JANUARY 2019 - NUMBER 22

PSB *et al*

Partnership for Structural Biology Newsletter

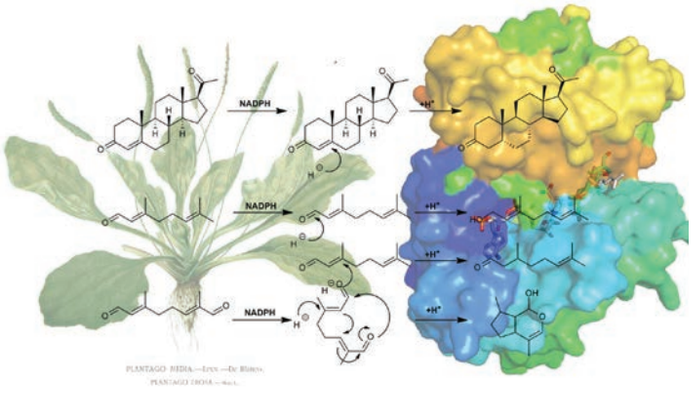
Scientific highlights

A multisubstrate reductase from *Plantago major*: structure-function in the short chain reductase superfamily

"Romeo: Your plantain leaf is excellent for that.
Benvolio: For what, I pray thee?
Romeo: For your broken shin."
Romeo and Juliet, Act 1, Scene 2.

Because plants are sessile, they have developed elaborate chemical means via secondary metabolism to respond to their environment, interact with the microbiome and defend against predators. Plants are able to produce thousands of different natural products as part of their complex secondary metabolism. These natural products constitute an important reservoir of new bioactive compounds of potential therapeutic use for humans. *Plantago major*, the common plantain plant, is a ubiquitously occurring weed that has been exploited medicinally for centuries. Throughout recorded history, *Plantago* has been used in poultices as a wound healing agent. One of the earliest written records was by the Greek physician Dioscorides in 'De materia medica', and subsequently appeared in numerous texts, even garnering mention by Shakespeare in "Romeo and Juliet" (see above quote) and "Love Labour's Lost". One of the bioactive compounds responsible for this therapeutic activity is the iridoid glycoside aucubin, with recognised anti inflammatory and antibacterial activity.

Iridoids constitute an important class of compounds that are found in other plants, such as the periwinkle. In 2012, the O'Connor group at the University of East Anglia identified the gene and protein responsible for the key step in iridoid synthesis: the iridoid cyclase. This critical work allowed the identification of a candidate gene from an EST database of *Plantago major*, which was then structurally and biochemically characterized. In the joint work by the team of Max Nanao at the ESRF and Chloe Zubieta at the CEA, Grenoble, the putative iridoid cyclase from *Plantago* was shown to exhibit not only iridoid cyclase activity, but also to promiscuously reduce carbon-carbon double bonds along with performing reductive reactions [1]. The *Plantago* iridoid cyclase has a very large and hydrophobic active site, which allows it to accept many different substrate molecules with different scaffolds, including the isoprenoid, citral, and the plant sterol, progesterone. In addition to protein crystallographic experiments, extensive site directed mutagenesis and enzymatic activity studies were performed by the researchers, revealing the catalytic and substrate specificity determining residues.



Left. Sketch of the plant *Plantago major*. Middle. NADPH-dependent reduction reactions of the substrates progesterone (top) citral (middle) and 8-oxogeranial (bottom) by the reductase enzyme PmMOR (*P. major* multisubstrate oxido-reductase). Right. Crystal structure of the reductase in complex with substrates.

An understanding of the biosynthetic pathways and enzymes involved will be crucial to address the need for new antimicrobials, anticancer and antiviral therapies. Studies such as these help reveal how plant enzymes are able to take common precursor molecules and perform elaborate chemical reactions to make new scaffolds for bioactive molecules. Indeed, using these studies as a foundation, reengineering the enzyme for increased iridoid synthase activity and the production of novel molecules with potential bioactive properties might be possible. The teams are planning to modify the activity of this critical enzyme in the iridoid biosynthetic pathway in order to produce new derivatives of aucubin with differing activity profiles.

C. Zubieta (CEA, CNRS, INRA, BIG), M. Nanao (ESRF)

[1] R. Fellows, CM. Russo, CS. Silva, SG. Lee SG, JM. Jez, JD. Chisholm, C. Zubieta, MH. Nanao (2018). Scientific Reports, **8**(1),14796.

CONTENTS

RIP2 filament formation is required for NOD2 dependent NF-κB signalling

The innate immune response to invading pathogens initiates with the detection of pathogen-specific molecules (PAMPs, pathogen-associated molecular patterns, e.g. bacterial cell-wall fragments or viral RNA) by host pattern recognition receptors (PRRs). PAMP binding to PRRs that contain an effector domain known as a death domain (DD, e.g. CARD or PYD) triggers receptor oligomerisation and recruitment of a downstream adaptor protein, which often contains a DD domain as well. The interaction of the PRR DD with that of the adapter in turn triggers polymerisation of the adapter protein, binding of downstream effectors, and formation of a higher order filamentous assembly called a signalosome [1].
Here, we focus on the PRR NOD2 (Nucleotide oligomerisation domain 2) and its adaptor kinase protein RIP2 (Receptor interacting protein 2). Binding of the bacterial peptidoglycan fragment muramyl-dipeptide (MDP) to NOD2, leads to receptor oligomerisation and recruitment of RIP2 *via* CARD-CARD interaction. Upon auto-phosphorylation and ubiquitination, RIP2 becomes a platform for downstream effector proteins, eventually triggering an inflammatory response, which protects the gut epithelial cells from both residual flora and pathogen invasion. Excessive or absent NOD2-RIP2 signalling is associated with several genetic and non-genetic inflammatory diseases, which lack specific and effective therapies, e.g. Crohn's disease, Blau syndrome, early-onset sarcoidosis, inflammatory arthritis, asthma, colorectal cancer and multiple sclerosis. This has made the NOD2-RIP2 signalling an attractive drug target for treating these inflammatory disorders [2].

