal awarded in 2020 focusing on this project. He is also the coordinator of a CPER project for IECB, including equipment that will be essential to this project.

**Selected publications**

1. Nottelet, P.; Bataille, L.; Gourgues, G.; Anger, R.; Lartigue, C.; Sirand-Pugnet, P.; Marza, E.; Fronzes, R.; Arfi, Y. The Mycoplasma Surface Proteins MIB and MIP Promote the Dissociation of the Antibody–Antigen Interaction. *Sci Adv* **2021**, *7* (10). <https://doi.org/10.1126/sciadv.abf2403>.
2. Flaugnatti, N.; Rapisarda, C.; Rey, M.; Beauvois, S. G.; Nguyen, V. A.; Canaan, S.; Durand, E.; Chamot-Rooke, J.; Cascales, E.; Fronzes, R.; Journet, L. Structural Basis for Loading and Inhibition of a Bacterial T6SS Phospholipase Effector by the VgrG Spike. *EMBO J* **2020**, *39* (11), e104129. <https://doi.org/10.15252/emboj.2019104129>.
3. Pony, P.; Rapisarda, C.; Terradot, L.; Marza, E.; Fronzes, R. Filamentation of the Bacterial Bi-Functional Alcohol/Aldehyde Dehydrogenase AdhE Is Essential for Substrate Channeling and Enzymatic Regulation. *Nat Commun* **2020**, *11* (1), 1426. <https://doi.org/10.1038/s41467-020-15214-y>.
4. Kumar, A.; Planchais, C.; Fronzes, R.; Mouquet, H.; Reyes, N. Binding Mechanisms of Therapeutic Antibodies to Human CD20. *Science* **2020**, *369* (6505), 793–799. <https://doi.org/10.1126/science.abb8008>.



**Dr. Yaser Hashem**  
Research Director (DR2), Inserm

Yaser Hashem obtained his PhD in 2010 in Strasbourg (France) in computational structural biology where he developed computational approaches for the study of bacterial ribosomal RNA interactions with several antibiotics. After his PhD he went on for a Postdoc at Columbia University in the city of New York with Prof. Joachim Frank (Nobel Laureate for Cryo-EM, 2017) where he worked on understanding the mRNA translation regulation using Cryo-EM and more specifically the translation initiation step in mammals. In 2014, Y. Hashem started his research group in Strasbourg (France) where he became expert in translation regulation in pathogenic parasites and their mammalian hosts. In 2017, Y. Hashem was awarded with the ATIP-Avenir grant followed shortly by the ERC (European Research Council) starting grant and the "Chair d'Excellence Junior" from the University of Bordeaux and joined the IECB as a Group Leader.

## Research team

**Dr. Yaser HASHEM** Research Director, DR2, (Inserm)

**Dr Marie SISSLER**, DR2, ARNA (CNRS)

**Mrs Stéphanie DURRIEU** Tech, ARNA (Univ. Bordeaux)

**Dr Ewelina GUCA** Postdoc (INSERM)

**Dr Aline RIMOLDI RIBEIRO** Postdoc (Université de Bordeaux)

**Ms Mayara DEL CISTIA** PhD student (Université de Bordeaux)

**Mr Anthony BOCHLER** PhD student (CNRS)

**Ms Margarita BELINITE** PhD student (CNRS)

**Dr Camila PARROT ATER**, Postdoc (Université de Bordeaux)

**Dr Hedyd SOUFARI** IR (INSERM)

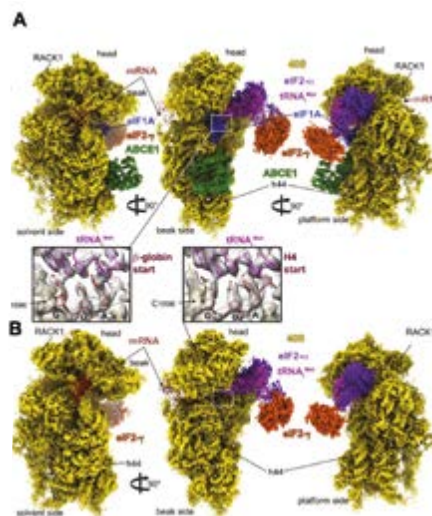
**Florian PIERRE** AI (CNRS)

**Dr Florent WALTZ** Postdoc (INSERM)

This team is part of Inserm, U1212

# RNA Processing and translation regulation in pathogens and hosts (RNA-PT)

The "mRNA translation regulation in pathogens and hosts" group endeavors to study at the molecular level the mRNA translation regulation in several species of pathogens, mainly eukaryotic (both cytosolic and mitochondrial mRNA translation), and their hosts. For several years already, the group has studied existing structural differences in the translation machinery between kinetoplastids and their mammalian hosts in order to discover new and more specific potential therapeutic targets that can be used for the development of safer therapeutic strategies against this family of dangerous parasites. One of the main focuses of the group is the translation initiation step that presents various important structural differences in kinetoplastids, such as Trypanosomes and Leishmanias, when compared to humans. The group is mainly specialized in cryo-electron microscopy, a technique that allows in principle to resolve molecular structures of large sizes to atomic resolutions.



## Translation initiation in mammals:

An efficient protocol allowing purification of mammalian initiation complexes from rabbit reticulocyte lysate (RRL) was setup. This protocol was termed grad-cryo, it has the advantage of enabling the purification of translation initiation complexes in near-native conditions at a stage where the start-codon has already been recognized, thus after the achievement of the scanning process on a chosen mRNA. The complexes are stalled with GMP-PNP, a non-hydrolysable analogue of GTP.

*Figure 1. Late-stage 48S initiation complexes (48SIC) from mammals. Thanks to grad-cryo protocol we were able to purify native IC in native conditions. A,  $\beta$ -globin IC. B, histon 4 IC. In boxes a blow up on the start-initiation codons for both complexes. From Simonetti & Guca et al. Cell Reports 2020.*

Thus, we have solved the structures of two native translation initiation complexes with two archetype abundant cellular mRNAs, the  $\beta$ -globin and histon 4. Our structures are at near-atomic resolution (3.0 to 3.5 Å, respectively, Figure 1) and reveal that depending on the mRNA sequence, its interactions with different components of the initiation complex such as eukaryotic initiation factors (eIFs) 1A, 2 and 3 can vary. These complexes can be directly compared with those from several species of pathogenic protozoa.

## Translation initiation in kinetoplastids:

The initiation stage is expected to represent numerous variations in pathogenic protozoa such as kinetoplastids (like *T. cruzi*, *T. brucei* and *L. major*) compared to its mammalian counterpart because of the presence of several large rRNA expansion segments at the binding site of several initiation factors such as eIF3. Protozoa like kinetoplastids present a complex life cycle where the parasite spends most of its life in an insect vector before being transmitted to a mammalian host upon biting. Moreover, in vitro growth of the parasite reveals various population regulation points aimed at optimizing the environmental resources such as oxygen and carbon resources. Therefore, we first attempted to study their in vitro growth in order to retrieve the best conditions allowing the purification of canonical translation initiation complexes.

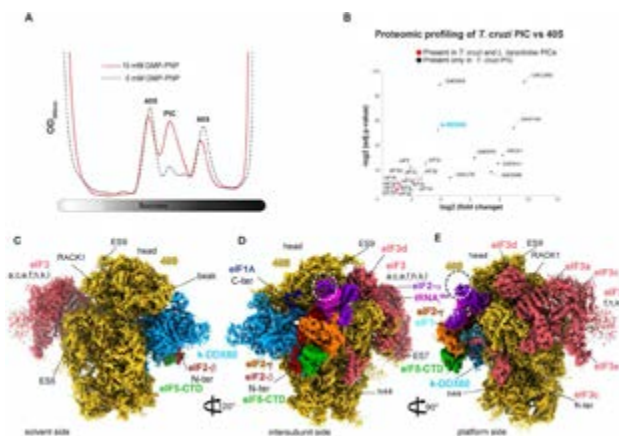


Figure 2. Composition and cryo-EM structure of the *T. cruzi* 43S PIC.

(A) The effect of the GMP-PNP treatment on the 43S PIC stabilization in the *T. cruzi* lysate assessed by UV absorbance profile analyses (B) Proteomic profiling of the endogenous pre-initiation complex in comparison with native 40Ss purified from the *T. cruzi* cell lysate (see methods for the validation). (C) The overall structure of the *T. cruzi* 43S PIC shown from the intersubunit side. The initiation factors are colored variably. (D) The 43S PIC reconstruction focused on the solvent side. Extra density of eIF2 $\alpha$  corresponding to the kinetoplastid specific N-terminal insertion is encircled by a dashed line. (E) The 43S PIC reconstruction focused on eIF3 and the 40S platform. From Boehler et al. 2020 (submitted).

The study of the dynamics of growth of epimastigote forms from *trypanosoma cruzi* and *Leishmania tarentolae* was carried out. After establishing the best growth conditions for both parasites, we have attempted the purification of initiation complexes using our grad-cryo protocol at different time-points of the growth curves and only at the 3rd growth day we were able to harvest the (pre)initiation complexes in sufficient yield to obtain a cryo-EM structure, the reason of which remains unknown.

The purified complexes were solved by cryo-EM at 3.3Å revealing the presence of several kinetoplastid-specific features such as the presence of an additional helicase involved in the initiation process that we have termed DDX60-like because of its faint homology to mammalian DDX60 (Figure 2).

### Transfection of *Leishmania tarentolae*:

In order to investigate the function of several kinetoplastid-specific protein that we have discovered during our investigation, such as DDX60-like, in regulating the initiation of translation in kinetoplastids, we set up a transfection system based on *Leishmania tarentolae*, a non-pathogenic kinetoplastid that also possesses DDX60-like in its initiation complex. Several mutants of DDX60-like will be transfected to *L. tarentolae* in order to inspect their impact on the efficiency of translation; but also on the formation of the translation initiation complexes.

We optimized the use of an integrative inducible system that includes a mutant strain *Leishmania tarentolae* T7-TR that constitutively expresses T7 RNA polymerase and TET repressor and the shuttle vector pLEXY\_I-blecherry3 in which the target genes are inserted into the expression cassette under control of T7 promoter with TET operator and is co-expressed with a blecherry marker that can be used to measure the expression level of the target protein by fluorescence microscopy (590 nm/ 620 nm). This work is still ongoing, in collaboration with Mélanie Bonhivers and Derrick Robinson.

### Mitochondrial translation regulation:

In the past year the team has heavily invested in investigating translation regulation in mitochondria in both pathogens and hosts. Thus, we have recently published the cryo-EM structure of the plant mitoribosome, as plants are also known to be hosts for several species of kinetoplastids (Waltz et al. Nature plants 2019, Waltz & Soufari et al. Nature plants 2020, Figure 3), but also from *T. cruzi* and *L. tarentolae* (manuscript in preparation) using purification protocols inspired from our grad-cryo protocol.

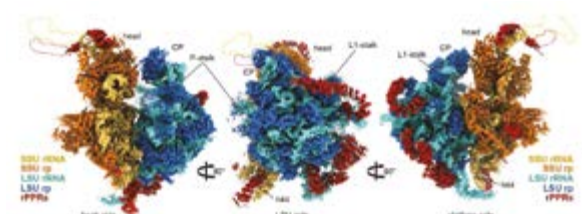


Figure 3. Overall structure of the plant mitochondrial ribosome.

rRNAs are colored in light blue (LSU) and yellow (SSU) and ribosomal proteins in blue (LSU) or orange (SSU). A dozen pentatricopeptide repeats proteins (PPR), specific to the plant mitoribosome were discovered and termed ribosomal PPR (rPPR), shown in red. From Waltz & Soufari et al. Nature plants 2020.

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- Waltz, F.; Soufari, H.; Boehler, A.; Giegé, P.; Hashem, Y. Cryo-EM Structure of the RNA-Rich Plant Mitochondrial Ribosome. *Nat. Plants* **2020**, *6* (4), 377-383. <https://doi.org/10.1038/s41477-020-0631-5>.



**Dr. Axel Innis**  
Research Director (DR2), Inserm

Axel Innis did his PhD in structural biology at the University of Cambridge, under the supervision of Prof. Tom Blundell (1998–2002). He then joined the group of Dr. R. Sowdhamini at the National Centre for Biological Sciences in Bangalore as a visiting fellow (2002–2004), where he developed a computational method for identifying functionally important sites in proteins. Following his time in India, Axel joined the laboratory of Prof. Thomas Steitz at Yale University (2004–2012) to work on what was, at the time, a little-known form of translational control: gene regulation by nascent polypeptides. He joined IECB as a group leader in January 2013, was awarded the 2017 Coups d'Élan Prize for French Research from the Bettencourt–Schueller Foundation and was selected as a 2017 EMBO Young Investigator. He is currently a research director (DR-2) affiliated to the ARNA laboratory (Inserm U1212).

## Research team

**Dr. Axel INNIS** Research Director (DR2), (Inserm)  
**Dr. Thibaud RENAULT** Researcher (CRCN)  
**Dr. Anne BOURDONCLE** Lecturer (MCU) (Univ. Bordeaux)  
**Dr. Fanny BOISSIER** Project engineer (visiting) (Univ. Bordeaux)  
**Ms. Mélanie GILLARD–BOCQUET** Project engineer Inserm – (Univ. Paris Descartes)  
**Dr. Mecit GÖKÇE** Postdoc (INSERM)  
**Dr. Aitor MANTECA** Postdoc (INSERM)  
**Dr. Thomas PERRY** Postdoc (INSERM)  
**Dr. Anne-Xander VAN DER STEL** Postdoc (INSERM)  
**Ms. Pauline COSSARD** PhD student (INSERM)  
**Ms. Elodie LEROY** PhD student (INSERM)

The team is part of Inserm U1212 / CNRS UMR 5320 (ARNA)

# Translational Regulation of Gene Expression

Ribosomes are the large macromolecular complexes responsible for accurately translating the genetic information contained in messenger RNA into protein. Our group seeks to understand how ribosomes make proteins, how their activity is regulated in response to different stimuli, and how a variety of small molecules are able to block these complex molecular machines. Our main focus is on the bacterial ribosome and how it is affected by nascent proteins known as arrest peptides, antimicrobial peptides produced by the host immune response, and antibiotics that target the translational machinery (Fig. 1). In order to determine how the ribosome is regulated at the molecular level, we use a combination of structural biology (cryo-EM), high-throughput functional characterization, biochemistry and computational biology.

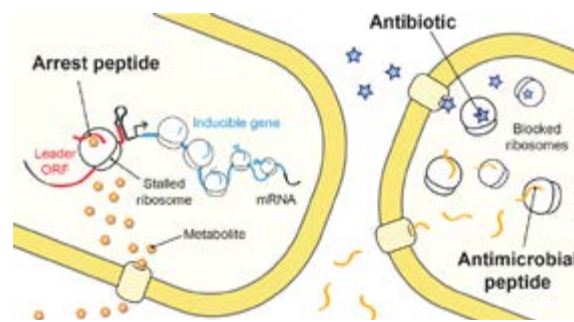


Figure 1 - Different types of molecules studied in the group that target the ribosome

## Arrest peptides

During translation, nascent proteins pass through a long cavity spanning the large subunit of the ribosome – known as the nascent polypeptide exit tunnel – before being released into the cytoplasm or delivered to the protein translocation machinery.

Although most proteins can easily complete this journey, interactions between specific nascent amino acid sequences and the exit tunnel result in impaired translation and ribosome stalling on the messenger RNA. These arrest peptides often block their own translation in response to a small molecule, such as a drug or metabolite, which is sensed by the ribosome nascent chain complex. Thus, arrest peptides are used for metabolite-dependent gene regulation in both prokaryotes and eukaryotes (Seip & Innis, 2016).

One of the group's main objectives is the large-scale identification and characterization of arrest peptides in bacteria, which we believe constitute a significant fraction of the hidden proteome of bacteria – the collection of short open reading frames (<75 amino acids) that have escaped genome annotation to date. For example, we have recently identified a new arrest peptide in  $\gamma$ -proteobacteria, called SpeFL, and have shown that the capture of a single molecule of the amino acid ornithine by a ribosome translating SpeFL is sufficient to activate polyamine biosynthesis. Using cryo-EM, we have shown that the bacterial ribosome and SpeFL form a highly selective binding pocket for L-ornithine, capable of discriminating between this amino acid and near-cognate ligands (Herrero del Valle et al., 2020). More recently, we have shown how one of the first arrest peptides identified, TnaC, regulates indole production in  $\gamma$ -proteobacteria by detecting a single molecule of the amino acid L-Tryptophan (van der Stel et al., 2021).

This and earlier studies have also shown that arrest peptides block translation by interfering with key aspects of ribosome function, such as tRNA accommodation, peptide bond formation or peptide release. However, the arrest code that determines whether a given nascent peptide is likely to inhibit its own synthesis remains to be elucidated, the range of metabolites that can be detected by the nascent peptide is unknown, and the molecular basis of metabolite sensing by the ribosome is only just beginning to be understood. Using cryo-EM and high-throughput tools developed in-house – such as inverse toeprinting (Seip et al., 2018) – we are systematically addressing these questions in order to reveal the true extent of metabolite sensing by arrest peptides.

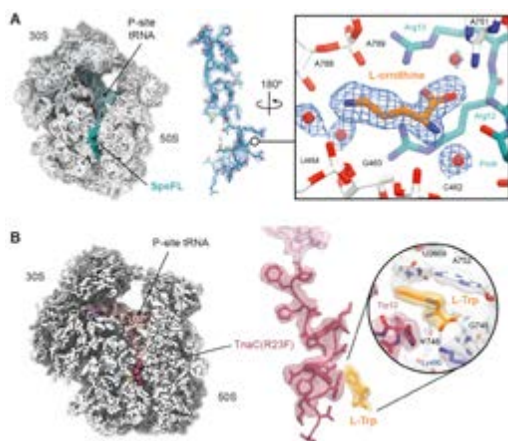


Figure 2 – Structural basis for the recognition of L-ornithine by a SpeFL-70S complex and of L-Trp by a TnaC-70S complex. (A, B) Transverse sections of cryo-EM density maps of the (A) SpeFL-70S (Herrero del Valle et al., 2020) and (B) TnaC-70S complexes (van der Stel et al., 2021), with a focus on the nascent peptides and bound (A) L-ornithine or (B) L-Trp.

### Antimicrobial peptides

Proline-rich antimicrobial peptides (PrAMPs) produced by the host immune response of insects and mammals display potent antimicrobial activity against Gram-negative bacteria and therefore represent a promising avenue for antibiotic development.

Although PrAMPs such as oncocin

(from the milkweed bug) or bactenecin-7 (from cows) were first thought to inhibit bacterial growth by binding to the chaperone protein DnaK, recent studies have shown that they have much greater affinity for the bacterial ribosome. However, their detailed mode of action remained unclear. In order to determine how these peptides inhibit translation, we determined the crystal structures of five different PrAMPs in complex with the *Thermus thermophilus* 70S ribosome. Our structures showed that these host defense molecules inhibit the transition from the initiation phase to the elongation phase of translation by binding to the nascent polypeptide exit tunnel and peptidyl transferase center of the ribosome (Seefeldt et al., 2015 and 2016, Mardirossian et al., 2018) (Fig. 3). Since these natural compounds share structural similarities with arrest peptides, the latter may prove helpful in steering the search for new peptide-based antimicrobials that are effective against resistant pathogens.

Using structural biology, droplet-based microfluidics, and biochemistry, we continue to explore the mechanisms by which naturally occurring antimicrobial compounds target and inhibit the bacterial translational machinery (Cytrinska et al. 2020).

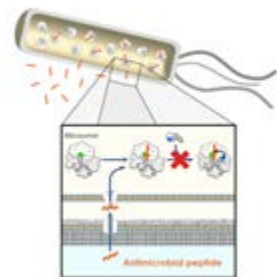


Figure 3 – Ribosome inhibition by antimicrobial peptides. The proline-rich antimicrobial peptides Onc112, Pyrrolicorin, Metalnikowin, Bac7 and Tur1A inhibit bacterial protein synthesis by blocking and destabilizing the translation initiation complex (Seefeldt et al. 2015, 2016; Mardirossian et al., 2018).

### Antibiotics

The threat of bacteria resistant to multiple antibiotics is a major public health challenge that must be tackled through coordinated action on multiple levels. As infectious pathogens have become increasingly resistant to the available drugs, antibiotic discovery programs in major pharmaceutical companies have struggled to produce new antibiotic scaffolds capable of sidestepping

current resistance mechanisms. Therefore, new strategies are needed to secure a steady supply of scaffolds and counter the spread of resistance.

The bacterial ribosome is a target for several major classes of antibiotics, including molecules that block peptide bond formation (chloramphenicol, oxazolidinones), impede the synthesis and movement of nascent proteins through the exit tunnel (macrolides), interfere with the decoding of messenger RNA (aminoglycosides) or prevent the translocation of tRNAs during the elongation step of protein synthesis (tuberactinomycins). Using structural biology and high-throughput approaches, we are (i) characterizing bacterial arrest peptides responsible for the induction of *erm* resistance genes in response to macrolide antibiotics, and (ii) revisiting the mechanisms of action of ribosome-targeting antibiotics, focusing on the defined functional states they target. This includes re-examining poorly characterized natural products from the golden age of antibiotic discovery (1950–1960s) to identify the detailed molecular mechanisms by which they block translation. A better understanding of how these antibiotics work could help design improved molecules that are effective against resistant pathogens.

In addition, we are developing high-throughput approaches that use the bacterial ribosome as a platform for the production and selection of translation inhibitors (Charon et al., 2019). We are particularly interested in molecules that target new sites on the ribosome, as they could be used as scaffolds to design novel drugs capable of bypassing known resistance mechanisms.

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**Dr. Petya Violinova KRASTEVA**  
Research Scientist (CRCN), CNRS  
UMR5248 CBMN

Dr. Petya V. KRASTEVA joined the IECB as a group leader in October 2019.

Her research focuses on cyclic dinucleotide signaling and extracellular matrix secretion in bacterial biofilm formation and pathogenesis. Combining X-ray crystallography, biophysical and biochemical assays, cryo-EM and in cellulo functional studies, her 'Structural Biology of Biofilms' team aims to provide a comprehensive view of bacterial social networks that spans the different resolution levels and presents molecular blueprints for the development of novel anti-infectives. Petya completed her PhD in Molecular and Cell Biology at Cornell University in 2011, after which she joined the editorial team of Nature Methods in New York. For her postdoc, she moved to the Institut Pasteur on 2012 and started her independent team as a CNRS CRCN and an ATIP-Avenir laureate in the end of 2016 at the I2BC, Gif-sur-Yvette. For her work on bacterial biofilms, Petya is the recipient of the Prix Jacques Monod (2016) and the CNRS Médaille de Bronze (2019).

