JULY 2017 - NUMBER 19

PSB *et al*

Partnership for Structural Biology Newsletter

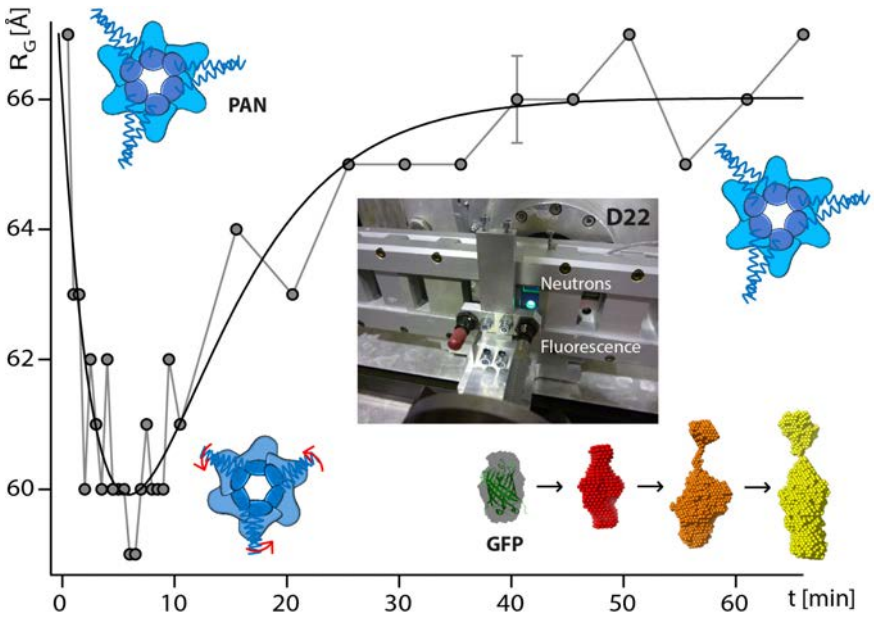
SCIENTIFIC HIGHLIGHTS

Neutron Scattering Provides New Insight into Protein Substrate Processing

AAA+ ATPases are a large family of ubiquitous enzymes with multiple tasks, including the remodelling of the cellular proteome. A subfamily, so-called unfoldases, recognize, unfold, and address misfolded or dysfunctional proteins towards proteolytic complexes, which eliminate these potentially toxic proteins for a healthy, functional state of the cellular proteome. Energy-dependent proteases are found across the three domains of life: bacteria, archaea and eukaryotes. In these systems the AAA+ proteins form a hexameric ring complex that associates with the catalytic core particles. The common feature believed to underlie the diverse functions of the AAA+ family of ATPases is their ability to undergo structural alterations during the ATP power stroke that cause unfolding of proteins or disassembly of protein complexes. Given the intrinsic flexibility of ATPases and the transient character of the interaction with their protein substrates, it is challenging in structural biology to follow the conformational changes of these enzyme-substrate complexes during the active unfolding process.

In a collaboration between ILL and IBS a novel approach was developed combining time-resolved small angle neutron scattering (TR-SANS) with online-fluorescence spectroscopy on D22 (Anne Martel), in order to monitor the PAN unfoldase from the deep-sea *Methanocaldococcus jannaschii* organism and a Green Fluorescent Protein (GFP) model substrate. By using alternating perdeuteration of both partners (ILL DLAB) and by controlling the enzymatic activity of the hyperthermophilic PAN system by temperature activation at 55-60°C, it was possible to follow conformational changes of both PAN and GFP separately and individually during the active unfolding process at a time resolution of 30 seconds.

The results show the progressive unfolding and aggregation of GFP as well as a reversible contraction of the PAN unfoldase during the active reaction. Concomitant with the unfolding of its substrate, the PAN complex underwent an energy dependent transition from a relaxed to a contracted conformation, followed by a slower expansion to its initial state at the end of the reaction. The results support a model in which AAA+ ATPases unfold their substrates in a reversible power stroke mechanism involving several subunits and demonstrate the general utility of this time-resolved approach for studying the structural molecular kinetics of multiple protein remodelling complexes and their substrates on the sub-minute time scale.
While the methodological approach developed has been designed for this specific project, it is expected that it can be applied to a wide range of biological macromolecular complexes, and provide structural information from individual partners at a time resolution of some seconds.



