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PSB et al

Partnership for Structural Biology Newsletter

SCIENTIFIC HIGHLIGHTS

Developing a molecular understanding of water purification with Moringa seed protein

Moringa oleifera is a tropical plant also called *The Miracle Tree* [1] because many of its parts have valuable applications (Figure 1). In particular, seed extracts from the plant have been used in traditional water treatment throughout Africa. The application of this extract to untreated water causes a 95% reduction of turbidity and decrease in particle and bacterial content. Previously, scientists have focused on the crude protein extract that was poorly characterised.

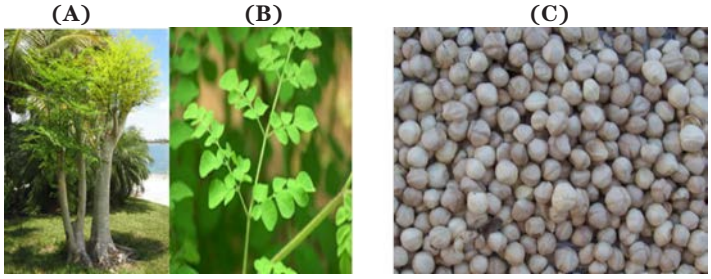


Figure 1: Photographs of the *Moringa oleifera* tree (A) and its leaves (B). On the right (C), Moringa seeds without kernels.

Given the interest in these seeds in terms of their potential for modern applications in water purification, we have isolated various components of the crude seed extract and characterised them using advanced biochemical and biophysical methods; these studies have been carried out in collaboration with the MS platform at IBS and EDyP platform at CEA and have allowed the identification of two main isoforms (*Mo*-CBP3-3 and *Mo*-CBP3-4)[2] of the *Moringa oleifera* chitin binding protein *Mo*-CBP3 as well as demonstrating flocculation properties.

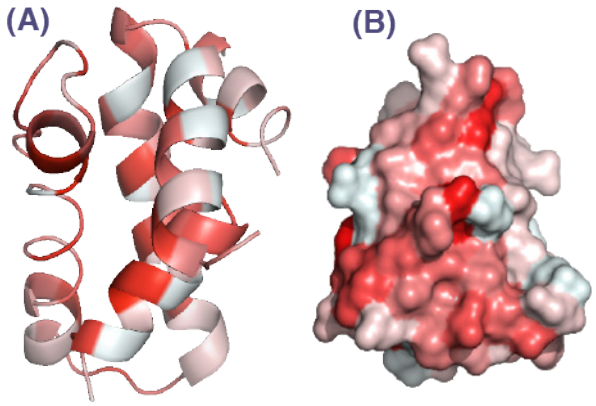


Figure 2: Schematic showing the crystal structure of *Mo*-CPB3-4 (helix) (A) and surface representation (B) showing the polar versus non-polar areas of the protein. Red represents the most hydrophobic and white the most hydrophilic regions according to the Eisenberg hydrophobicity scale (Eisenberg *et al.*, 1984).

The X-ray crystallographic structure of one isoform, *Mo*-CBP3-4 was determined to a resolution of 1.68 Å (beamline ID29 at ESRF). This protein structure shows a compact fold due to the presence of a small chain and a large chain linked together by four disulfide bonds. This arrangement is the key to the remarkable stability of the protein as well as its heat- and proteolysis resistance properties; moreover the charge distribution shows a contrast between the core of the protein which is hydrophobic and the solvent exposed region, which contains a large number of arginine residues and is highly hydrophilic (Figure 2). These features of the structure explain the colloidal interactions involved in the flocculation process.

In addition to the biochemical, biophysical and structural studies, a reflectometry study was performed on both charged (e.g silica) and uncharged (e.g alumina) surfaces in order to elucidate mechanisms relating to flocculation. Neutron reflection measurements performed on instrument D17 at ILL have revealed the nature of the interaction based on the positively charged nature of the protein (consistent with the crystal structure) and the interface behavior as a thin monolayer [3].

This work provides a first step in understanding the molecular basis of water purification applications of the *Moringa* seed protein system. The development of a recombinant expression system is in progress to enable an *in vitro* biosynthetic production route.

This project was part of an international collaboration between ILL, ESRF, and Universities in Europe and Africa. Special thanks to Dr Kwaambwa (Namibia) and Prof A. Rennie (Uppsala) whose previous work on the crude protein extract of *Moringa* seeds led to this unique project.

M. Moulin (ILL) & V. T. Forsyth (ILL/Keele University)

[1] Shindano, John 'Moringa (Moringa oleifera) *ACS symposium Series* [0097-6156] (2009) **1021**, 421-467.
[2] J. Freire *et al.*, (2015). PLoS One; **10** (3), 1–24.
[3] M. Moulin *et al.*,(2019); Journal of Colloid and Interface Science **554**:296–304.

CONTENTS

Function of an elusive bacterial toxin uncovered

Legionella pneumophila is a pathogenic bacterium that causes acute pneumonia called Legionnaires' disease. Pneumonia resulting from exposure to *Legionella*, although uncommon in Europe (affecting 1 in 100K), has a >10% fatality rate. SidJ is an important toxic protein of legionella that is injected into the human cytoplasm to enable successful bacterial infection and replication. The inhibition of SidJ is considered a viable option to curb legionella infection, but the lack of a molecular understanding of SidJ's function hindered these efforts. SidJ has no sequence similarity to proteins of known function, an aspect that has made the biochemical characterization of SidJ a challenging task.

In a collaborative effort involving Bhogaraju and Galej groups from EMBL Grenoble, and the team of Prof. Ivan Dikic from Frankfurt, Germany, a study describing the detailed molecular function of SidJ has been published in Nature [1]. They discovered that SidJ is a glutamylating enzyme that attaches the amino acid glutamate to the target proteins at the expense of an ATP molecule. This is the first observation of a bacterial protein possessing glutamylation activity. Interestingly, *Legionella* has evolved to use human calmodulin to trigger SidJ's activity, and as a result, SidJ only gets activated once it enters the human cytoplasm during infection. Using mass spectrometry, the study also uncovered numerous human substrates of SidJ during infection.

Michael Adams, a graduate student in the Bhogaraju group at EMBL Grenoble performed biochemical and biophysical studies to understand the SidJ mediated glutamylation and the effect of calmodulin-binding. Teams from EMBL Grenoble have extensively used PSB platforms to perform isothermal titration calorimetry, fluorescence-based NAD^+ hydrolysis, protein production and cryo-EM. Even though the complex of SidJ and calmodulin is only ~100kDa in size, we succeeded in determining the cryo-EM structure of SidJ/calmodulin complex to understand the atomic details of calmodulin-dependent activation of SidJ's enzymatic activity. Wojtek Galej and his graduate student Moritz Pfleiderer collected single particle cryo-EM data on a Talos Glacios microscope fitted with Falcon III EC detector at EMBL Grenoble. These data yielded the 4.1Å cryo-EM structure of SidJ-calmodulin complex (Figure 1).