In our work, the result of a fruitful and highly interdisciplinary collaboration between EMBL Grenoble, EMBL Heidelberg, IBS and the solid-state NMR group of Dr Oschkinat at the Leibniz-Institut in Berlin, we investigated whether the recruitment of RIP2 by NOD2 could lead to the formation of a signalosome ('nodosome') [3]. We started with the observation by negative-stain electron microscopy (EM) that phosphorylated and active full-length RIP2 (RIP2fl) forms filaments *in vitro* in the presence of ATP and magnesium (Figure 1A). The subsequent observation that the RIP2 CARD domain also spontaneously forms more slender filaments, suggests that the CARD domain are the core of the RIP2fl filaments, while the kinase domain (RIP2K) is on the exterior (Figure 1B). Pull-down experiments combined with immuno-gold labelling show that NOD2 tandem CARDs bind to one end of the RIP2 CARD filament, suggesting a mechanism for polar filament nucleation by activated NOD2 (Figure 1C). By combining X-ray crystallography of the RIP2 CARD monomer, high-resolution cryo-EM (using data collected on the IBS Polara) and solid-state NMR, we determined the atomic structure of the helical RIP2 CARD filament at 3.9 Å of resolution, which reveals the intermolecular interactions that stabilize the assembly (Figure 1D). Using structure-guided mutagenesis combined with a cellular reporter assay, we further demonstrate that RIP2 polymerization is essential for NF-κB activation by NOD2. Therefore our data are in favour of formation of a nodosome, whose assembly might occur as follows (Figure 1E): (1) binding of MDP to NOD2 causes derepression of the NOD2 CARDs; (2) NOD2 oligomerises and (3) recruits RIP2 via its CARD domain (4) cumulative binding of RIP2 promotes filament elongation to form the helical assembly; (5) polymerization of RIP2CARD in the presence of ATP stabilises the active antiparallel dimeric form of RIP2K; (6) E3 ligases bind the active form of RIP2K; (7) RIP2 becomes K63-ubiquitinated enabling it to recruit downstream effector proteins.

Our results could be of use to develop new pharmacological strategies to treat inflammatory diseases characterised by aberrant NOD2 signalling.

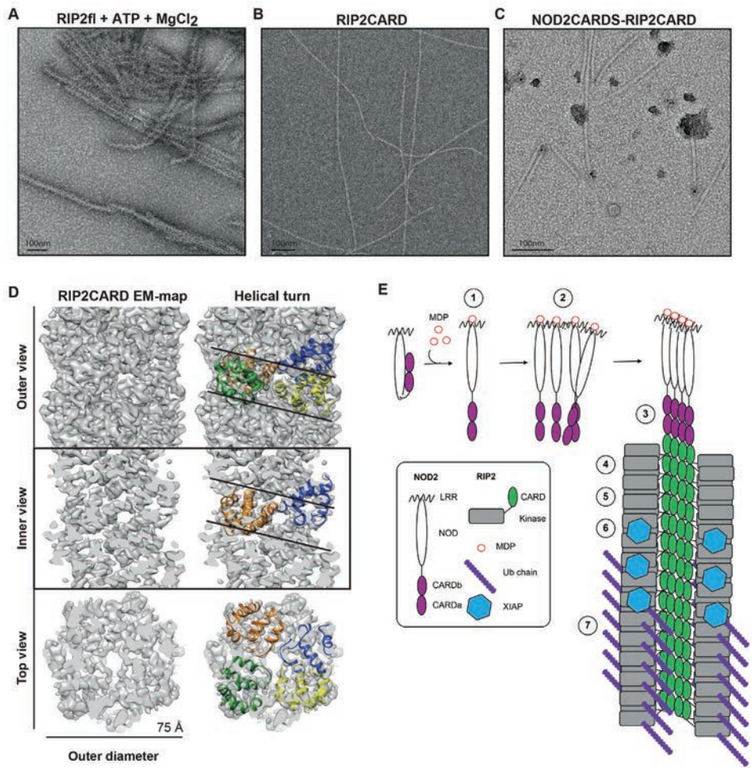


Figure 1. Visualization and structure of RIP2 filaments
A-B) Negative-stain micrographs of (A) RIP2 filament and (B) RIP2CARD filament
C) Negative-stain micrograph showing immuno-gold labeling (black dots) results on the NOD2CARD-RIP2CARD filaments
D) Final cryo-EM map of the three-dimensional RIP2CARD filament. Outer, inner and top view without (left) and with (right) RIP2CARD models fitted into the helical turn.
E) Model of Nodosome assembly, based on our results, published data and analogy to other signalosome systems.

E. Pellegrini (EMBL), A. Desfosses (IBS), and S. Cusack (EMBL)

[1] J. D. Nanson, M.H. Rahama, T. Ve & B. Kobe (2018) Semin. Cell Dev. Biol. (doi.org/10.1016/j.semcdb.2018.05.002)
[2] D. Philpott *et al.* (2014) Nat. Rev. Immunol. **14**, 9-23.
[3] E. Pellegrini *et al.* (2018) Nat. Commun. **9**:4043.

Structure of an enzyme complex essential for the metabolism of the bacterial cell wall of important pathogens

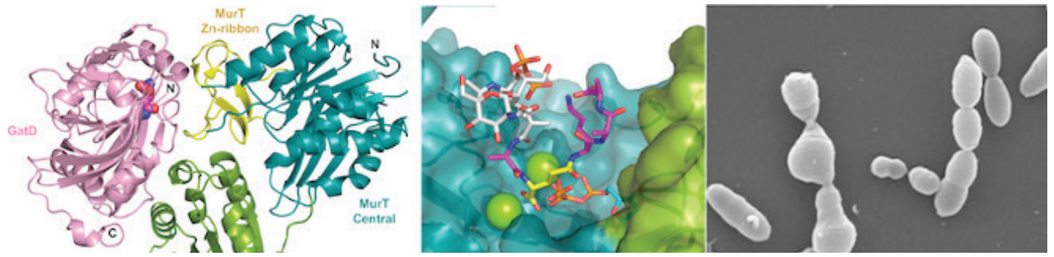


Figure 1. Left: Crystal structure of MurT/GatD. Center: Model of substrates in the active site. Right: Morphological aberrations in *Streptococcus pneumoniae* upon depletion of MurT/GatD.