Reversible contraction of the PAN unfoldase during the active unfolding process of GFP. The picture inset shows the instrumental setup at D22 which allows the study of biomacromolecules in solution simultaneously by neutrons and by optical spectroscopy. A single, typical experimental error bar is shown for the RG data at 40 min. Bottom right: *ab initio* envelopes of GFP at different times during the unfolding reaction.

Z. Ibrahim & F. Gabel (ILL/IBS)

[1] Z. Ibrahim *et al.* (2017). Scientific Reports, **7**, 40948.

CONTENTS

How toxoplasmosis subverts MAPK signaling to survive in the host cell

Grenoble is host to many different research institutes, providing an opportunity for exchange and collaboration between scientists with different profiles. This was of crucial importance to this work carried out in collaboration with the team of Ali Hakimi at the IAB, Hassan Belrhali and Andres Palencia from the EMBL and Ulrike Kapp from the ESRF. Remarkably, this collaboration started following a dinner in 2013, where Hassan and Matthew (Bowler) discovered a shared interest in the same protein kinase: p38α, which is involved in several cellular functions and is a drug target for cancer and inflammatory disorders. Activation of p38α depends on a specific dual phosphorylation at the so called activation loop, usually promoted by another upstream kinase. Previous experiments have shown that during infection *Toxoplasma gondii* binds and activates p38α using an effector protein, called GRA24, overcoming classical MAPK activation by the upstream kinase [1]. This step promotes the subsequent GRA24-p38α complex entering the host cell nucleus initiating the inflammatory response that guarantees both control of the parasite population and survival of the host. Ali and Hassan were looking to solve the structure of p38α with the bound kinase recognition motif of GRA24 (GRA24 KIM domain). After initial trials at the HTX platform, we obtained crystals and this was the starting point for a fruitful collaboration between the EMBL, the ESRF and the IAB which finally led to the molecular description of how GRA24 sequesters p38α and leads to kinase activation (Fig 1A) [2].

The crystal structure of the GRA24KIM-p38α complex shows that GRA24 uses all the typical binding motifs used by kinases, phosphatases and scaffolding proteins to bind MAPKs, with a high K_D of 1.6 µM (measured by ITC) (Fig 1B). Binding of the GRA24 KIM promotes rearrangement of the two kinase lobes, eventually making the ATP binding site prone to nucleotide binding. Therefore, GRA24 binds with high specificity, which is essential to sequester the kinase from other regulatory proteins, and promotes activation of p38α without kinase phosphorylation. To further investigate the mechanism of activation of p38α by GRA24, we produced a recombinant complex of p38α and the N-terminal truncation of GRA24 (GRA24ΔN), which contains two KIM domains, by co-expression in bacteria. The overall structure of the complex, obtained through a combination of SAXS (Small angle X-ray scattering), AFM (Atomic Force Microscopy) and negative stain EM data, shows that GRA24ΔN is intrinsically disordered and one molecule of GRA24 binds two molecules of p38α independently, which is in agreement with the fact that one KIM domain is enough for GRA24 activity (Fig 1 C-E). Mass spectrometry confirmed that p38α in the GRA24ΔN-p38α complex is in the active state as being dually phosphorylated at the activation loop. *In vivo* experiments indeed show GRA24ΔN is the only factor required to stimulate the kinase response, leading to the important discovery that the complex is a powerful tool *in vitro* to unambiguously evaluate the pharmacological inhibition of constitutively active p38α.

E Pellegrini (ESRF, EMBL), M W Bowler (EMBL)

[1] L. Braun, M. *et al.* (2013). J. Exp.Med., **210**, 2071-2086.
[2] E. Pellegrini, *et al.* (2017). Structure, **25**, 16-26