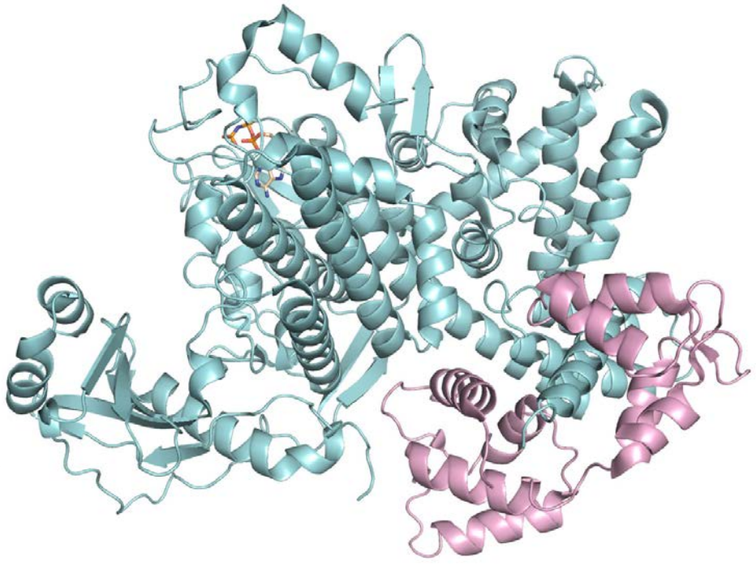


Figure 1: Cryo-EM structure of SidJ-calmodulin complex. SidJ is in cyan and calmodulin is in pink. ATP analogue, AMPPNP, is in stick representation and colored orange. (Courtesy EMBL Grenoble).

Surprisingly, the Cryo-EM structure of SidJ-calmodulin revealed that SidJ adopts a kinase-like fold with two potential nucleotide-binding sites. Mutation of residues in both nucleotide-binding pockets results in inactivation of SidJ indicating that both these sites are co-ordinating in an unknown manner to achieve glutamylation. The cryo-EM structure also revealed that calmodulin binds to the C-terminal α-helical region of SidJ and stabilizes the catalytic pocket of SidJ by an allosteric mechanism.

In summary, researchers from PSB and Frankfurt have elucidated the biochemical function of an elusive bacterial toxin-SidJ. The cryo-EM structure of SidJ-calmodulin complex revealed druggable pockets of SidJ, which will pave the way for novel therapeutic strategies to curb Legionnaires' disease. Of note, similar results have been obtained by three other research groups coming from the USA and China [2-4].

S. Bhogaraju (EMBL)

[1] S. Bhogaraju *et al.* (2019) Nature **572**, 382-386.
[2] M.H. Black *et al.* (2019) Science **364**, 787-792.
[3] N. Gan *et al.* (2019) Nature **572**, 787-792.
[4] A. Sulpizio *et al.* (2019) Elife **8**, e51162.

Bacterial nucleoids are more complex and dynamic than anticipated...

Deinococcus radiodurans is well known for its outstanding radiation resistance and has been reported to possess a compact, ring-like nucleoid, which has been proposed to contribute to its unusual phenotype [1]. The objective of our study [2] was to assess the shape and dynamics of *D. radiodurans* nucleoids as a function of the cell cycle and to determine the extent of compaction of these nucleoids. Imaging small entities such as bacterial nucleoids, which are less than a micron in size, remains a real challenge. In collaboration with D. Bourgeois from the PIXEL team and J.-P. Kleman from the M4D imaging platform [3] at IBS, we used a combination of spinning-disk confocal time-lapse microscopy and single-molecule localisation microscopy to visualise the cells and nucleoids and probe their conformational dynamics in dividing cells. *D. radiodurans* is a relatively large bacterium and its cell cycle lasts two hours, which is ideal to capture the changes occurring at the cell and nucleoid level during growth and division.

Our images revealed that *D. radiodurans* nucleoids do not only adopt a ring shape, but instead follow a well-defined choreography in which the ring-like structures are just one act of this ballet. Starting from a highly compact conformation just after cytokinesis, the nucleoids then expand to adopt a toroidal shape which progressively opens up into a crescent as the next round of cell division is initiated (Figure 1A). As septal growth progresses the crescent-shaped nucleoids stretch along the longest axis of the cell and align orthogonally to the future division axis. At this stage the replicated genomes are pulled into the two daughter cells before septal closure and the newly formed nucleoids are ready to start a new cycle again. Chromosome condensation and segregation is well characterised in eukaryotes, but such processes had never been visualised in bacteria. In this study, we clearly evidenced that bacterial nucleoids are highly complex structures that are tightly coupled to cell cycle progression.

These remarkable images allowed us to extract the dimensions of both the cells and their associated nucleoids at the different stages of the cell cycle. Nucleoids of exponentially growing cells were found to occupy 35% of the cell volume in *D. radiodurans* compared to 65% in *E. coli*. In stationary phase cells, this value was found to be less than 20%. These measurements clearly confirmed that the *D. radiodurans* possesses a condensed genome. Our images also revealed that the major nucleoid-associated protein of *D. radiodurans*, namely the histone-like protein, HU, largely colocalises with the genomic DNA, but only binds transiently to the DNA, as determined by single-particle tracking PALM (photoactivated localization microscopy) and FRAP (fluorescence recovery after photobleaching) experiments (Fig. 1B-C). Further studies will be needed to determine precisely the role of this key protein, but these features may contribute to the remarkable plasticity of *D. radiodurans* nucleoids. This work is clearly only the start of the story and in the future, we hope to shed light on the mechanisms underlying nucleoid organisation and dynamics.