Bacteria are protected by a sturdy cell wall that is made of a polymer called peptidoglycan. The universality of peptidoglycan in bacteria underlies the broad spectrum of many successful antibiotics that prevent its synthesis (e.g. penicillin). However, in our times of widespread antibiotic resistance, the diversity of peptidoglycan modifications found in different bacterial species offers a variety of potential targets for new drugs with restricted antibacterial spectrum.

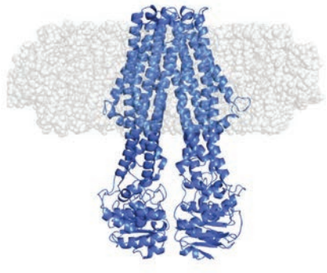
The peptidoglycan is assembled at the bacterial cell surface from a precursor building block which consists of a disaccharide moiety and short peptide stem. In some Gram-positive pathogenic bacteria such as *Streptococcus pneumoniae, Staphylococcus aureus* or *Mycobacterium tuberculosis*, the second residue of the peptidoglycan precursor, D-glutamate, is amidated into iso-D-glutamine by an essential enzyme: the amidotransferase MurT/GatD, which consists of two subunits. The modification conferred by MurT/GatD is the replacement of an oxygen by a nitrogen atom in the peptidoglycan precursor. This seemingly minor difference has important consequences, since strains of pathogenic bacteria created in the laboratory without MurT/GatD cannot survive, demonstrating that MurT/GatD is a potential target for novel antibiotics. In *Streptococcus pneumoniae*, partial depletion of MurT/GatD causes important morphological aberration, indicating that the cell wall is altered.

We have solved the crystal structure of the MurT/GatD complex from *Streptococcus pnuemoniae* at 3.0 Å resolution (Figure 1). The structure reveals that MurT has central and C-terminal domains similar to other Mur ligases with a cysteine-rich insertion, which probably binds zinc, contributing to the interface with GatD. The mechanism of amidation by MurT is likely similar to the condensation catalyzed by other Mur ligases, as was confirmed by site-directed mutagenesis. GatD is a glutaminase providing ammonia from the hydrolysis of glutamine. Ammonia is likely channeled from GatD to the MurT active site through an internal network of cavities. The crystal structure and *in vitro* enzymatic assay that we have set up in this study constitutes a basis for future drug development studies.

C. Morlot and A. Zapun (IBS)

[1] C. Morlot, D. Straume, K. Peters *et al.* (2018). Nature Communications, **9**, 3180.

Stealth nanodiscs – a novel approach for the study of integral membrane proteins



The integral membrane protein MsbA (blue) embedded in a 'semi-transparent' membranous nanodisc (grey). The nanodisc itself is, through selective deuteration, rendered invisible in SANS experiments that focus on the conformation of the protein in its native context.

Membrane proteins are a crucially important class of macromolecule in living systems where they play key roles in signalling, transport, enzyme catalysis. Over 60 % of modern drugs target membrane proteins. Structural studies of integral membrane proteins (IMPs) are extremely challenging, since many of them are inactive or insoluble in the absence of a lipid environment. Using a sophisticated deuterium labelling strategy, the ILL Life Sciences Group and Copenhagen University pioneered an approach making use of stealth carrier nanodiscs that are effectively rendered invisible to low-resolution neutron diffraction by fractional deuterium labelling, enabling structural studies of IMPs in a lipidic native-like solution environment [1]. Now, for the first time, Josts *et al,* have applied this method in a joint small-angle neutron scattering (SANS) and X-ray scattering (SAXS) study of the ATP-binding cassette (ABC) transporter protein MsbA solubilized in the stealth nanodiscs, using the SANS-SAXS platform of the Partnership for Structural Biology (PSB) [2]. The study was carried out by an international group of researchers led by Henning Tidow, University of Hamburg. The data, mostly acquired using the D11 instrument at ILL, has allowed direct observation of the signal from the solubilized membrane protein without contribution from the surrounding lipid nanodisc. Not only was the overall shape of the protein determined but also differences between conformational states of MsbA were reliably detected from the scattering data, demonstrating the sensitivity of the approach and its general applicability to structural studies of IMPs. This is the first account of a structural study of an integral membrane protein using the stealth nanodisc deuteration strategy in conjunction with SANS – an approach that is likely to become increasingly important in the future studies of these difficult but crucially important biological macromolecules.

T. Forsyth and S. Prevost (ILL)

[1] S. Maric *et al.* (2014). Acta Cryst. D, **70**, 317-328.
[2] I. Josts *et al.* (2018). Structure, **26**, 1072-1079.

Seeing 5-HT3 serotonin receptors in action

Ion channels are membrane proteins that under certain conformations (usually called 'open states') form hydrophilic pathways allowing high fluxes of ions through lipid bilayers and therefore control the membrane potential of living cells. Discovering how these proteins go from closed to open states in response to various stimuli is fundamental to understand cellular electrical activity.

Despite an impressive increase in the number of ion channel structures solved in the last two decades, a complete understanding of gating mechanisms at the atomic level is not yet available, because up to now, for most channels, only a single conformation has been obtained.

We tackled this question by solving with cryo-electron microscopy several structures of 5-HT3 serotonin receptors, which belong to the family of pentameric ligand-gated ion channel receptors involved in fast neuro-transmission. The structures reveal how the neurotransmitter serotonin and the anti-emetic drug tropisetron bind to that receptor. The structures also unveil how this receptor moves during its working cycle and therefore help to understand how it operates at the molecular level.