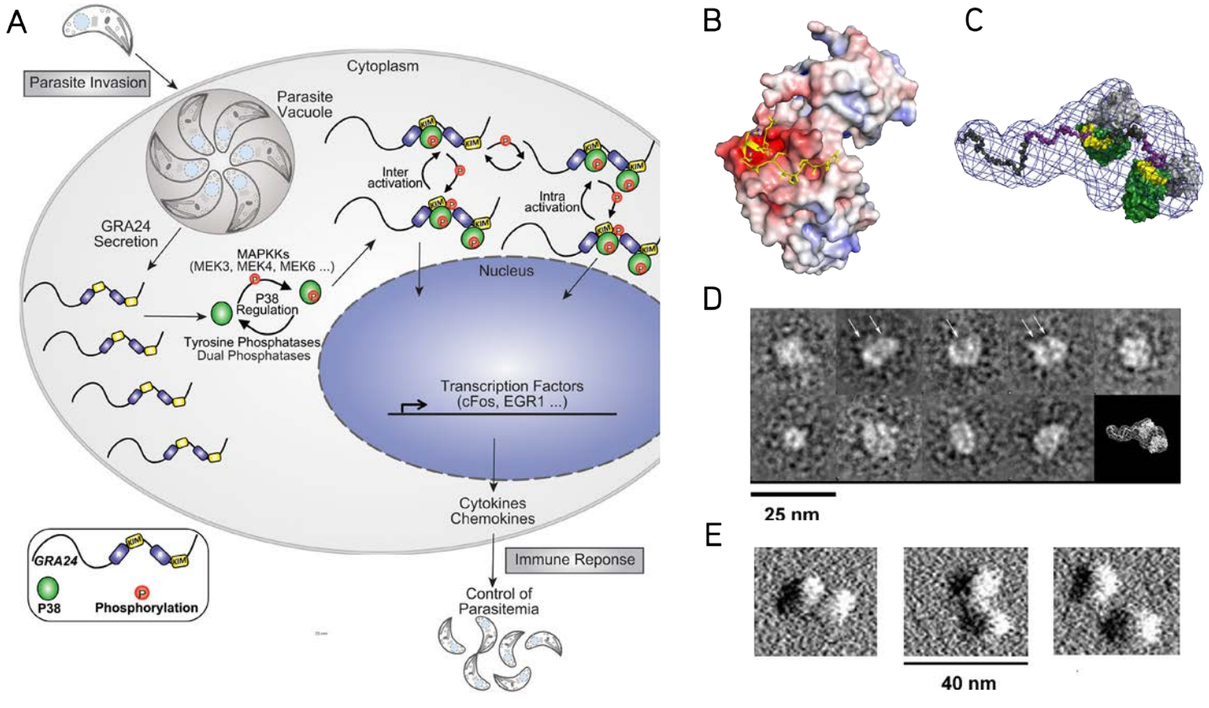


Figure 1. (A) Model of how GRA24 subverts MAP kinase signaling. GRA24 is secreted from the parasitophorous vacuole into the host cell cytoplasm where its high-affinity KIM sequence will recruit p38α. On binding GRA24, p38α is sequestered from regulatory kinases and phosphatases and can bind ATP allowing phosphorylation of the activation loop between and within complexes. The p38α-GRA24 complex is now a highly efficient active complex that can be translocated to the nucleus allowing a sustained and regular inflammatory response. (B) Structural overview of binding of GRA24 KIM motif to p38α kinase. (C) Model of the GRA24ΔN-p38α complex and its SAXS envelop. p38α gray (N-terminal lobe) and green (C-terminal lobe); KIM yellow, activation loop blue. (D) Class averages from negative-stain EM of the GRA24ΔN-p38α complex. Arrows indicate class averages where the two p38α molecules are separated, considerable variety in the orientation of the two kinases is observed. The SAXS model is shown to scale for reference. (E) The GRA24ΔN-p38α complex visualized by AFM, kinase pairs are observed in multiple conformations.

Mechanism of transmembrane signalling by sensor histidine kinases elucidated via iodide-SAD crystallographic studies