### Research team

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**Wiem ABIDI** Ph.D. candidate CBMN (CNRS)  
**Lucia TORRES-SANCHEZ** Ph.D. candidate CBMN (CNRS)  
**Dr. Axel SIROY** Post-Doc CBMN (CNRS)  
**Dr. Dursun KORKUT** Post-Doc CBMN (CNRS)  
**Marion DECOSSAS** Research engineer (CNRS)

This team is part of the mixed research unit "CBMN : Chimie et Biologie des Membranes et des Nano-Objets", UMR 5248, CNRS/Université de Bordeaux/Bordeaux INP

# Structural Biology of Biofilms

Currently the team's research is focused on two main scientific questions: i) what are the intracellular signaling mechanisms that regulate bacterial biofilm formation and, ii) what are the structure, function and dynamics of the membrane-embedded biosynthetic platforms for the secretion of extracellular matrix components. Both projects are a direct continuation of the PI's interests and scientific accomplishments from the beginning of her academic career during which she has gained strong expertise in a plethora of techniques: from bacteriology, molecular biology and recombinant DNA technologies, to protein science, membrane protein biology, X-ray crystallography, cryo-EM and other biochemical and biophysical approaches. Most recently, the 'Structural Biology of Biofilms' team provided unprecedented insights in the structural biology of bacterial cellulose secretion (BCS) in biofilms with complete structure-function analyses of a megadalton-sized membrane-embedded Bcs secretion macrocomplex, as well as multiple regulatory subcomplexes.

Most bacteria respond to surfaces by the biogenesis of intracellular c-di-GMP that acts at the transcriptional, translational and post-translational levels to inhibit motility and biofilm formation via secreted adherence factors. Many free-living and pathogenic enterobacteria secrete biofilm-promoting cellulose using a multicomponent, envelope-embedded Bcs secretion system under the control of intracellular second messenger c-di-GMP. The molecular understanding of system assembly and cellulose secretion has been largely limited to the crystallographic studies of a distantly homologous BcsAB synthase tandem and a low-resolution reconstruction from our group (Krašteva @ et al., Nature Communications 2017) of an assembled BcsRQABEF macrocomplex encompassing most of the inner-membrane and cytosolic subunits in an as-yet unknown stoichiometry.

We recently demonstrated (Zouhir, Abidi et al., mBio 2020) that essential for secretion BcsR and BcsQ regulate each other's folding and stability and are recruited to the inner membrane via c-di-GMP-sensing BcsE and its intraoperon partner, BcsF. Crystallographic data showed that BcsE's predicted GIL domain is a degenerate Receiver-GGDEF domain tandem, where the divergent diguanylate cyclase module binds both c-di-GMP and BcsQ. Interestingly, the remaining N-terminal domain - while indispensable for BcsERQ membrane targeting - also interacts with transcription antitermination complex components, raising the possibility that BcsE acts on multiple levels to fine-tune bacterial cellulose secretion: from co-transcriptional control via anti-termination, though secretion system assembly, to maintaining a pool of c-di-GMP for processive synthase activation.

To further decipher the secretion system's architecture, we then moved onto extremely challenging single-particle cryo-EM studies on the assembled megadalton-sized Bcs macrocomplex (Abidi, Zouir et al., Science Advances 2021). We characterized the mechanism for asymmetric periplasmic crown polymerization: we identified beta-strand insertions in the enterobacterial BcsB homologs that are absent in the well-studied Rhodobacter protein and that lead to superhelical polymerization via beta-sheet complementation between subunits. Another surprise came from the fact that rather than having the postulated one-to-one BcsA:BcsB ratio, we observed a non-canonical synthase assembly, with a single BcsA subunit underlying the multimeric BcsB crown and being buttressed by the regulatory BcsRQ tandem. As the cytosolic components featured significant conformational heterogeneity in the cryo-EM datasets, we also undertook a bottom up approach in resolving the secretion system's assembly by solving the crystal structure of the essential for secretion BcsRQ complex and showing functional data

indicating that the tandem can act as a secretion system-specific membrane protein sortase. We further solved several structures of BcsE in complex with the BcsRQ tandem and identified multiple sites for c-di-GMP recognition, which would allow the creation of a c-di-GMP proximity pool for processive dinucleotide recycling and synthase activation at each substrate polymerization cycle.

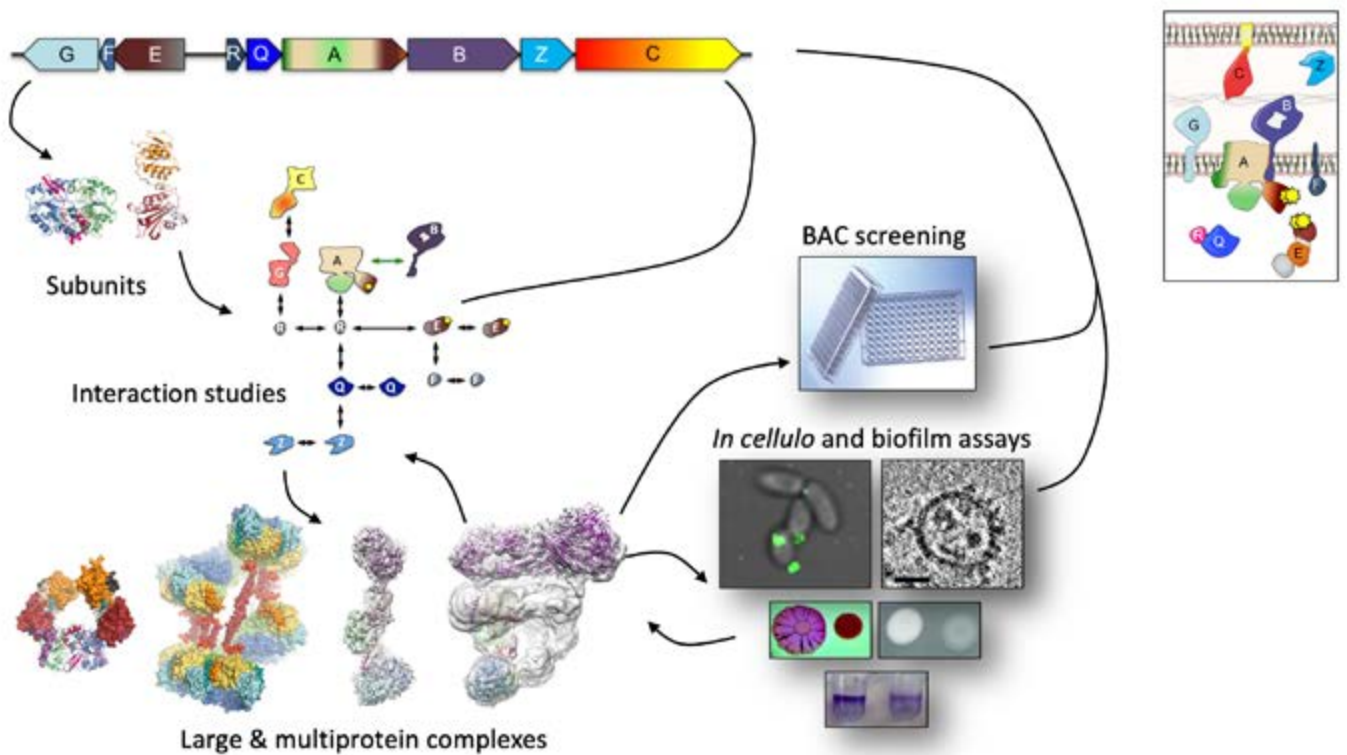
As a summary our main project combines biochemistry, structural biology and microbiology to provide comprehensive models of bacterial exopolysaccharide secretion that span the different resolution levels: from individual subunit to protein complexes to observing the structure, function and dynamics of these systems in cells and biofilms.

### Selected publications

1. Zouhir, S.; Abidi, W.; Caleechurn, M.; Krasteva, P. V. Structure and Multitasking of the C-Di-GMP-Sensing Cellulose Secretion Regulator BcsE. *mBio* **2020**, *11* (4). <https://doi.org/10.1128/mBio.01303-20>.
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## Exopolysaccharide secretion – an integrated structure-function approach





**Dr. Cameron Mackereth**  
Research Director (DR2), INSERM

Cameron Mackereth began his scientific training at the University of Waterloo (Canada) where he completed a degree in Biochemistry in 1996. His Ph.D. at the University of British Columbia (Canada) under the supervision of Dr. Lawrence McIntosh dealt with the structural investigation of a domain common to several protein families involved in transcription and cellular signaling. He continued to use nuclear magnetic resonance (NMR) spectroscopy at the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany, where he looked at domain arrangements of large protein-RNA splicing complexes in the group of Dr. Michael Sattler. In the fall of 2007, he joined the IECB as a group leader. In 2011 he was also recruited as a senior research associate (chargé de recherche, CR1) within the French National Institute of Health and Medical Research (Inserm), and in 2017 became an Inserm research director (directeur de recherche, DR2).

### Research team

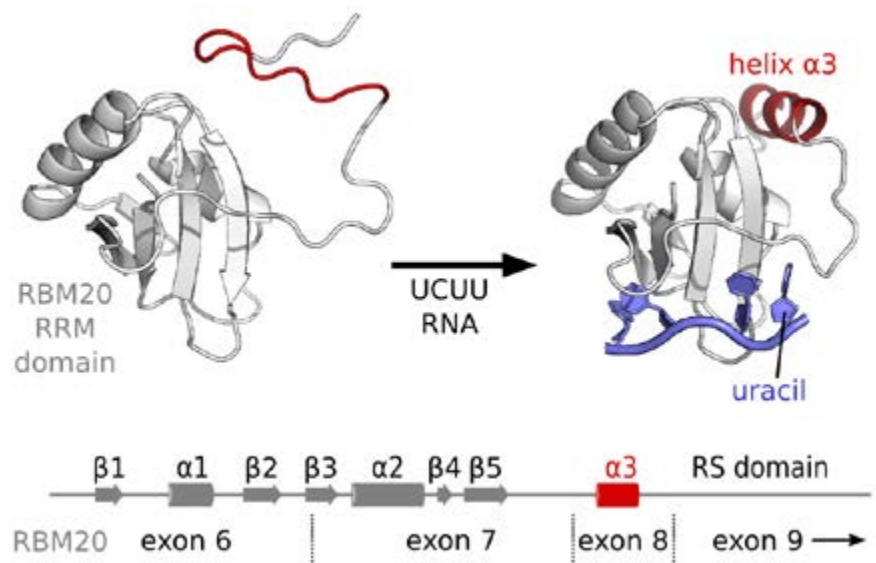
**Dr. Cameron MACKERETH** Research Director, DR2, (INSERM)  
**Dr. Samir AMRANE** Researcher, CRCN, (INSERM)  
**Dr. Pierre BONNAFOUS** Associate Professor, MCU (Univ. Bordeaux)  
**Dr. Gilmar SALGADO** Associate Professor, MCU (Univ. Bordeaux)  
**Dr. Julien MARQUEVIELLE** Postdoc (INSERM)  
**Dr. Somnath MONDAL** Postdoc (INSERM)  
**Vincent TALENTON** Research Engineer, IE (INSERM)  
**Amani KABBARA** PhD (INSERM)  
**Dorian LECERF** Masters student, M2 (INSERM)  
**Camille CHARLES** Masters student, M1 (INSERM)

This team is part of the unit "Acides Nucléiques: Régulations Naturelles et Artificielles" (ARNA), Inserm U1212/CNRS UMR5320/Univ. Bordeaux

# NMR Spectroscopy of Protein-Nucleic Acid Complexes

The group studies molecular details of protein-nucleic acid macromolecules and other complexes using a variety of new NMR techniques as well as established biophysical approaches. Equally important to the lab is the traditional strength of NMR as a tool to probe the dynamics of biological samples, the characterization of transient interactions, and the possibility to look at structures that exhibit a significant amount of unstructured elements. The goal is to connect protein structure and dynamics to the selective binding of nucleic acids, and thus understand essential biomolecular interactions and to model disease mutations. Current topics include alternative splicing factors, proline isomerases, DNA mismatch repair, as well as nucleic acid structures in viral infection and cancer.

Cardiac development and a healthy heart are reliant upon the production of specific splice isoforms of numerous proteins such as titin and the ryanodine receptor Ryr2. One of the key proteins that regulate heart-specific alternative splicing is the vertebrate splicing factor RBM20 (RNA Binding Motif protein 20). RBM20 regulates protein isoforms of titin and other genes important for heart development and function, with mutations in the gene linked to cardiomyopathy. Previous studies have identified the four base RNA motif UCUU as a common element in pre-mRNA targeted by RBM20. Using NMR spectroscopy, we have published the structure of the RNA Recognition Motif (RRM) domain from mouse RBM20 bound to RNA containing a UCUU sequence. The main finding is an unexpected mechanism that couples RNA binding with partial protein folding in the creation of the 3' uracil recognition (see figure).



In order to interact with the complete UCUU motif, a well-folded C-terminal helix (encoded by exon 8) is critical for high affinity binding. A construct with this helix removed binds only weakly to the RNA motif, and has lost preference for the 3' uracil. This is unusual since the construct nonetheless retains all of the residues and backbone atoms that directly contact RNA. Structure determination of the unbound form of the larger RBM20 RRM domain (encoded by exons 6, 7 and 8) shows that the C-terminal helix

is absent, and thus the uracil binding pocket is also missing. It therefore appears that a combination of the transient helix, RRM-helix interactions, and loop-uracil contacts are required to form a high affinity complex. Absence of any one of these three factors prevents protein-RNA complex formation. Similar binding mechanisms by which protein secondary structure is stabilized and essential for RNA selectivity is rare in protein-RNA complex formation.

Animal mitochondrial DNA (mtDNA) has a higher substitution rate than nuclear DNA, with the accumulation of mtDNA mutations being one of the hallmarks of ageing. Octocorals have a reduced rate of mitochondrial evolution and encode a MUTS-like protein (mt-MutS) in their mtDNA. Its function, however, remains unknown. An international project has been selected as a Program Grant from the Human Frontiers Research Program in collaboration with the group of J. Stewart (previously at MPI for Biology of Ageing, Germany; now at Univ. Newcastle, UK) and D. Lavrov (Iowa State University, USA). Comparative, structural, and experimental approaches will be used to investigate the function of mt-MutS and to use engineered mt-MutS as a tool to determine if lower mutation rates in mtDNA improve health in ageing.

We are also continuing work in collaboration with the group of Matthieu Sainlos at the Institute of Interdisciplinary Neuroscience here in Bordeaux. In the past we have developed and extensively characterized protein-based binders modeled on the fibronectin FN3 domain that are able to bind to a single member of a highly conserved protein family. Specifically, these binders recognize the PSD-95 protein and not the highly similar PSD-93, SAP-97 or SAP-102 proteins. A new avenue of study targets other proteins in the DLG family by using a similar approach, and indeed several promising candidates have been identified. In addition, a future aim will be to develop gene encodable photocontrollable PDZ domain-mediated binders, as well as using an approach that aims at an affinity-clamp mechanism.

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**Dr. Nicolas Reyes**  
Research Director (DR2),  
CNRS/Univ. Bordeaux

Nicolas Reyes's research interests focus on understanding the molecular mechanisms of function and pharmacology of human membrane proteins. He has a multidisciplinary background in membrane protein biophysics spanning from single-molecule and electrophysiological measurements (Nature 2006, Nature 2008) to solving three-dimensional structures of membrane transporters (Nature 2009). As an independent researcher, he successfully set up laboratories at the Pasteur Institute and then, the European Institute of Chemistry and Biology, and made important contributions to the molecular mechanisms of an essential component of the synaptic machinery in the human brain, namely excitatory amino acid transporters (Nature 2017), as well as the binding mechanisms of therapeutic antibodies broadly used in cancer immunotherapy to its human receptor (Science 2020). To achieve this, he secured and coordinated long-term national (INCA, ANRS), and international (ERC-Starting/ERC-Consolidator) funding awards.

### Research team

**Dr. Nicolas REYES** Research Director, DR2 (CNRS)  
**Dr. Juan CANUL-TEC** Postdoc (CNRS)  
**Dr. Kapil GOUTAM** Postdoc (CNRS)  
**Dr. Maria MARTINEZ** Postdoc (CNRS)  
**Dr. Miryam VILLALBA** Postdoc (Univ. Bordeaux)  
**Shashank KHARE** PhD Student (Univ. Bordeaux)

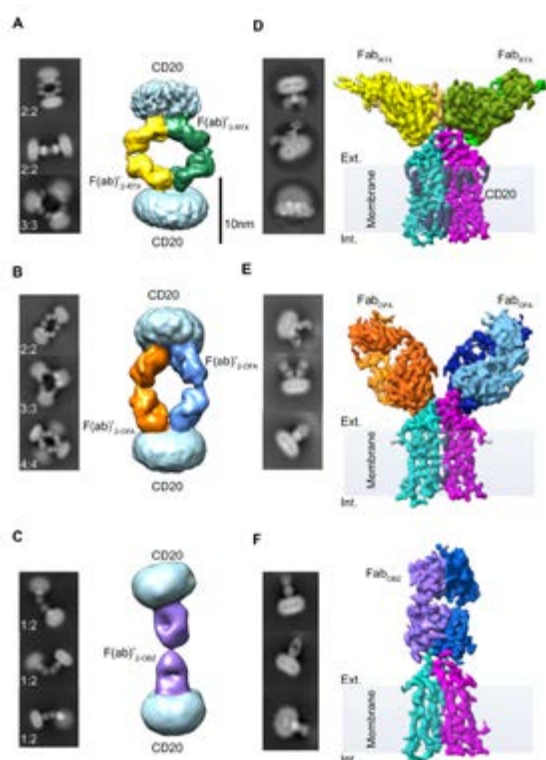
This team is part of the unit "Fundamental Microbiology and pathogenicity" (MFP), CNRS UMR5234/CNRS/Univ. Bordeaux

# Membrane Protein Mechanisms

Human solute carriers (hSLC) form a superfamily of integral membrane proteins that transport essential molecules and ions across membranes, and are the cellular receptors of human and pathogenic proteins.

The transport and receptor functions of hSLC are involved in a wide range of pathological conditions that make them important emerging drug targets in neurodegeneration, cancer, and infectious diseases, among others.

Our research program aims to unravel novel transport, receptor and pharmacological mechanisms of medically important hSLCs using a multidisciplinary biophysical approach. To achieve this, we determine high-resolution structures of hSLCs and their macromolecular complexes with other membrane, as well as soluble proteins. Moreover, we complement and challenge the structural data with functional approaches to probe the proteins dynamics and thermodynamics.



**Figure 1** Cryo-EM analysis of CD20-(Fab)'2 complexes. A-C, 3D reconstructions of 2:2 (Fab)'2:CD20 assemblies with lipid-detergent micelle colored light-blue and individual (Fab)'2 molecules color coded. 2D classes are also shown for 2:2, 3:3 and 4:4 assemblies. D-F, Cryo-EM maps of a single-copy of CD20 bound to Fab (individual CD20 subunits in cyan and pink, respectively) within the CD20-(Fab)'2 assemblies with Fab (heavy and light chains in dark and light colors, respectively) and lipid (grey) molecules bound. Examples of 2D classes in different orientations are shown.

### Binding mechanisms of therapeutic antibodies to human CD20

Human cluster of differentiation 20 (CD20) (Ishibashi et al., 2001; Liang et al., 2001), is a membrane receptor expressed during B-lymphocyte development (Einfeld et al., 1988). Its cellular function is poorly understood, and it is involved in calcium transport and intracellular calcium signaling associated with the B-cell receptor (Petrie et al.,

2002). CD20 is also expressed in malignant B cells and is the target of approved therapeutic monoclonal antibodies (mAbs) that are divided into two groups, type I and type II, based on two signature differences (Deans et al., 1998; Cragg et al., 2003; Cragg et al., 2004; Marshall 2017): type I mAbs recruit complement more potently than type II and therefore, induce robust complement-dependent cytotoxicity (CDC); type I binds twice as many type II mAbs to a given B cell type. However the molecular mechanisms underlying these differences remain unknown.

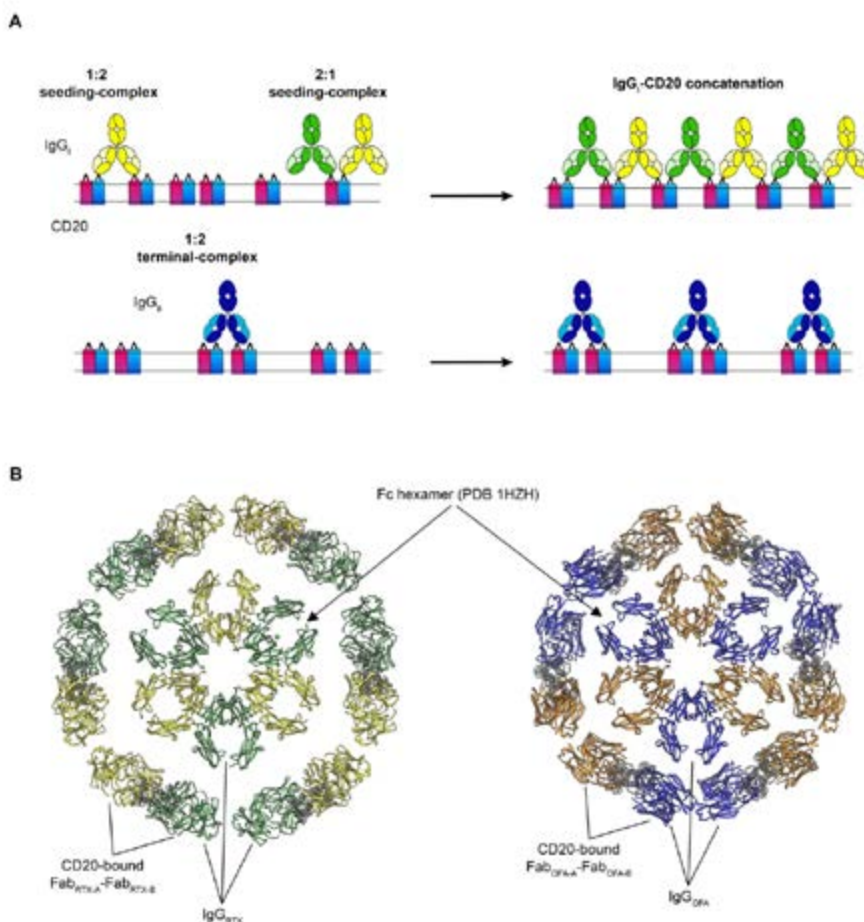
During the reporting period, we determined cryo-electron microscopy (cryo-EM) high-resolution structures of full-length human CD20 complexes with divalent-Fab fragments (F(ab)'2) from three major therapeutic mAbs: RTX, OFA, and OBZ (Fig. 1). The

structures and binding thermodynamics demonstrate that dimeric CD20 is able to bind two RTX or OFA Fab molecules, but only one OBZ Fab, due to extensive interactions with the two CD20 subunits and steric hindrance. These results provide long-sought atomic-level understanding on the ability of RTX and OFA, so-called type I mAbs, to activate complement potentially compared to OBZ (type II) (Fig. 2).

