JANUARY 2018 - NUMBER 20

PSB et al

Partnership for Structural Biology Newsletter

Scientific highlights

A structural snapshot of bacterial elongation

The bacterial cell wall is essential for survival, and the peptidoglycan (PG) is one of its most important components. PG plays a key role in cell shape maintenance, resistance to osmotic pressure, and cell division; not surprisingly, its biosynthetic machinery has been a preferential target for antibiotic development for decades [1]. Proteins that are involved in PG biosynthesis associate into multi-membered complexes that regulate cell division and elongation, and their inhibition or deregulation can lead to defects in cell shape and often cell wall lysis and death [2].

Penicillin-Binding Proteins (PBPs) catalyze the two last reactions in PG biosynthesis and interact with several members of the cell division and elongation complexes during the bacterial cell cycle [3]. One of these partners is MreC, a membrane-associated protein that forms fibers in certain bacteria and is thought to serve as a scaffold for the cell wall elongation complex. However, since proteins associate and dissociate according to the phase of the cell cycle, these interactions are fleeting, and their complexes are fragile and difficult to isolate in the lab. Here, we have overcome these difficulties and isolated the PBP2:MreC core elongation complex from the human pathogen *Helicobacter pylori* [4]. We solved the structure of both the complex and of the apo form of PBP2, with all data being collected at the ESRF. In the apo structure of PBP2, the N-terminal region (blue and red in Fig. 1) is 'closed', mimicking two clasped hands. It turns out that in the complex, however, this region opens through a 'hinge' that moves by approximately 80 Å, permitting binding of two MreC 'butterflies'. This conformational modification reveals a highly hydrophobic platform that, when associated to MreC, forms a hydrophobic zipper (Fig. 2).

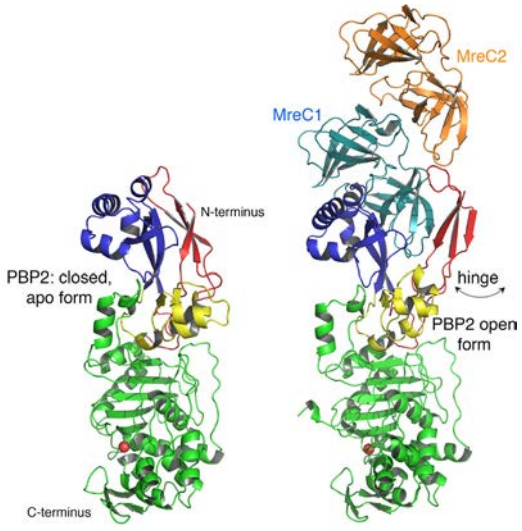


Figure 1. Architecture and structural arrangement of PBP2 and the PBP2:MreC complex from *Helicobacter pylori*. In the structure of the complex, the N-terminus of PBP2 (red and blue) opens through a hinge region, exposing a previously hidden hydrophobic region that allows binding of MreC.

We first verified the importance of the stability of the zipper by introducing mutations and testing the interaction *in vitro*. Isothermal titration calorimetry (ITC) experiments were performed using the biophysical platform of the ISBG with the help of Caroline Mas, and indicated that MreC forms that carried mutations in the hydrophobic zipper did not bind to PBP2. Subsequently, *H. pylori* cells that were modified to express the same mutations in their genome were shown to lose their characteristic elongated shape and rapidly increase in diameter, indicating that the PBP2:MreC hydrophobic zipper is essential for shape maintenance in bacteria.

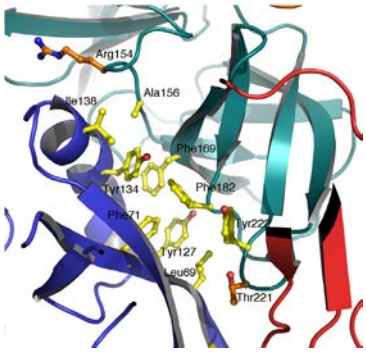


Figure 2: Details of the interaction region between PBP2 and MreC. The 'hydrophobic zipper' is essential for complex stability and maintenance of bacterial cell shape.

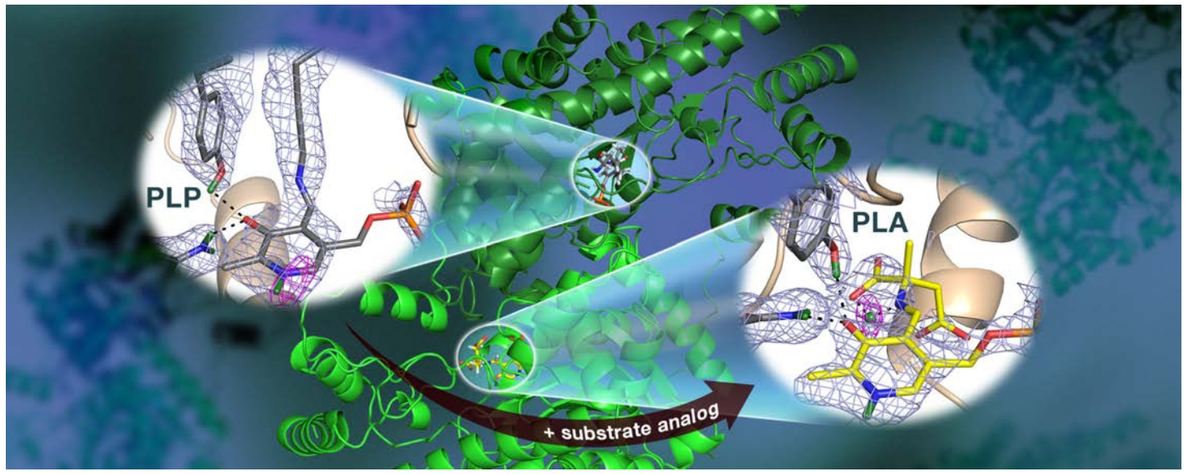
This work thus provides the first visualization of the core region of a bacterial elongation complex, and reveals a novel region that could be targeted for the development of antibiotics that act by inhibiting cell wall elongation. This could lead to the generation of totally new molecules that are structurally distinct from antibiotics used in clinics now, that face severe problems of resistance.