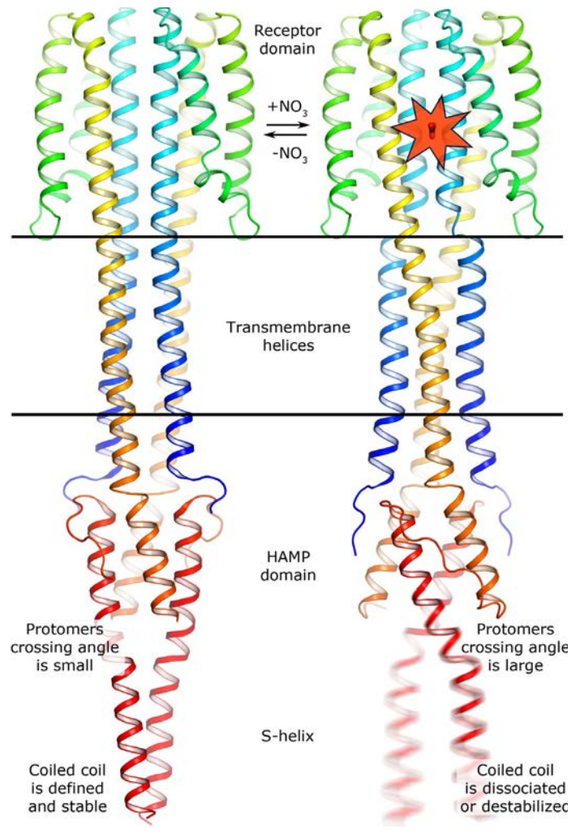
Sensing the environment is an ability of every living organism which is essential for survival. Sensor histidine kinases (HK) are a wide class of membrane receptor proteins; in bacteria many of these are involved in the nutrition and pathogenicity processes which makes them attractive studying targets to cure bacterial infections. Various HK of different species are expected to share similar mechanisms of functioning due to the same domain architecture and dimeric working state. Although HK are widespread, little is known regarding the molecular mechanism of sensory signal transduction through the membrane.

The collaboration between the structural biology group at the ESRF and the group of Valentin Gordeliy at IBS managed to shed light on the principles of signal transduction by HK. The structure of the truncated construct of NarQ – nitrate/nitrite-dependent membrane HK of *E. coli* – was elucidated in both ligand-bound and ligand-free states. In *E. coli* NarQ senses nitrate and nitrite in the periplasm and regulates the metabolism of these which are used as electron acceptors in the respiratory chain in the absence of oxygen. The two structures determined by X ray crystallography confirmed the concept of piston-like motion of a transmembrane α-helix as the main signal transmission mechanism across the membrane in HK previously proposed with the structure of the truncated sensor domain of homologous protein [1]. It was revealed that binding of nitrate or nitrite to the periplasmic sensor module of the protein causes structural rearrangements resulting in a symmetric relative displacement of ~1 Å of two out of four transmembrane α-helices in the dimer perpendicular to the membrane. On the cytoplasmic side this relatively small displacement of 1 Å is transmitted to the amplifying HAMP domain (named after being conserved in histidine kinases, adenylyl cyclases, methyl-accepting proteins and phosphatases) undergoing a scissoring motion which results in the maximum displacement amplitude of 7 Å at the end the HAMP output helices (see figure). The latter phenomenon is assumed to impact the stability of the downstream helices thus modulating the efficiency of kinase function.

The structure of the NarQ construct was determined using single-wavelength anomalous dispersion (SAD) as the molecular replacement technique provided unsatisfactory results [3]. SAD was performed on iodide ions which were incorporated into the crystal structure via simple and fast cryo-soaking of protein crystals in sodium iodide solution. This method [2] turned out to be a robust technique when applied to the case of membrane protein crystal structure investigations and this was tested on four target structures including the one of NarQ. All four structures were readily solved using iodide-SAD. The study also showed the possibility of applying the method in serial crystallography experiments carried out on membrane protein crystals. Because of its simplicity and effectiveness this method can become a viable tool in the arsenal of membrane protein crystallographers.

I. Melnikov[1], V. Polovinkin[2], G. Leonard[1], V. Gordeliy[2] & A. Popov[1] ([1]ESRF; [2]IBS)

[1] Cheung J, Hendrickson WA (2009). Structure, 17(2): 190-201;
[2] Melnikov I, Polovinkin V, Kovalev K, Gushchin I, *et al.* (2017) Science Advances 3(5):e1602952;
[3] Gushchin I, Melnikov I, Polovinkin V, Ishchenko A, *et al.* (2017) Science doi: 10.1126/science.aah6345



Mechanism of NarQ transmembrane signalling. Binding of the ligand results in a piston-like displacement towards the periplasm of the helices TM1 relative to TM2 and consequent lever-like conformational changes in the HAMP domain, which cause dissociation or destabilization of the signalling helix.