J. Timmins (IBS)

[1] S. Levin-Zaidman, J. Englander, E. Shimoni *et al.* (2003). Science, **299**, 254-256.
[2] K. Floc'h, F. Lacroix, P. Servant *et al.* (2019). Nat. Comm., **10**, 3815.
[3] M4D platform: http://www.isbg.fr/analyses-cellulaires/imagerie-cellulaire-m4d/

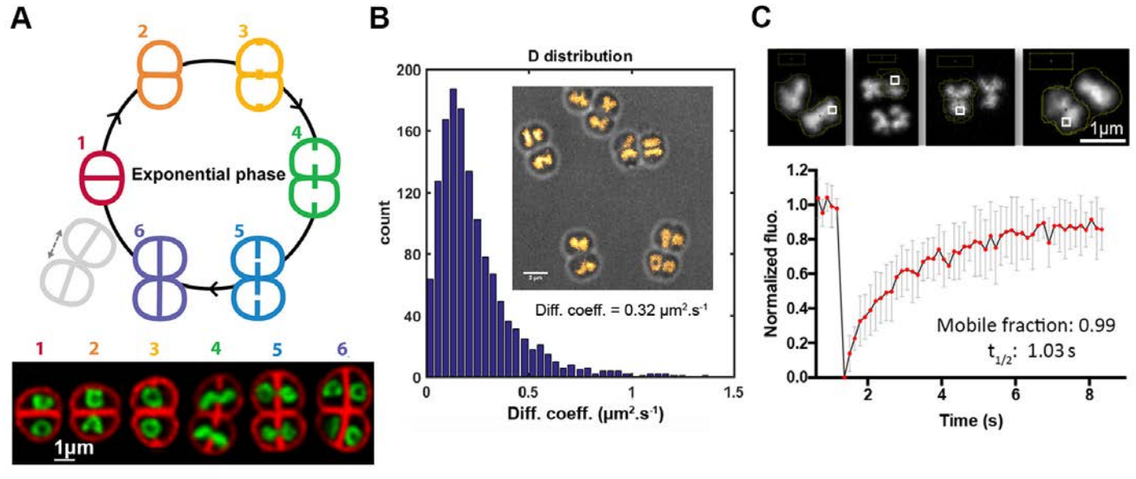


Figure 1: (A) Schematic representation of the different phases of the *D. radiodurans* cell cycle in exponentially growing bacteria and representative images of dual labelled *D. radiodurans* (membrane dye, Nile Red; DNA dye, Syto 9) at each of the six phases of the cell cycle. (B) Distribution of the apparent diffusion coefficients of 1,355 single tracks of HU-PAmCherry measured by single-particle tracking PALM in live, exponentially growing HU-PAmCherry expressing cells. Inset: reconstructed PALM image of HU-PAmCherry expressing cells. Scale bar: 2 µm. (C) Ensemble measurements of HU-mCherry mobility probed by FRAP on HU-mCherry expressing live cells. Top: examples of HU-mCherry labelled nucleoids used for FRAP experiments. The photobleached region is indicated with a white box. Scale bar: 1 µm. Bottom: analysis of the recovery of the fluorescence signal of HU-mCherry after photobleaching.

Specific radiation damage is a lesser concern at room temperature

There are two types of radiation damage in protein X-ray crystallography [1]. The first one, global damage, has been known since the beginning of X-ray crystallography. Global damage accounts for the decrease in the diffraction properties of a crystal during data collection due to the interaction of X-rays with the atoms of the crystal, which, in particular, generates free electrons and radicals in the bulk solvent that progressively destroy the crystalline order. Global damage is slowed down by roughly two orders of magnitude on the dose scale when the diffraction experiment is performed at cryogenic rather than at room temperature. Cryo-crystallography has led to the explosion of protein structure determination in the 1990's as it allows determining a protein structure from a single crystal. However, it was then realized that a second type of radiation damage was at play in cryogenic experiments: specific damage. This damage affects certain specific chemical groups that are sensitive to electrons, for instance disulphide bonds, carboxylate groups or metal cations, which can be found in protein active sites. This can be explained by the fact that X-ray induced free electrons can still diffuse at cryogenic temperature. Therefore, specific damage may lead to artefacts in structural analysis of reaction intermediate states and thus in mechanistic interpretation. The discernibility of specific damage at cryogenic temperature means that there is a significant difference between the rates of the two types of damage, i.e. a 'decoupling' between the two phenomena. As room temperature protein crystallography is fast developing thanks to the development of faster, noiseless detectors, of improved sample environment and of serial crystallography, the question of the comparison of the respective rates of specific and global damage build-up at room temperature has become a hot topic. We have compared the rates of both damage build-up at cryogenic and room temperature for three different proteins, including the reaction intermediate state of a fragment of a photoreceptor [2]. While the two types of damage are largely decoupled at cryogenic temperature (decoupling factor between 12 and 1600), they occur on a similar dose scale at room temperature (decoupling factor between 1 and 8) (Figure 1). This indicates that depending on the studied protein, specific damage may not be a primary concern in crystallographic structure determination at room temperature, provided diffraction data can be collected from a single crystal. This should stimulate the development of time-resolved crystallography experiments at synchrotrons.

G. Gotthard (ESRF), S. Aumonier (ESRF), D. De Sanctis (ESRF), G. Leonard (ESRF), D. von Stetten (ESRF) & A. Royant (ESRF /IBS)

[1] J. M. Holton (2009) J. Synchrotron Radiat. **16**, 133–142.
[2] G. Gotthard *et al.* (2019). IUCrJ, **6**, 665-680.

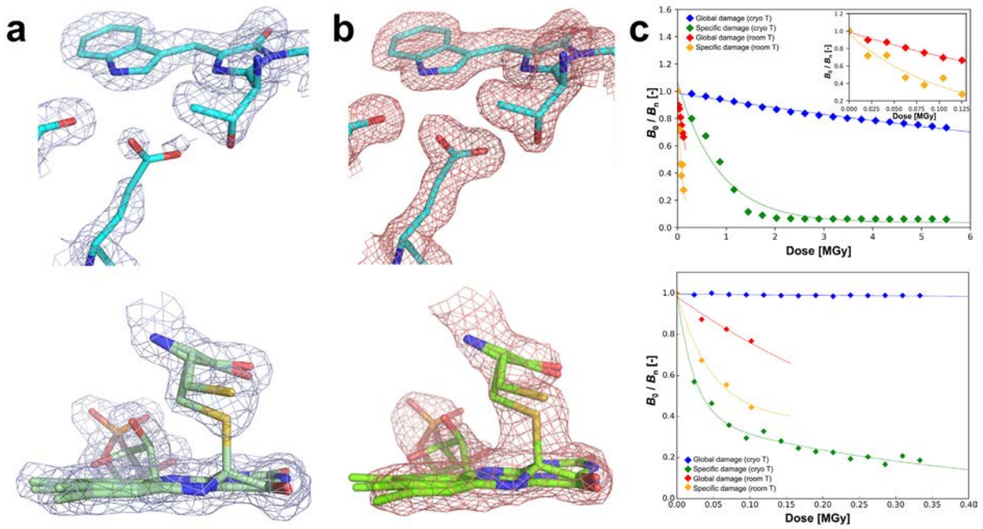


Figure 1: (**a**) Specific damage at cryogenic temperature to the structures of Cerulean (top, decarboxylated glutamate) and of a photoreaction intermediate state of a LOV2 domain AtPhot2LOV2 (bottom, reduced sulphur-carbon bond). (**b**) Room temperature structures of Cerulean and of AtPhot2LOV2. (**c**) Evolution of various B-factors illustrating the rates of global and specific damage at cryogenic (blue, green) and room temperature (red, orange) for Cerulean (Top) and AtPhot2LOV2 (Bottom).