After a long optimisation of the sample preparation performed at the PSB facilities, we could obtain four conformations at resolutions ranging from 3.2 Å to 4.5 Å [1]. For instance, one inhibited conformation reveals the details of the binding site when the antagonist tropisetron is present (Figure 1a left). Another conformation obtained in the presence of serotonin (Figure 1a right) shows how the binding site is re-arranged when the agonist is bound and also features an open transmembrane pore permeant to cations (Figure 1b right). Of the four conformations imaged, the one with the best resolution (3.2 Å) was obtained using data from the ESRF Titan Krios cryo-electron microscope (beamline CM01). In that structure the location of bound serotonin and its interactions can be seen in detail and without ambiguity. Serotonin fits tightly within its binding pocket in an orientation consistent with functional and binding studies, surrounded by a "cage" of aromatic residues (Figure 1c inset).

While it was straightforward to assign the inhibited and one of the serotonin-bound conformations to physiological resting closed and activated open states, respectively, two other "intermediate" structures -obtained in complex with serotonin, but featuring a closed - were more complex to interpret. By integrating the structures with complementary functional experiments, where we measured the electric function of mutant receptors to probe motions in the pore, and with *in silico* molecular dynamics simulations, we propose that these "intermediate" structures correspond to pre-active states.

In 2018 alone, while we solved structures of the 5-HT3 receptors, other researchers elsewhere solved structures of acetylcholine- or GABA-activated cousin receptors [2,3], marking a notable acceleration in the pace of research in this field. While altogether these results will help the rational design of new drugs targeting this important family of receptor, a complete understanding of the molecular mechanism of operation for these ligand-gated receptors still awaits time-resolved structural approaches.

J. Neyton and H. Nury (IBS)

[1] L. Polovinkin, G. Hassaine, J. Perot, *et al.* (2018) Nature, **563**, 275-279.
[2] Walsh RM *et al.* (2018) Nature, **557**, 261-265.
[3] Miller, P. *et al.* (2018) bioRxiv. doi:10.1101/338343

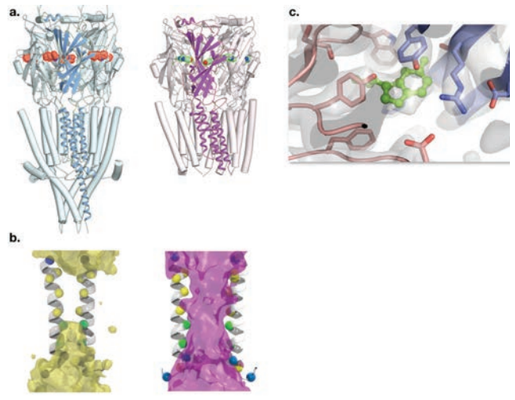


Figure 1: (a) Two of the four conformations obtained by cryo-electron microscopy are shown. The conformation on the left was obtained in the presence of the inhibitor Tropisetron. The conformation on the right was obtained from a subset of particles prepared in the presence of serotonin. (b) A molecular dynamics study shows an interruption of the water column in the pore of the Tropisetron-bound structure, which thus cannot let ions go through. This channel is closed. On the contrary, the serotonin-bound structure keeps a fully hydrated pore throughout the MD trajectory allowing high flux of ions to pass through. This channel is open. (c) Thanks to the high resolution of the structure that was obtained at the ESRF CM-01 microscope a non-ambiguous positioning of the serotonin in its binding site was achieved.

News from the platforms

"Autoflex maX": a new mass spectrometer for sequencing intact proteins

Mass spectrometry (MS) can assess the mass of biomolecules with high accuracy, sensitivity and speed [1-3]. On November 19th, 2018 a state-of-the-art mass spectrometer was installed at the IBS MS platform. Specifically, the new mass spectrometer is a Matrix Assisted Laser Desorption Ionisation (MALDI) time-of-flight (TOF/TOF) instrument and its commercial name is "Autoflex maX" (Bruker Daltonics). The IBS mass spectrometer is the first "maX" installed by Bruker in France.

The use of maX will allow the MS facility to sequence intact proteins, to determine the type, number and position of post-translational modifications (PTMs) and to characterise protein–protein interactions (PPIs). These types of MS analyses are known as "top-down approaches". The facility intends to establish sensitive and accurate MS methods to study both soluble and membrane proteins. The MS-based sequencing method aims to replace the Edman degradation service offered by the IBS protein sequencing platform, which will close at the end of 2018.

After an initial testing phase (i.e., a "set up and development phase"), the "MS sequencing" facility will be available for academics and industry (i) on a fee-per-service basis for routine analyses and (ii) on a collaborative basis.

To conclude, the maX is the first MALDI-mass spectrometer to be installed in the Grenoble area that performs "top-down approach" to sequence proteins and characterise their PTMs and thus represents a novel asset for many local projects.

L. Signor and E. Boeri Erba (IBS)
ibs-plateforme-ms.contact@ibs.fr

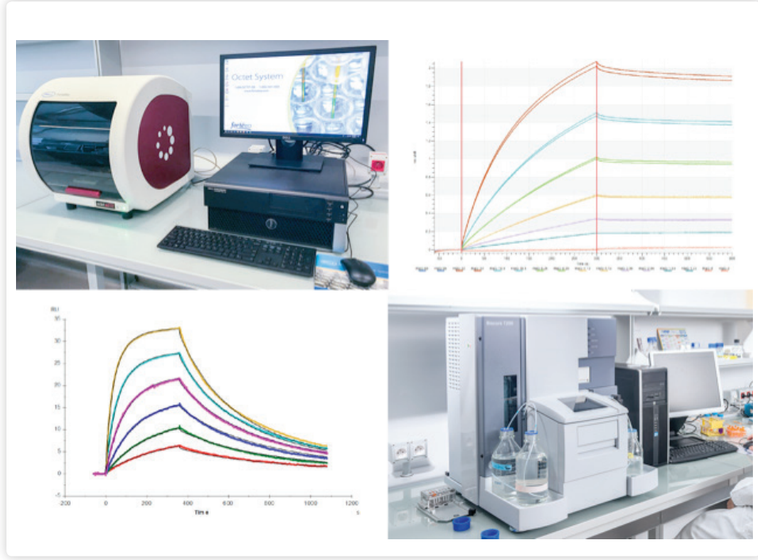
[1] Boeri Erba E, Klein PA, Signor L (2015). *J Mass Spectrom.*, 50, 1114-1119
[2] Boeri Erba E (2014). *Proteomics.* 14, 1259-70
[3] Signor L, Boeri Erba E (2013). *J Vis Exp.* 9, doi: 10.3791/50635.