A. Dessen and C. Contreras-Martel (IBS)

[1] L. Silver. (2013) *Ann. N. Y. Acad. Sci.* **1277**, 29-53.
[2] T. den Blaauwen, *et al.* (2008). FEMS Microbiol. Rev. **32**, 321-344.
[3] P.-J. Matteï, D. Neves, & A. Dessen. (2010). *Curr. Opin. Struct. Biol.* **20**, 749-766.
[4] C. Contreras-Martel, A. Martins, C. Ecobichon, D. Maragno Trindade *et al.* (2017) Nature Commun. **8**, 776.

CONTENTS

Neutrons observe vitamin B6-dependent enzyme activity useful for drug development



Neutron crystallography was used to study aspartate aminotransferase (AAT), a vitamin B6-dependent protein. The structure showed that the chemical reaction had occurred in only one active site and revealed the positions of critical H-atoms, including a low-barrier H-bond that may be crucial for catalysis. $2F_o$–F_c nuclear scattering length density maps are shown as light blue mesh, omit F_o–F_c nuclear scattering length density maps (magenta mesh) highlight the positions of critical H-atoms. [Credit: J. Hemman/A. Kovalevsky, Oak Ridge National Laboratory, U.S. Dept. of Energy]

This article presents a neutron study of a vitamin B6-dependent protein performed on the ILL LADI-III protein crystallography instrument [1]. This study has the potential to open avenues for new antibiotics and drugs to battle diseases such as drug-resistant tuberculosis, malaria and diabetes. The neutron crystallographic study allowed the location of hydrogen (H) atoms in aspartate aminotransferase, or ATT, an enzyme vital to the metabolism of certain amino acids, to be determined.

Vitamin B6-dependent proteins are part of a diverse group of enzymes that conduct over a hundred different chemical reactions in cells. The enzymes are of interest to biomedical, as well as bioenergy, researchers because of their role in metabolizing amino acids and other cell nutrients.

These enzymes each perform a specific chemical reaction with exquisite accuracy, while suppressing other viable chemical transformations. Their mechanism of action is not well understood, but it is of great significance for drug design.

The team's previous research predicted that H-atoms move in and around the enzyme's active site, where the chemical reaction takes place, indicating that the positioning of the H-atoms controls the reaction type. Knowing the precise location of H-atoms can explain why the behaviour of these enzymes is so specific, but hydrogen atoms positions are difficult to detect using standard methods such as X-ray crystallography. To directly determine the positions of H-atoms within AAT, we therefore turned to neutron diffraction techniques.

AAT crystals were exposed to neutrons using the LADI-III beamline at the Institut Laue-Langevin. Surprisingly, a reaction within one AAT monomer was observed while the other AAT monomer was unchanged, providing a before-and-after perspective of the enzyme-catalyzed chemical reaction.

The data revealed that in one of the enzyme's monomers the covalent bonds reorganized after a chemical reaction that occurred in the active site whereas, in another, the reaction had not taken place. Essentially, we were able to obtain two structures in one crystal, which has never been done before for any protein using neutrons.

With this knowledge, we will run molecular simulations to determine the hydrogen atoms' specific behaviour when interacting with the enzyme. The results could be useful in guiding the future design of novel medicines against multidrug-resistant tuberculosis, malaria, diabetes and antibiotic-resistant bacteria.

This study highlights how neutrons are an unrivalled probe for identifying the location of hydrogen atoms in biological systems, providing us with an unprecedented level of structural detail for this important enzyme.

M. Blakeley (ILL) & A. Kovalevsky (ORNL, USA)

[1] S. Dajnowicz *et al.* (2017). Nature Comm., **8**, 955.

The neuronal guidance receptor Robo1 forms inactive dimers in the absence of Slit2

An enduring mystery of our central nervous system (CNS) is how the precise wiring of so many neurons into complex functional neuronal circuits is achieved. The CNS of bilateral species is especially complex, requiring a connection between the two symmetric halves. Here, a specialised group of neurons, the so-called 'commissural neurons', must navigate along specific routes and cross the midline, or bilateral division, before continuing to their intended target (Figure 1). For this connection, the leading edge of growing neurons use specific cell surface receptors to sense their environment for guidance cues to enable their successful navigation along complex paths. At the midline, commissural neurons require the Roundabout (Robo) receptor, and its guidance ligand, Slit, to achieve this feat and prevent any re-crossing. Robo receptors are large multi-domain single pass membrane proteins that require binding of Slit for activation. Until now it was largely unknown how this binding was transmitted across the cell membrane.

Robo1 is composed of five immunoglobulin (Ig), three fibronectin (FnIII) domains, and a largely unstructured intracellular C-terminal region. By combining X-ray crystallography and biological small angle X-ray scattering (bioSAXS) with a negative stain electron microscopy (EM) reconstruction we were able to propose a molecular mechanism for Slit induced Robo signaling [1]. Here, access to the EMBL HTX crystallization facility, the joint structural biology group beamlines, and the EM and biophysical platforms of the PSB were critical to tackle this difficult structural biology problem.