Kissing RNA stimulates p53 for tumor prevention

A team of EMBL researchers in the Marcia group – in collaboration with the IBS platform for atomic force microscopy led by Jean-Luc Pellequer and with the support of PSB facilities and ESRF BioSAXS beamline BM29 – discovered unexpected molecular properties in an RNA molecule that acts as a tumor suppressor in human brain and endocrine glands [1].

RNA does not just transmit genetic information to proteins: Nature has selected highly-structured RNA molecules to perform other vital cellular functions, too. For instance, catalytic introns and spliceosomal RNAs perform splicing, ribosomal and transfer RNAs synthesize proteins, riboswitches recognize metabolites and environmental stimuli, and structured motifs in untranslated mRNA regions regulate maturation, translation and degradation of protein-coding transcripts. But besides these "housekeeping" RNAs – for which detailed mechanistic information and high resolution three-dimensional structures are known – it has recently emerged that the cells produce a stunning number of other regulatory, non-protein-coding RNAs. Many such regulatory RNAs are extremely large (1,000s of nucleotides) and are thus broadly classified as the "long non-coding RNA", lncRNA for short. While it is becoming evident that lncRNAs are essential to prevent diseases, their specific functions are still poorly characterized and it has so far been impossible to study lncRNA structures, because of their huge size and unprecedented molecular complexity.

One lncRNA essential for human health is the maternally-expressed gene 3, or MEG3, which is abundantly expressed in the adult brain and pituitary gland. In these tissues, MEG3 potentiates protein p53, a key transcription factor controlling cell proliferation, whose role is to arrest the growth of unhealthy cells before they degenerate into cancerous tissues. MEG3-dependent stimulation of p53 specifically prevents pituitary adenoma and meningioma, two high-incidence intracranial tumors that can currently only be cured by surgery.

Using an original approach – crucially fostered by the PSB environment – the Marcia team integrated biochemistry, cell biology and single-particle atomic force microscopy imaging to identify specific MEG3 building blocks essential for p53 stimulation and for the control of cell proliferation. Interestingly, these functional building blocks comprise RNA hairpins connected by so-called "kissing loops", which are three-dimensional motifs characteristic of highly-structured RNAs. The researchers demonstrated that disruption of these kissing loops resulted in concomitant disruption of both the overall 3D structure and the tumor suppression function of MEG3 (Figure 1). Their findings prove experimentally what had so far remained a speculative molecular mechanism assigned to lncRNAs: tertiary structure motifs can guide lncRNA function.

The work, which also includes contributions from colleagues at the CIBIO (University of Trento, Italy) and the Max Delbrück Center (Berlin, Germany), presents the first visualization of an lncRNA 3D structure and demonstrates the surprising precision by which the MEG3 function is regulated at the molecular level. While future research should dissect the exact cascade of events that governs MEG3-dependent stimulation of p53, the current work sets important bases for characterizing the MEG3 structure by integrated structural biology, and for exploring ways of tuning MEG3 pharmacologically to arrest tumor growth. More broadly, the method employed here for MEG3 excitingly paves the way for a detailed molecular characterization of many other medically-relevant human lncRNAs in the years to come.

M. Marcia (EMBL)

[1] T. Uroda, E. Anastasakou, A. Rossi, J.M. Teulon *et al.* (2019). Molecular Cell, **75**, 982-995.

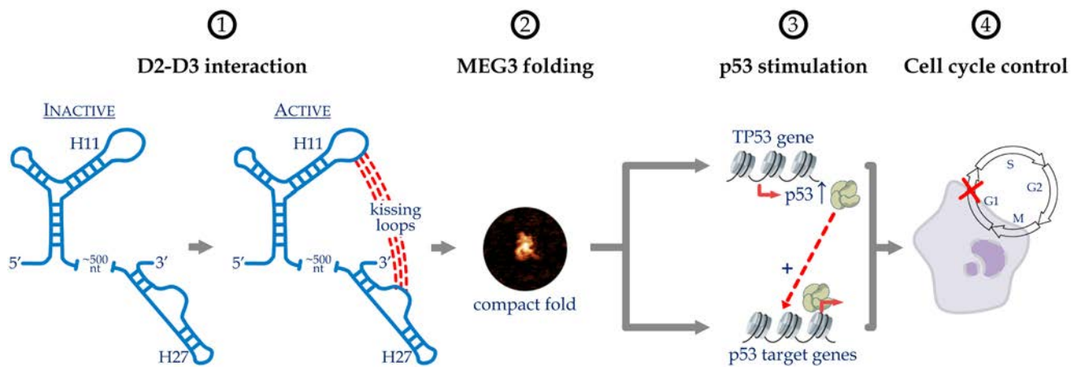


Figure 1: Structure-functional relationships in human lncRNA MEG3.
Formation of an intramolecular "kissing loop" interaction (1) is necessary for MEG3 folding into a compact, globular structure (2), which is responsible for stimulation of p53 and p53 target genes (3) and ultimately for inducing cell cycle arrest or apoptosis (4).