Our structures also show that OFA binds with strikingly different geometry compared to RTX and completely lacks homotypic Fab-Fab interaction, as observed for RTX and for another FDA-approved murine mAb, ocrelizumab. Consistently, our binding thermodynamic analysis further show that homotypic Fab-Fab interactions contribute marginally to RTX binding energy. Finally, our CD20-RTX and CD20-OFA structures shed light on the importance of structural factors other than binding stoichiometry to

## Selected publications

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determined complement recruitment potency.

*Figure 2 Binding mechanism and hexameric CD20-IgG model. A, IgG:CD20 "seeding" and "terminal" complexes mechanisms for type I and II mAbs, respectively. B, Structural model built with the symmetric Fc hexamer from PDB 1HZH and six copies of CD20-FabRTX (left) or CD20-FabOFA (right) structures determined in this work, arranged symmetrically and concentric to the Fc ring. The space between CD20-Fab complexes was minimized to avoid clashes between Fab domains bound to different CD20 molecules. Fc domains and the two corresponding Fab domains are shown in the same color.*

Taken all together, our work constitutes the first structural comparison of major anti-CD20 mAbs used in clinic bound to its full-length receptor, and unravels key differences between first- and second-generation mAbs, as well as a murine and human mAbs at atomic level. Importantly, based on our results, we are able to explain well-known differential cytotoxic properties between therapeutic mAbs, namely their potency to activate the complement pathway as immunological effector mechanism. Hence, the work represents a major advance in the molecular understanding of extensively used human cancer immunotherapies, and it should facilitate rational design of next-generation anti-CD20 mAbs.

This was the work of a PhD student in my group, Anand Kumar, and it has been recently published as a full-article in *Science*, as well as presented at an international symposium (cryoEM Symposium EMBL Heidelberg). An invited-speaker presentation at an international conference (Gordon Research Conference) was cancelled due to COVID-19.



**Dr. Frédéric Friscourt**  
ATIP-Avenir Fellow, CNRS-Univ. Bordeaux

Frédéric Friscourt received his PhD from the University of Glasgow, UK in 2009, under the guidance of Prof. P. Kočovský, on the development of novel chiral ligands for enantioselective catalysis. He then joined the group of Prof. G-J. Boons at the Complex Carbohydrate Research Center, GA, USA, as a post-doctoral research associate (2009–2014) in order to transition to chemical biology research. There, he became involved in the design of probes for imaging the glycome. In 2014, he obtained a Junior Chair of Excellence from the University of Bordeaux and was soon after recruited as a group leader at the IECB in Bordeaux. He recently received the prestigious CNRS-ATIP-Avenir award (2017). His current research focuses on using organic chemistry to develop novel tools that can probe the influence of glycans in the brain, notably in neuro-disorders.

## Research team

**Dr. Frédéric FRISCOURT** ATIP Avenir Team Leader (CNRS/Univ. Bordeaux)  
**Dr. Jürgen SCHULZ** IR2 (CNRS)  
**Dr. Zoeisha CHINOY** PostDoc (Univ. Bordeaux)  
**Khalaf TAREK** PhD Student (Univ. Bordeaux)  
**Václav NĚMEC** Visiting Scientist Masaryk University

This team is part of the unit "Institut de Neurosciences Cognitives et Intégratives d'Aquitaine" (INICIA), CNRS UMR5287/Univ. Bordeaux

# Chemical Neuroglycobiology

Glycans are chains of monosaccharides that are covalently linked to cell surface proteins and lipids. They have been recognized as key participants in cell-cell communications and for instance, in the brain, are crucial mediators in neurite outgrowth, synapse formation and plasticity. From a pathological point of view, changes in the neuro-glycome of cells are associated with developmental disorders, can mark the onset of glioma and neuro-inflammation. Despite these intriguing observations, the molecular mechanisms by which these complex carbohydrates influence neural cells are not well understood due to a lack of suitable biochemical methods. We aim at unravelling the functional roles of glycans in the nervous system by exploiting organic chemistry to develop novel tools that can probe glycans in the brain.

## Imaging glycans: a daunting task.

Although protein tracking in living cells has become routine experiments in cell biology laboratories thanks to the utilization of genetic reporters, glycans are, unfortunately, not amenable to these imaging techniques, as they are not directly encoded in the genome. As an emerging alternative, the **bioorthogonal chemical reporter strategy**, which elegantly combines the use of metabolically labeled azido sugars and highly reactive cyclooctyne probes, through strain-promoted alkyne azide cycloadditions (SPAAC), is a versatile technology for labeling and visualizing glycans. However, the biological stability of chemical reporters is not always up to par and cyclooctyne probes are often highly hydrophobic, which can lead to increase background signal.

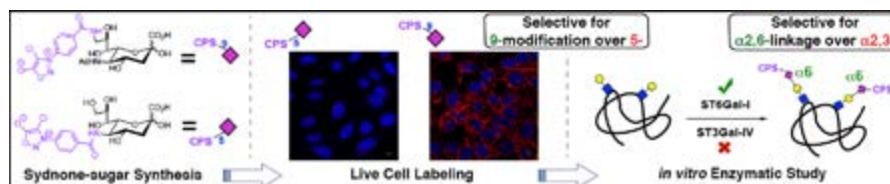
To address these difficulties, we are developing fluorogenic bioorthogonal systems, in which non- or weakly fluorescent reagents produce highly fluorescent products as well as more stable chemical reporters.

## Sydnonones as novel bioorthogonal chemical reporters.

To circumvent the stability issue of azides, we recently developed modified **sydnonones**, highly stable aromatic mesoionic 1,3-dipoles and employed them as chemical reporters for the challenging detection of modified-proteins in complex cellular extracts (J. Org. Chem. **2018**, *83*, 2058).

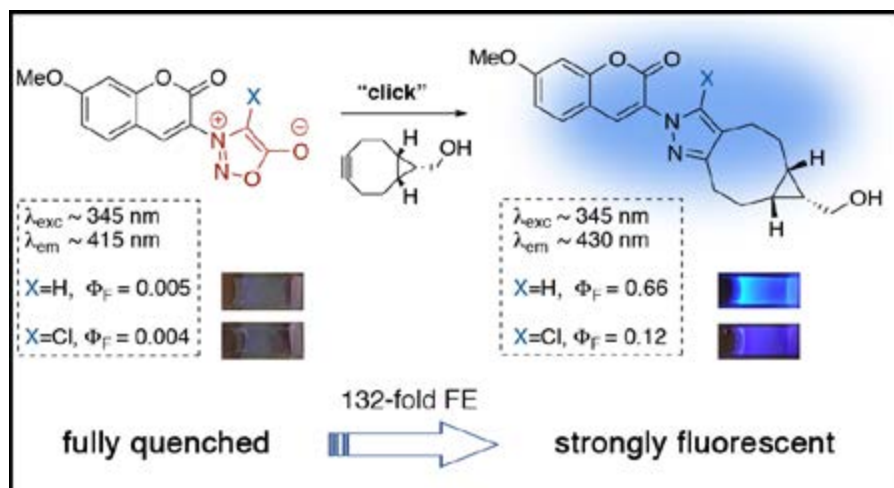
Next, we developed **modified monosaccharides with sydnonones for the tagging of complex glycans in living mammalian cells** (*Angew. Chem. Int. Ed.* **2019**).

We showed that the positioning of the reporter on the sialic acid scaffold significantly altered its metabolic fate. While employing the 9-modified neuraminic acid led to robust cell-surface labeling, the 5-modified analog was not metabolized by cells and we identified CMP-sialic acid synthetase as the enzymatic roadblock. Further in vitro enzymatic assays also revealed that the 9-modified neuraminic acid is preferentially accepted by the sialyltransferase ST6Gal-I over ST3Gal-IV, leading to the favored incorporation of the reporter into linkage-specific  $\alpha$ 2,6-N-linked sialoproteins, making 9-sydnone modified neuraminic acid a valuable probe to selectively track and capture a subset of sialosides.



### Novel Fluorogenic Systems.

In our effort to develop novel fluorogenic reagents, we recently showed that the sydnone moiety could also efficiently quench the fluorescence of coumarin, which could be restored, with a 132-fold enhancement, upon cycloadditions with cyclooctynes and were successfully applied, in a biochemical context, for the highly specific labeling of proteins in no-wash conditions. TD-DFT calculations suggested that the fluorescence quenching of the sydnone-modified coumarins was likely due to the presence of an energetically low-lying non-emissive charge-separated state (Org. Lett. 2018).



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- Chinoy, Z. S.; Friscourt, F. Bioorthogonal Chemical Ligations Towards Neoglycoproteins. *In Reference Module in Chemistry, Molecular Sciences and Chemical Engineering*; Elsevier, 2021; p B9780128194751000000. <https://doi.org/10.1016/B978-0-12-819475-1.00080-8>.



**Dr. Gilles Guichard**  
Research Director (DR1), CNRS

Gilles Guichard graduated in chemistry from the Ecole Nationale Supérieure de Chimie in Toulouse (1991) and Univ. Montpellier (1992) in France. He received his PhD from the Univ. Strasbourg (1996), working on immune recognition of pseudopeptides and synthetic vaccines. Following post-doctoral research with Prof. Dieter Seebach at the ETH in Zürich (1997) in the field of  $\beta$ -peptide foldamers, he joined the Institut de Biologie Moléculaire et Cellulaire (IBMC) in Strasbourg as a CNRS Chargé de Recherche (1998). Since 2006, he has been a CNRS Research Director. In 2009, he joined CBMN and moved as a new group leader to the Institut Européen de Chimie et Biologie (IECB) in Bordeaux. His current research focuses on biomimetic chemistry of peptides, foldamer chemistry, molecular recognition, bioinspired nanostructures and structure guided design of modulators of protein-protein interactions.

### Research team

**Dr. Gilles GUICHARD** DR1 (CNRS)  
**Dr. Christel DOLAIN** MCU (Univ. Bordeaux)  
**Dr. Morgane PASCO** CRCN  
**Dr. Guillaume COMPAIN** MCU (Univ. Bordeaux)  
**Dr. Guillaume NAULET** ATER (Univ. Bordeaux)  
**Dr. Jérémie BURATTO** Postdoctoral fellow (Univ. Bordeaux)  
**Dr. Sung HYUN YOO** Postdoctoral fellow (Univ. Bordeaux)  
**Dr. Bo LI** Postdoctoral fellow (CNRS)  
**Dr. Chiranjit DUTTA** Postdoctoral fellow National Univ. Singapore (NUS)  
**Dr. Roxanne ORSTEIN** Postdoctoral fellow (CNRS)  
**Clément MONSARRAT** PhD student (Univ. Bordeaux)  
**Antoine HACIHASANOGLU** PhD student (Univ. Bordeaux)  
**Maxime NEUVILLE** PhD student (Univ. Bordeaux)  
**Aline DELAMARE** PhD student (Univ. Bordeaux)

This team is part of the unit "Chimie et Biologie des Membranes et Nanoobjets" (CBMN), CNRS – Université de Bordeaux – Bordeaux INP (UMR 5248)

# Peptidomimetic Chemistry

We are interested in designing and elaborating systems with protein like structure and functions and to investigate and develop their biological and biomedical applications. Although centered on chemical synthesis, our research program is based on a multidisciplinary approach involving spectroscopic studies, crystallographic analyses, combinatorial techniques, and binding studies.

Our main line of research focuses on Peptidomimetic & Foldamer Chemistry. We are using the knowledge gained from structural studies conducted in the past years to develop functional foldamers. We have recently designed cationic amphiphilic helices that can deliver plasmid DNA or siRNA into cells (*Bioconjug Chem* 2019, *Chem Commun* 2021). Amphiphilic water-soluble foldamer sequences can also be used to create precise nanometer scale assemblies mimicking protein quaternary structures (*ChemPlusChem* 2021, **Highlight #1**).

The finding that oligourea foldamers can be interfaced with natural peptide helices and that the two helical forms do communicate within a single strand (*Angew Chem Int Ed* 2015) is of particular interest for applications of foldamers in biology. Recent applications developed in our group include the design of  $\alpha$ -helix mimics to inhibit protein-protein interactions (PPIs) (*Angew Chem Int Ed* 2021, *Sci Adv* 2021, **Highlight #2**) or activate receptors (*Nat Commun* 2019 and *Chem Sci* 2019).

Besides our work on foldamers, we are engaged in two programs dedicated to the structure-guided design of antibacterial molecules that specifically block protein synthesis and DNA replication in bacteria via inactivation of the corresponding molecular machineries: (i) the bacterial ribosome (coll. A. Innis, IECB, Pessac) and (ii) the bacterial sliding clamp (coll. D. Burnouf, IBMC, Strasbourg). We have recently reported the antibacterial activities of dual peptides targeting both the E. coli sliding clamp and the ribosome (*RSC Chem Biol*, 2020)

### RECENT HIGHLIGHTS:

#### Highlight #1: Nanotubular Assemblies from Oligourea Foldamers in Aqueous Conditions (*ChemPlusChem* 2020 – selected for the cover)

Previously, we have reported amphiphilic foldamer sequences that self-assemble into extended supramolecular tubular structures in aqueous conditions. By using a combination of electron microscopy, X-ray crystallography and circular dichroism, we have now shown that it is possible to control the shape and morphology of the foldamer tubular assemblies – from single short tubules to extended tubule bundles (i.e., fibres) – by modulating the polarity of the aqueous environment through the use of alcohol additives (Fig. 1).

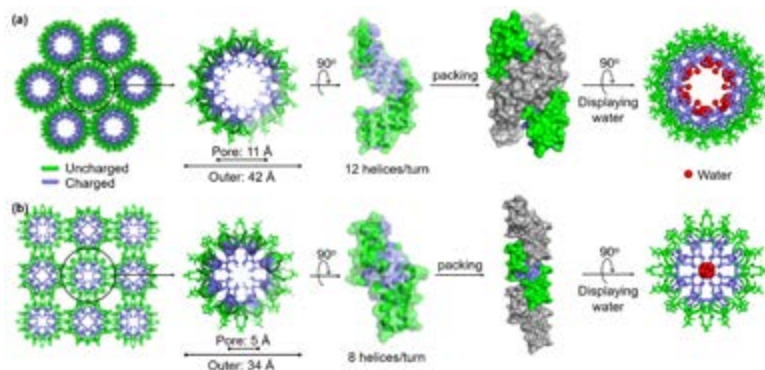


Fig. 1. Nanotubular structures formed by self-assembly of amphiphilic helical oligourea foldamers. The structures were determined from crystals grown in the presence of (a) 20% IPA and (b) 35% IPA.

**Highlight #2: Structural basis for Protein surface recognition by oligourea-peptide hybrids (Angew Chem Int Ed 2021, collaborative work with UREKA and N. Rochel at IGBMC)**

Interfacing oligoureas with peptides was used for the first time in this project to design peptide-oligourea hybrids that disrupt PPIs. High affinity binders to ubiquitin ligase MDM2 and vitamin D receptor (VDR) were obtained within few rounds of optimization by carefully designing oligourea sequences. We also obtained an ensemble of X-ray structures of foldamer ligands bound to their respective target proteins MDM2 and VDR. The high resolution structures collected in this project confirm that a high degree of  $\alpha$ -helix mimicry was achieved in the two series of ligands (Fig. 2). By enabling the introduction of foldamer inserts into a peptide sequence, the strategy reported here, whereby an  $\alpha$ -helical segment is replaced by a foldamer insert, may thus yield peptide analogues with substantial resistance to proteolytic degradation, a feature which is often desirable when developing peptide therapeutics.

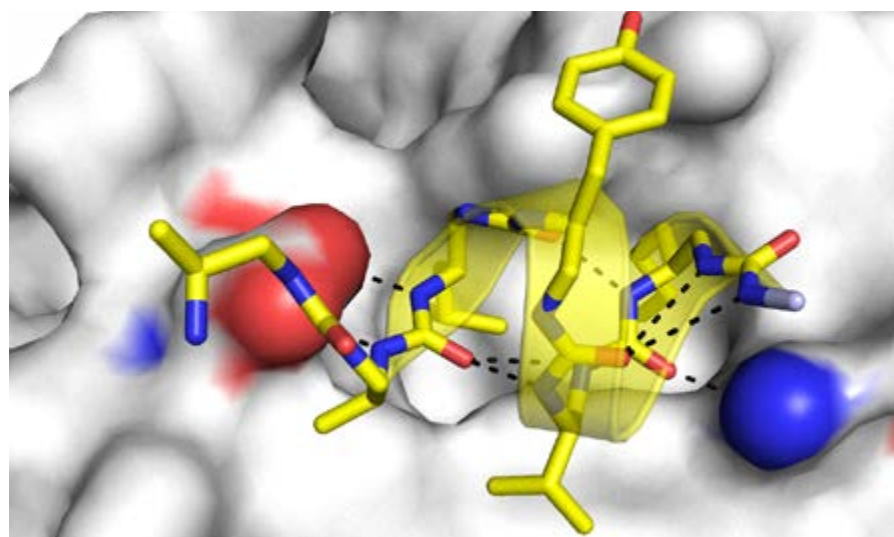


Fig. 2. Crystal structure of an oligourea helix in complex with the ligand binding domain of VDR. Intramolecular H-bonds within the oligourea backbone and intermolecular H-bonds with the residues of the VDR charge clamp are shown as black dotted lines.

## Selected publications

1. Fremaux, J.; Venin, C.; Mauran, L.; Zimmer, R.; Koensgen, F.; Rognan, D.; Bitsi, S.; Lucey, M. A.; Jones, B.; Tomas, A.; Guichard, G.; Goudreau, S. R. Ureidopeptide GLP-1 Analogues with Prolonged Activity *in Vivo* via Signal Bias and Altered Receptor Trafficking. *Chem. Sci.* **2019**, *10* (42), 9872–9879. <https://doi.org/10.1039/C9SC02079A>.
2. Fremaux, J.; Venin, C.; Mauran, L.; Zimmer, R. H.; Guichard, G.; Goudreau, S. R. Peptide-Oligourea Hybrids Analogue of GLP-1 with Improved Action *in Vivo*. *Nat Commun* **2019**, *10* (1), 924. <https://doi.org/10.1038/s41467-019-08793-y>.
3. Mbianda, J.; Bakail, M.; André, C.; Moal, G.; Perrin, M. E.; Pinna, G.; Guerois, R.; Becher, F.; Legrand, P.; Traoré, S.; Douat, C.; Guichard, G.; Ochsenbein, F. Optimal Anchoring of a Foldamer Inhibitor of ASF1 Histone Chaperone through Backbone Plasticity. *Sci. Adv.* **2021**, *7* (12), eabd9153. <https://doi.org/10.1126/sciadv.abd9153>.
4. Douat, C.; Bornerie, M.; Antunes, S.; Guichard, G.; Kichler, A. Hybrid Cell-Penetrating Foldamer with Superior Intracellular Delivery Properties and Serum Stability. *Bioconjugate Chem.* **2019**, *30* (4), 1133–1139. <https://doi.org/10.1021/acs.bioconjchem.9b00075>.
5. Yoo, S. H.; Collie, G. W.; Mauran, L.; Guichard, G. Formation and Modulation of Nanotubular Assemblies of Oligourea Foldamers in Aqueous Conditions Using Alcohol Additives. *ChemPlusChem* **2020**, *85* (10), 2243–2250. <https://doi.org/10.1002/cplu.202000373>.
6. Lombardo, C. M.; Kumar M V, V.; Douat, C.; Rosu, F.; Mergny, J.-L.; Salgado, G. F.; Guichard, G. Design and Structure Determination of a Composite Zinc Finger Containing a Nonpeptide Foldamer Helical Domain. *J Am Chem Soc* **2019**, *141* (6), 2516–2525. <https://doi.org/10.1021/jacs.8b12240>.
7. Bornerie, M.; Brion, A.; Guichard, G.; Kichler, A.; Douat, C. Delivery of siRNA by Tailored Cell-Penetrating Urea-Based Foldamers. *Chem. Commun.* **2021**, *57* (12), 1458–1461. <https://doi.org/10.1039/D0CC06285E>.
8. Aisenbrey, C.; Douat, C.; Kichler, A.; Guichard, G.; Bechinger, B. Characterization of the DNA and Membrane Interactions of a Bioreducible Cell-Penetrating Foldamer in Its Monomeric and Dimeric Form. *J. Phys. Chem. B* **2020**, *124* (22), 4476–4486. <https://doi.org/10.1021/acs.jpcc.0c01853>.
9. André, C.; Veillard, F.; Wolff, P.; Lobstein, A.-M.; Compain, G.; Monsarrat, C.; Reichhart, J.-M.; Noûs, C.; Burnouf, D. Y.; Guichard, G.; Wagner, J. E. Antibacterial Activity of a Dual Peptide Targeting the *Escherichia Coli* Sliding Clamp and the Ribosome. *RSC Chem. Biol.* **2020**, *1* (3), 137–147. <https://doi.org/10.1039/DOCB00060D>.