More information: http://www.isbg.fr/samples-preparation/mass-spectrometry/

Overview of the Matrix Assisted Laser Desorption Ionisation time-of-flight (MALDI-TOF/TOF) instrument installed at the IBS.

The SPR platform is evolving and is becoming the SPR/BLI platform

The ISBG platform dedicated to the characterization of biomolecular interactions by Surface Plasma Resonance (SPR) is evolving to offer access to a new technology for real-time detection and measurement without labelling: the BioLayer interferometry technology (BLI).



Thanks to its two state-of-the-art instruments (a Biacore T200 for SPR and an OctetRED96e for BLI), the platform allows the characterisation of the interactions between biological molecules of very diverse nature: small molecules (> 200 Da), sugars, lipids, peptides, proteins, nucleic acids to more complex objects like liposomes, viral particles and even bacteria and cells. Real-time measurement by SPR or BLI allows accurate determination of the molecule quantities on the sensor surfaces, the monitoring of complex formation and thus, the measurement of the parameters governing it (kinetics rates, affinities, thermodynamics, concentrations).

The platform offers an autonomous service, i.e. users work independently after:
- A meeting organized with the scientific and technical managers to assess the feasibility of the project, to guide the user towards the most appropriate technology, to define the experimental protocols, and to inform the user about the platform's operation usage and costs,
- User training in the use of the instrument, data acquisition and data analysis,
- Reservation by referenced and trained users.

The staff also remains available to assist users in the experimental development and data analysis. Depending on our involvement in the projects, a collaboration can also be envisaged.

The newly installed OctetRED96e system (November 2018) will be available to all PSB users from January 1st, 2019. The Biacore T200 system is still available and opened to everyone.

The overall cost corresponding to the actual use of the instruments will be charged and includes the cost of common consumables provided by the platform (tubes, plates, chemicals and weekly maintenance kits) and the maintenance costs. Sensorchips (SPR) or Biosensors (BLI) and kits specific for the experiments must be purchased and supplied by the user. For PSB users, the costs are directly subsidized (about 50%).

For further information, you can consult the webpages on the ISBG website dedicated to each instrument (http://www.isbg.fr/caracterisations-biophysiques/) or you can contact the platform staff.

J.-B. Reiser & A. Chouquet (IBS, IRPAS Group – ISBG, SPR/BLI platform)

Events

PSB Spotlight on Light Scattering

The Fifth PSB Spotlight meeting on Light Scattering took place on November 20th, 2018.

Around 40 participants attended the morning talks in the Chadwick amphitheater, ILL. Three external speakers (Marguerite Rinaudo – UGA Grenoble, Dan Some – Wyatt Technology Israel and Frédéric Violleau – PURPAN/INP Toulouse) and three local speakers (Caroline Mas – ISBG/IBS, Martha Brennich – EMBL and Sylvain Prevost – ILL) presented the theory of light scattering and new application tools for the study and characterisation of macromolecules.

This included the presentation of exceptional technologies such as CG-MALLS for absolute characterization of biomolecular interactions and the presentation of a unique Field Flow Fractionation centre in Toulouse.

In the afternoon, 18 people participated in the practical session animated by Caroline Mas (ISBG/IBS) and Thierry Azoulay (Wyatt Technology, Paris) on the CIBB biophysical platform.

From left to right, Top: Dan Some, Margerite Rinaudo and Frédéric Violleau. Bottom: Caroline Mas, Sylvain Prevost and Martha Brennich

The purpose of these tutorials was to give an overview of typical sample analysis using Static Light Scatering (analysis of membrane protein) and Dynamic light scattering (using a DLS Dynaproplate reader). The organizers wish to thank all the speakers and participants for making this day a success.

C. Mas (ISBG/IBS)

ADTB-2018 - Advanced Diffraction Techniques for Biology

The ADTB workshop, that took place from the 5th to 9th of November 2018, was dedicated to the newest diffraction techniques in the field of structural biology. It gathered the MXIS workshop organized until 2017 and dedicated to *in situ* diffraction, with special thematic sessions focused on crystallization of macromolecules, serial crystallography at the synchrotron and with XFEL X-ray sources, and electron and neutron diffraction. It therefore covered a large range of techniques and lasted one week.
It included full lectures at the IBS (day 1 and 2), practical sessions at the ESRF beamlines (FIP-BM30A, ID30B and ID23), the ILL, the EMBL and the CIBB computer room (days 3 and 4), with in addition a series of visits of the PSB platforms (day 5).

15 participants from 12 different countries attended this workshop.
ADTB-2018 was the opportunity for participants to discover the extent of possibilities offered by diffraction techniques, such as ligand dry-coating, *in situ* diffraction for structure resolution at room temperature or for ligand screening, diffraction on micro or nano crystals, coupled neutron and X-ray diffraction experiments, etc.

In addition, the ADTB allowed participants to become familiar with crystallization techniques that address current challenges in structural biology, such as growth of uniformly-sized population of small crystals (0.2-10 µm) required for XFEL or electron diffraction or of large single crystals (with volumes ~0.1-1.0 mm^3) required for neutron crystallography, and also to experience crystallization on micro-chips.