News from the platforms

MASSIF-1 and the lessons from the automatic processing of 56 000 samples

MASSIF-1 allows a wide range of projects to use Macromolecular Crystallography (MX) beamlines, from those that require extensive screening to find the best diffracting crystal to small-molecule fragment screening and experimental phasing at high and low resolutions, without any human intervention. Between September 2014 and December 2018, the beamline received 56,459 samples [1]. A large number of characteristics is recorded from each sample, from crystal dimensions to the results of automatic processing. Put together, they provide a general overview of the crystal morphology of biological macromolecules and how these properties relate to the macromolecule itself. This is the first study of its kind in the history of macromolecular crystallography. The results allow many long-held assumptions to be tested experimentally and provide a framework to direct the development of future beamline facilities.
All of the information gathered and used in the automatic location, characterization and data collection are stored in databases. Crystal dimensions are measured from the X-ray centring routine. The volume distribution (Figure 1) of the crystals is lognormal and the mode volume is equivalent to a cube with edges of 27µm. The plot of the surface area against the volume demonstrates that most crystals have a surface area greater than that expected for a cube, with crystals with very large volumes being more cuboid. This implies that using X-ray beams of larger than 100µm will have limited returns and most crystals will require diameters of 10–50µm with, of course, the possibility of collecting data from multiple volumes in plate-shaped or needle-shaped crystals. Surprisingly, the MOSFLM estimated mosaic spread value from four characterization images is independent of the crystal shape and size, but there is a good correlation of resolution with low mosaic spread and/or molecular weight. The variation of diffraction quality within a crystal has also been analysed and reveals that most crystals are quite homogenous but there are a large number of observations where diffraction quality varies enormously. The analysis presented has only started to delve into the data and the modern data-science techniques could help further improve the measurement of diffraction data from protein crystals.

D. Nurizzo (ESRF) & M. Bowler (EMBL)

[1] O. Svensson, M. Gilski, D. Nurizzo and M. W. Bowler. (2019) IUCrJ, 6 (5), 822-831

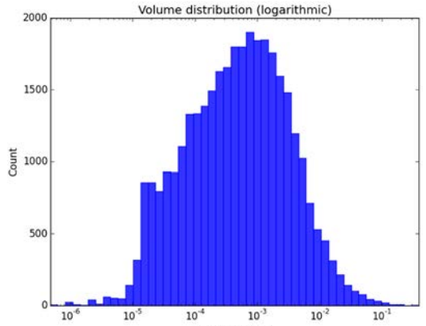


Figure 1: Histogram of crystal volumes. The histogram shows the distribution of crystal volumes measured on MASSIF-1. Note the logarithmic scale. N=33 905.

CRIMS V4, your tool for *Online Crystallography*

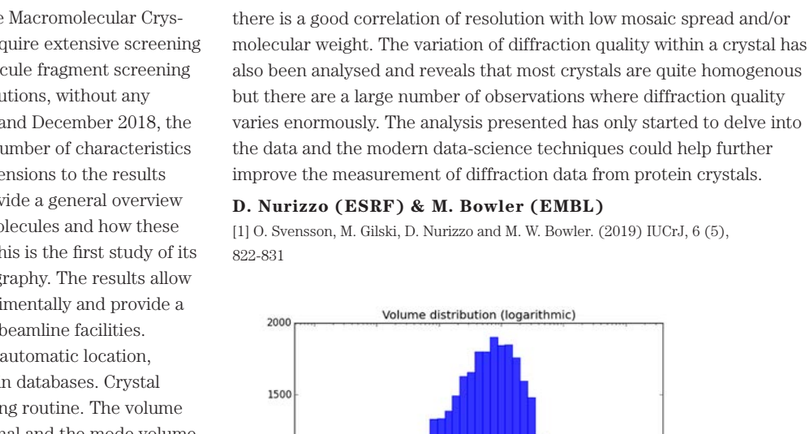
If you have recently used the HTX lab platform, you may have noticed changes in the look-and-feel of our web pages. Well, there is more than meets the eye. Actually, this is CRIMS V4, a new version of our crystallography software bringing completely new technology and functionalities.

The Crystallography Information Management System (CRIMS), was initially created to support the operation of the HTX lab in Grenoble, but was soon adopted by other laboratories as their main information system, including two EMBL sites and multiple other crystallization facilities across Europe. CRIMS was already providing a good number of functionalities, including web-based experimental design and real-time tracking of crystallization experiments. However, crystallography keeps evolving at an incredible pace, for example, the CrystalDirect technology for automated crystal harvesting and cryocooling is in full production since a couple of years and is now being implemented at other labs, such as Petra III. On the other hand, new applications like fragment screening, are becoming more mainstream. The new CRIMS V4 provides access to all the CrystalDirect functionalities enabling crystal harvesting and cryocooling over the web. Moreover, it supports automated communication with three synchrotrons, ESRF, Petra III and SLS. This enables scientist to send crystal information to the synchrotron of their choice (via ISPyB), associate it to their experimental number, and recover information automatically after diffraction experiments. Thanks to this, the HTX lab now provides access to a unique Online Crystallography pipeline. A fully automated and continuous workflow, including crystallization screening, crystal optimization, automated sample mounting and diffraction data collection controlled remotely through specially designed web interfaces. CRIMS V4 also includes support for large scale fragment screening, which is now in high demand at the facility. This was possible thanks to a collaboration with the Global Phasing Consortium, and implementation of the PIPEDREAM software suite, providing automated hit identification and structure refinement. This makes it possible to rapidly evaluate over hundreds to thousands of results in the context of a fragment screening project.

CRIMS V4 has also changed a lot under the hood. The whole software has been recoded and now uses microservice architecture. Yes, this is how Netflix is able to serve movies over the internet to millions of clients. In our case this technology does not only add performance, but also allows software to be split into smaller, more manageable units, significantly cutting development time. Moreover, the new CRIMS is cloud compatible and able to work in a distributed infrastructure.

CRIMS V4 was possible thanks to the effort and dedication of Peter Murphy, with important contributions from Raphaël Bourgueas and Gael Seroul. We also take this opportunity to thank all CRIMS users for their support and very valuable feedback over the last years. We hope you enjoy it !

J. A. Marquez (EMBL)



The Crystallographic Information Managemet System (CRIMS)

News from ISBG platforms

The activities of the ISBG in a few figures:
- Approximately 450 distinct users per year with platform time use : 76% PSB, 21% external academics and 3% industry.
- 90 user publications clearly acknowledging the platforms each year
- 4000 days of user activity annually including 900 samples in mass spectrometry, 3000 hours of optical microscopy on M4D instruments, 600 days of use of biophysical characterisation (AUC/PAOL, SPR, CIBB platform).
- 4 new instruments became available to users in 2019: a MALDI-TOF/TOF mass spectrometer, an Orbitrap mass spectrometer, a biolayer interferometer (BLI) and a Thermo Fisher Glacios electron microscope.

Data on platform use are collected annually as part of the Quality Management program involving annual internal and external audits. The ISBG platforms at the IBS have received ISO 9001 certification since 2011 and national NF-X50-900 certification since 2016.

D. Hart (IBS-ISBG)

nanoESI-Orbitrap Velos Pro mass spectrometer (from Thermo Fisher) coupled to liquid chromatography capabilities acquired by EMBL and installed in the ISBG mass spectrometry platform at the IBS.