**Dr. Emmanuelle THINON**  
Research scientist (CRCN), CNRS

Emmanuelle Thinon completed her PhD in Chemical Biology at Imperial College London in 2014, under the supervision of Prof. Ed Tate. For her postdoc, thanks to a Marie Skłodowska-Curie Global fellowship, she worked at the Rockefeller University (USA) with Prof. Howard Hang and The Francis Crick Institute (UK) with Dr. Sharon Tooze. In 2019, she was recruited as a research associate (chargé de recherche, CRCN) within the French National Centre for Scientific Research (CNRS), to join the CBMN (Institute of Chemistry & Biology of Membranes & Nanoobjects) in Bordeaux. In November 2019, she joined IECB as a group leader.

### Research team

**Dr. Emmanuelle THINON** Research scientist  
CRCN, (CNRS)

**Loris VERRON** Research Engineer (UB)

**Dr. Elena CESAR-RODO** Postdoc (UB)

This team is part of the unit "Chimie des membranes et des nanoobjets" (CBMN), CNRS UMR5248/INP/ Univ. Bordeaux

# Chemical Biology of membrane proteins

The study of small transmembrane proteins can often be challenging. In particular, it can be difficult to tag these proteins with a fluorophore in cellulo without perturbing the proteins localization and function, or to extract and purify them from membranes, without disturbing their structure or interactions with other proteins, for structure determination or for mass spectrometry interaction proteomics. Additionally, these proteins can be post-translationally modified by lipids, but proteomics methods to precisely identify and quantify some of these modifications are noticeably lacking. The "Chemical Biology of membrane proteins group" endeavors to develop and/or apply a combination of chemical and biological approaches to facilitate the study of these small membrane proteins.

The first aim of our work is to use a combination of chemical approaches (site-specific chemical labelling, crosslinking interaction proteomics, chemical proteomics, genetic code expansion, solid state NMR) to characterize small transmembrane proteins involved in viral infections. Some of these proteins are post-translationally modified by S-palmitoylation, which corresponds to the reversible addition of a C16 fatty acid to Cys, often adjacent to the protein transmembrane domain. S-palmitoylation is essential for protein localization and regulation, but its precise function, notably during viral infection, remains unknown for some proteins.

Using a combination of methods, we are currently characterising a small protein involved in viral infections by studying the role and regulation of its S-palmitoylation, its structure in interaction with membranes and by identifying its interaction partners. These studies will help us to understand if this protein could be new antiviral drug target.

The second aim of our work is to develop new methods to tag proteins at the endogenous level (no overexpression). The addition of a tag to a protein, such as GFP (Green Fluorescence Protein) for immunofluorescence studies, can sometimes perturb the protein biophysical properties, localization and/or function. The tag is often added by overexpressing the protein of interest, which can sometimes lead to toxicity, hence the development of new methods to tag proteins at the endogenous level is essential. The tag could be a small fluorophore (BODIPY etc) for live cell imaging studies or a crosslinking moiety to enable the identification of the interactome of the protein of interest by mass spectrometry-based proteomics. These new methods will be applied to facilitate the study of small membrane proteins.

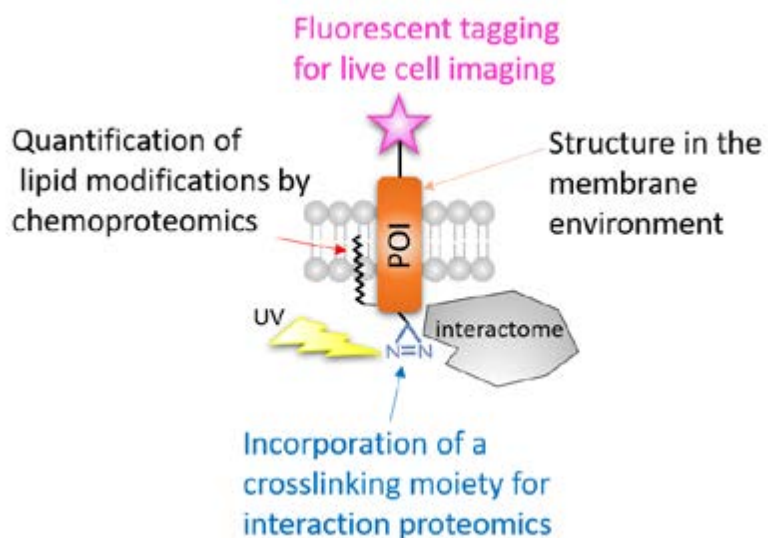


Figure 1. Characterization of small membrane proteins using a combination of chemical and biological methods.

### Selected publications

1. Spence, J. S.; He, R.; Hoffmann, H.-H.; Das, T.; Thinon, E.; Rice, C. M.; Peng, T.; Chandran, K.; Hang, H. C. IFITM3 Directly Engages and Shuttles Incoming Virus Particles to Lysosomes. *Nat Chem Biol* **2019**, 15 (3), 259-268. <https://doi.org/10.1038/s41589-018-0213-2>.
2. Thinon, E.; Hang, H. C. Chemical Proteomic Analysis of S-Fatty Acylated Proteins and Their Modification Sites. *Methods Mol Biol* **2019**, 2009, 45-57. [https://doi.org/10.1007/978-1-4939-9532-5\\_4](https://doi.org/10.1007/978-1-4939-9532-5_4).
3. Tapodi, A.; Clemens, D. M.; Uwineza, A.; Jarrin, M.; Goldberg, M. W.; Thinon, E.; Heal, W. P.; Tate, E. W.; Nemeth-Cahalan, K.; Vorontsova, I.; Hall, J. E.; Quinlan, R. A. BFSP1 C-Terminal Domains Released by Post-Translational Processing Events Can Alter Significantly the Calcium Regulation of AQP0 Water Permeability. *Exp Eye Res* **2019**, 185, 107585. <https://doi.org/10.1016/j.exer.2019.02.001>.



**Dr. Mikayel AZNAURYAN,**  
Research Associate (CRCN), Inserm

Mikayel Aznauryan obtained his PhD from Yerevan State University in Armenia (2008–2011). Then, he moved to Switzerland (2012–2014) as a postdoctoral researcher at the Department of Biochemistry of the University of Zurich (Group of Prof. B. Schuler), to work on protein folding and dynamics with single-molecule FRET spectroscopy. Afterwards, Mikayel Aznauryan did a second postdoc (2014–2018) at the Department of Chemistry and the Interdisciplinary Nanoscience Center of Aarhus University in Denmark, where he used single-molecule methods to study the dynamics of nucleic acid structures. Mikayel Aznauryan has joined IECB at the end of 2018 and has been awarded FRM Jeune equipe and IdEx Chaire Junior grants to start his group. Shortly after, he obtained INSERM researcher position (CRCN) within ARNA laboratory.

## Research team

**Dr. Mikayel AZNAURYAN** Research associate  
CRCN (Inserm)

**Dr. Carmelo DI PRIMO** Research associate  
CRHC (Inserm)

**Mme. Sabrina ROUSSEAU** Engineer, IE (Inserm)

**Dr. Laurent FERNANDEZ** Postdoc (Inserm)

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This team is part of the unit “Acides Nucléiques: Régulations Naturelles et Artificielles” (ARNA), Inserm U1212/CNRS UMR5320/Univ. Bordeaux

# Single-molecule Biophysics

Intrinsically disordered proteins (IDPs) or proteins containing intrinsically disordered regions (IDRs) are ubiquitous in eukaryotic proteome. They typically lack persistent structure in their native form and do not necessarily require defined structure for molecular recognition and specific function. They are frequently involved in liquid-liquid phase separation (LLPS) driven assembly of various cellular condensates. We use a large variety of biochemical and biophysical tools, among which particularly single-molecule FRET spectroscopy, in order to understand the mechanisms of IDP interactions and to reveal the basis of specificity for molecular recognition and particular function of IDPs, as well as uncover how certain IDP interactions define and modulate the LLPS and formation of cellular condensates.

The key expertise of the group is centered on in vitro and in-cell single-molecule FRET spectroscopy, which is our main tool to look at IDPs and their interaction. For this purpose, we also use a variety of other state-of-the-art biochemical and biophysical techniques, such as biomolecular nuclear magnetic resonance (NMR) spectroscopy, surface plasmon resonance (SPR), isothermal titration calorimetry (ITC), live-cell imaging and others, to obtain a comprehensive picture of mechanisms of IDP interactions and to reveal the basis of specificity for molecular recognition and particular function of IDPs.

Currently, in the group we are working on the following research projects:

- investigation of eukaryotic translation initiation and especially the role of disordered translation initiation factors in this process;
- understanding the molecular mechanisms of function of disordered RNA-binding proteins in LLPS and cellular condensates;
- measuring biomolecular binding kinetics, including ternary complexes, fragile targets, strong non-specific binding, thanks to original methodological developments in SPR.

From detection of single biomolecules to characterization of their conformational distributions, fluctuations and dynamics

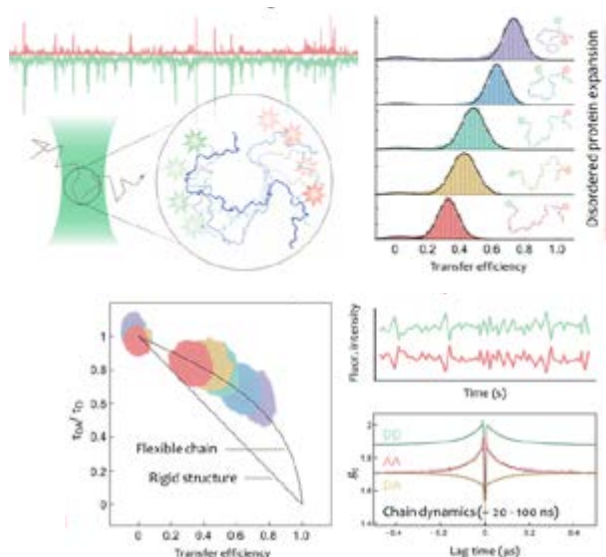


Figure 1. From detection of single biomolecules to characterization of their conformational distributions, fluctuations and dynamics

### Disordered initiation factors – fine-tuners of the early steps of translation initiation

Translation initiation is directly regulated by the cap-binding complex (eIF4F), which recognizes and associates with the 5' terminal cap of mRNA and prepares it for the recruitment of the ribosome. eIF4F consists of three different proteins, so called translation initiation factors eIF4E, eIF4G and eIF4A. eIF4E is responsible for direct recognition of mRNA methyl-guanosine cap. eIF4A is an ATP-driven RNA helicase that unwinds secondary structures surrounding the 5'-end of mRNA. eIF4G serves as a scaffold for assembly of eIF4F, directly interacting with both eIF4E and eIF4A (Jackson, Hellen et al. 2010). In addition, eIF4F complex associates with eIF4B and eIF4H, which possess several crucial regulatory roles in translation initiation (Parsyan 2014). In a concerted action, these factors prepare a "landing pad" and facilitate the recruitment of the 43S pre-initiation complex and scanning the mRNA towards the start-codon recognition and ribosome assembly.

Despite the large number of binding partners (initiation factors, mRNA, rRNA) and important functionality *in vivo*, eIF4B (and eIF4H) are predicted to be predominantly disordered (Uversky 2014) and can be termed as disordered translation initiation factors (DisIFs). We focus on investigation of these proteins, and aim at understanding their behavior, interactions and mechanistic basis for function in translation initiation.

### Disordered initiation factors and their relation to LLPS and stress granules

It is currently emerging that many aspects of intracellular organization are performed through formation of membrane-less condensates (Banani, Lee et al. 2017). The main driving force for condensate assembly is believed to be the intracellular LLPS (Shin and Brangwynne 2017). Currently many independent observations point that IDPs or proteins with long IDRs are key drivers of cellular LLPS (Dignon, Best et al. 2020).

Cellular condensates form under action of various stimuli. For example, stress granules (SGs) form during cellular stress and are responsible for subcellular storage of stress-inhibited translation machinery and stabilization of naked mRNAs (Protter and Parker 2016).

Recent evidences indicate an enrichment of translation initiation factors, among which DisIFs (eIF4B, eIF4H, eIF4G (contains several IDRs)), within the proteome of SGs (You, Huang et al. 2020), whereas their exact roles therein are currently unknown. We primarily focus on understanding of the behavior and function of DisIFs in LLPS and assembly of SGs. On the other hand, we aim to expand the current understanding of the general laws determining IDP-driven condensate formation and underlying specificity and selectivity mechanisms.

### Selected publications

1. Hellenkamp, B.; Schmid, S.; Doroshenko, O.; Opanasyuk, O.; Kühnemuth, R.; Rezaei Adariani, S.; Ambrose, B.; Aznauryan, M.; Barth, A.; Birkedal, V.; Bowen, M. E.; Chen, H.; Cordes, T.; Eilert, T.; Fijen, C.; Gebhardt, C.; Götz, M.; Gouridis, G.; Gratton, E.; Ha, T.; Hao, P.; Hanke, C. A.; Hartmann, A.; Hendrix, J.; Hildebrandt, L. L.; Hirschfeld, V.; Hohlbein, J.; Hua, B.; Hübner, C. G.; Kallis, E.; Kapanidis, A. N.; Kim, J.-Y.; Krainer, G.; Lamb, D. C.; Lee, N. K.; Lemke, E. A.; Levesque, B.; Levitus, M.; McCann, J. J.; Naredi-Rainer, N.; Nettels, D.; Ngo, T.; Qiu, R.; Robb, N. C.; Röcker, C.; Sanabria, H.; Schlierf, M.; Schröder, T.; Schuler, B.; Seidel, H.; Streit, L.; Thurn, J.; Tinnefeld, P.; Tyagi, S.; Vandenberk, N.; Vera, A. M.; Weninger, K. R.; Wünsch, B.; Yanez-Orozco, I. S.; Michaelis, J.; Seidel, C. A. M.; Craggs, T. D.; Hugel, T. Precision and Accuracy of Single-Molecule FRET Measurements—a Multi-Laboratory Benchmark Study. *Nat Methods* **2018**, 15 (9), 669–676. <https://doi.org/10.1038/s41592-018-0085-0>.
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**Dr. Valérie Gabelica**  
 Research Director (DR2), INSERM

Valérie Gabelica studied Chemistry and obtained her PhD in Sciences in 2002 at the University of Liège. After a postdoc in Frankfurt as Humboldt fellow, she rejoined the Mass Spectrometry Laboratory in Liège where she obtained a permanent position as FNRS research associate in October 2005. She joined the IECB in 2013 with the support of an Atip-Avenir grant, and became an Inserm research director (DR2) in December 2013. She obtained an ERC Consolidator grant in 2014. Her main research interests are fundamental aspects of mass spectrometry and its application to non-covalent complexes in general and nucleic acid complexes in particular, with research themes spanning from physical chemistry to biophysics and structural chemistry and biology.

### Research team

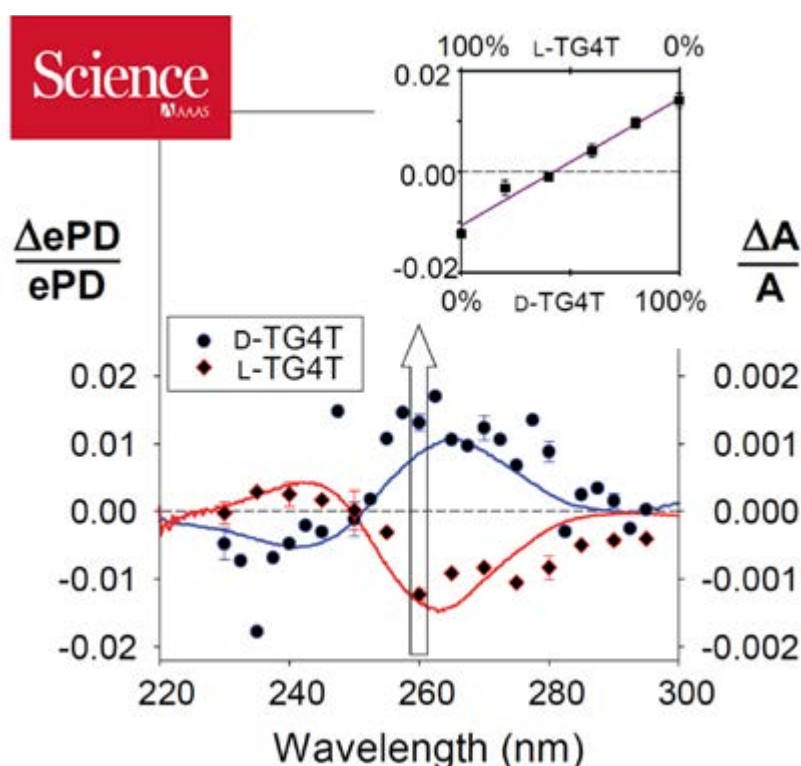
**Dr. Valérie GABELICA** Research Director  
 DR2 (INSERM)  
**Dr. Eric LARGY** Maître de Conférences  
 (Univ. Bordeaux)  
**Dr. Steven DALY** Post-doc (INSERM)  
**Dr. Anirban GHOSH** Post-doc (INSERM)  
**Dr. Nina KHRISTENKO** Post-doc (INSERM)  
**Dr. Sanae BENABOU ZDAOU** IdEX post-doc  
 (Univ. Bordeaux)  
**Dr. Debasmita GHOSH** Post-doc (INSERM)  
**Alexander KÖNIG** PhD student  
 (Univ. Bordeaux)  
**Matthieu RANZ** M1 student  
 (ENSCBP Bordeaux)

This team is part of the unit "Acides Nucléiques: Régulations Naturelles et Artificielles" (ARNA), Inserm U1212/CNRS UMR5320/Univ. Bordeaux

# Mass Spectrometry of Nucleic Acids & Supramolecular Complexes

Our team focuses on the measurement sciences applied to non-covalent interactions. Nucleic acids (and more recently, foldamers and protein therapeutics) are our model systems and systems on which we learn new things. Our aim is to decipher the relationships between structures and energetics—Angstroms and Calories—in non-covalent complexes. Non-covalent interactions govern the structure and function of myriads of systems, from supramolecular assemblies to biological complexes. High-resolution structural methods help to understand what interactions are at stake in specific states of well-defined assemblies. Yet function is linked to energetics: How prevalent is a structural form? How does it switch to other states? How fast? To bridge the gap between structure and energetics, our team develops new mass spectrometry approaches to separate, quantify, and structurally characterize the different ensembles of structures (the different states) simultaneously present in solution.

In mass spectrometry, our team contributes to establish more solid ground to interpret ion mobility spectrometry experiments in terms of structure. Unexpected results have led us to challenge the paradigm claiming that non-covalent interactions are always well maintained in the gas phase. It turns out that this assertion was based on a bias favoring the publication of positive results. We highlighted the importance of structural changes related to the electrospray ionization process (*Khristenko et al.*, JASMS 2019). The dissemination of these research results at numerous international conferences, as well as the publication of recommendations (Gabelica et al., *Mass Spectrom. Rev.* 2019), has had an impact on many developers and users of these methodologies, both academic and industrial.



Another highlight is the first ever circular dichroism spectra of biomolecules trapped in a mass spectrometer. This is a totally new way to characterize chirality using mass spectrometry. This work has been published in *Science* (2020). We used mass spectrometry to isolate ions of guanine-rich DNA sequences that form G-quadruplexes, then irradiate the DNA with laser pulses of given wavelength, polarization, and energy. The laser light causes electrons to detach from the DNA ions, a process that changes the ions' charge state. We switch between different polarizations of light and a CD spectrum from difference in electron photodetachment as a function of the polarization and wavelength. The shapes of gas-phase CD spectra of various DNA structures were similar to conventional solution-phase CD spectra of the same structures, which suggests that the gas- and solution-phase structures have similar base-stacking patterns. Using the new method, we could distinguish between antiparallel and hybrid conformations of the same DNA sequence. The new approach can be extended to study proteins and protein aggregates, for example to determine the  $\alpha$ -helix/ $\beta$ -sheet fraction in distinct oligomer stoichiometries, but such use will require overcoming challenges such as signal-to-noise issues.

In the field of nucleic acid biophysics, our team demonstrated the feasibility of probing nucleic acid structures by in-solution hydrogen/deuterium exchange mass spectrometry (*Largy & Gabelica, Anal. Chem. 2020*). This approach has a bright future to help us obtain dynamical insight into nucleic acid complexes, through the quantitative analysis of the exchange rates. Besides, we published a comprehensive database of G-quadruplexes for native mass spectrometry in potassium (*Ghosh et al., NAR 2021*), wherein we thoroughly document the solution topology of G-quadruplexes in MS-compatible conditions.

## Selected publications

1. Gabelica, V.; Shvartsburg, A. A.; Afonso, C.; Barran, P.; Benesch, J. L. P.; Bleiholder, C.; Bowers, M. T.; Bilbao, A.; Bush, M. F.; Campbell, J. L.; Campuzano, I. D. G.; Causon, T.; Clowers, B. H.; Creaser, C. S.; De Pauw, E.; Far, J.; Fernandez-Lima, F.; Fjeldsted, J. C.; Giles, K.; Groessl, M.; Hogan, C. J.; Hann, S.; Kim, H. I.; Kurulugama, R. T.; May, J. C.; McLean, J. A.; Pagel, K.; Richardson, K.; Ridgeway, M. E.; Rosu, F.; Sobott, F.; Thalassinou, K.; Valentine, S. J.; Wyttenbach, T. Recommendations for Reporting Ion Mobility Mass Spectrometry Measurements. *Mass Spectrom Rev* **2019**, 38 (3), 291–320. <https://doi.org/10.1002/mas.21585>.
2. Khristenko, N.; Amato, J.; Livet, S.; Pagano, B.; Randazzo, A.; Gabelica, V. Native Ion Mobility Mass Spectrometry: When Gas-Phase Ion Structures Depend on the Electrospray Charging Process. *J Am Soc Mass Spectrom* **2019**, 30 (6), 1069–1081. <https://doi.org/10.1007/s13361-019-02152-3>.
3. Largy, E.; Gabelica, V. Native Hydrogen/Deuterium Exchange Mass Spectrometry of Structured DNA Oligonucleotides. *Anal Chem* **2020**, 92 (6), 4402–4410. <https://doi.org/10.1021/acs.analchem.9b05298>.
4. Daly, S.; Rosu, F.; Gabelica, V. Mass-Resolved Electronic Circular Dichroism Ion Spectroscopy. *Science* **2020**, 368 (6498), 1465–1468. <https://doi.org/10.1126/science.abb1822>.
5. Ghosh, A.; Largy, E.; Gabelica, V. DNA G-Quadruplexes for Native Mass Spectrometry in Potassium: A Database of Validated Structures in Electrospray-Compatible Conditions. *Nucleic Acids Res* **2021**, 49 (4), 2333–2345. <https://doi.org/10.1093/nar/gkab039>.