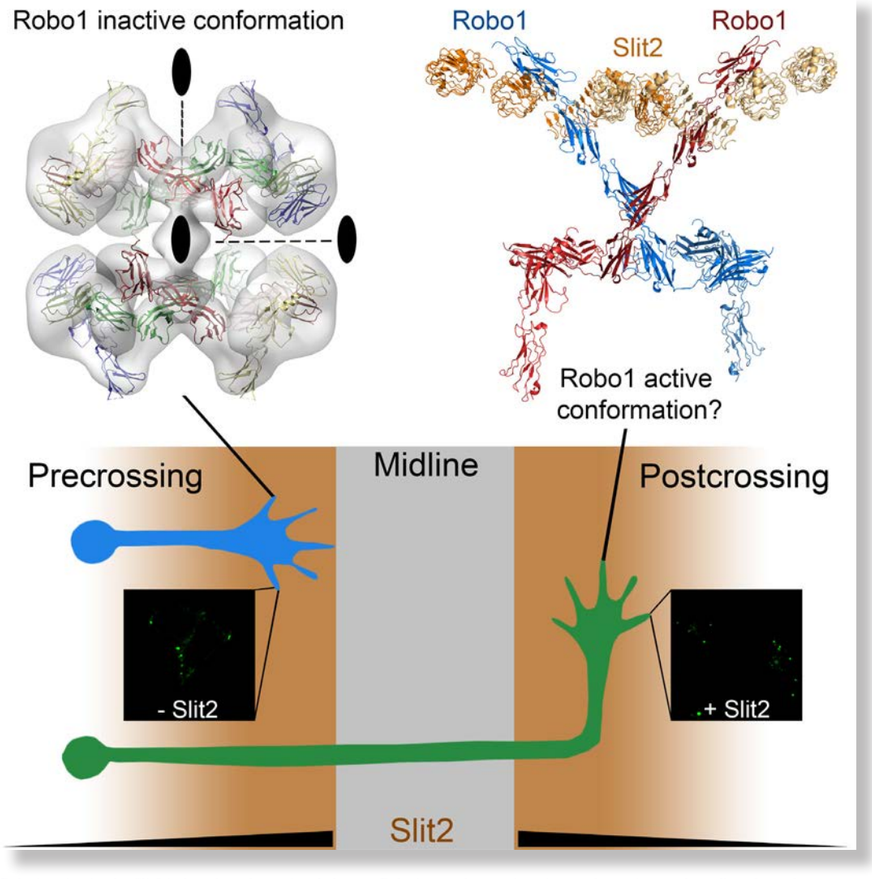
In collaboration with Irina Gutsche's group at the IBS, who performed the negative stain EM reconstruction of the Robo1 ectodomain, we observed that Robo1 forms a tetrameric assembly (Figure 1). The low resolution EM reconstruction enabled us to generate a Robo1 model by fitting the X-ray structure of the Ig1-4 domains, a bioSAXS model of the Ig1-5 domains, and a previous crystal structure of the Fn2-3 region [2]. We observed that Robo1 adopts a compact dimeric conformation mainly mediated by the central Ig domain region that is inconsistent with Slit binding, most likely representing an inactive conformation.

These inactive dimers further oligomerise in a 'back to back' fashion to generate a 'dimer of dimers' assembly reminiscent of cell-cell contacts. Complementary fluorescence light microscopy experiments further showed that Robo1 does not undergo any oligomeric change upon Slit2 binding (Figure 1).

Taken together these results are consistent with a mechanism in which Robo1 undergoes a conformational change upon Slit2 binding for signaling (Figure 1). Future work will be required to better understand the structural changes Robo1 undergoes upon Slit2 binding. Because the Slit-Robo signaling pathway has been hijacked in several cancers the inactive Robo1 conformation we observe could be exploited for the development of therapeutics stabilising this interaction.

A. McCarthy (EMBL)

[1] Aleksandrova *et al.* (2017) Structure. DOI: 10.1016/j.str.2017.12.003
[2] Barak *et al.* (2014) J. Struct. Biol. **186**, 283-291.



Robo1 activation by Slit2 induced conformational change. Robo1 can adopt an inactive conformation on the cell surface of commissural neurons as they approach the midline, as determined by a negative stain EM reconstruction. Following exposure to an increased Slit2 concentration at the midline Robo1 does not undergo a change in oligomerization state as previously thought, as shown by light microscopy. A Slit2 induced conformational change of Robo1 is most likely required for activation to prevent re-crossing. Our proposed model is based on the determined Robo1 Ig1-4 X-ray structure.

A blue light-driven enzyme produces hydrocarbons

To date, if we exclude the photosynthetic reaction centers, there are only two families of enzymes using light as a source of energy to perform their catalytic activity: the light-dependent protochlorophyllide reductases and the DNA photolyases. The discovery of the fatty acid photodecarboxylase (FAP) [1] adds a third member to this list of light driven enzymes (Figure 1). The FAP was identified in the green microalgae *Chlorella variabilis* NC64A as a member of the glucose-methanol-choline (GMC) oxidoreductases, a family of flavoproteins comprising a wide range of oxidases and dehydrogenases.

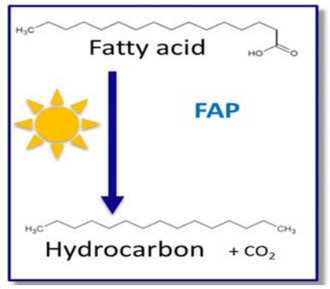


Figure 1. Reaction catalyzed by the fatty acid photodecarboxylase (FAP). The enzyme is not active in absence of light.

The gene encoding for FAP was expressed in *E. coli* and the recombinant protein was purified to further characterize its hydrocarbon-forming activity. The polypeptide chain contains 654 amino acids for a molecular weight of ~69 kDa. Mass spectrometry analysis revealed the presence of a flavin adenine dinucleotide (FAD) cofactor, as observed in other characterized GMC oxidoreductases. Characterization of the decarboxylase activity of the recombinant protein shows that it is optimum around pH 8.5 with a higher affinity for substrates with C16-17 chains. The catalytic activity stops immediately after switching from blue light to red light.

The purified FAP was crystallized in monoclinic space group and a complete data set was collected on the automated beamline MASSIF-1 at ESRF. The structure was solved by molecular replacement using domains from two GMC oxidoreductases (*E. coli* choline oxidase and *Aspergillus flavus* glucose dehydrogenase) at 3.15 Å resolution. The overall structure is folded in two domains. The first domain stabilizes the FAD cofactor and the C-terminal domain participates in the substrate binding. A narrow tunnel, mainly hydrophobic, links the solvent to the FAD cofactor and a C16 saturated fatty acid has been modeled in the electron density with its carboxylate moiety pointing towards the FAD cofactor (Figure 2).

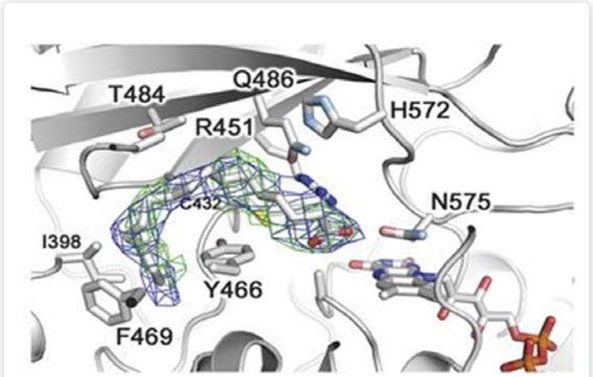


Figure 2. Details of the palmitic acid–binding site with the side chains of residues within 4 Å of the substrate shown in stick. The omit map electron density associated to palmitic acid is also shown and contoured at 0.5σ ($2F_o$-F_c; blue) and 2σ (F_o-F_c; green).