EVENTS

ADTB-2019 - Advanced Diffraction Techniques for Biology

The ADTB workshop, that took place from the 19th until the 22nd of November, was dedicated to the newest diffraction techniques in the field of structural biology. It covered serial crystallography using synchrotron and XFEL sources, *in situ* diffraction; electron and neutron diffraction as well as rational crystallization. It included full lectures at the IBS (day 1 and 2), practical sessions at the IBS, ILL, ESRF and EMBL/CIBB computer room (days 3 and 4), with in addition a series of PSB platform visits (day 4). With 15 participants from 7 different countries attending the workshop, ADTB-2019 was an opportunity for them to discover the extent of possibilities offered by emerging diffraction techniques, such as serial crystallography, ligand dry-coating, *in situ* diffraction for structure resolution at room temperature or for ligand screening, electron and X-ray diffraction on micro- or nano-crystals, coupled neutron and X-ray diffraction experiments, crystallization using conventional and non-conventional crystallization techniques (based on the knowledge of phase diagram, temperature control, dialysis, batch, counter-diffusion, microfluidics), etc. This workshop was entirely organized on the EPN campus, Grenoble, by the IBS, ILL, CBS (Montpellier), EMBL and ESRF.

We thank, in addition to the institutions above, the Association Française de Cristallographie, Grenoble-Alpes university, Grenoble-INP, and the FRISBI program for their financial support.

The ADTB organizing committee

3rd practical workshop on sample preparation for Single Particle cryo-EM

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All four EPN institutes, ESRF, IBS, EMBL and ILL worked together for the third practical hands-on workshop (November 5th -7th) on sample preparation for single particle cryo electron microscopy. Out of more than 80 applications, 12 participants were selected following lectures, demonstrations and practical sessions held at ESRF, IBS and the EMBL. As for the other workshops, participants were given the opportunity to come with their own samples to translate tips and tricks given by six tutors to prepare their own sample grids. At first though, samples were quality checked using negative staining at the IBS using the T12 microscope located there. Groups of two participants then prepared grids with tutors' explaining how to best use a Vitrobot device. Grids were subsequently screened using three microscopes located at the EMBL, ESRF and the IBS. Special thanks go to Claudine Romero for helping again to organize the workshop and to Thermo Fisher for both financially and personally supporting the workshop.

E. Kandiah (ESRF), G. Effantin (IBS), M. Hons (EMBL), G. Schoehn (IBS), D. Traore (ILL) & C. Mueller-Dieckmann (ESRF)

Short Pulse Science for Synchrotron Serial Crystallography

In the context of the Extremely Brilliant Source (EBS) dedicated workshop series, the "Short Pulse Science with the Extremely Brilliant Source" took place in the ESRF auditorium on the 28th and 29th of October 2019. A total of 21 invited speakers and 5 ESRF scientists, animated the discussion on different topics ranging from fundamental physics to structural biology that will take advantage of short intense X-ray pulses to perform fast measurements and study processes as a function of time. Structural biology applications covered the use of pulsed beam in solution scattering and pink beam experiments for serial crystallography. The status of the EBSL8 project, the upgrade of ID29 that is currently under construction, was also presented. The new ID29 will be entirely dedicated to Synchrotron Serial Crystallography. The beamline will deliver a photon flux of 10^15 – 10^16 ph/s in a submicron spot size. Two experimental hutches are being built in the Chartreuse experimental hall, near to a new dedicated sample preparation facility and a data processing area.

D. de Sanctis (ESRF)

ID29 beamline layout after the EBS.

11th AFMBioMed international summer school

The AFMBioMed summer school offers an introduction to atomic force microscopy (AFM) in life sciences and medicine. The school allows students, postdocs, technicians, engineers, and researchers to learn fundamental principles of AFM with an emphasis on practical training. The 11th AFMBioMed school organized at IBS from October 21st to 25th 2019 received 30 students from 16 different countries. This year the school was shared with 15 Early Stage Researchers of the European Union's Horizon 2020 research and innovation programme, under the Marie Skłodowska-Curie grant agreement N° 812772 (project Phys2BioMed). Six AFM instruments were available during the 9 possible practical training sessions including high-resolution imaging, cellular imaging and mechanics and single protein pulling experiments. The AFMBioMed summer school remains a unique opportunity for students to get in depth knowledge of AFM practice and theory. This all-inclusive event is organized on the EPN campus with a special event at the Grenoble Bastille. The school was organized by Jean-Luc Pellequer and Jean-Marie Teulon (IBS/MEM) in collaboration with Trevor Forsyth (ILL). All the information can be found on our website: www.afmbiomed.org/grenoble-2019.aspx

J.-L. Pellequer (IBS)

Fête de la Science 2019

Top left: © ILL. Top right: © IBS/ O. Cavoret. Bottom left: © ESRF/ C. Argoud. Bootom right: © IBS/ O. Cavoret.

This year the 28th national science festival « Fête de la Science » was a special edition for the EPN Campus. For the first time, the official opening of this event in Isère was held on our Campus on October 3rd, 2019 in the presence of the directors of the four institutes and several representatives of the Grenoble area (C. Lombard, representing the Préfet de l'Isère, V. Henry representing the Rectrice de l'Académie de Grenoble, C. Ferrari, President of Grenoble-Alpes-Métropole, E. Piolle, Mayor of Grenoble and J. Jean-Baptiste, Director of the Casemate). All PSB partners (EMBL, ESRF, IBS and ILL) were involved in this event and visits of platforms were organized in each of the institutes.
Later in the week, activities for elementary and secondary school classes were proposed by ESRF and IBS. But the highlight of the week was undoubtedly the animation prepared by our four communication officers for the EPN common stand at the Parvis des Sciences in Minatec. An escape game on the scale of the stand offered the general public the opportunity to better understand the periodic table of elements, a major tool for scientists and to answer questions such as how is it organized, how are new elements discovered and what are their applications. Enthusiastic families spent a great moment looking for clues and the game ended with a photo of the participants dressed up as special researchers. Congratulations to all volunteers for sharing their enthusiasm with different audiences and contributing to this ambitious and very successful edition!

O. Cavoret (IBS)

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

The biannual EMBO Practical Course on Small Angle X-ray and Neutron Scattering (SAXS/SANS) from bio-macromolecules in solution took place on the EPN campus from September 23rd-27th 2019. It welcomed 19 participants (PhD students, postdocs and senior scientists) from Europe and overseas.