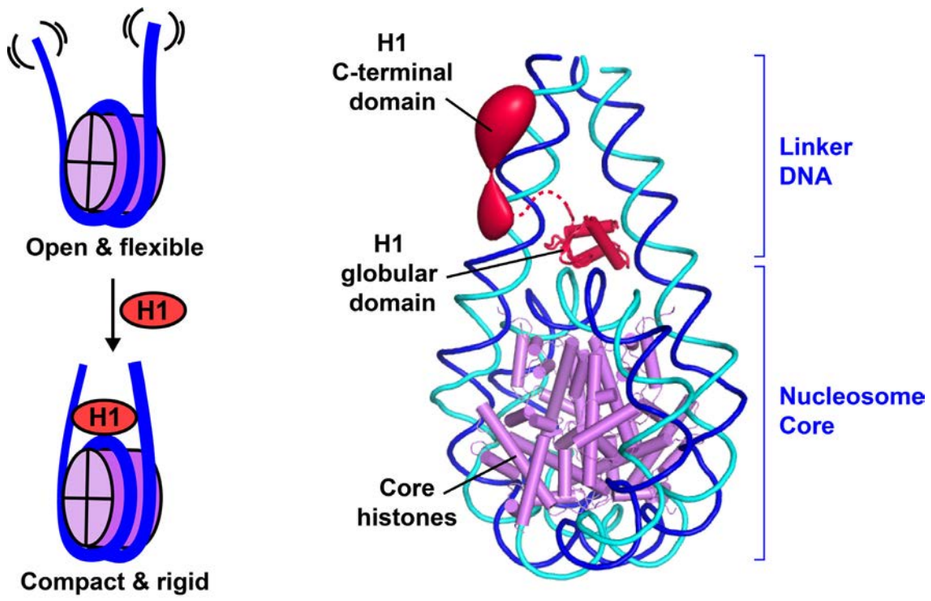
Structure of a fundamental particle of chromatin

DNA is packaged in the cell nucleus as chromatin, which contains positively charged histone proteins that bind the negatively charged DNA and allow it to adopt a compact structure. The degree of DNA compaction varies greatly, from the relatively open structure of an actively transcribed gene to the highly condensed structure of a mitotic chromosome. The basic structural unit of chromatin is the nucleosome core particle: 147 base pairs of DNA wrapped around an octamer of core histones (two copies each of histones H2A, H2B, H3 and H4). The next level of chromatin organization is the intact nucleosome, comprising the core particle plus a variable length of linker DNA, as well as an additional histone, the linker histone H1. Linker histones are critical for the assembly and maintenance of higher-order chromatin structures. Although the atomic structure of the nucleosome core particle has been known for two decades, that of the intact nucleosome has proved elusive.

As part of a consortium of researchers from Grenoble, Lyon, Strasbourg, USA and Japan, we determined the structure of a nucleosome containing 50 base pairs of linker DNA, both in the unbound state and in complex with H1 [1]. These structures were determined by cryo-EM and X-ray crystallography and were validated in biochemical experiments. The study revealed that the binding of H1 makes the nucleosome significantly more rigid and compact. Moreover, although the core particle has two-fold symmetry, the structure of the intact nucleosome is surprisingly asymmetric. The reason is that the positively charged C-terminal domain of H1 associates primarily with only one of the two DNA linkers. Both these observations are expected to have important consequences for how nucleosomes assemble to form a condensed fibre. Our structure will facilitate future efforts to elucidate the mechanism of chromatin condensation and the architecture of higher-order chromatin structures, which in turn will provide insights into key nuclear processes such as gene expression, DNA replication, epigenetic modifications and DNA repair.

I. García-Sáez & C. Petosa (IBS)

[1] J. Bednar, I. Garcia-Saez, R. Boopathi, A.R. Cutter, *et al* (2017). Molecular Cell, 66(3), 384-397