**Dr. Antoine Loquet**  
Research Director (DR), CNRS

Antoine Loquet graduated from the University of Lyon / Ecole Normale Supérieure de Lyon. He did his PhD (2006–2009) under the guidance of Anja Böckmann (IBCP Lyon), working on the development of Solid-State NMR to solve protein structures. In 2008 he joined the group of Beat Meier (ETH Zürich) to study prion fibrils by Solid-State NMR. He then focused his research on molecular assemblies by Solid-State NMR as an EMBO postdoctoral fellow with Adam Lange at the Max Planck Institute for Biophysical Chemistry (Göttingen, Germany). There, he developed Solid-State NMR methods to determine atomic structures of large biological supramolecular assemblies. He obtained a CNRS position in 2013 at the CBMN (Institute of Chemistry & Biology of Membranes & Nanoobjects) in Bordeaux. In 2014, he was recruited as a group leader at the IECB and since 2016, he is leading the group “NMR of Membranes and Protein Assemblies” at CBMN. His current research concentrates on the structural investigation of molecular assemblies using Solid-State NMR. He obtained an ERC Starting Grant in 2015. He is CNRS Research Director since 2020.

## Research team

**Antoine LOQUET** Research Director, DR (CNRS)  
**Dr. Erick DUFOURC** Emeritus Research Director, (CNRS)  
**Dr. Corinne SANCHEZ** Assistant Professor, McF (Univ. Bordeaux)  
**Dr. Yann FICHO** Researcher, CRCN (CNRS)  
**Axelle GRÉLARD** Research Engineer, IR CNRS  
**Mélanie BERBON** Engineer, IE (CNRS)  
**Dr. Birgit HABENSTEIN** Researcher, CRCN (CNRS)  
**Alix REYNAUD** Engineer (CNRS)  
**Dr. Arpita TAWANI** Postdoc (CNRS)  
**Dr. Alons LENDS** Postdoc (CNRS)  
**Coralie ROBERT** PhD (CNRS – Univ. Bordeaux)  
**Bilal MUHAMMED** PhD (CNRS)

This team is part of the unit “CBMN”, UMR5248 CNRS – U. Bordeaux – Bordeaux INP

# Solid-state NMR of Molecular Assemblies

Self-assembly is a fundamental process by which individual subunits assemble into ordered macromolecular entities, such as filaments, fibrils, oligomers, tubes or nanomachines. In biology, protein assemblies are involved in crucial cellular processes, ranging from the propagation of neurological disorders to viral and bacterial infections. The group aims at investigating atomic structures, and assembly processes of such sophisticated assemblies. We develop and apply solid-state NMR to capture structural and dynamic details at the atomic scale. Our group is also involved in the production of large protein assemblies to solve their structures based on solid-state NMR methods. Molecular assemblies either involved in cellular processes or engineered by supramolecular chemistry constitute the current research activities.

## Structural study of amyotrophic lateral sclerosis-associated TDP-43:

We use a combination of electron microscopy, X-ray fiber diffraction, Fourier-transform infrared spectroscopy analysis, and solid-state NMR spectroscopy to investigate the molecular organization of TDP-43 amyloid aggregates.

## Development of solid-state NMR methods based on cryoMAS probe:

In collaboration with Bruker and other pilot laboratories, we present the proof-of-concept of protein studies by a prototype probe operating at cryogenic temperatures for the sample coil and the electronics.

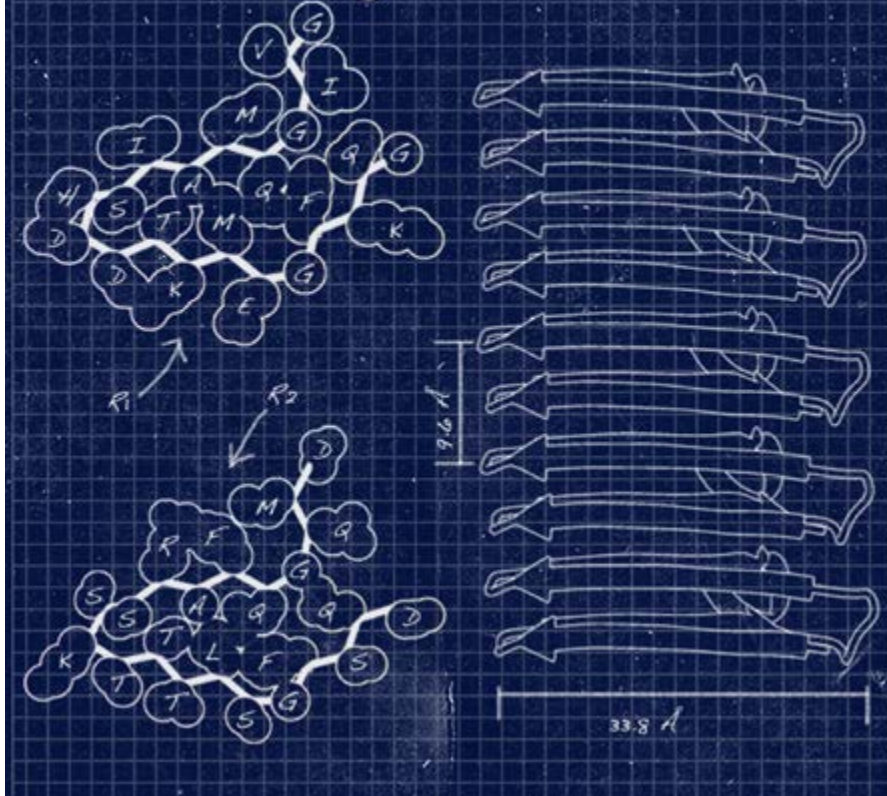
## Structural characterization of $\alpha$ -synuclein polymorphs:

We set up a robust methodology to investigate the molecular conformation of  $\alpha$ -synuclein amyloid polymorphs by fast magic-angle spinning NMR.

## Structural and molecular basis of amyloid-amyloid cross-seeding:

We show that virtually identical amyloid backbone structures might not be sufficient for cross-seeding and that critical side-chain positions could determine the seeding specificity of an amyloid fold. Our work redefines the conceptual boundaries of prion strain and sheds light on key molecular features concerning an important class of pathogenic agents.

## Molecular basis of amyloid cross-seeding



## Selected publications

- Shenoy, J.; El Mammeri, N.; Dutour, A.; Berbon, M.; Saad, A.; Lends, A.; Morvan, E.; Grélard, A.; Lecomte, S.; Kauffmann, B.; Theillet, F.; Habenstein, B.; Loquet, A. Structural Dissection of Amyloid Aggregates of TDP-43 and Its C-terminal Fragments TDP-35 and TDP-16. *FEBS J* **2020**, *287* (12), 2449–2467. <https://doi.org/10.1111/febs.15159>.
- Daskalov, A.; Martinez, D.; Coustou, V.; El Mammeri, N.; Berbon, M.; Andreas, L. B.; Bardiaux, B.; Stanek, J.; Noubhani, A.; Kauffmann, B.; Wall, J. S.; Pintacuda, G.; Saupe, S. J.; Habenstein, B.; Loquet, A. Structural and Molecular Basis of Cross-Seeding Barriers in Amyloids. *Proc Natl Acad Sci USA* **2021**, *118* (1), e2014085118. <https://doi.org/10.1073/pnas.2014085118>.
- De Giorgi, F.; Laferrière, F.; Zinghirino, F.; Faggiani, E.; Lends, A.; Bertoni, M.; Yu, X.; Grélard, A.; Morvan, E.; Habenstein, B.; Dutheil, N.; Doudnikoff, E.; Daniel, J.; Claverol, S.; Qin, C.; Loquet, A.; Bezar, E.; Ichas, F. Novel Self-Replicating  $\alpha$ -Synuclein Polymorphs That Escape ThT Monitoring Can Spontaneously Emerge and Acutely Spread in Neurons. *Sci. Adv.* **2020**, *6* (40), eabc4364. <https://doi.org/10.1126/sciadv.abc4364>.
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- Latxague, L.; Benizri, S.; Gaubert, A.; Tolchard, J.; Martinez, D.; Morvan, E.; Grélard, A.; Saad, A.; Habenstein, B.; Loquet, A.; Barthélémy, P. Bolaamphiphile-Based Supramolecular Gels with Drugs Eliciting Membrane Effects. *Journal of Colloid and Interface Science* **2021**, *594*, 857–863. <https://doi.org/10.1016/j.jcis.2021.03.026>.



**Dr. Derek McCusker**

Research Director (DR2), CNRS

Derek McCusker studied Immunology at Glasgow University and focused on the role of the proteasome in immunity in Prof. John Trowsdale's lab at Cancer Research UK for his thesis. During postdoctoral work with Dr Robert Arkowitz at the Laboratory of Molecular Biology in Cambridge he became interested in the control of cell growth. He then joined Prof. Douglas Kellogg's group at the University of California, Santa Cruz, where he investigated how cells coordinate cell growth and cell division. He was recruited by CNRS in 2009 and joined IECB as a group leader. Here, he obtained his habilitation in 2013 from the University of Bordeaux and since 2017 he has been a Director of Research with the CNRS. The group uses interdisciplinary approaches to study the dynamics of cell growth during the cell cycle.

### Research team

**Dr. Derek McCusker** Research Director DR2 (CNRS)

**Landry PEYRAN** PhD student (Univ. Bordeaux)

**Aurelie MASSONI-LAPORTE** Assistant Engineer (CNRS)

This team is part of the unit "Institut de Biochimie et Genetiques Cellulaire" (IBGC), CNRS UMR5095/Univ. Bordeaux.

# Dynamics of Cell Growth & Division

Cells grow, duplicate their genome and divide via a series of events collectively termed the cell cycle. Coordination between the cell cycle machinery and proteins that regulate cell growth ensure the fidelity of cell division; however, the underlying mechanisms are unclear. In humans, failure of these control mechanisms has been directly linked to tumour formation. The goal of the Cell Growth and Division Laboratory is to understand how cell growth is controlled and how growth is coordinated with cell cycle progression in the model eukaryote *Saccharomyces cerevisiae*. These fundamental questions are addressed using cutting-edge interdisciplinary approaches.

## 1. Cellular Self-organization – generating order from the abyss.

Living matter can be distinguished from inanimate matter such as liquids and gases by virtue of the propensity of living systems to self-organize. This entails the use of energy dissipation to drive multiple components within living cells far from thermodynamic equilibrium (e.g. polymers of tubulin or actin). By coupling polymerization/depolymerization cycles to regulatory components, living cells are endowed with remarkable and unique properties: the ability to grow, move and self-replicate. Some signaling components within cells that display characteristics of self-organization are becoming amenable to purification and reconstitution, and thus a more thorough understanding. Examples of self-organized processes within cells and the regulatory mechanisms controlling them are discussed in this review. This invited Perspective was published in MBoC in 2020.

## 2. Phosphatidylserine and GTPase activation control Cdc42 nanoclustering to counter dissipative diffusion.

All cells establish a single polarity axis that enables chromosomes to be equally partitioned into the mother and daughter cell at the end of each cell cycle. Defects in the establishment of a single polarity axis are directly linked to tumorigenesis. Cdc42 is an essential, conserved polarity regulator in all eukaryotes examined. In budding yeast, Cdc42 localization defines the cell's polarity axis by determining where the cell will grow and divide during the cell cycle. The mechanisms ensuring that Cdc42 concentrates at a single site to define the cell's polarity axis are incompletely understood. Here, we used high-density single protein tracking combined with photoactivation localization microscopy (sptPALM) to monitor Cdc42 dynamics and organization at single molecule resolution in budding yeast (Figure 1). We found that the mobility of Cdc42 was reduced at the pole of the cell compared with other regions of the membrane. This is important, since reduced mobility would stabilize the protein at the pole, helping to concentrate it there and establish a robust polarity axis. We found that Cdc42 is organized in very small "nanoclusters" and that these clusters are larger at the cell pole than elsewhere on the plasma membrane (Figure 1, inset). Two factors were identified that control Cdc42 mobility and promote its nanoclustering: the activation of the GTPase and a specific lipid called phosphatidylserine that is enriched at the cell pole. Phosphatidylserine appears to promote Cdc42 nanoclustering via a scaffold protein called Bem1 that interacts with Cdc42, and that we previously demonstrated boosts the activation of Cdc42. These studies reveal how the mobility of a Rho GTPase is controlled to counter the depletive effects of diffusion, thus stabilizing Cdc42 on the plasma membrane and sustaining cell polarity. This work was published in MBoC in 2018.

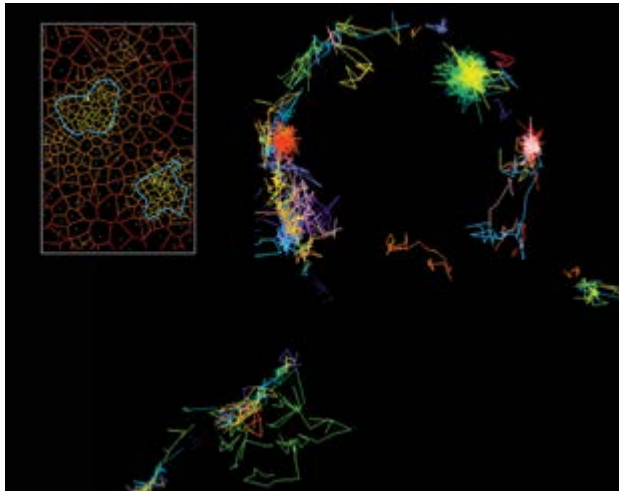


Figure 1. Single molecule imaging of Cdc42 in budding yeast. On the right, each track displays a single molecule localization. Note how some Cdc42 molecules are highly confined (green, pink and orange) while others are free to diffuse. Inset displays single molecule localizations in white and Cdc42 nanoclusters in blue.

### 3. Avidity-driven polarity axis establishment via multivalent lipid-GTPase module interactions.

While Rho GTPases are indispensable regulators of cellular polarity, the mechanisms underlying their anisotropic activation at membranes have been elusive. Using the budding yeast Cdc42 GTPase module, which includes a Guanine nucleotide Exchange Factor (GEF) Cdc24 and the scaffold Bem1, we found that avidity generated via multivalent anionic lipid interactions is a critical mechanistic constituent of polarity establishment. We identified basic cluster (BC) motifs in Bem1 that drive the interaction of the scaffold-GEF complex with anionic lipids including phosphatidylserine at the cell pole. This interaction appears to influence lipid acyl chain ordering, thus regulating membrane rigidity and feedback between Cdc42 and the local membrane environment. Sequential mutation of the Bem1 BC motifs, PX domain and the PH domain of Cdc24 led to a progressive loss of cellular polarity stemming from defective Cdc42 nanoclustering on the plasma membrane and perturbed GTPase signaling (Figure 2). Our work demonstrates the importance of avidity via multivalent anionic lipid interactions in the spatial control of GTPase activation. Dr. Julien Meca, a PhD student in the lab, was awarded the 2019 Monique Garnier-Semancik prize for the best Life Science thesis by the Univ. of Bordeaux for his contribution to this work. The study was a collaboration between the McCusker and Loquet teams at IECB. The study was also selected as being of outstanding interest to the field by a 2020 review in Current Opinion in Cell Biology (together with the Rapali et al paper published by the team in eLife in 2017).

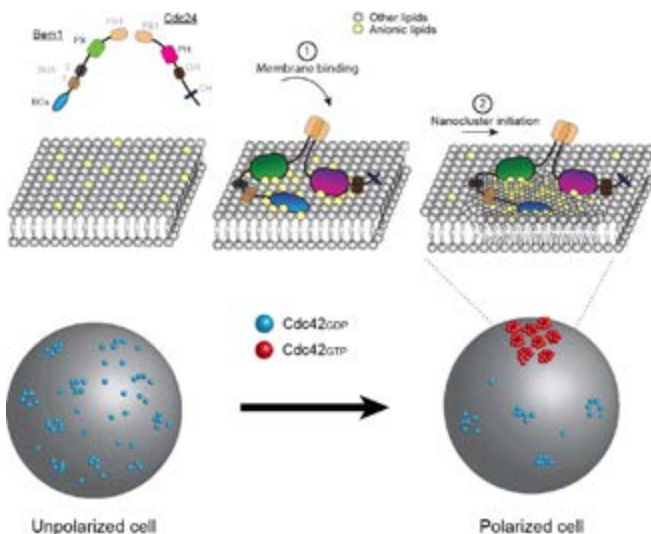


Figure 2. Schematic illustrating the relationship between Cdc42 regulators and the membrane environment during the establishment of a polarity axis. 1) The Bem1-Cdc24 complex is recruited to the plasma membrane via multivalent interactions with anionic lipids such as phosphatidylserine. The BC motifs in Bem1 provide the strongest affinity for anionic lipids at this step. 2) Upon their recruitment to anionic lipids, the Bem1 BC motifs may influence the local membrane environment, contributing to local Cdc42 activation by Cdc24 and Cdc42 nanoclustering.

### Selected publications

1. Rapali, P.; Mitteau, R.; Braun, C.; Massoni-Laporte, A.; Ünlü, C.; Bataille, L.; Arramon, F. S.; Gygi, S. P.; McCusker, D. Scaffold-Mediated Gating of Cdc42 Signalling Flux. *Elife* **2017**, 6. <https://doi.org/10.7554/eLife.25257>.
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3. McCusker, D. Cellular Self-Organization: Generating Order from the Abyss. *Mol Biol Cell* **2020**, 31 (3), 143–148. <https://doi.org/10.1091/mbc.E19-04-0207>.
4. Meca, J.; Massoni-Laporte, A.; Martinez, D.; Sartorel, E.; Loquet, A.; Habenstein, B.; McCusker, D. Avidity-Driven Polarity Establishment via Multivalent Lipid-GTPase Module Interactions. *EMBO J* **2019**, 38 (3). <https://doi.org/10.15252/emj.201899652>.



**Dr. Anne Royou**  
Research Director (DR2), CNRS

Following a bachelor degree in physiology and cell biology, Anne Royou did a postgraduate degree in molecular and cellular genetics at the Université Paris XI. She did her PhD thesis under the guidance of Dr. Roger Karess, at the Centre de Génétique Moléculaire in Gif-sur-Yvette, studying the role of non-muscle myosin II during development in *Drosophila*. Following her PhD, she joined Dr. William Sullivan's lab at the University of California, Santa Cruz, as a post-doctoral fellow. There, she became interested in the mechanisms that preserve genome integrity during cell division. She obtained a CNRS permanent position in 2009, an ATIP/Avenir grant in 2010 and was recruited as a team leader at IECB in 2011. In 2013 she was awarded an ERC starting grant. In 2016 she was promoted DR2 by the CNRS.

### Research team

**Dr. Anne ROYOU** Team Leader (CNRS)  
**Marie-Charlotte CLAVERIE** Assistant Engineer (Univ. Bordeaux)  
**Dr. Emilie MONTEBAULT** Researcher (CNRS)  
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# Control & Dynamics of Cell Division

The mechanisms that safeguard cells against aneuploidy are of great interest as aneuploidy contributes to tumorigenesis. Using live imaging approaches, we have identified two novel mechanisms that permit the accurate transmission of chromosomes during cell division. The first mechanism involves the faithful segregation of damaged chromosomes. Our studies reveal that chromosome fragments segregate properly to opposite poles. This poleward motion is mediated through DNA tethers that connect the chromosome fragments. The second mechanism involves the coordination of chromosome segregation with cell cleavage. We found that cells can adapt to trailing chromatids by elongating transiently during anaphase. This Myosin-mediated mechanism ensures the clearance of chromatids from the cleavage plane at the appropriate time during cytokinesis, thus preserving genome integrity.

Mitosis is the final stage of the cell cycle where a copy of the duplicated genome condensed into chromosomes is transmitted to each daughter cell. Failure to do so produces daughter cells with an inappropriate genome content, also called aneuploidy. The mechanisms that safeguard cells against aneuploidy are of great interest as aneuploidy contributes to tumorigenesis. Our group has identified two novel mechanisms that permit the accurate transmission of chromosomes during cell division. The first mechanism involves the faithful segregation of damaged chromosomes. The second mechanism coordinates chromosome segregation with cell cleavage.

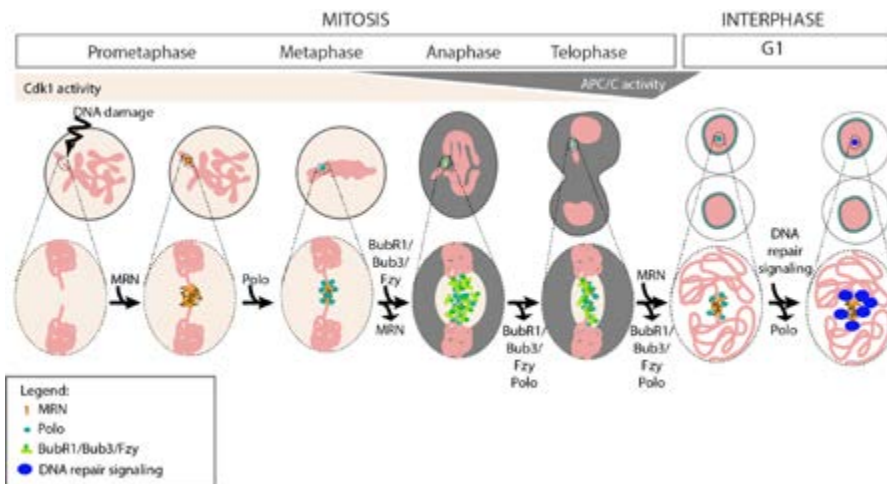
### Mechanism that permits faithful transmission of broken chromosomes

The presence of DNA damage, such as DNA double-strand breaks (DSB), triggers the activation of the DNA Damage Response, which delays the cell cycle and promotes DNA repair. While the DNA damage response is well documented in interphase, less is known about the response to DSB during mitosis. The presence of DSB during mitosis is particularly challenging for the cell as it produces broken chromosomes lacking a centromere. This situation can cause genomic instability due to improper segregation of the broken fragments into daughter cells. Our team has uncovered a process by which broken chromosomes are faithfully transmitted, via the tethering of the two broken chromosome ends. We demonstrate that the mitotic proteins Polo, BubR1 and Bub3 accumulate on DSB during mitosis and facilitate the proper segregation of the broken chromosome fragments. This requires the BubR1-mediated sequestration Fizzy, a co-factor of the E3 ubiquitin ligase Anaphase-promoting-complexe/cyclosome (APC/C) and the subsequent local inhibition of the APC/C at the site of damage.