This workshop was jointly organized by IBS, EMBL, ESRF, ILL and the CBS Institute (CNRS, Montpellier). ADTB-2018 was made possible thanks to financial support from IBS, EMBL, AFC, Cristech, UGA, Grenoble-INP, and the FRISBI program.

The ADTB organizing committee

Parvis des Sciences 2018

EMBL together with its EPN partners, IBS, ILL and ESRF, participated in the 11th edition of the Parvis des Sciences organized on October 13th at Minatec, Grenoble. This event takes place every year in the context of the National Science festival and aims at showing the public what science is all about.

Several activities were proposed to a large public. For the youngsters, a memory game was created with beautiful pictures of protein crystals and structures, instruments like the latest electronic microscopes. Young children tried their best to cut and fold proteins, while the teenagers had a hard job folding DNA.

Another activity that had a lot of success was the fishing of crystals. Two microscopes were available and people could observe crystals on plates and had to fish them with loops. Once they realized that it's not such an easy task and requires a lot of concentration, a short movie on CrystalDirect was shown to explain how EMBL scientists have improved the process. The Cryo-EM activity enabled the public to observe the 3D structure of proteins. The public was very enthusiastic; in total we received more than 1600 visitors. Special thanks to all the volunteers who enjoyed explaining about their daily work.

E. Bralet (EMBL)

ILL ESS user meeting

The joint ILL/ESS user meeting took place from October 10th to 12th at the world trade center in Grenoble. This was the very first joint user meeting organized by both institutes.

The ILL has been at the forefront of neutron science for the last 50 years and the commissioning of the European Spallation Source (ESS) in the near future strengthens the importance of the field in the European scientific landscape. With 10 parallel focus sessions and workshop events, the 3 day long meeting offered cutting edge scientific talks in all areas of neutron science as well as important technical and instrumental advancements.

510 scientists were present and close to 160 talks (plenary or in the parallel sessions) were given. This first edition was a great success and sets the tone for future meetings. It will undoubtedly strengthen the relationship between both institutes by offering a platform for collaboration, and exchange of ideas. The next edition will be held in Lund in 2020.

E. Mossou (ILL)

'Structural Glycoscience' Summer School

The 4th edition of the workshop entitled "Structural Glycoscience" took place from the 2nd to 4th of July 2018 and was co-organized and hosted both at CERMAV and IBS by Anne Imberty, Cédric Laguri, Franck Fieschi and Annabelle Varrot. It was supported jointly by the Glyco@Alps CDP project from Idex UGA and the H2020-MSCA-EJD network "PhD4GlycoDrug". 37 PhD students and 3 post-doctoral fellows from the glycoscience field and originating from 21 different countries attended. Protein-carbohydrates complexes are key structural elements in cell and in signaling events on their surface. This summer school gave an integrative/multidisciplinary overview on the approaches used to determine and characterize protein-carbohydrate interactions at the molecular and atomic levels. Those included biophysical methods such as AUC, ITC, SPR structural methods: SAXS, microscopy, X-ray crystallography, NMR and molecular modelling. 3 mornings were dedicated to conferences by international and local speakers and 2 afternoons to practicals, posters and visits. The feedback was highly positive both on the contents and organization. Attendees appreciated the wealth and expertise available for glycosciences in Grenoble as well as the visits and demonstrations in particular of IBS plateforms (NMR, microscopy, AUC and SPR) and ESRF beamlines (ID17, ID29, BM29 and BM30A).

A. Varrot (CERMAV)

PSB Student Day 2018

Every year all the students working in structural biology units on the EPN Campus are invited to the PSB Student Day. This event, which is organized by students for students, offers not only the possibility to network and improve one's presentation skills but also to exchange experience and knowledge across the PSB institutes. This year the event took place on 28th June 2018 in the IBS seminar room featuring clip presentations from 1st and 2nd PhD students, and 3rd year PhD students (ESRF: Sylvain Aumonier, ILL: Sarah Waldie, IBS: Rana El Masri, EMBL: Yan Li) presented the interesting results they obtained during their PhD projects. Keynote talks were given by Eva Kowalinski, new Group Leader at EMBL, and Rime Kerfah shared her experience in technology transfer (C.E.O. of NMR-Bio). In addition, PhD students participated in the competition for the best poster and the best flash presentation. Following the assessment of a jury, Tina Uroda (EMBL) was awarded the best poster prize, while the best flash presentation was given to Pauline Juyoux (EMBL) based on an audience vote out of 27 competitors. The event was organized by the PSB Student Committee: Wiktor Adamski (IBS), Sylvain Aumonier (ESRF), Sarah Waldie (ILL) and Joanna Wandzik (EMBL), with help from Florent Bernaudat (PSB). The organizers wish to thank all speakers and participants for this wonderful scientific day and look forward to seeing everyone next year.

W. Adamski (IBS)

PSB CryoEM school

The first PSB CryoEM school took place on 14-20 June 2018 on the EPN campus. This one week course consisted of both lectures focusing mainly on single particle analysis, but other cryo-electron microscopy techniques were also described (cellular EM; FIB-SEM; electronic micro diffraction, tomography), and of practical sessions on sample preparation, data collection and data analysis. The morning lectures were given in the Chadwick amphitheatre in order to be open to everyone (30-40 attendees on average) but the practicals were restricted to a selection of participants composed of students and postdocs from the PSB as well as from other institutes in Grenoble. The course also involved 17 instructors from the IBS, EMBL and ESRF.
The organizers wish to thank all the instructors and participants for making this first edition of the school a success and the course is likely to be reorganized in the near future.

F. Bernaudat (PSB Coordinator)

PROFILE

Sigrid Milles

Sigrid Milles is a CNRS researcher in the Flexibility and Dynamics of Proteins (FDP) Group at the IBS. She has secured an ERC starting grant (1.6M€) for 5 years to study intrinsically disordered proteins involved in endocytosis by single-molecule fluorescence and nuclear magnetic resonance spectroscopy.

Could you tell us a little bit about your scientific background and career prior to your arrival at the IBS 5 years ago?