Time-resolved optical spectroscopy studies in correlation with the three-dimensional structure lead to a catalytic mechanism where FAD is excited by blue light to reach an excited state. This intermediate state abstracts an electron from the carboxylate group of the fatty acid substrate stabilized in the hydrophobic tunnel. The radical fatty acid would then decarboxylate spontaneously and the alkane would be released before the entrance of a new substrate.

D. Nurizzo (ESRF)

[1] D. Sorigué, B. Légeret, S. Cuiné, S. Blangy *et al.* Science. 357 903-907 (2017); doi: 10.1126/science.aan6349.

News from the platforms

CM01, the Cryo-electron microscope at the ESRF

The new state-of-the-art Titan Krios cryo electron microscope (cryo-EM) was officially inaugurated on November 10th, 2017 in the presence of former Nobel laureate Ada Yonath, representatives of the local and national authorities and the directors of all 4 institutes located on the EPN campus. This latter aspect nicely reflects the fact that scientists from all EPN institutes will jointly operate the microscope: Eaazhisai Kandiah (ESRF), Gregory Effantin (IBS), Michael Hons (EMBL) and a scientist from the ILL.
During the last few years, cryo-EM has undergone a change that has been described as the "resolution revolution" [1]. During this time, the average resolution for cryo-EM determined structures deposited in the Protein Data Bank has fallen from 15 - 20 Å to around 8 Å with many of the most recent depositions exhibiting 'atomic' resolution (i.e. 3 – 4 Å). The Titan Krios microscope, equipped with a Quantum LS imaging filter coupled to a K2 Summit direct electron detector and a Volta Phase Plate will be used exclusively for single particle cryo-EM experiments and complements the techniques already offered by the ESRF to its international Structural Biology user community. In particular, combining high-resolution structures of individual components obtained by X-ray diffraction experiments with data from cryo-EM experiments, will help to better answer questions related to structure and function of larger molecular complexes and membrane proteins. The 1st user experiment was carried out on CM01 on November 24th, 2017 and the microscope is in full user operation since then.

The microscope will be operated in the same way as the ESRF Structural Biology group MX and BioSAXS beam lines with the exception that access will be via Rolling Access proposals evaluated by the ESRF's external beam time allocation panel. Proposals can be submitted at any time during the year and an additional advantage for ESRF users is that the microscope will stay operational during the long ESRF-EBS shutdown. Initially, only samples that have been pre-characterised and for which frozen grids have been prepared and are available will be accepted for experiments (see http://www.esrf.fr/home/UsersAndScience/Experiments/MX/About_our_beamlines/CM01.html for details). At a later stage, less advanced experiments can also be accepted. This is possible thanks to the availability of laboratory space and a Vitrobot® to prepare grids but more importantly, due to the availability of a PSB cryo-EM platform composed of microscopes located at the IBS (Polara, F20 and T12), the EMBL Grenoble (T12) and the Titan Krios. This service is currently being set in place and carefully tested before it will be officially opened to a wider scientific community foreseen in the second half of 2018.

C. Mueller-Dieckmann (ESRF), G. Schoehn (IBS)

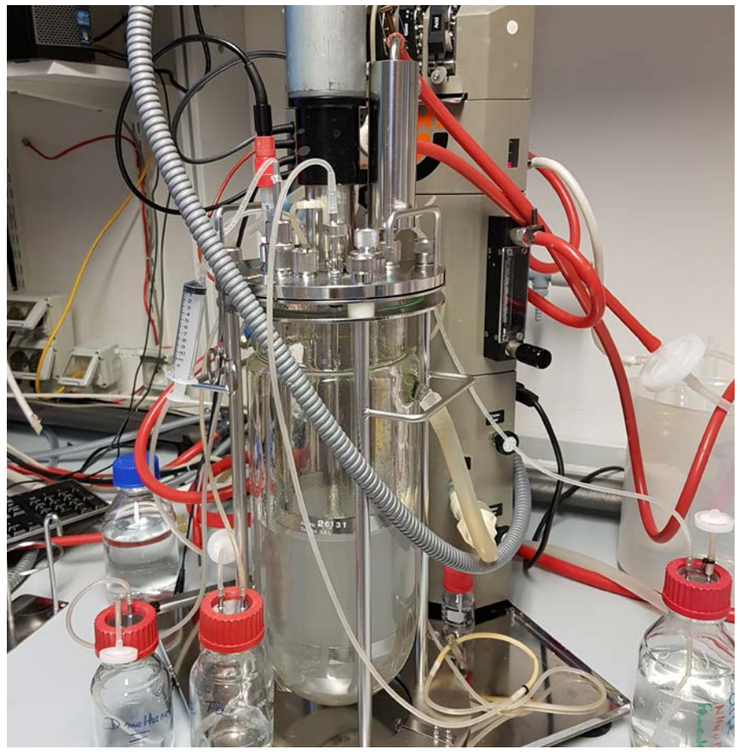
[1] Callaway E. The revolution will not be crystallized: a new method sweeps through structural biology. Nature. 525(7568):172-4 (2015). doi: 10.1038/525172a

The traditional ribbon was cut by the Directors of EPN campus institutes, representatives of French national, regional and local authorities and A. Yonath. **Credits: ©ESRF/P. Jayet**

The CM01 team with, from left to right: Guy Schoehn (Cryo-EM platform leader, IBS), Grégory Effantin (Scientist, IBS), Michael Hons (Scientist, EMBL), David Flot (BLOM, ESRF), Isai Kandiah (Scientist, ESRF), Christoph Mueller-Dieckmann (Principal Beamline Scientist, ESRF). **Credits: ©ESRF/S. Candé**

Heavy hydrogen cholesterol for Neutron Scattering studies and Raman microscopy

Cholesterol - best known as something to avoid - is a major component of mammalian cells playing a critical role in permeability, rigidity and membrane dynamics and contributes to specific membrane structures such as lipid rafts. Cholesterol is also a main cargo in lipoprotein complexes (i.e. LDL, HDL) and is directly implicated in several pathogenic conditions such as coronary artery disease. Neutron scattering studies on membranes or membrane protein complexes exploiting contrast variation have up to now been limited by the lack of availability of fully deuterated (perdeuterated) cholesterol.