The DSB sensor, Mre11-Rad50-Nbs1 complex, and Polo kinase are recruited to DNA lesions during mitosis. However, their mechanism of recruitment is elusive. Using live-cell imaging combined with the micro-irradiation of single chromosomes, we analysed the dynamics of Polo and Mre11 at DNA lesions during mitosis. The two proteins display distinct kinetics. While Polo kinetics at DSBs are Cdk1-driven, Mre11 promptly but briefly associates with DSBs regardless of the phase of mitosis and re-associates with DSBs in the proceeding interphase. Mechanistically, Polo kinase activity is required for its own recruitment and that of the mitotic proteins BubR1 and Bub3 to DSBs. Moreover, depletion of Rad50 severely impaired Polo kinetics at mitotic DSBs. Conversely, ectopic tethering of Mre11 to chromatin is sufficient to recruit Polo. Our study highlights a novel pathway that links the DSB sensor MRN complex and Polo kinase to initiate a prompt, decisive response to the presence of DNA damage during mitosis.

## Selected publications

1. Landmann, C.; Pierre-Elies, P.; Goutte-Gattat, D.; Montebault, E.; Claverie, M.-C.; Royou, A. The Mre11–Rad50–Nbs1 Complex Mediates the Robust Recruitment of Polo to DNA Lesions during Mitosis. *Journal of Cell Science* 2020, jcs.244442. <https://doi.org/10.1242/jcs.244442>.



**Figure 1 | Model for the DNA damage response in mitosis**  
 After DNA damage, the MRN complex is promptly but transiently recruited to the site of damage and targets Polo. Polo subsequently promotes the recruitment of the BubR1/Bub3 complex, which sequesters Fzy, the co-factor of the Anaphase Promoting Complex/Cyclosome (APC/C) at the site of DNA lesions. At anaphase, the Polo/BubR1/Bub3-dependent local inhibition of the APC/C is critical for tethering the two broken chromosome fragments during poleward movement of sister chromatids. At telophase, after the broken fragments have properly segregated the BubR1/Bub3 complex dissociate completely from the DNA lesions, while a pool of Polo remains associated with DNA breaks into the next interphase. Meanwhile, MRN re-accumulates at the site of damage, promoting the activation of downstream DNA repair components.

### Mechanism that coordinates chromosome segregation with cell cleavage

Chromosome segregation must be coordinated with cell division to ensure proper transmission of the genetic material into daughter cells. Our group identified a novel mechanism by which *Drosophila* neuronal stem cells coordinate chromosome segregation with cell division. Cells adapt to the presence of trailing chromatids at the site of division by transiently, but dramatically, elongating during anaphase, thus facilitating the clearance of the trailing chromatids from the cleavage plane. This adaptive elongation depends on myosin activity and the Rho Guanine-nucleotide exchange factor, Pebble. Cells promote the clearance of trailing chromatids from the cleavage site by undergoing two phases of adaptive elongation. The first phase relies on assembly of a wide contractile ring at the onset of cytokinesis. The second phase requires outward flux of myosin from the ring toward the polar cortex during ring constriction. Myosin efflux is a novel feature of cytokinesis and its duration is coupled to nuclear envelope reassembly (NER) and the ensuing nuclear sequestration of Pebble. Trailing chromatids induce a delay in NER concomitant with a prolonged period of cortical myosin activity, thus providing forces for the second adaptive elongation. The cytoplasmic retention of Pebble is sufficient to prolong myosin efflux and promote elongation in the absence of trailing chromatids. We propose that the modulation of cortical myosin dynamics is part of the cellular response triggered by an anaphase checkpoint that delays NER when trailing chromatids are present at the midzone.



**Dr. David Santamaría**  
Group leader (DR2), INSERM

David Santamaría received his PhD from University Autónoma of Madrid (Spain) in 1999, under the guidance of Prof. Jorge B. Schwartzman, studying replication fork barriers. He then joined the laboratory of Prof. Ronald A. Laskey, (1999–2003) at the Wellcome/CRC Institute (Cambridge, UK) where he dealt with the initiation of DNA replication and its connection with cell cycle control. He returned to Spain (2003–2016) as a staff scientist in Prof. Mariano Barbacid group (CNIO, Madrid) where he used mouse genetics to conduct a comprehensive analysis of the Cyclin Dependent Kinase family and to identify therapeutic targets in lung adenocarcinoma. He joined the IECB in 2016 and obtained a DR2 INSERM position starting January 2018.

### Research team

**Dr. David SANTAMARIA** Research Director DR2 Inserm U1218/ (Univ. Bordeaux)  
**Dr. Marie-Julie NOKIN** Postdoc (Univ. Bordeaux)  
**Dr. Tra-Ly NGUYEN** Postdoc (Univ. Bordeaux)  
**Elodie DARBO** Ingénieur de Recherche en Bioinformatique Inserm U1218/(Univ. Bordeaux)  
**Sonia SAN JOSÉ** Assistante Ingénieur (Univ. Bordeaux)  
**Sergio DE HITTA** PhD student (Univ. Bordeaux)

This team is part of the unit Actions for onCogenesis understanding and Target Identification in Oncology) "ACTION", Inserm U1218/Univ. Bordeaux

# Novel Mediators in Lung Oncogenesis

We use mouse models to characterize new signalling pathways and oncogenic functions that govern the onset of lung adenocarcinoma (LUAD). We have a particular interest in the mechanisms that regulate the initiation, intensity and duration of the RAS-ERK signalling output. The activity of this pathway is an essential feature controlling tumour initiation, disease progression and drug resistance to several targeted agents. Our work has identified a key role of KRAS membrane dimerization/clusterization in this process. Our immediate goal is to understand the molecular basis underlying this feature and to functionally characterize yet unknown protein factors required to assemble a KRAS-dependent signalling platform on the inner plasma membrane. As a whole, this approach may identify novel therapeutic targets with low toxicity and potential clinical applicability in LUAD.

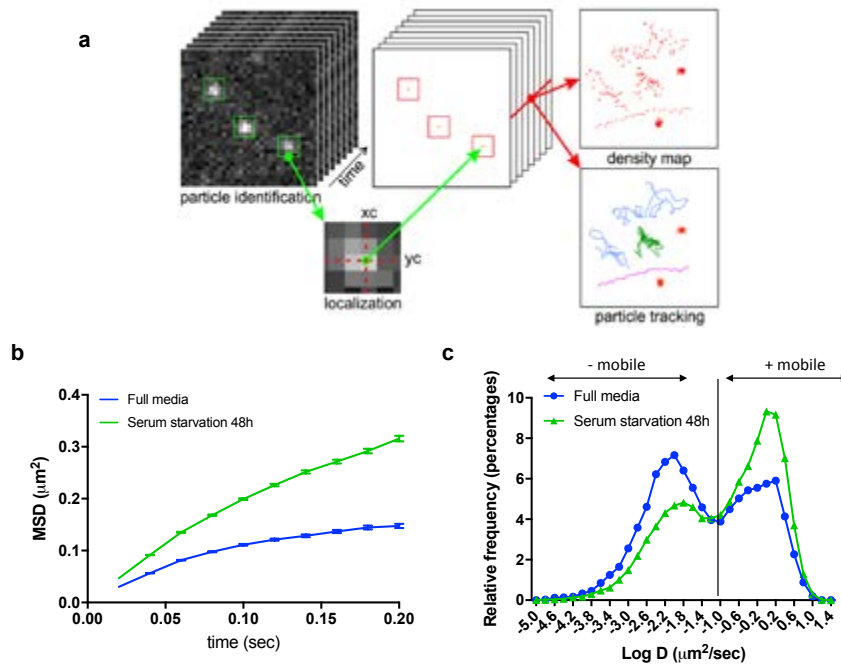
As an illustrative example of our still limited understanding of KRAS biology we have provided the first biological evidence suggesting that KRAS monomers on the cell membrane are functionally inert and that dimerization is an essential requirement for the activation of downstream signalling and the establishment of an oncogenic output. Mounting evidences indicate that KRAS dynamics at the inner cell membrane are an important regulatory node controlling its activity both in health and disease. These observations potentially identify KRAS dimerization as a novel therapeutic target for the treatment of KRAS driven cancers.

We are optimizing the conditions to identify and characterize structural co-factors that are required for the formation and/or stabilization of KRAS-dimers and the signalling clusters that depend on them. To this end we have started a collaboration with experts at the Bordeaux Imaging Center (BIC) to optimize FLIM/FRET and single-molecule localization microscopy approaches (SPT) to follow the stability and membrane trajectories of KRAS-containing complexes (Figure 1). Also, in collaboration with colleagues at the IECB Fred Friscourt and Emmanuelle Thinon we are using protein-specific localized crosslinking (based on amber suppression) to identify new membrane interactors of KRAS using cross-linking/mass spectrometry.

Also, in collaboration with Stephanie Cabantous (CRCT, Toulouse) we are using a flexible fluorescent cellular system compatible with high-throughput approaches that will be key to monitor and quantify KRAS dimerization in cells. This will be key not only to validate by gain & loss of function experiments the candidates identified in our crosslinking approach but also for the identification of compounds preventing KRAS dimerization.

Finally, we have used a genetic approach to demonstrate a quantitative relationship between the activity of RAS-ERK (MAPK pathway) signalling with cancer transformation and progression. It is also a key factor implicated in resistance to targeted therapies and disease relapse. We have developed a genetic cellular system that provides the right selective pressure to carry out an unbiased identification of novel regulators of the MAPK transcriptional output by Crispr/Cas9. Likewise, we are interrogating publicly available LUAD datasets to identify recurrent transcriptional changes that correlate with MAPK quantitative output and that may help identify novel regulators of the pathway. We hope this combined approach will contribute to elucidating new control mechanisms with cancer relevance. For instance, reactivation of MAPK signalling output is a key event in the onset of resistance to agents targeting this pathway in the clinic. We have a special interest in LUAD driven by BRAF activating mutations. These patients are treated with a combination of BRAF (dabrafenib) and MEK (trametinib) inhibitors.

Unfortunately, tumours inevitably relapse and there are no therapeutic alternatives for these patients. We are combining established cell lines as well as patient derived xenografts from paired biopsies pre- & post-treatment in collaboration with Sophie Cousin (Institut Bergonié, Bordeaux) and Ernest Nadal (Idibell, Barcelona, Spain). We are using these models to investigate potential vulnerabilities acquired during the onset of drug resistance.



**Figure 1:** a) Schematic representation of the SPT approach utilized to investigate KRAS membrane diffusion dynamics. Live cells are imaged every 20 ms and dedicated algorithms are utilized for the analysis of the serial images. This determines protein trajectories and dynamic parameters. b) Experimental data demonstrating a confined behavior of membrane-bound KRAS upon mitogenic (serum) stimulation. The MSD analysis indicates the area explored by each molecule suggesting that a higher fraction of immobile KRAS when activated. c) The diffusion coefficient confirms lower KRAS mobility in the inner membrane upon activation.

## Selected publications

1. Esteban-Burgos, L.; Wang, H.; Nieto, P.; Zheng, J.; Blanco-Aparicio, C.; Varela, C.; Gómez-López, G.; Fernández-García, F.; Sanclemente, M.; Guerra, C.; Drosten, M.; Galán, J.; Caleiras, E.; Martínez-Torrecedradora, J.; Fajas, L.; Peng, S.-B.; Santamaría, D.; Musteanu, M.; Barbacid, M. Tumor Regression and Resistance Mechanisms upon CDK4 and RAF1 Inactivation in KRAS/P53 Mutant Lung Adenocarcinomas. *Proc Natl Acad Sci USA* **2020**, *117* (39), 24415–24426. <https://doi.org/10.1073/pnas.2002520117>.
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### Pr. Léon Ghosez

Professor Emeritus UCL, Visiting Scientist IECB, Univ. Bordeaux

Léon Ghosez was born in Aalst, Belgium, in 1934. He studied at the University of Louvain where he got a PhD in 1958 under the supervision of Prof. G. Smets. He then spent 2 years as postdoctoral researcher at Harvard University (Prof. R.B. Woodward) and also collaborated for a few months with Prof. R. Huisgen in the Department of chemistry of the University of Munich. He got his "Habilitation" at the age of 32 for his independent work on the stereochemistry of synthesis and rearrangement of halocyclopropanes. In 1969 he became "Professeur Ordinaire" at the University of Louvain where he created the laboratory of organic synthesis. During his career in Louvain (1963–1999) he supervised the research of 125 PhD students and 135 postdoctoral associates. He also held appointments at the University of Liège (1969–1999) and the Ecole Polytechnique in Palaiseau (1993–1999). He took an active part in the creation of IECB where he established a research group in 1998. Since 2000, he shared the directorship of IECB with Dr J.J. Toulmé. Since 2011 he is an invited scientist in the same Institute. His current research interests include the design and total synthesis of biologically active molecules and the search of mild, efficient and "green" Lewis acid catalysts. In 2007, he received the medal of the Société Française de Chimie as a recognition of his support to the development of organic chemistry in France. Léon Ghosez is an emeritus member of the Royal Academy of Sciences of Belgium and a fellow of the Royal Society of Chemistry. In 2017 he received the title of "Chevalier de la Légion d'Honneur".

### Research team

Dr. Léon GHOSEZ Prof. Emeritus, Invited scientist (CNRS–Univ. Bordeaux)

The team is part of the CNRS/University of Bordeaux UMR 5144 CBMN.

# Organic & Medicinal Chemistry

## 1. Sustainable electrophilic catalysts for the activation of highly functionalized and sensitive molecules

The project aims at finding solutions to the often encountered problems associated with the use of many electrophilic catalysts : chemoselectivity, low turnover, too high molecular weight, in particular for asymmetric catalysis, toxicity and generation of much waste. New ionic solvents and silicon-derived Lewis superacids have been found to provide solutions to these problems. New electrophilic catalysts for cycloaddition and alkylation reactions involving highly functionalized acid-sensitive molecules have been developed.

## 2. Deoxy substitution of hydroxyl-containing compounds under mild and sustainable conditions

The project aims at finding milder conditions for the replacement of an hydroxyl group by a nucleophile. present methods often required acidic conditions or the use of toxic reagents or (and) lead much waste ( eg Mitsunobu reaction) The concept is based on earlier findings of the group on the deoxychlorination-, bromination- and iodination reactions with the readily available haloenamines : conditions are mild, atom economy is high and no reagent is toxic. The reaction was recently extend to the often pharmacologically interesting replacement of an hydroxyl group by fluorine. The plan is to extend the method to a wide variety of nucleophilic reagents including carbon nucleophiles.

The last postdoctoral associate left at the end of 2019. All negotiations related to new collaborations were postponed as a result of the pandemic. As a result, no experimental work was performed in 2020. Some theoretical calculations on the mechanism of deoxyfluorination reactions were performed in collaboration with Dr. Frédéric Robert of ISM, CNRS/UBx and a publication is in preparation. Most of the time was devoted to writing publications. Part 2 and 3 of a detailed account of our work on deoxyhalogenation ( Cl, Br, I) were published in special issues of Tetrahedron. A first publication of our collaborative studies with Institut Servier on privileged scaffolds inspired by natural products also appeared in 2020 in the anniversary issue of Chemistry, a European Journal, at the invitation of the Editor-in-Chief.

As a result of a long-standing collaboration of the group with Prof. K. Houk at UCLA, one more paper dealing with a refined theoretical study of keteniminium cycloadditions was published in the Journal of Organic Chemistry. The results of another collaborative study with K. Houk dealing with an unprecedented regiochemistry of ketene cycloadditions to olefins has been submitted to J. Amer. Chem. Soc.

A full paper including experimental and theoretical studies of the deoxyfluorination of carboxylic acids and alcohols will be completed at the beginning of fall.

Invitations to submit "feature articles" to synthesis and chem. comm. have been accepted. Both should be published in 2021.

## Selected publications

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47. André, C.; Veillard, F.; Wolff, P.; Lobstein, A.-M.; Compain, G.; Monsarrat, C.; Reichhart, J.-M.; Noûs, C.; Burnouf, D. Y.; Guichard, G.; Wagner, J. E. Antibacterial Activity of a Dual Peptide Targeting the Escherichia Coli Sliding Clamp and the Ribosome. *RSC Chem. Biol.* **2020**, *1* (3), 137–147. <https://doi.org/10.1039/D0CB00060D>.
48. Mateus, P.; Chandramouli, N.; Mackereth, C. D.; Kauffmann, B.; Ferrand, Y.; Huc, I. Allosteric Recognition of Homomeric and Heteromeric Pairs of Monosaccharides by a Foldamer Capsule. *Angewandte Chemie – International Edition* **2020**, *59* (14), 5797–5805. <https://doi.org/10.1002/anie.201914929>.
49. Ceschi, S.; Largy, E.; Gabelica, V.; Sissi, C. A Two-Quartet G-Quadruplex Topology of Human KIT2 Is Conformationally Selected by a Perylene Derivative. *Biochimie* **2020**, *179*, 77–84. <https://doi.org/10.1016/j.biochi.2020.09.015>.
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# Invited Peer-Reviewed Articles

1. Nokin, M.-J.; Ambrogio, C.; Nadal, E.; Santamaria, D. Targeting Infrequent Driver Alterations in Non-Small Cell Lung Cancer. *Trends in Cancer* **2021**, 7 (5), 410–429. <https://doi.org/10.1016/j.trecan.2020.11.005>.
2. Di Primo, C. Surface Plasmon Resonance for Investigating Molecular Interactions with RNA. *Methods Mol Biol* **2020**, 2113, 73–88. [https://doi.org/10.1007/978-1-0716-0278-2\\_6](https://doi.org/10.1007/978-1-0716-0278-2_6).
3. Habenstein, B.; El Mammeri, N.; Tolchard, J.; Lamon, G.; Tawani, A.; Berbon, M.; Loquet, A. Structures of Type III Secretion System Needle Filaments. In *Bacterial Type III Protein Secretion Systems*; Wagner, S., Galan, J. E., Eds.; Current Topics in Microbiology and Immunology; Springer International Publishing: Cham, 2019; Vol. 427, pp 109–131. [https://doi.org/10.1007/82\\_2019\\_192](https://doi.org/10.1007/82_2019_192).
4. Allison, T. M.; Barran, P.; Benesch, J. L. P.; Cianferani, S.; Degiacomi, M. T.; Gabelica, V.; Grandori, R.; Marklund, E. G.; Menneteau, T.; Migas, L. G.; Politis, A.; Sharon, M.; Sobott, F.; Thalassinos, K. Software Requirements for the Analysis and Interpretation of Native Ion Mobility Mass Spectrometry Data. *Anal Chem* **2020**, 92 (16), 10881–10890. <https://doi.org/10.1021/acs.analchem.9b05792>.
5. Herrero del Valle, A.; Innis, C. A. Prospects for Antimicrobial Development in the Cryo-EM Era – a Focus on the Ribosome. *FEMS Microbiology Reviews* **2020**, 44 (6), 793–803. <https://doi.org/10.1093/femsre/fuaa032>.
6. Auguste, P.; Leitinger, B.; Liard, C.; Rocher, V.; Azema, L.; Saltel, F.; Santamaria, D. Meeting Report – First Discoidin Domain Receptors Meeting. *Journal of Cell Science* **2020**, 133 (4), jcs243824. <https://doi.org/10.1242/jcs.243824>.
7. Vicens, Q.; Boehler, A.; Jobe, A.; Frank, J.; Hashem, Y. Interaction Networks of Ribosomal Expansion Segments in Kinetoplastids. In *Macromolecular Protein Complexes III: Structure and Function*; Harris, J. R., Marles-Wright, J., Eds.; Subcellular Biochemistry; Springer International Publishing: Cham, 2021; Vol. 96, pp 433–450. [https://doi.org/10.1007/978-3-030-58971-4\\_13](https://doi.org/10.1007/978-3-030-58971-4_13).
8. Carvalho, J.; Mergny, J.-L.; Salgado, G. F.; Queiroz, J. A.; Cruz, C. G-Quadruplex, Friend or Foe: The Role of the G-Quartet in Anticancer Strategies. *Trends Mol Med* **2020**, 26 (9), 848–861. <https://doi.org/10.1016/j.molmed.2020.05.002>.
9. Allison, T. M.; Barran, P.; Cianferani, S.; Degiacomi, M. T.; Gabelica, V.; Grandori, R.; Marklund, E. G.; Menneteau, T.; Migas, L. G.; Politis, A.; Sharon, M.; Sobott, F.; Thalassinos, K.; Benesch, J. L. P. Computational Strategies and Challenges for Using Native Ion Mobility Mass Spectrometry in Biophysics and Structural Biology. *Anal Chem* **2020**, 92 (16), 10872–10880. <https://doi.org/10.1021/acs.analchem.9b05791>.
10. Chinoy, Z. S.; Friscourt, F. Bioorthogonal Chemical Ligations Towards Neoglycoproteins. In *Reference Module in Chemistry, Molecular Sciences and Chemical Engineering*; Elsevier, 2021; p B9780128194751000000. <https://doi.org/10.1016/B978-0-12-819475-1.00080-8>.
11. Nguyen, P. H.; Ramamoorthy, A.; Sahoo, B. R.; Zheng, J.; Faller, P.; Straub, J. E.; Dominguez, L.; Shea, J.-E.; Dokholyan, N. V.; De Simone, A.; Ma, B.; Nussinov, R.; Najafi, S.; Ngo, S. T.; Loquet, A.; Chiricotto, M.; Ganguly, P.; McCarty, J.; Li, M. S.; Hall, C.; Wang, Y.; Miller, Y.; Melchionna, S.; Habenstein, B.; Timr, S.; Chen, J.; Hnath, B.; Strodel, B.; Kaye, R.; Lesné, S.; Wei, G.; Sterpone, F.; Doig, A. J.; Derreumaux, P. Amyloid Oligomers: A Joint Experimental/Computational Perspective on Alzheimer's Disease, Parkinson's Disease, Type II Diabetes, and Amyotrophic Lateral Sclerosis. *Chem. Rev.* **2021**, 121 (4), 2545–2647. <https://doi.org/10.1021/acs.chemrev.0c01122>.