I was interested in different types of sciences, so I decided to study biophysics in Berlin. I had studied French at school and loved Paris, so I didn't think twice when I was offered the opportunity to do a 6-months internship in a biochemistry lab in Paris as part of the Erasmus programme. During my studies, I also worked part-time in a biophysics laboratory in Berlin and that is actually how I got into science. The lab was in the same building as where I had most of my classes, so in between lectures I could go to develop western blots and take care of my cells…. I also did my master thesis in this lab in fluorescence and came across a few people who did spectroscopy and were developing their own machines - this really fascinated me.

This is what then led me to Edward Lemke's lab at EMBL Heidelberg for a PhD. At the time they were a brand new group setting up a single-molecule spectroscopy laboratory. So we started completely from scratch, both in the wet lab and in terms of microscopy, and I was the first PhD student, so this was a great training opportunity. It was during my PhD that I became interested in intrinsically disordered proteins or IDPs (i.e. proteins with no secondary structure). The lab worked on various IDPs involved in the Nuclear Pore Complex (NPC). My project was centred around intrinsically disordered nucleoporins, so I was doing a lot of wet lab, but mainly spectroscopy and I was also responsible for the instrumentation of our single molecule fluorescence spectrometer.

After my PhD, I wished to learn complementary methods that I could apply to the study of IDPs. I wanted to gain more insight into the dynamics of the protein backbone (eg. whether there's a transient alpha helix, or whether the stiffness of a given region changes). Fluorescence is useful for long range measurements, but not for the dynamics at the scale of the individual residues. This is what brought me to NMR and to Martin Blackledge's lab here in Grenoble.

You have a background in Biophysics, how difficult was it to be involved in methodology and instrumentation development during your PhD?

That was actually completely fine. I was a little worried about the instrumentation part to start off with, however, I was very familiar with the theory behind it - actually I was much more familiar with this than the wet lab part! - I only started learning cloning and protein expression during my PhD. The projects that I had worked on in Berlin were pure biophysics and spectroscopy projects, so I had more knowledge in the spectroscopy side than the biochemistry side when I started my PhD. Looking back on it, I am very happy that I had the opportunity to learn both as this gives me a better grasp and liberty when building and conducting a project from scratch.

The topic of your ERC grant is fairly different from the work you have done in the past. How did the idea for the project come along?

I worked on several biological topics during my PhD and my postdoc, so I wasn't really fixed on a specific biological topic. I knew I wanted to work on IDPs and I knew I wanted to combine single-molecule fluorescence and NMR. I also really wanted to look at structure and dynamics inside the cell, which is in theory possible with both techniques, but single-molecule fluorescence is particularly well suited to study proteins at low concentrations and hence to work in native conditions. So I looked for a topic fulfilling all these conditions, on which there hadn't been a lot of research done already and that was independent, but nevertheless related, from the ones I had worked on. That's how I came across clathrin-dependent endocytosis, which is a highly regulated process responsible for the entry of molecules into eukaryotic cells. A lot of IDPs are involved in this process and they notably play very important roles in regulating the onset of endocytosis.

I first had the idea of this project about 2 years before starting to write the ERC. At the time, I didn't know I would apply one day for an ERC grant. I started with a few ideas, and it really developed very slowly, especially as I come from a more technological background, but the more I read about endocytosis the more I thought that this was a very interesting biological project.

How has the PSB and in particular the platforms helped you in your research?

The platforms are absolutely crucial for an interdisciplinary project like mine. I have obviously been using the NMR spectrometers at IBS, the biophysical platform intensively (in particular fluorescence lifetime spectroscopy). I also interact with people at the ESRF through the SAXS beamline. The PSB and the access to the platforms is one of the main reasons why Grenoble is a very attractive place to carry out this project.

Do you see where you are going beyond the ERC?

I would like to stay in academia, set up a successful team that is sustained, become known in combining these 2 techniques. If I could become established in the field of endocytosis in addition, it would be fantastic. However, it is clear that my lab will be very methodology-oriented. In the future, I hope to establish solid collaborations with endocytosis specialists and in particular with cell biologists and imaging groups.

What advice would you give a young researcher looking to apply to an ERC?

Find a project that you are really passionate about. Apply for an ERC not because you want to apply for an ERC, but because you have an idea and a project that you think is great. Then think thoroughly about the project and ask yourself critical questions. For me, rehearsing my audition many times with people with different backgrounds helped me tremendously to clarify my ideas and express them in a language that was understandable to everyone. I made 14 different versions of my interview presentation and changed the last one 2 days before the interview.

What would you say your best quality is?

I am very passionate, I enjoy going to work, and I really like talking about science. Science lives from people talking to each other and that's why those PSB events are so important.

E. Mossou (ILL) & J. Timmins (IBS)

NEWCOMERS

Sagar Bhogaraju
Has joined the EMBL Grenoble as a group leader in October 2018. Using structural and biochemical approaches, his team will study the role of ubiquitin signaling in human physiology and disease. Sagar obtained his PhD at Max Planck Institute of biochemistry, Munich, Germany where his research focused on understanding the process of protein transport in eukaryotic antenna like organelle called cilium. He went on to pursue postdoc in the lab of Prof. Ivan Dikic in Frankfurt, Germany where he focused on cellular mechanisms of ubiquitination especially focusing on atypical ubiquitin ligases from pathogenic bacteria such as *Salmonella* and *Legionella*.
Contact: bhogaraju@embl.fr

Shibom Basu
Has joined the synchrotron crystallography team at the EMBL in December 2018. He will contribute to the operation and development of methods for micro- and serial-crystallography on the ESRF beamline ID23-2 and participate in the ID29 upgrade project. Shibom obtained his PhD in Structural Biology on time resolved crystallography using X-ray free electron lasers from Arizona state university (US). This was followed by a postdoc in the MX group at the Swiss Light Source, where he worked on experimental strategies and data processing methods for conventional and serial crystallography.
Contact: shbasu@embl.fr