A perdeuterated high cell-density culture carried out in the ILL-Deuteration Laboratory platform

In collaboration with Harald Pichler's group from the Institute of Molecular Biotechnology in Graz, Austria, the Deuteration Laboratory in ILL's Life Sciences group has developed a protocol for *in vivo* production of perdeuterated recombinant cholesterol in lipid engineered *Pichia pastoris*. Complete deuteration of cholesterol via chemical synthesis is challenging and has never been described. On the other hand, cholesterol cannot be isolated from mammalian cells grown in deuterated media due to the high toxicity of deuterium for these type of cells. The strategy we applied was to use yeast cells, which can be adapted and grown in deuterated minimal media. However, yeast cells in contrast to animal cells do not synthesize cholesterol, but a related sterol, ergosterol. The Graz collaborators succeeded in lipo-engineering *Pichia pastoris* through multiple gene insertions and gene knock-outs in a way that their recombinant system produces cholesterol instead of ergosterol [1]. In the ILL Deuteration Laboratory this lipo-engineered yeast strain has been adapted to growth in deuterated minimal medium and used in high cell density fermenter cultures to incorporate deuterium from heavy water and deuterated glycerol into cholesterol with yields sufficient for structural studies. Perdeuteration of the purified cholesterol was verified by mass spectrometry [2]. The deuterated molecule has since been produced by the Deuteration laboratory platform for several peer-reviewed proposals and used successfully in neutron scattering experiments [3]. Besides its usefulness for neutron work the perdeuterated cholesterol is being evaluated for its use in Raman microscopy to study cellular lipid uptake.

M. Moulin and M. Haertlein (ILL)

[1] M. Hirz, *et al.* (2013). Appl Microbiol Biotechnol 2013, **97**, 9465-9478
[2] M. Moulin *et al.* Perdeuteration of cholesterol for neutron scattering applications using recombinant Pichia pastoris, Chemistry and Physics of Lipids under review.
[3] S. Waldie *et al.* (2017) Localisation of Cholesterol within Supported Lipid Bilayers Made of a Natural Extract of Tailor-Deuterated Phosphatidylcholine. Langmuir in press

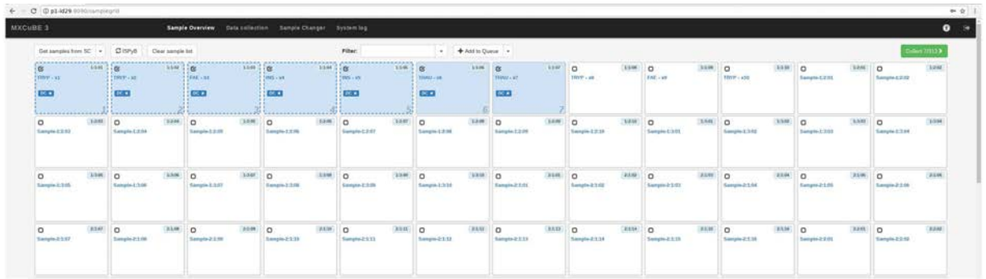
Introducing MXCuBE3 - MX goes web

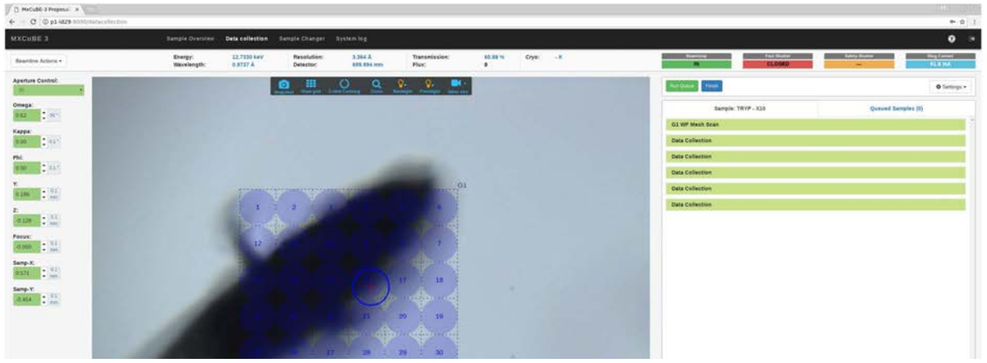
Since 2005 MXCuBE has been the invaluable companion of Structural Biologists. An intuitive and responsive GUI has facilitated user experiments and driven the evolution towards automation and high throughput. In 2013 the second generation of MXCuBE was deployed and became eventually the core of a fruitful international collaboration with EMBL, DESY, HZB, SOLEIL, ALBA, MAX-lab and Global Phasing. MXCuBE2 introduced some major user features and technical advancement that made it popular among the European user community. A programmable queue, accessible to external routines, enabled the implementation of completely automated data collection, as on MASSIF, and facilitated the design of elaborated experiments. Moreover, the tracking of centered positions boosted the application of microbeam, simplified the data collection sequence and fully exploited mesh scans.

During the last couple of years, the ESRF, in collaboration with MAX-lab, has developed a completely new user interface, MXCuBE3, as a web application. A web application can be accessed from almost any computer or mobile device without needing additional software.

MXCuBE3 interface is written in Javascript, HTML5 and CSS and uses the same hardware control layer of its predecessor. This ensures a smooth transition to the new interface and facilitates the deployment on the beamlines. In this way, it maintains the strengths of MXCuBE2 and further simplifies the user operation of complex experiments. The user interface presents two main views to prepare and run the experiments, which are Samples Overview and Data Collection. Samples Overview represents each sample as a card, containing the sample details and the information of the data collections performed or scheduled to be performed on that sample and that are synchronized with ISPyB. The cards can be sorted and filtered by different criteria, such as, for example, dewar location, or sample name. The Data Collection gives access to the control to perform an interactive data collection, maximizing the interaction with the sample view, from where position can be saved and data collection parameters be defined. MXCuBE3 is currently under severe testing on ID29 and will be progressively deployed on the other end stations in the coming months.