## Patents

- Burnouf D; Compain, G.; [G. Guichard](#) ; Wagner J – Antibacterial Peptides EP Application EP/20305722.9 (29/06/2020)
- Burnouf D; [G. Guichard](#) ; Wagner J – New fusion peptides as antimicrobial agents, International publication WO002020148420A1 (23/07/2020)

## Evaluation Boards

- Membre de section, Comité National de la Recherche scientifique – Section 16, 2019, [G. Guichard](#)
- Panel member, ERC AdG 2020, [V. Gabelica](#)
- Member of Axe1, grant evaluator, GSO canceropole, 2020, [D. Santamaria](#)
- Remote evaluator Panel ERC AdG 2020, [Y. Hashem](#)

## Prizes, Awards

- Médaille de Bronze, CNRS, 2019, [P.V. Krasteva](#)
- IDEX Senior Chair, Univ Bordeaux, 2019, [N. Reyes](#)
- Monique Garnier–Semancik prize for the best thesis in Life Sciences. Univ. Bordeaux, 2019, [J. Meca](#), [D. McCusker](#)
- AMPERE Prize for Young Investigator, Ampere Society, 2021, [A. Loquet](#)
- EBSA Prize and Medal for Young Investigator, European Biophysical Societies Association, 2021, [Y. Hashem](#)
- Prix de la Division de Chimie Organique (DCO), Société Chimique de France, [G. Guichard](#)

## Journal & Scientific Society Boards

- Associate Editor, Biochemistry and Cell Biology, 2015, [C. Mackereth](#)
- Scientific advisor, Société de Chimie Thérapeutique (SCT), 2020, [G. Guichard](#)
- Vice President, Société Chimique de France (SCF) – Section Aquitaine, 2020, [G. Guichard](#)
- Associate Editor, Analytical Chemistry (ACS), 2021, [V. Gabelica](#)
- Member of the Board, Molecules, 2020, [A. Loquet](#)
- Review Editor, Frontiers in Cell & Developmental Biology, 2021, [D. McCusker](#)
- Grant reviewer, Canceropole Sud–Oest, 2020, [D. McCusker](#)
- Grant reviewer, Marie–Curie postdoc Fellowships, 2019, [D. McCusker](#)
- Grant reviewer, Canceropole Sud–Oest, 2019, [D. McCusker](#)
- Review Editor, Journal of Biological Chemistry, 2018–Present, [M. Sissler](#)
- Member of the Board, Société Française de Biochimie et Biologie Moléculaire (SFBBM), 2018–Present, [M. Sissler](#)

# Teaching

- Microbiology 2H, Cours de microbiologie générale Institut Pasteur, 2020, [R. Fronzes](#)
- Structural biology (3 hours of lectures + 4 hours of TD), Master 1 students ("Biologie, Santé"), University of Bordeaux, 2020, [A. Innis](#)
- Structural biology (3 hours of lectures), Master 1 students (CBio), University of Bordeaux, 2020, [A. Innis](#)
- 192 h
- Biology (Drug delivery, Nucleic acids, Thermodynamics kinetics and physical chemistry of solutions, Biomolecular chemistry and structure, Metabolic biochemistry and enzymology, Introduction to research); Medical Sciences (Instrumentation, Kinetics, Physical chemistry of lipids, Organic chemistry minerals thermodynamics and kinetics); Chemistry (Theoretical chemistry analytical, General chemistry), Pharmacy (Physical chemistry of interfaces and separation), Undergraduate (Job opportunities), L1, L2, L3, M1, M2 (Univ. Bordeaux), 2020, [G. Salgado](#)
- Structural biochemistry – 43h, First year undergraduate (L1), 2019, [P. Bonnafous](#)
- Methodology – 40h, Second year undergraduate (L2), 2019, [P. Bonnafous](#)
- Bioinformatics – 40h, Third year undergraduate (L3), 2019, [P. Bonnafous](#)
- Spectroscopy – 12h, Third year undergraduate (L3), 2019, [P. Bonnafous](#)
- Bioinformatics – 50h, First year Masters (M1), 2019, [P. Bonnafous](#)
- Master M2 – 2h, First year Masters (M1), 2019, [C. Mackereth](#)
- Chimie générale, Chimie organique, Biomolécules du vivant, Biologie Chimique : 192 HETD, BSc Level (L1 SVSTC Chimie, L2 SVSTC Chimie), MSc Level (M1 MMF/COSV Chimie du vivant, M2 COSV biologie chimique, 2020, [C. Dolain](#)
- Chimie générale, chimie organique, vectorisation, peptides bioactifs, sondes en imagerie, chimie thérapeutique : 192 HETD, PACES (Première Année Commune aux Etudes de Santé), cursus pharmacie 2ème année et 3ème année, module chimie du double cursus "Ecole Santé Sciences" (2ème année de pharmacie, odontologie et médecine), Master 2 TECSAN. Coresponsabilité du module de chimie du double cursus "Ecole Santé Sciences", 2020, [G. Compain](#)
- Responsible for internships, Master 2 de Chimie Univ. Bordeaux, 2020, [M. Pasco](#)
- 2nd year ENSTBB – 1h30, Characterization of biomolecules by SPR, 2020, [DI PRIMO](#)
- Master 1 Biochimie Univ. Bordeaux – 4h, Analysis of the interactions by SPR, 2020, [DI PRIMO](#)
- L3 TecSan Univ. Bordeaux – 14h, Instrumentations: SPTR technology, 2020, [DI PRIMO](#)
- Docteur en pharmacie: Sciences analytique – 96 h, Acid/base, oxidoreduction, potentiometry, buffers, precipitation, coordination, UV-vis spectroscopy, IR spectroscopy, GC, HPLC, liquid-liquid extraction, 2020, [E. Largy](#)
- Docteur en pharmacie: Apprentissage des techniques et gestes de base – 18 h, Basic lab technics, 2020, [E. Largy](#)
- DEUST Production, contrôles et qualité des produits de santé : Chimie analytique – 25.5 h, Acid/base, oxidoreduction, potentiometry, buffers, precipitation, coordination, 2020, [E. Largy](#)
- DEUST Production, contrôles et qualité des produits de santé : Réglementaire – 10.5 h, Regulatory aspects of drug quality control in the pharma industry, 2020, [E. Largy](#)
- Master 2 Analyse Chimique et Contrôle Qualité des Médicaments et Autres Produits de Santé & Master 2 Analytical chemistry for Drugs and Natural Products : Qualification – 3 h, Qualification of analytical instruments in regulated environment, 2020, [E. Largy](#)
- Master 2 Analyse Chimique et Contrôle Qualité des Médicaments et Autres Produits de Santé & Master 2 Analytical chemistry for Drugs and Natural Products : Recherche et développement analytique – 9 h, HPLC/UPLV analytical method development and transfer in pharma R&D. Detectors (MS, UV, fluorescence, Corona, LS,...), pumps, advanced column applications, 2020, [E. Largy](#)
- Master 2 Analyse Chimique et Contrôle Qualité des Médicaments et Autres Produits de Santé & Master 2 Analytical chemistry for Drugs and Natural Products : Contrôle qualité appliqué au produit fini – 15 h, Characterization and quantitation of drug substance and impurities by HPLC, 2020, [E. Largy](#)
- Master 2 Analyse Chimique et Contrôle Qualité des Médicaments et Autres Produits de Santé : Projet tuteuré en laboratoire ou contrat en entreprise – 35 h, Mentoring of students in the pharmaceutical industry, 2020, [E. Largy](#)
- Master 2 Analyse Chimique et Contrôle Qualité des Médicaments et Autres Produits de Santé : Validation – 5 h, Analytical method validation, 2020, [E. Largy](#)
- Master 2 Analyse Chimique et Contrôle Qualité des Médicaments et Autres Produits de Santé : Management et gestion de projet – 12 h, Project management, 2020, [E. Largy](#)
- MAPI – 4 h, Supporting the emergence and restructuring of new projects – Health sciences, 2020, [E. Largy](#)
- Projet SAN STEP – 14 h, Pedagogical innovations: Teaching online, on modern media (online quizzes, video presentations, analytical toolbox website,...), 2020, [E. Largy](#)
- Jury VAE – 1 h, Jury for the validation of knowledge acquisition through professional experience (pharmaceutical analysis), 2020, [E. Largy](#)
- IDEX/ANA: Projet IDEX Analytical Chemistry for drugs and natural product – 7.5, Pedagogical innovations: teaching in English, 2020, [E. Largy](#)
- Préparateurs en pharmacie hospitalière – 3 h, Solution chemistry, 2020, [E. Largy](#)
- NMR 4 hours, ENSTBB, 2020, [A. Loquet](#)
- Cell polarity & the cell cycle ~4h, Cancer Cell Biology. Genetics, 2020, [D. McCusker](#)
- Cell Polarity & Cancer – ~8h, Cancer Cell Biology Masters program, 2019, [D. McCusker](#)
- Drosophila as a model organism, Master 1 program, 2020, [E. Montembault](#)
- Master 2 Université de Bordeaux, Génétique Moléculaire et Cellulaire (GMC), 2020, [D. Santamaria](#)
- Master 2 Université de Bordeaux, UER Cytogénétique et Biologie Moléculaire des tumeurs, 2020, [D. Santamaria](#)

# PhD Theses

- Pauline Pony "Etude structural et fonctionnelle des spiroosomes bactériens", [R. Fronzes](#), University of Bordeaux, 2020
- Nadia El Mammeri "Study of biological supramolecular assemblies by solid-state NMR", [A. Loquet](#), U. Bordeaux, 2020
- Jayakrishna Shenoy "Structural characterization of amyloid fibrils by solid-state NMR", [A. Loquet](#), U. Bordeaux, ERC, 2020
- Gaëlle Lamon "Structural studies of fungal cell walls by solid-state NMR", [A. Loquet](#), U. Bordeaux, ANR, 2020
- Anthony Legrand "Studies of plant REMORINS" [B. Habenstein](#), U. Bordeaux, 2020
- Anthony Bochler « Structural differences and specificities of mRNA translation initiation complexes between trypanosomes and their hosts », [Y. Hashem](#), University of Strasbourg, 2020

# Science & Society

- Fêtes de la Science, Bordeaux, France, octobre 2020, [R. Fronzes](#)

# Team Funding

## European and International fundings

Coordinated by IECB researchers/IECB researchers as participants

IECB Researcher(s)	Funding body	Research project	Period
R. Fronzes	ERC Consolidator Grant	Structure and Function of the Bacterial Transformasome	2017-2022
Y. Hashem	ERC Starting Grant	Translation regulation in eukaryotic pathogens and hosts	2018-2023
A. Innis	ERC Consolidator Grant	Ribosome inhibition by nascent or antimicrobial peptides	2017-2022
A. Innis	EMBO	EMBO Young Investigator Award	2018-2020
A. Innis	EMBO	EMBO Long Term Fellowship	2018-2020
A. Innis	JPI-AMR	Development of novel ribosome-targeting antibiotics	2019-2022
P.V. Krasteva	ERC Starting Grant	Structural Biology of Exopolysaccharide Secretion in Bacterial Biofilms	2018-2023
C. Mackereth	Human Frontiers Research Program	Enhancing mitochondrial DNA fidelity to improve mammalian lifespan and healthspan	2019-2022
C. Mackereth	France-Canada Research Fund	Structure and binding kinetics of the cocaine-binding aptamer	2019-2022
G. Salgado	CNRS	Emergence International	2020
N. Reyes	ERC Consolidator Grant	Transport and Receptor Mechanisms of Human Solute Carriers	2018-2023
N. Reyes	NIH-RO1	Mechanisms of allosteric modulation of glutamate transporters	2019-2024
G. Guichard	EU	Metal-Foldamer Porous Frameworks	2021-2023
M. Aznauryan	Euskampus	Fundamental insights into binding mechanisms for the rational design of sensors for the detection of SARS-CoV-2	2021-2022
M. Aznauryan	EuroNanomed	Monitoring of Acquired Brain Injury and recovery biomarkers by the combined label-free nanoSensing of multiple circulating molecules	2019-2022
V. Gabelica	ERC Consolidator Grant	Advanced mass spectrometry approaches to reveal nucleic acid folding energy landscapes	2014-2020
V. Gabelica	H2020-MSCA-IF	Folding Pathways of DNA G-quadruplexes in Crowding Conditions, and Implications for Mass Spectrometrybased Ligand Screening Assays	2018-2020
D. Santamarina	Fundacio La Marato	Modulating glycemia to improve benefit of chemoradiotherapy and immunotherapy in Non- Small Cell Lung Carcinoma	2020-2022

## National funding

Coordinated by IECB researchers/IECB researchers as participants

IECB Researcher	Funding body	Research project	Period
R. Fronzes	ANR	Dissecting the Antibody Cleavage System of Mycoplasmas	2018-2021
R. Fronzes	ANR	Structural basis of Helicobacter pylori type IV secretion system	2019-2022
R. Fronzes	ANR	Une approche multidisciplinaire pour comprendre la structure et la dynamique du système de sécrétion de type VI	2020-2024
R. Fronzes	EquipEX+	Microscopie corrélative à haute résolution en conditions cryogéniques	2021-2027
Y. Hashem	ANR	Translation initiation in plant mitochondria	2021-2025
Y. Hashem	ANR	ABC-F mediated antibiotic resistance	2018-2022
Y. Hashem	ANR	Mitochondrial translation in plants	2016-2020
Y. Hashem	ATIP-Avenir	Translation regulation in Hosts and Pathogens	2018-2021
N. Reyes	Institut National du Cancer (INCA) PLBIO	Molecular pharmacology of ASCT2, a novel therapeutic target in human cancer	2017-2020
F. Friscourt	CNRS ATIP-Avenir	Making the invisible, visible, detecting traumatic brain injury	2017-2021
G. Guichard	ANR PRC	Therapeutic targeting and chemical biology of histone chaperone using rationally designed medium-size inhibitors	2021-2025
G. Guichard	INSERM PCSI 2020	Inhibition et dégradation ciblée du chaperon d'histone ASF-1 par des molécules synthétiques dans les cellules cancéreuses	2020-2022
G. Guichard	CNRS MITI (DEFI Biomimétisme)	Precise Pore-forming Assemblies of Bioinspired Oligomers with High Folding Fidelity	2020
G. Compain	ANR JCJC	Selective and directional supramolecular interactions based on highly polar fluorinated synthons	2021-2025
C. Dolain	CNRS EMERGENCE@INC2020	Vers la synthèse de structures beta artificielles multi-brins	2020-2021
E. Thinon	ANR	Chemical approaches to study the S-palmitoylation of a host factor in Influenza A virus infection	2021-2024
M. Aznauryan	ANR	Probing the molecular mechanisms of function of disordered translation initiation factors: from in vitro to in-cell	2021-2025
V. Gabelica	ANR	Understanding Native Electrospray of Artificial and Natural Polymers	2019-2022
A. Loquet	U. Bordeaux	PhD	2020-2023
A. Loquet	ANR	PhD	2020-2023
A. Loquet	FranceAgrimer	Postdoc	2020-2022

IECB Researcher	Funding body	Research project	Period
A. Loquet	MITI CNRS	Consummables	2020-2021
L. Peyran	Ministry of research	Cell polarity & nuclear dynamics	2020-2023
D. McCusker	CNRS	Testing the role of cooperative lipid interactions as a source of non-linearity in polarity establishment	2018
D. Santamarina	INCA-PIbio	Targeting KRAS dimerization in advanced lung adenocarcinoma	2019-2021

## Regional funding

Coordinated by IECB researchers/IECB researchers as participants

IECB Researcher	Funding body	Type of funding	Period
Y. Hashem	IdEx Bordeaux	IdEx Junior Chair: Translation régulation in pathogens and hosts	2017-2021
P.V. Krasteva	IdEx Bordeaux	IdEx Junior Chair ExoPol	2019-2022
F. Friscourt	LabEx TRAIL	Traumatic Brain Injury Glycobiomarker	2019-2021
F. Friscourt	Canceropole GSO	Chemo-enzymatic elaboration of well-defined oligosaccharides as galectin inhibitors for cancer therapy	2019-2021
G. Guichard / M. Khatib	IdEx Bordeaux	A chemical biology approach to study inhibition and targeted degradation of proprotein convertases in cancer cells	2020-2023
G. Guichard / C. Palomo	IdEx Bordeaux	Bioinspired Foldamer-based Asymmetric Catalysis at low catalyst loading	2018-2021
G. Guichard	Regional Council/DGA	Nouveaux Peptides Antibiotiques	2018-2021
J. Buratto	Univ Bordeaux - SPARK BORDEAUX	Development of a biomolecular platform for rapid identification and structural analysis of novel peptide-based inhibitors of SARS-CoV-2 fusion with target cells	2020-2021
E. Thinon	University of Bordeaux	Chemical approaches to decipher the role of a host factor in Influenza A virus internalization	2019-2022
E. Thinon	Region Nouvelle Aquitaine	Caractérisation d'une nouvelle cible thérapeutique antivirale	2020-2022
M. Aznauryan	Region Nouvelle Aquitaine	Caractérisation des mécanismes moléculaires gouvernant la fonction d'eIF4B : vers de nouvelles cibles contre le cancer	2020-2023
V. Gabelica / S. Benabou	IdEx Bordeaux	Exploring the biophysical properties of DNA i-motif structures by native mass spectrometry and ion mobility spectrometry	2019-2021
V. Gabelica	Region Nouvelle Aquitaine	Caractériser le repliement de protéines thérapeutiques recombinantes par spectrométrie de mobilité ionique	2019-2024
A. Loquet	Region Nouvelle Aquitaine	PhD	2020-2023

## Charity-funded research projects

Coordinated by IECB researchers/IECB researchers as participants

IECB Researcher	Charity	Research project	Period
A. Loquet	CNIV	PhD	2020-2023
A. Loquet	IFV	PhD	2020-2023
A. Royou	Ligue	Etude de FANCD2 au niveau des ponts ultrafins d'ADN en anaphase	2018-2019

## Contracts with the industry

Coordinated by IECB researchers/IECB researchers as participants

IECB Researcher	Company	Research contract	Period
V. Gabelica	Merck Biodevelopment SAS	Caractériser le repliement de protéines thérapeutiques recombinantes par spectrométrie de mobilité ionique	2019-2022

# Collaborations

## Pole 1 – Structural biology

### Structure and Function of Bacterial Nanomachines

Dr. Rémi Fronzes

- Dr. Yonathan Arfi, INRAE, Biologie du Fruit et Pathologie, UMR 1332, Villenave D'ornon, France
- Dr. Eric Cascales, LISM CNRS UMR7255, Marseille, France
- Dr. Laure Journet, LISM CNRS UMR7255, Marseille, France
- Dr. Laurent Terrdot, IBCP, Lyon, France

### RNA Processing and translation regulation in pathogens and hosts

Dr. Yaser Hashem

- Dr. Philippe Giegé, CNRS, Strasbourg, France
- Dr. Hakim Mireau, INRA, Versailles, France
- Dr. Tatyana Pestova, SUNY Downstate Medical Center, Brooklyn, NY, USA
- Christopher Helen, SUNY Downstate Medical Center, Brooklyn, NY, USA

### Translational Regulation of Gene Expression

Dr. Axel Innis

- Prof. Andreas Vilcinskas, Justus-Liebig-University of Giessen, Giessen, Germany

### NMR Spectroscopy of Protein–Nucleic Acid Complexes

Dr. Cameron Mackereth

- Dr. Ivan Huc, Ludwig Maximilian University, Munich, Germany
- Dr. Yann Ferrand, CNRS UMR 5248 (CBMN), Pessac, France
- Dr. Juan Ausio, University of Victoria, Victoria, Canada
- Dr. Santosh Kumar Upadhyay, CSIR–Institute of Genomics and Integrative Biology, Delhi, India
- Dr. Carla Cruz, CICS–UBI, Covilha, Portugal
- Dr. Jean-Louis Mergny, Laboratory of Optics and Biosciences, Palaiseau, France
- Dr. Fabien Darfeuille, Inserm U1212 (ARNA), Bordeaux, France

### Organic & Medicinal Chemistry

Dr. Nicolas Reyes

- Dr. Hugo Mouquet, Institut Pasteur, Paris, France

## Pole 2 – Organic & bioorganic chemistry

### Peptidomimetic Chemistry

Dr. Gilles Guichard

- Dr. Collie Gavin, AstraZeneca, Cambridge, UK
- Prof. Bechinger Burkhard, Univ. Strasbourg, Institut de chimie, Strasbourg, France
- Dr. Kichler Antoine, Univ. Strasbourg, Faculté de Pharmacie
- Dr. Wagner Jérôme, Univ. Strasbourg, Ecole Supérieure de Biotechnologie de Strasbourg (ESBS), Illkirch, France
- Dr. Burnouf Dominique, Univ. Strasbourg, Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France

## Pole 3 – Biophysics

### Single-molecule Biophysics

Dr. Mikayel Aznauryan

- Prof. Sébastien Lecommandoux, LCPO, University of Bordeaux, Pessac, France
- Prof. Stéphane Quideau, CNRS, University of Bordeaux, Talence, France

### Mass Spectrometry of Nucleic Acids & Supramolecular Complexes

Dr. Valérie Gabelica

- Dr. Marie-Paule Teulade-Fichou, Institut Curie, CNRS UMR176, Centre Universitaire Paris XI, Orsay, France
- Dr. Anton Granzhan, Institut Curie, CNRS UMR176, Centre Universitaire Paris XI, Orsay, France
- Dr. Yann Ferrand, UMR 5248 CBMN, Pessac, France
- Prof. Antonio Randazzo, University Federico II, Naples, Italy
- Prof. Concetta Giancola, University Federico II, Naples, Italy
- Prof. Claudia Sissi, University of Padova, Padova, Italy
- Dr. Hélène Lavanant, Université de Rouen Normandie, Rouen, France
- Prof. Edwin De Pauw, Université de Liège, Liège, Belgium

### Solid-state NMR of Molecular Assemblies

Dr. Antoine Loquet

- Prof. Philippe Barthélemy, ARNA, Bordeaux, France
- Prof. Philippe Derreumax, IBPC, Paris, France
- Dr. Sébastien Mongrand, LBM, Villenave d'Ornon, France
- Dr. Sven Saupe, IBGC, Bordeaux, France
- Dr. Joseph Wall, Brookhaven laboratory, Upton NY, USA
- Dr. Guido Pintacuda, ENS Lyon, Lyon, France
- Dr. Benjamin Bardiaux, Institut Pasteur, Paris, France
- Prof. Banafshe Larijani, Bath University, Bath, UK
- Dr. Alia Hassan, Bruker, Zurich, Switzerland
- Dr. Guillaume Duhamel, Aix Marseille University, Marseille, France
- Dr. FX Theillet, IBIC, Gif/Yvettes, France
- Dr. Fabien Darfeuille, ARNA, Bordeaux, France