Noredine Mahdjoub
Has joined EMBL Grenoble in July 2018 to become the new service technician of the CIBB building. Noredine is a service technician in automation and industrial electricity by training, and previously he has worked as such in the industrial sector (Schneider élec.) and the tertiary sector (Crédit Agricole Rhône Alpes HQ), but also as a technical manager in the health sector (Clinique du Mail) and the shopping centre Grand Place.
Contact: nmahdjoub@embl.fr

Ebru Oner
Has joined the laboratory service team of the CIBB building in September 2018. As a laboratory steward, she takes care of the washing and sterilisation of laboratory glassware and material, but also helps to manage the CIBB common store. Previously, Ebru has worked similar positions at the Institute for Advanced Biosciences (IAB) and the Institut de Biologie Structurale (IBS)
Contact: ebru.oner@ibs.fr

DATES FOR YOUR DIARY

February 4th to 6th 2019 - ESRF User meeting

The ESRF User Meeting 2019 will be held on the EPN campus during the first of February. Monday 4 February: tutorials - Tuesday 5 February: User Meeting plenary session - Wednesday 6 February: 3 microsymposia (UDM) including "ID29: Tunable past and time-resolved future". More information and registration details: http://www.esrf.eu/UM2019

February 11th 2019 - PSB Student Day

The 10th edition of the annual PSB Student Day will take place this year on Monday 11 February in the Chadwick amphitheatre. More information will be sent by mail and on: http://www.psb-grenoble.eu

March 11th 2019 - PSB seminar

PSB Seminar by Janet Iwasa (University of Utah) in the Chadwick amphitheatre. Janet Iwasa is a molecular animation and data visualization expert, renowned for her contributions to molecular and cellular visualizations. More information on: http://www.psb-grenoble.eu

March 19th 2019 - PSB Spotlight on Imaging

The PSB Spotlight on Imaging will take place on 19 March on the EPN campus. This meeting will present the use and combination of various microscopy and tomography techniques for biological studies. Practical sessions on various techniques (AFM, super-resolution microscopy, cryoEM, X-ray/neutron tomography, ...) will also be offered. Two other PSB Spotlight meeting will be organised this year on Macromolecular Interactions (Spring 2019) and Mass Spectrometry (Autumn 2019). More information and registration details: http://www.psb-grenoble.eu

March 26th to 29th 2019 - Advanced isotope labelling Methods for Integrated Structural Biology at the EPN Science Campus in Grenoble.

The AILM workshop will focus on the development of isotopic labelling techniques and their application to the study of biomolecular structure and dynamics. More information and registration details: https://www.ailm2019.org

April 2nd- 3rd 2019 - PSB Scientific Advisory Board review

The PSB Scientific Advisory Board (SAB) review will take place on 2-3 April 2019. The SAB is an international committee composed of eminent scientists in the field of structural biology and the current members are: Jean Cavarelli (Institut de Génétique et de Biologie Moléculaire et Cellulaire, FR), Robert Gilbert (U. of Oxford, UK), Guillermo Montoya (Copenhagen U., DK), Helen Saibil (Birkbeck College, UK), Titia Sixma (Chair, Netherlands Cancer Institute, NL), Vladimír Sklenář (Mazarik U., CZ), Jeremy C. Smith (U. of Tennessee/ORNL, USA) and Henning Stahlberg (Biozentrum - U. of Basel, CH).

May 27th to June 7th 2019 - Spring school entitled 'Biology at different scales, an interplay between physics and biology', in Les Houches, France.

The school is addressed to graduate students and is at the interface between biology and physics. More information and registration details: http://www.leshouches.strikingly.com.

July 4th- 5th 2019 - PSB Symposium 'Macromolecules in Action'

This meeting aims to illustrate how major biological questions can be resolved in structural biology through the application of interdisciplinary approaches, enhancing our understanding of the dynamic behaviour of macromolecular complexes in the cell. More information and registration details: http://www.esrf.eu/psbsymposium

ANNOUNCEMENTS

Cécile Morlot (IBS/Pneumococcus group) is the recipient of a bronze medal of the CNRS 2018. This distinction rewards her on-going and fruitful research activity, which makes her a specialist with talent within a particular research field. Cécile Morlot studies bacterial morphogenesis and division, using structural biology approaches combined with super-resolution fluorescence microscopy.

The European Research Council (ERC) has awarded a "Starting Grant" to **Sigrid Milles** from the 'Flexibility and dynamics of proteins' group at the IBS for her project 'MultiMotif'. This ERC project aims at developing an integrated approach using single molecule fluorescence and NMR spectroscopy to study intrinsically disordered proteins and understand the molecular mechanism by which linear motifs regulate the process of endocytosis.

On November 27th 2018, ESRF celebrated its 30th anniversary in the presence of the representatives of its 22 partner countries. On December 10th, the ESRF entered a 20-month shutdown, during which its flagship storage ring will be dismantled to make way for a revolutionary X-ray source, an Extremely Brilliant Source (EBS), open for users in 2020.

On August 22nd 2018, former ESRF Director of Research, **Sine Larsen**, was awarded the Max Perutz Prize from the European Crystallographic Association (ECA). This award comes in recognition of her multi-faceted contributions to crystallography, including crystal structure analyses of organic molecules and proteins, charge density studies, and the development of synchrotron radiation facilities.

Edith Heard, Professor at the College de France and Director of the Genetics and Developmental Biology Unit at Institut Curie in Paris, has been appointed as the fifth Director General of EMBL. Her mandate will start on January 1st 2019. Edith Heard's areas of research include epigenetics and developmental biology and she has strong expertise in chromosome and RNA biology.

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The Partnership for Structural Biology (PSB) is a collaboration between a number of prestigious European and French scientific laboratories in Grenoble. The PSB is unique in combining world leading user facilities for synchrotron X-ray and neutron scattering with NMR, electron microscopy, molecular biology and high throughput techniques on a single site together with strong projects in a broad range of structural biology.