D. de Sanctis (ESRF)





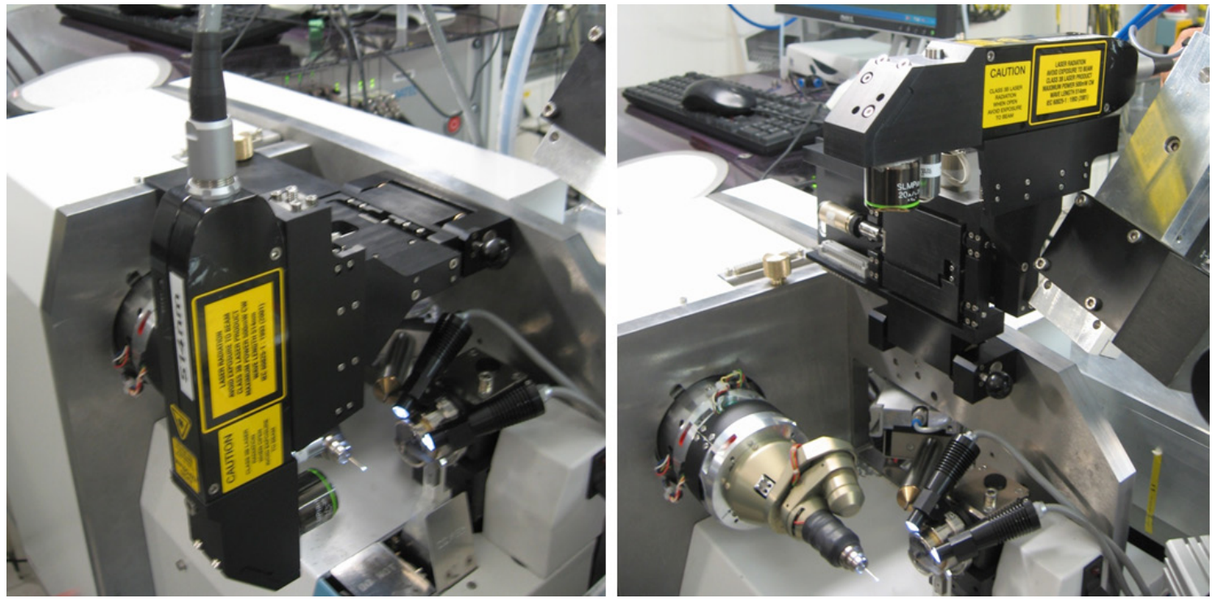
The Samples Overview (top) and the Data Collection (bottom) views in the new version of MXCuBE3.

Online Raman spectroscopy on ID29

Raman spectroscopy is a vibrational spectroscopic technique that probes the conformation of specific chemical groups within proteins. It relies on measuring visible or near infrared photons that are inelastically scattered by the molecule. It can be used as a technique complementary to X-ray crystallography to help resolve ambiguities in the interpretation of enzymatic or X-ray induced processes. The concomitant use of Raman spectroscopy with X-ray crystallography has been coined 'online Raman spectroscopy'. The original version of the technique developed in the context of the Cryobench platform [1] had the Raman probe (which focuses light from the excitation laser to the sample and records the Raman signal on the way back) perpendicular to the X-ray beam, which made alignment of the X-ray irradiated and laser-irradiated volumes difficult. We have designed and built an online Raman setup in an on-axis geometry, i.e. with the laser objective facing the X-ray beam [2]. It allows for straightforward interleaved Raman spectra acquisition and X-ray diffraction measurements with fast probe exchange and simple alignment. The device was successfully used in the characterization of a covalent intermediate in the O2-dependent breakdown of uric acid by the cofactor-free enzyme urate oxidase [3], and in the elucidation of the discoloration of crystals of the fluorescent protein mNeonGreen [4]. In both cases, the method was instrumental in finely monitoring the decay of each species induced by exposure to minute X-ray doses as low as a few kilograys.

D. von Stetten (ESRF), T. Giraud (ESRF), D. de Sanctis (ESRF), A. Royant (IBS/ESRF)
Contacts: antoine.royant@ibs.fr or desancti@esrf.fr

[1] http://www.esrf.eu/UsersAndScience/Experiments/MX/Cryobench/
[2] D. von Stetten *et al.* (2017) J. Struct. Biol. 200, 124-127.
[3] S. Bui *et al.* (2014) Angew. Chem. Int. Ed. Engl. 53, 13710-13714.
[4] D. Clavel *et al.* (2016) Acta Crystallogr. D Struct. Biol. 72, 1298-1307.



Online Raman setup mounted on the MD2 microdiffractometer of beamline ID29. Left: Raman probe in the on-axis position for the recording of Raman spectra. Right: Raman probe in the upward postion allowing for the recording of diffraction data.

Events

MXIS-2017 - Part 2- Practical course on in-plate crystallography and *in situ* diffraction

The MXIS training course was dedicated to *in situ* protein crystallography. The Part 2 of the 2017 session, lasting 3-days from 29th November to 1st December, included full lectures at the IBS (day 1) and practical sessions at the ESRF on beamlines FIP-BM30A and ID30B (day 2 to day 3).
8 participants from 7 different countries attended this workshop. This session was preceded by the Part 1 session at the CBS (Montpellier) from 8th to 10th November, for plate preparation and initial evaluation of diffraction.
MXIS-2017 was the opportunity for participants to test the large number of possibilities offered by *in situ* crystallography and associated techniques, such as ligand dry-coating, crystallization, *in situ* diffraction for crystal screening, structure determination and ligand screening. The participants were given the opportunity to practice on their own samples.
This workshop was organized by IBS (Grenoble), ESRF (Grenoble), EMBL-Grenoble and CBS (Montpellier). MXIS-2017 was made possible thanks to financial support from IBS, EMBL, and the FRISBI program.

J-L. Ferrer (IBS)

PSB Spotlight meeting on Molecular Dynamics Simulations

The third PSB Spotlight meeting on Molecular Dynamics Simulations took place on 10th November 2017. Around 60 participants attended the morning talks in the IBS seminar room and the four speakers, Benjamin Bouvier (U. Picardie), François Dehez (U. Lorraine), Alessandro Barducci (CBS Montpellier) and Kresten Lindorff-Larsen (Copenhagen U.) presented the theory of molecular dynamics and illustrated its applications through examples from their own research. These included studies of the dynamics of protein/nucleic acid complexes and membrane proteins, metadynamics and the combination of simulated and experimental data obtained by NMR. In the afternoon, 25 people participated in the practical session organized by Nicola Salvi (IBS) in the CIBB graphics room. The purpose of this tutorial was to give an overview of typical steps involved in the analysis of molecular dynamics simulations of biomolecules using the Gromacs software.
The organizers wish to thank all the speakers and participants for making this day a success.