## Pole 4 – Molecular & cellular biology

### Novel mediators in lung oncogenesis

Dr. David Santamaria

- Dr. Ernest Nadal, Idibell, Barcelona, Spain
- Dr. Chiara Ambrogio, Molecular Biotechnology Center (MBC) University of Torino, Torino, Italy
- Prof. Mariano Barbacid, Spanish National Cancer Centre (CNIO), Madrid, Spain

# Invited Conferences

## Pole 1 – Structural biology

### RNA Processing and translation regulation in pathogens and hosts

- ZOMES X CONFERENCE 2019, Akko, Israel, February 4–7 2019, Y. Hashem
- The RIBOSOME 2019 meeting, Merida, Mexico, January 6–10 2019, Y. Hashem

### Translational Regulation of Gene Expression

- EMBO YIP RNA and Structural Biology Sectoral Meeting, Virtual Conference 02/09/20, A. Innis

### Structural Biology of Biofilms Group

- Microbiology Day, France (Virtual Mini-symposium), March 2021, Petya V. Krasteva
- Microbiology Day, France (Virtual Mini-symposium), March 2021, W. Abidi

### NMR Spectroscopy of Protein–Nucleic Acid Complexes

- 14th NMR Retreat of Protein–RNA Interaction, Parpan, Switzerland, 01/2020, C. Mackereth

### Organic & Medicinal Chemistry

- Transmembrane Transporter Society International Meeting, Copenhagen, Denmark, 2020 (Pending due to COVID-19), N. Reyes
- Gordon Research Conference “Ligand Recognition and Gating”, Toscana/Italy, 2020 (Pending due to COVID-19), N. Reyes
- CryoEM symposium EMBL, Heidelberg/Germany, February/2020, N. Reyes

## Pole 2 – Organic & bioorganic chemistry

### Chemical Neuroglycobiology

- Society for Glycobiology SFG2020, Virtual Meeting, hosted USA, November 2020, F. Friscourt

### Peptidomimetic Chemistry

- Scéance thématique de l'Académie Nationale de Pharmacie, Paris, France, September/2020, G. Guichard
- 2èmes Journées Scientifiques De Chemobiologie : la Chimie à la rencontre de la Biologie, Nice, France, January/2020, G. Guichard

## Pole 3 – Biophysics

### Mass Spectrometry of Nucleic Acids & Supramolecular Complexes

- G4 series, international, 09/2020, V. Gabellica
- G4 series, international, 12/2020, E. Largy
- GDR EMIE – Réunion plénière 2020, Oleron, France, 10/2020, V. Gabellica
- 1st Annual Scientific Meeting of GDR “RNA”, Online meeting, 11/2020, V. Gabellica

### Solid-state NMR of Molecular Assemblies

- ACS Spring, Virtual (Texas), 2021, A. Loquet
- Emerging Topics in Biomolecular Magnetic Resonance, Virtual (Göttingen), 2021, A. Loquet
- Biophysics of amyloid formation, Ulm (Germany), 2020, A. Loquet

## Pole 4 – Molecular & cellular biology

### Dynamics of cell growth & cell division

- Oncosphere Seminar Series. Bordeaux, 2019, D. McCusker
- Laboratory of Membrane Biogenesis. Bordeaux, 2019, D. McCusker

# Conference Organisation

- Bordeaux Fly Meeting, Bordeaux, January 2020, A. Royou



### Access to the platform:

The platform is accessible to researchers from the public and from the private sector. All information on available equipment and process to request services or contact experts can be found on the BPCS web page:

<http://www.iecb.u-bordeaux.fr/index.php/en/structural-biophysico-chemistry>

Three types of services are offered:

(1) Instrument access time: duly trained users can request machine time, perform the experiments, and interpret the data. Office space is available to accommodate external users.

(2) Routine services: samples are submitted, the platform personnel performs the assays and sends the analysis report to the user. Experiments for which the data interpretation is routine fall into this category.

(3) Collaborative projects: all requests that require the platform personnel's scientific expertise and/or methodological developments in instrumentation, experiment design, or data interpretation, fall into this category (Bordeaux Recherche Oncologie).

# Technology Platforms



### Dr. Brice Kauffmann

Head of IECB's Biophysical and Structural Chemistry platform, IR, CNRS

Head of IECB's Biophysical and Structural Chemistry platform, IR, CNRS After a PhD in protein crystallography (2003, University of Nancy I), Brice Kauffmann spent three years at the European Molecular Biology Laboratory (EMBL) in Hamburg (Germany) working on the development of a new macromolecular crystallography beamline (X12, DESY). He joined the European Institute of Chemistry and Biology in January 2006 as a staff Scientist.

### Selected publications

1. Dazzoni, R.; Grélard, A.; Morvan, E.; Bouter, A.; Applebee, C. J.; Loquet, A.; Larjani, B.; Dufourc, E. J. The Unprecedented Membrane Deformation of the Human Nuclear Envelope, in a Magnetic Field, Indicates Formation of Nuclear Membrane Invaginations. *Sci Rep* **2020**, *10* (1), 5147. <https://doi.org/10.1038/s41598-020-61746-0>.
2. Arévalo-Ruiz, M.; Amrane, S.; Rosu, F.; Belmonte-Reche, E.; Peñalver, P.; Mergny, J.-L.; Morales, J. C. Symmetric and Dissymmetric Carbohydrate-Phenyl Ditrizole Derivatives as DNA G-Quadruplex Ligands: Synthesis, Biophysical Studies and Antiproliferative Activity. *Bioorganic Chemistry* **2020**, *99*, 103786. <https://doi.org/10.1016/j.bioorg.2020.103786>.
3. Liu, P.; Chen, W.; Okazaki, Y.; Battie, Y.; Brocard, L.; Decossas, M.; Pouget, E.; Müller-Buschbaum, P.; Kauffmann, B.; Pathan, S.; Sagawa, T.; Oda, R. Optically Active Perovskite CsPbBr<sub>3</sub> Nanocrystals Helically Arranged on Inorganic Silica Nanohelices. *Nano Lett.* **2020**, *20* (12), 8453-8460. <https://doi.org/10.1021/acs.nanolett.0c02013>.
4. Duan, H.; Donovan, M.; Hernandez, F.; Di Primo, C.; Garanger, E.; Schultze, X.; Lecommandoux, S. Hyaluronic-Acid-Presenting Self-Assembled Nanoparticles Transform a Hyaluronidase HYAL1 Substrate into an Efficient and Selective Inhibitor. *Angew. Chem. Int. Ed.* **2020**, *59* (32), 13591-13596. <https://doi.org/10.1002/anie.202005212>.
5. Misuraca, L.; Caliò, A.; Grillo, I.; Grélard, A.; Oger, P. M.; Peters, J.; Demé, B. High-Temperature Behavior of Early Life Membrane Models. *Langmuir* **2020**, *36* (45), 13516-13526. <https://doi.org/10.1021/acs.langmuir.0c02258>.
6. Pappas, C. G.; Mandal, P. K.; Liu, B.; Kauffmann, B.; Miao, X.; Komáromy, D.; Hoffmann, W.; Manz, C.; Chang, R.; Liu, K.; Pagel, K.; Huc, I.; Otto, S. Emergence of Low-Symmetry Foldamers from Single Monomers. *Nat. Chem.* **2020**, *12* (12), 1180-1186. <https://doi.org/10.1038/s41557-020-00565-2>.
7. Glavier, M.; Puvanendran, D.; Salvador, D.; Decossas, M.; Phan, G.; Garnier, C.; Frezza, E.; Cece, Q.; Schoehn, G.; Picard, M.; Taveau, J.-C.; Daury, L.; Broutin, I.; Lambert, O. Antibiotic Export by MexB Multidrug Efflux Transporter Is Allosterically Controlled by a MexA-OprM Chaperone-like Complex. *Nat Commun* **2020**, *11* (1), 4948. <https://doi.org/10.1038/s41467-020-18770-5>.

## Biophysical & Structural Chemistry platform (BPCS)

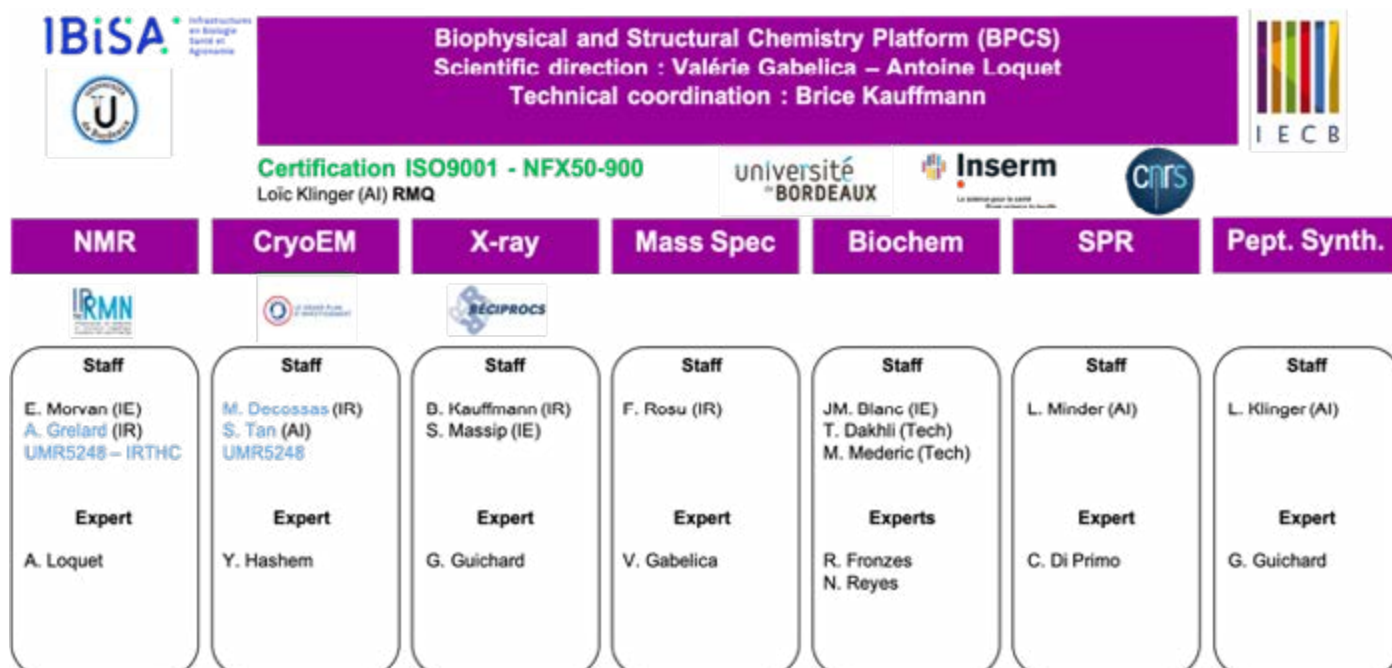
IECB's core facility in biophysical and structural chemistry aims at answering structural and functional questions on molecules and complexes of biomedical interest. IBISA-labelled since 2011, this facility provides a privileged access to state-of-the-art instruments as well as dedicated scientific expertise from scientists leading research programs either at IECB or in partner labs on the Bordeaux campus. The IECB Facility serves to nucleate the development of a supportive local community with expertise in structural biology and increases the attractiveness of the University of Bordeaux for talented scientist in this field from all over the world.

In 2019, the IECB core facility has joined the new "Core Facilities" department at Bordeaux University. At the forefront of methodological developments in Structural Biochemistry and Biophysics, the facility is gathering on the same site a coherent set of techniques and expertise to investigate from a structural and biophysical perspectives molecular recognition processes and structure of supramolecular assemblies.

Importantly, the facility stands at the frontiers between chemistry and biology, by focusing both on biological molecules and on synthetic molecules (foldamers) conceived to fold and self-assemble like biological molecules, and/or interact with biological systems. It is no longer sufficient to determine simply the structure and biochemical properties of macromolecules in vitro. In line with the trend towards systems biology and integrated structural biology initiative in Europe (Instruct), a major challenge now is understanding how that macromolecules functions dynamically within a larger macromolecular assembly or in a cellular pathway or even at the organism level. Understanding dynamic processes is not possible using a single technology, but becomes potentially accessible through the integration of a number of approaches, spanning different resolution scales.

The IECB facility follows that development strategy by regrouping expertise and state-of-the-art instruments in Biochemistry (production and purification of recombinant proteins, peptide synthesis...), NMR spectroscopy (liquid and solid state with 8 spectrometers from 100 MHz to 800 MHz), X-ray crystallography (from crystallization to atomic resolution structure on single crystals or powder samples with a high flux X-ray source and Hybrid direct detector), CryoEM (with a 200kV FEI Talos Arctica microscope equipped with a GATAN K2 summit camera), mass spectrometry (the facility has a strong specificity in studying non covalent complexes by ion mobility mass spectrometry with an Agilent 6560 ESI-IMS-Q-TOF), surface plasmon resonance and spectroscopy (absorption and circular dichroism spectroscopy, SPR exploiting a T200 instrument from Biacore).

## Organization



## Figures for 2020

Users of the platform: In 2020, the platform contributed to 107 projects for more than 60 different public or private laboratories or companies.

Key figures:

- More than 50 people trained per year (students, technicians, researchers)
- 81 publications with staff members as co-author or with acknowledgments for the BPCS.

## 2020 Highlights:

**Impact of the COVID-19 pandemic on the BPCS activities.**

The BPCS has suffered a considerable negative impact from the COVID-19 pandemic, with the reduction in research activities primarily impacting the 2020 budget. The balance sheet of the whole platform shows a deficit of almost 35 k€. The impact on the scientific production will most probably be observed only in the next few years.

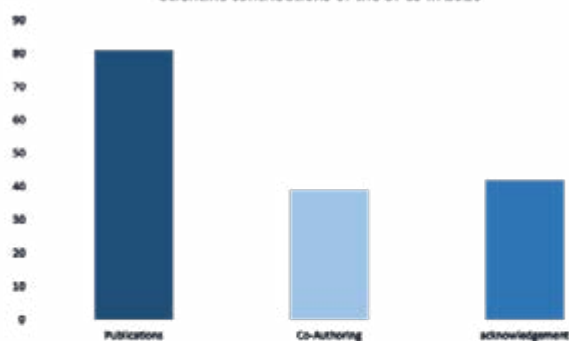
**Renewal of ISO9001 and NFX-50-900 certification.**

Following the audit conducted by the independent agency LRQA, the BPCS platform has renewed its certifications for 3 years. Thanks to the great involvement of the staff, the platform continues its continuous improvement process.

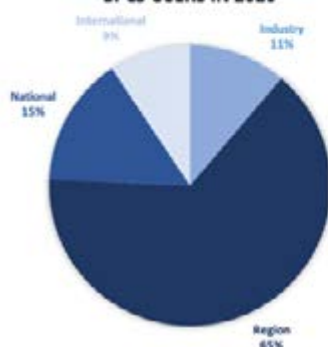
**Installation of a new Fourier Transform Ion Cyclotron Resonance (FT-ICR) mass spectrometer.**

In November 2020, a new 7TESI MALDI Solarix XR FT-ICRMS (Bruker Daltonics, Germany) was installed at the platform. The instrument will enable high-resolution analysis of non-covalent complexes and detailed analysis of biomolecule fragmentation with a variety of techniques (IR and UV lasers, collisions, ion-ion reactions, etc...)

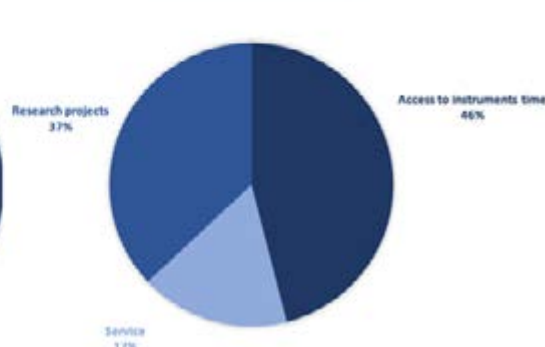
Scientific contributions of the BPCS in 2020



BPCS USERS IN 2020



BPCS ACTIVITIES IN 2020





# Technology Transfer & Start-ups

The scientific breakthroughs achieved at IECB are meant to nurture technological innovation. The skills, knowledge and technologies developed at the institute are transferred to economic players via different routes:

### Collaborative research

Servier, UreKa, DART Neurosciences... Several key industry players work with IECB teams. In 2018, the institut totalized 3 on-going projects with industrial partners.

### Contract services and consulting

The IECB brings together a wide range of scientific equipments and expertise in chemistry and biology. Such resources are made available to public and private research centers through IECB's Biophysical and Structural Chemistry platform.

### Technology transfer

IECB researchers are strongly encouraged to patent their discoveries.

The technology transfer unit Novaptech that was hosted at IECB in 2008-2013 is now a promising biotech company headquartered in Bordeaux.

### Incubating start-ups

IECB has 300 m2 work space dedicated to start-ups. Ureka created in 2010 is located at the institute since 2014. Until 2018, a part of this area was also occupied by Fluofarma, created in 2003 by two team leaders from IECB.





Established in the region of Bordeaux since March 2014, UREkA, a subdivision of ImmuPharma, propose to revolutionize the way we make peptide-based drugs.

Coming from the vision of Robert Zimmer, director of ImmuPharma and Gilles Guichard, professor at the IECB, UREkA is the result of many years of research of foldamer chemistry in the laboratory of Gilles Guichard. UREkA is now performing research programs to apply its Urelix™ technology for the discovery of innovative therapeutics in close collaboration with IECB group leader Gilles Guichard.

#### Medicinal chemistry – diseases of interest

- Diabetes
- Hypoglycemia
- Obesity
- Non-alcoholic steatohepatitis (NASH)
- Cancer

#### Collaborative research projects

- Implementation of Urelix™ technologies in partners projects.
- Design and synthesis of bioactive foldamers.
- Hit to lead
- SAR
- Development



Dr. Sébastien Goudreau  
Ureka Research Director

**Year of creation** 2010

**Staff** 5

**Collaborative projects with IECB teams in 2018:** 2

**Website** [www.urekapharma.com](http://www.urekapharma.com)

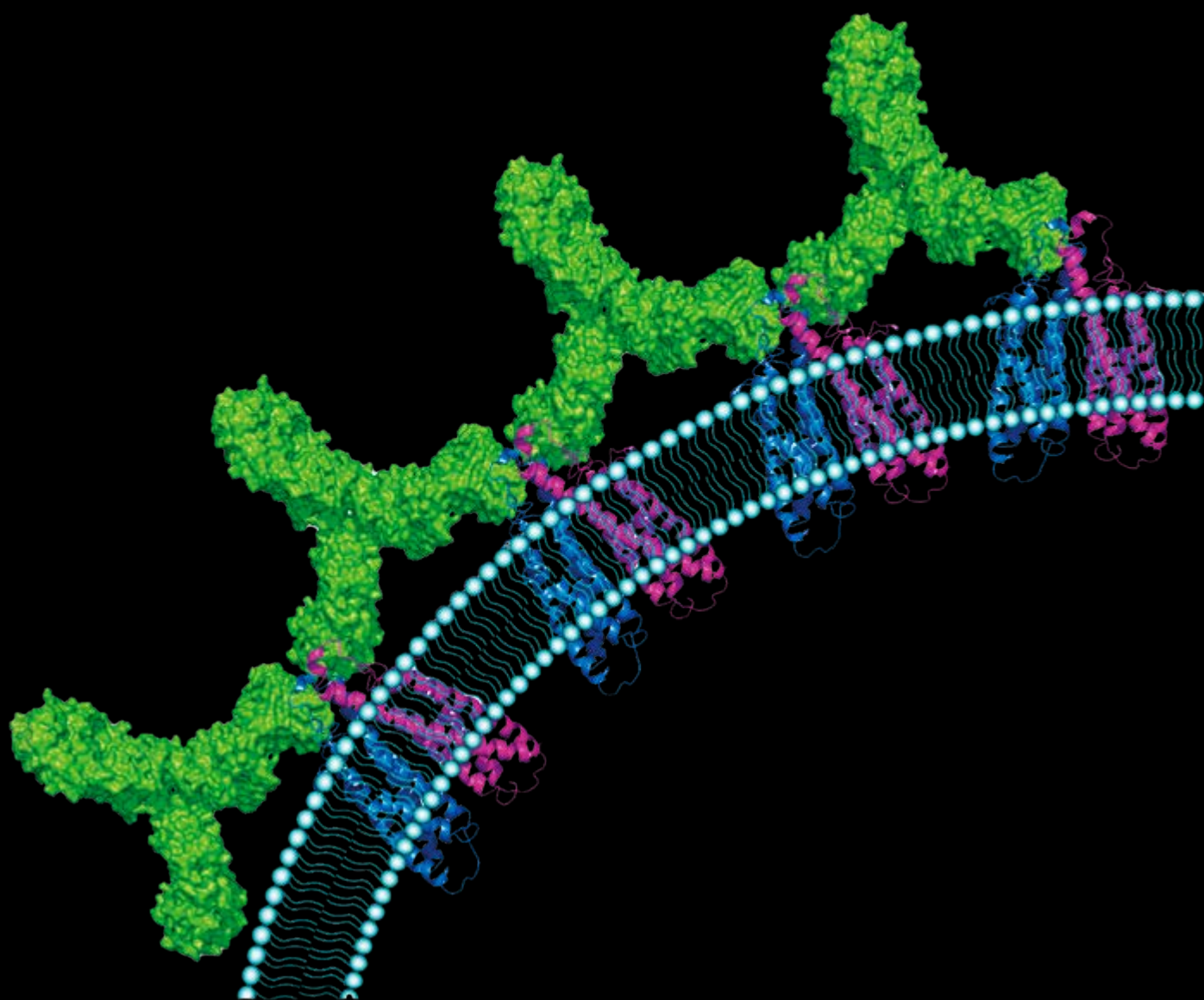
**Contact**  
[sebastien.goudreau@immupharma.com](mailto:sebastien.goudreau@immupharma.com)







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