The course included lectures, computer tutorials, a neutron practical at ILL and a remote SAXS experiment at the P12 beamline at PETRA-III (Hamburg). The major aim was to provide the participants with the basics but also with state-of-the-art knowledge to design and carry out SAXS/SANS experiments successfully in the framework of integrative structural biology projects. A highlight was the keynote lecture by Dr. Gregory Hura (Advanced Light Source, Berkeley) which was open to all PSB members.

The event was organized by F. Gabel (IBS), P. Pernot (ESRF) and A. Martel (ILL), with the highly appreciated support of A. Mader (ILL), S. Claisse (ILL) and all of the invited speakers.

F. Gabel (IBS)

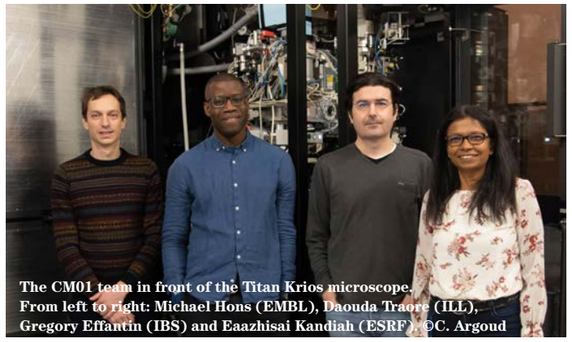
PSB Symposium "Macromolecules in action"

After the success of its first symposium on cryo-EM in 2017, the Partnership for Structural Biology (PSB) organised a second symposium on "Macromolecules in action" which took take place on the EPN campus on 4th-5th July 2019. The aim of this meeting was to illustrate how the big 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.
The meeting gathered 143 registered participants, including 16 internationally renowned experts which were invited to present their outstanding work during five scientific sessions: “Molecular machines”, “Machines on genes”, “Machines on RNA”, “Cross membrane talk” and “Technology advancements”. Eight submitted abstracts were selected for short talks and another eight for flash presentations, and three best poster prizes were awarded. The organisation of the symposium was also the occasion to showcase the EPN campus and tours of the PSB platforms were organised for external participants on the first day. In addition, prior to the meeting a two-day PSB tutorial course “Exploring Hollywood's Tools to Visualize Biology” was organised for local scientists to train on the animation software Maya/mMaya, thanks to the kind contribution of one of the symposium’s invited speakers, Gaël McGill (Harvard Medical School & Digizyme Inc), who is a leading expert on molecular animation.
The organisers wish to thank all the participants for making a lively and successful event and all the sponsors (BioXFEL, EMBO, GRAL, Molecular Dimensions, Quantifoil, Thermofisher scientific, and UGA) for their financial support, and look forward to the third edition in 2021.

F. Bernaudat (PSB)

PROFILE

The CM01 team



The CM01 team in front of the Titan Krios microscope. From left to right: Michael Hons (EMBL), Daouda Traore (ILL), Gregory Effantin (IBS) and Eaazhisai Kandiah (ESRF). ©C. Argoud

CM01, the Titan Krios cryo-electron microscope (cryo-EM) at the ESRF was inaugurated in November 2017, and is the first platform to be operated by all four PSB Partners and thus provides an excellent example of a PSB collaboration benefitting a wide range of European users. PSB *et al* met with the four scientists who provide user support on CM01, to know more about them, how they work together and what their views on cryo-EM are.

Can you say a few words about your background/career path and how you came to electron microscopy?

Eaazhisai Kandiah (EK): I am a physicist by education. I was interested in solid state physics and I did both my PhD and postdoc in crystallography, at the Indian Institute of Science in Bangalore and at ETH Zurich, respectively. During the postdoc I was working on nucleosome remodeling proteins, but their crystallization was very difficult so at that time the lab was setting up an EM facility and group. The scientist who was setting up EM at ETH (Takashi Ishikawa), encouraged me to go to his previous lab (led by Alasdair C. Steven at the National Institutes of Health, NIH) to learn and get a good training in EM. This is how it got started and I’ve now been working with EM for the last 12 years. I then came to Imre Berger’s lab in EMBL Grenoble, further moved to Irina Gutsche group at IBS and finally started at ESRF in September 2017.

Daouda Traore (DT): I graduated in chemistry (Université Joseph Fourier Grenoble), but from day one structural biology was what I had in mind. During my PhD I worked on both X-ray crystallography and X-ray absorption spectroscopy, in the Synchrotron Group at IBS. Then my first postdoc was mainly focused on X-ray crystallography at Monash University in Australia (which I started only a week after defending my thesis). During my second postdoc, I was working on pore forming toxins which form giant barrels and punch holes on the surface of cell membranes. Those molecules can simply not be crystallized. This was the main motivation which led my boss (Prof. James Whisstock, Monash University) to the purchase of the first Australian Titan Krios microscope and that is how I got interested in cryo-EM.

Michael Hons (MH): I graduated in molecular biomedicine at the University of Bonn and gradually moved via chemical biology and biophysics to structural biology. I started in a chemical biology lab (led by Michael Famulok, University of Bonn) on a project on aptamer-small GTPase interactions involving several biophysical methods. At that time, I became interested in structural biology and did an industrial internship on protein crystallography at Bayer. In 2011, as I knew it was hard to grow crystals of protein complexes, I started to learn cryo-EM in Holger Stark’s lab (Max Planck Institute for Biophysical Chemistry). There I worked on dynamical studies of the effect of antibiotics on ribosomes and on the structure of cohesin which holds sister chromatins together. I stayed six years in his lab before moving to Grenoble to start at EMBL Grenoble and take part in the CM01 team at ESRF.

Gregory Effantin (GE): I have been working in the EM field since 2002. I did my first training in EM with James Conway at the IBS, and I then continued working in the same group for my PhD. At that time, EM was not as popular as it is today but I really liked it and, even more than 15 years ago, there was already lots of potential for EM as we could do things that were not possible using crystallography or NMR. Then I did a postdoc in cryo-EM at the NIH near Washington DC, and then came back to Grenoble to do more cryo-EM, cryo-elecron tomography and cellular electron microscopy alternating between the groups of Winfried Weissenhorn and Guy Schoehn at IBS.

The resolution revolution in cryo-EM took place just a few years ago. Did you expect it or was it also a surprise for you?

GE: I always believed in cryo-EM even before the revolution. The resolution was steadily improving, even though it was mainly for large and/or highly symmetrical molecules. But when the first direct electron detector was installed at the IBS in 2014, we immediately got a 4Å resolution on the very first sample we analysed, and in just one week of work! That is when I really realised that it would change everything, but I did not really see it coming.

EK: It is during my days at ETH (2004) that I first started hearing enthusiastic seminars from pioneers in the field, such as Wolfgang Baumeister and others, about bringing the future of cryo-EM closer and therefore believing that some important developments would come up. But I thought it would develop like any other technique and didn’t expect it to come that fast and to this extent.