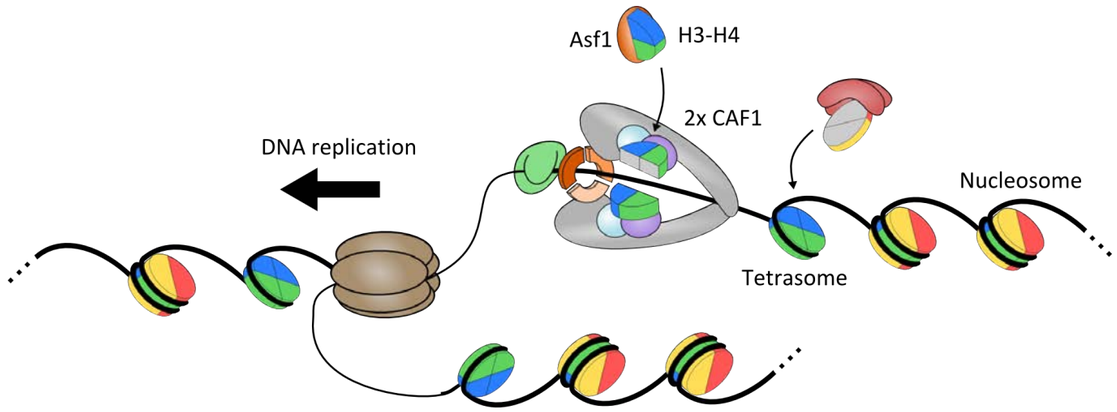
Nucleosome structure bound to linker histone H1. Left: The binding of histone H1 induces the nucleosome to adopt a more compact and rigid conformation. Right: The H1 globular domain binds on the nucleosome two-fold axis, while the highly basic C-terminal domain localizes to a single DNA linker, conferring polarity to the nucleosome.

The mechanism of nucleosome assembly by CAF1

Animal and plant cells contain very long DNA molecules that are tightly packaged by being wrapped around proteins called histones to form structures known as nucleosomes. While this is a useful way to store DNA, it also makes it inaccessible to many proteins and other molecules that activate genes, copy DNA or perform other important cell processes. To enable these processes to take place, the cell can selectively disassemble particular nucleosomes and remove the histone proteins. Afterwards, the nucleosomes must reassemble to repackage the DNA.
A single nucleosome contains four pairs of histones, with two pairs consisting of a H3 and a H4 histone, each. Histone chaperones assemble nucleosomes in a two-step process. First, two of these histone H3-H4 pairs (collectively known as a tetramer) interact with DNA to form a DNA-histone complex. Then, in the second step, two more pairs of different histones bind to complete the nucleosome. An enzyme called CAF1 is known to attach H3-H4 tetramers onto DNA as the DNA is being copied, performing the first step of the process described above. Only by the activity of CAF1 nucleosomes can form on the newly made DNA. How CAF1 deposits H3-H4 tetramers onto the DNA is not known. In a collaboration with colleagues from I2BC in Gif-sur-Yvette (Liu and Ochsenbein), Institut Curie in Paris (Sitbon and Almouzni), IBS in Grenoble (Boeri-Erba) and the German cancer research center in Heidelberg (Mücke and Langowski) we explored how yeast CAF1 works by carrying out a series of biochemical and biophysical experiments [1]. Using the MassSpec and the biophysical PSB platforms we showed that each CAF1 enzyme binds to a single H3-H4 pair. SAXS experiments at BM29 confirmed this result and revealed the overall shape of the protein complex. Biochemical experiments showed that two CAF1 enzymes, each loaded with its histone cargo, bind to DNA and attach a H3-H4 tetramer onto it. The tetramer has to form in this way for histones to be correctly delivered to DNA after the DNA has been copied. We also identified a new region of the CAF1 enzyme that binds to DNA. Together with another region, this enables CAF1 to bind to an extended stretch of DNA that is of sufficient length to accommodate the H3-H4 tetramer.
Together, the findings explain the sequence of events that take place when CAF1 begins to assemble new nucleosomes. Future work will be required to understand the structure of CAF1 in different situations and to find out how the cell targets this enzyme to stretches of DNA that have just been copied.

P. V. Sauer & D. Panne (EMBL)

[1] Sauer PV, Timm J, Liu D, Sitbon D, *et al* (2017) Elife 6, e23474



Cartoon schematic of CAF1 function. During ongoing DNA replication (arrow shows direction of replication), two CAF1 proteins (grey-blue-purple) bind to extended stretches of newly made DNA while each one is loaded with a H3-H4 histone pair (blue, green). Together, they deposit their cargo and make a tetrasome. Each histone pair has been delivered to CAF1 by another, unspecialized protein named Asf1. To make nucleosomes out of tetrasomes, different histones (yellow, red) need to be added by other proteins (red beans).