F. Bernaudat (PSB coordinator)

From left to right: M. Blackledge, N. Salvi, F. Bernaudat, B. Bouvier, K. Lindorff-Larsen, A. Barducci and F. Dehez

Fête de la Science

The annual science festival "Fête de la Science" was celebrated all over France last October. Throughout the month, the ESRF and IBS welcomed several primary and high school classes for practical sessions and on Saturday 21st October, over 30 scientists from the EPN Science Campus met with the public at the Parvis des Sciences next to MINATEC.
For the third year in a row, the EPN institutes shared a common stand entitled "Toute la lumière sur la matière" to promote the excellent science performed onsite, inform the public about their infrastructures, and to demonstrate their complementary and the advantages of their close proximity. Structural biology was highly represented on the stand where visitors could choose from several hands-on activities such as crystal fishing, DNA and protein folding, or the discovery of archeal and fluorescent proteins. In relation with the installation of the new cryo-electron miscroscope at the ESRF, an important focus was also dedicated to cryo-EM to present the opportunities offered by this remarkable instrument.
With over 1800 visitors in a single day at the Parvis des Sciences, there is no doubt that the outreach must have been significant and we highly encourage PSB scientists to participate to future editions of the "Fête de la Science", in order to meet with a very interested audience in an enjoyable environment.

© C. Argoud

F. Bernaudat (PSB coordinator)

EMBO practical course on small angle neutron and X-ray scattering from proteins in solution

The biennial EMBO Practical Course on Small Angle X-ray and neutron scattering (SAXS/SANS) from proteins in solution took place on the EPN campus from September 11th-15th 2017. It welcomed 23 participants (PhD students, postdocs and senior scientists) from Europe and overseas.

The course included lectures, computer tutorials and practicals at ILL and ESRF beamlines. The major aim was to provide the participants with the basics but also with state-of-the-art knowledge to carry out SAXS/SANS experiments successfully in the framework of integrative structural biology projects. A highlight was the keynote lecture by Dr. Dmitri Svergun (EMBL Hamburg) which was open to all PSB members.
The event was organized by F. Gabel (IBS and ILL), P. Pernot (ESRF), M. Brennich (EMBL) and A. Martel (ILL), with the highly appreciated support of A. Mader (ILL), S. Claisse (ILL) and all of the invited speakers.

More information can be found at the course webpage:
http://meetings.embo.org/event/17-small-angle-scattering

Group photo ©S. Claisse

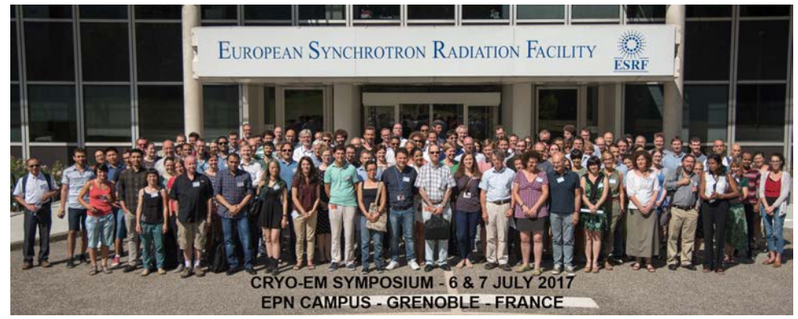
F. Gabel (IBS - ILL)

9th AFMBioMed international summer school

The AFMBioMed summer school offers an introduction to atomic force microscopy in life sciences and medicine. The school allows students, postdocs, technicians, engineers, and researchers to learn fundamental principles of AFM with a strong emphasis on practical training. The 9th AFMBioMed school took place from August 21st to 25th at IBS and received 23 students from 17 different countries. Five AFM instruments from two major manufacturers (Bruker and Oxford Instruments) were available during the 10 possible practical training sessions including high-resolution imaging, high-speed cellular mechanics, and single protein pulling experiments. The school was organized by Jean-Luc Pellequer and Jean-Marie Teulon (IBS/MEM) in collaboration with ILL.
More information can be found on our website: www.afmbiomed.org/grenoble-2017.aspx.

J. L. Pellequer (IBS)

Cryo -EM Symposium on the EPN campus



A symposium comprising plenary talks, as well as scientific and methodological sessions from worldwide renowned experts in the field of single particle cryo-electron microscopy (EM) was held in the ESRF auditorium on July 6-7th. The aims of this conference were to promote the exciting opportunities in structural biology opened by the advances in EM, to introduce the Titan Krios microscope that just arrived at the ESRF, and to describe the newly established cryo-EM facility at the EPN campus to the international user community.
This symposium brought together 170 registered leading experts, researchers and representatives of industrial partners that presented the latest developments in cryo-EM as well as outstanding research in structural biology. It also provided a forum for discussion among established and junior scientists from different disciplines keen in implementing cryo-EM for addressing their biological challenges. A submitted abstract was selected for a short talk and two posters were awarded.
The Symposium was live-streamed through the ESRF YouTube channel and viewers commented in real-time during the presentations.
It was organized by all institutes of the EPN campus (ESRF, EMBL, IBS and ILL), financially supported by the organizations PSB, CEA, GRAL, FRISBI, UGA and sponsored by Thermo Fisher, Gatan, Molecular Dimensions, Mitegen and Quantifoil.

M. Marcia (EMBL), G. Schoehn (IBS), M. Soler-Lopez (ESRF)

PROFILE

Eva Kowalinsky

Could you tell us a few words about who you are and where you are from?

Originally from Germany, I studied Molecular Biotechnology at the Universities of Heidelberg, Germany, and Uppsala, Sweden. During my Master's studies, for the first time, I came in touch with Grenoble and the ESRF, as we went to the synchrotron for data collection. Then, for my PhD studies, EMBL Grenoble appeared to be the perfect fit for me - science wise, as EMBL is one of the best European institutes for life science research - but also the alpine surroundings attracted me a lot!