MH: In 2011, when I arrived in Holger Stark’s lab they had already impressive results. To me the revolution has indeed been a steady process, as before 2015 there were breakthroughs on many levels allowing the revolution in EM. But what came unexpected to me is that it would go so fast to smaller and smaller molecules. When I started, 300 kDa was considered to be small, but with the new detectors people quickly started working on much smaller stuff.

How is CM01 operated and how do you share the workload?

EK: The role of each person is very well defined and that is something I appreciate. The ESRF User Office and the Safety Unit take care of all the administrative issues for the user support. Management of CM01 is ensured by Christoph Mueller-Dieckmann (ESRF), the Beamline Responsible, David Flot (ESRF), the Beamline Operation Manager (BLOM), Thierry Giraud (ESRF), for technical support, Deborah Davison (ESRF) for the scheduling and Guy Schoehn (IBS) for crucial scientific consultation. The data processing pipeline is handled by Olof Svensson (ESRF). So it’s a much bigger team and we have a smooth operation that was set like any other ESRF beamline. For us four, the common and most important function is user support, which we equally share.

MH: We schedule our availabilities in order to divide the user support between the four of us. One experiment usually lasts three days, and we can support from one to four experiments in a row, but we try to avoid having only one person in charge constantly for two weeks as this is quite intense.

GE: The arrival of Daouda in the team last summer made a big difference as it is allowing to spread the user support workload over four persons rather than three, and thus enabled us to dedicate more time for other tasks either related to CM01 or to our other duties in our respective institutes.

Do you also carry out your own research and what are your other duties?

GE: I have some projects within the IBS EM group which are long term projects, and some collaborations mostly with other groups at IBS that need some EM expertise. My involvement depends on the need for the project, it can be any of the typical cryo-EM tasks: sample preparation, imaging, image analysis and interpretation of results. Projects are coming and ending so this is also very dynamic and it opens different opportunities.

MH: In the last 3 years, the demand for cryo EM has increased strongly at EMBL Grenoble so I was giving a lot of training and took care of the EMBL microscopes (a T12 and a Glacios). Also the Glacios was just installed last year. These were interesting times, but I had no time for my own project. Now I am starting my own research, mainly through various collaborations.

DT: My contract is 50% ILL and 50% Keele University. For the university side I have some teaching duties in the UK and I also have to have my own research programme. I study the bacterial conjugation machinery and this project involves a lot of structural biology including cryo-EM. For the ILL part of my contract, 25% of my time is dedicated to provide support on CM01 and the remaining 25 % is to link up the neutrons with EM.

EK: The main function of my job is the maintenance and operation of the Krios microscope. I perform my own research as well. I am co-supervising a PhD student who is shared between Sanofi and ESRF. Besides, I have a few collaborative projects on the microscopy side (data collection, data analysis), some of which came from users but mostly from people I met at meetings and conferences.

In your view what are the advantages of working in the PSB environment and how does it benefit CM01?

EK: Many of the external users of CM01, were previous users of PSB facilities like the Synchrotron, the SANS/SAXS platform and the cryoEM platform of the IBS. The Krios was installed as a complementary technique to the MX beamlines and the microscope is only used for high end data collection. The users support requires a lot of knowledge sharing and thus I think that making the four institutes come together to run CM01 was really an important decision.

DT: I think it is an advantage to work with people from different backgrounds as they do things slightly differently but in the end we all have the same goal. For me it was a good thing to see different approaches with no right or wrong way, but just having different perspectives.

GE: An advantage for CM01 to be within the PSB is the EM expertise on the campus. Guy Schoehn (IBS) was involved in the setup of the Krios and his contribution (when the ESRF bought the Krios) has been very important. It is clear that without the ESRF there would be no Krios, but without the other institutes how would it run? The provision of manpower and expertise from the other institutes is thus a big help.

MH: Initially, we had all different knowledge and background and during these years working together we learned things from each other and now use this common knowledge to run the platform. Also, the evolution of EM on the campus has been quite dramatic. At first it was just the IBS being strong in cryo-EM and today we have a large community of people doing EM in the four institutes, and three infrastructures gathering 6 EM microscopes which is one of the largest cluster of cryo-EM in France. All of this in less than three years, I find it very impressive!

Florent Bernaudat (PSB Coordinator) and Mizar Oliva (ILL)

DATES FOR YOUR DIARY

26th to 31st January 2020 - FEBS INSTRUCT MOBIEU HyThaBio Practical Course
More information: https://biomacromoldynamics2020.febsevents.org

3rd to 5th February 2020 - ESRF User meeting
More information: http://www.esrf.eu/UM2020

17th February 2020 - PSB Student day
More information: http://www.psb-grenoble.eu/

2nd March to 3rd April 2020 – Hercules European School
More information: http://hercules-school.eu

16th March to 20th March 2020 – Tutorial in Macromolecular Crystallography 2020 edition
For more information please contact the course organiser, Wim Burmeister: Wim.Burmeister@ibs.fr

29th March to 3rd April 2020 – Les Houches Winter School on Fluorescence Markers for Advanced Microscopy
More information: https://fluorescenceleshouches.wordpress.com

3rd April 2020 – EMBL Science & society talk: Challenging the Misrepresentation of Science and Evidence in Public Life by Tracy Brown OBE
More information: https://senseaboutscience.org

7th to 12th June 2020 – Les Houches TSRC Protein Dynamics Workshop
More information: www.tinyurl.com/protdyn2020

30th June to 3rd July 2020 – AFC2020 French Crystallography Association Congress
More information: https://www.afc.asso.fr/agenda-afc/1449-colloque-2020-de-l-afc

ANNOUNCEMENTS

On the 30th October 2019, **Sigrid Milles**, researcher at IBS, received the Paoletti Prize 2019 for her work on intrinsically disordered proteins studied by single molecule fluorescence and nuclear magnetic resonance spectroscopy. This prestigious award is presented in memory of Claude Paoletti, former Head of the CNRS Life Sciences Department who took many initiatives to support young researchers.

Contacts
Editors: Andrew McCarthy (EMBL), Mizar Oliva (ILL)
and Montserrat Soler Lopez (ESRF)
Student representative: Lena Anastasakou (EMBL)
Editor-in-chief: Joanna Timmins (IBS) and Florent Bernaudat (PSB)
Design: Virginie Guerard
Photos: Serge Claisse
Email: cisbnewsletter@embl.fr
www.psb-grenoble.eu

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.