You did your PhD in Stephen Cusack's lab. Where did you go after your PhD and how did your PhD experience help you as a post-doc?

After my predoc studies, I went to the Max Planck Institute of Biochemistry in Munich for postdoctoral training in the lab of Professor Elena Conti. While in my PhD studies, I worked on a single protein, the innate immune receptor RIGI, here, I had to handle a complex consisting of 15 recombinantly produced subunits!
I studied how the exosome, a multi-protein assembly for cellular RNA degradation, is regulated by the so-called Ski-complex in the cytosol. I continued to solve protein structures by x-ray crystallography, but also gained experience in electron microscopy.

What are your research plans for the coming years?

In the future, I plan to study the structures of cellular complexes that are involved in modification and editing of RNAs. I find it very fascinating that there are mechanisms that enable the re-writing of the genetic information that is encoded by the DNA genome. In my group, we will combine structural biology methods like x-ray crystallography and cryo electron microscopy with biophysical and biochemical methods to look at the protein complexes involved. We want to understand how RNA modification is regulated and how specific sequences can be targeted for modification.

How do you think the PSB will help you?

EMBL Grenoble and the EPN campus including the PSB are a have-it-all place to advance any structural biology project at its optimum! On the hardware side, within PSB there is the access to state-of-the-art facilities (protein expression, high throughput crystallization, ESRF beamlines, the ESRF Titan Krios, NMR, mass spectrometry, biophysics equipment, etc.). Additionally, I see manifold benefits from the scientific expertise available on campus. Scientific exchange is happening amongst the groups, there are various networking possibilities and applied training in structural biology provided from Master's to expert level.

Would you have any advice to give to students who wish to become successful in science one day?

Be passionate, be curious, work hard, appreciate your colleagues and see the fun in what you are doing! And to draw a conclusion that fits somehow to Grenoble: Advancing a project is like climbing a difficult mountain. On the way, there are very pleasant stretches but also hard passages to overcome; concentrate always on your very next step, but have the big aim in mind!

J. Timmins (IBS) and F. Bernaudat (PSB coordinator)

Newcomers

Eaazhisai Kandiah
I joined the structural biology group at the ESRF in September 2017 and I am the beamline scientist for the new cryo-EM beamline, CM01 at the ESRF. I received my PhD at the Indian Institute of Science, Bangalore, India in Macromolecular Crystallography. I kept my interest in Structural Biology during all my postdoctoral studies while combining the revolutionary technique, cryo-EM with X-ray crystallography to address diverse biological projects including HDAC related nucleosome remodelling, RNAPII transcription specifically on TFIID pre-initiation complex, virus infections (HBV, HIV and Polio) and bacterial stress response.
Contact: kandiah@esrf.fr

DATES FOR YOUR DIARY

5th to 7th February 2018 - ESRF User meeting

The annual ESRF User Meeting 2018 will take place on the EPN Campus . The Monday 5th will be dedicated to tutorials on a range of different scientific topics and the Plenary Session will be held on Tuesday 6th and will include a Poster Session. Three dedicated microsymposia will be organized on Wednesday 7th including UDM3. *Understanding Neurological Diseases: Synchrotron Science in a Multidisciplinary Approach* (Venue: IBS Seminar Room). More information at http://www.esrf.eu/UM2018

26th Feb. to 2nd March 2018 - Tutorial in Macromolecular Crystallography 2018 edition

Fundamental aspects of crystallography will be treated in both theoretical and practical sessions, including data collection on a synchrotron beamline. The theoretical sessions will be a mix of lecture and problem solving. The tutorial is aimed in the first place at graduate students from Université Grenoble-Alpes and PSB, but is also open to post-docs and staff of the EPN/PSB Partners. **Registration by a simple mail to** wim.burmeister@ibs.fr **is open and limited to 24 participants.**

19th to 21st March 2018 – 3rd Annual Users Meeting of iNEXT

This meeting will take place on the EPN campus and is intended to bring together iNEXT users and iNEXT partners to exchange on recent scientific advances made in the field of structural biology. **Registration is free of charge and deadline is February 5th**. Additional information and registration at: http://www.esrf.eu/inext-annual-users-meeting-2018

This Meeting will be followed by a dedicated "iNEXT meets Industry" workshop from 21st to 23rd March. More information at: http://www.esrf.eu/inext-meets-industry-workshop

26th to 27th March 2018 - GRAL 48h

The third edition of the GRAL 48h meeting will take place in Autrans on March 26th and 27th 2018. This meeting is open to all GRAL scientists (BIG, IBS, EMBL and their Grenoble partners) within the limit of 150 participants. The meeting will consist of short talks and poster presentations concerning the major research themes of the LabEx, as well as keynote lectures by invited speakers. For further information, please contact: manel.boumegoura@cea.fr

12th to 19th May 2018 - EMBO Practical Course on Structural Characterization of Macromolecular Complexes by integrative structural biology

This course will take place on the EPN Campus. It aims to teach how to expedite structural biology projects involving macromolecular complexes by combining multiple experimental approaches. The course is primarily intended for advanced PhD students and early-stage postdocs (limited to 20 registered participants), but the lectures will be open to everyone. More information at http://meetings.embo.org/event/18-characterization

11th to 15th June 2018 – PSB Tutorial in Cryo-Electron Microscopy

The very first PSB Tutorial in Cryo-Electron Microscopy will take place on the EPN Campus. The course will consist of morning lectures focusing mainly on single particle analysis, but will also give an introduction to other techniques. In the afternoons, several practical sessions will be organized on image analysis, sample preparation and data collection at a microscope. Further information will be announced soon.

ANNOUNCEMENTS

Renewal of the Joint Structural Biology Group Agreement between ESRF and EMBL

On November 10th, the ESRF and EMBL renewed their Joint Structural Biology Group Agreement until 2021. The signature ceremony took place at the ESRF Auditorium in the presence of the ESRF Science Advisory Committee (SAC) members.
This agreement was signed (from left to right) by Luis Sanchez-Ortiz (ESRF Director of Administration), Francesco Sette (ESRF Director General), Silke Schumacher (EMBL Director of International Relations representing the EMBL Director) and Stephen Cusack (EMBL Head of Grenoble outstation).

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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.