News from the platforms

CrystalDirect to beam

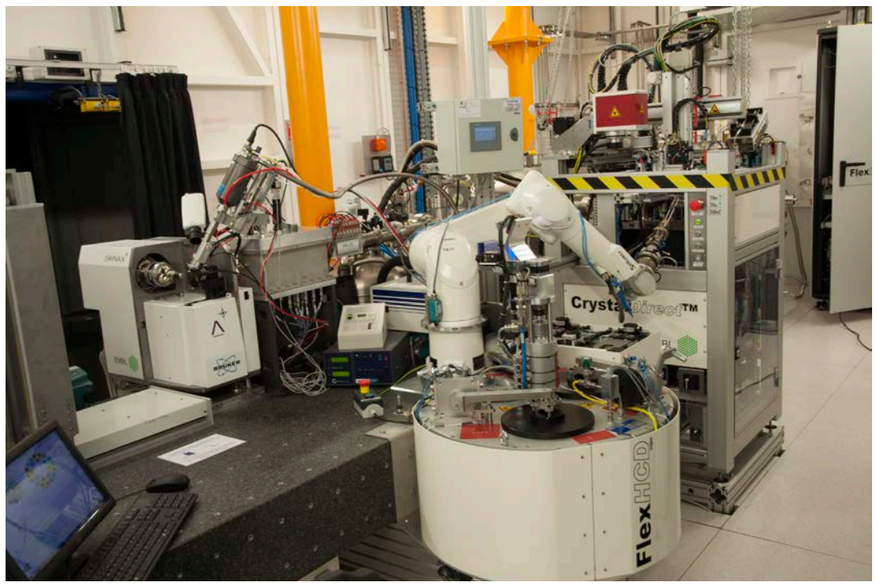
CrystalDirect™ (CD) is a new crystal harvesting robotic device developed by the High Throughput Crystallization (HTX) and Instrumentation teams at the EMBL allowing the automated harvesting of crystals without human intervention [1, 2]. Recent improvements at the HTX platform include the installation of a FlexED cryo-storage system coupled to a CD version2 harvester, allowing a completely automated pipeline from pure protein through to automated crystal harvesting. Other features of the new CD2 crystal harvester include flexible shape selection for mounting, ligand soaking, and CRIMS-ISPyB connections for data collection. While this is a significant advancement in sample handling that will improve the efficiency of many structural biology pipelines, especially for drug discovery projects when many data collections are necessary, it still requires the storage and transfer of crystals to MX beamlines for evaluation and data collection. As anticipated in the early days, the natural progression was to evaluate if having a CD harvester on a beamline would be beneficial to the structural biology community.

The installation of the advanced CD2 harvester at the HTX facility made the CD1 harvester prototype available, which was modified to operate together with the Flex robotics of a FlexHCD sample changer and installed on ID30B at the ESRF during spring 2017. The system was successfully integrated into the experimental setup over the following months to enable a harvested sample to be directly mounted on the MD2-S diffractometer by the FlexHCD. A new MXExpressR protocol was subsequently developed to allow the complete automation of harvesting to data collection pipeline by building on the automation workflows developed on MASSIF-1 and the other MX beamlines at the ESRF [3].

The first experiments included automated crystal harvesting, transfer to the diffractometer, diffraction evaluation and optimized data collection under cryogenic conditions, which were successful and enable direct diffraction analysis of multiple crystals from a crystallization plate. More complicated data collection protocols such as 'Mesh-and-collect', experiments at room temperature using a humidified air stream, and automated dehydration pipelines were also performed. Such options would allow ESRF users to explore more parameters, such as crystal hydration and ligand soaking times, to improve crystal diffraction for experimental success. These experiments were very useful in exploring the integration of a CD harvester into a beamline environment and will continue during the summer, after which it will be recovered. In the future CrystalDirect to beam could offer the shortest path from crystal to data to exploit the X-ray beam characteristics available after the ESRF-EBS upgrade.

J. Sinoir[1], G. Papp[1], F. Felisaz[1], A. Pica[1], G. Hoffmann[1], M. Bowler[1], U. Zander[1], T. Giraud[2], G. Leonard[2], C. Mueller-Dieckmann[2], A. Popov[2], O. Svensson[2], J. Marquez[1], A. McCarthy[1], F. Cipriani[1] ([1]EMBL; [2]ESRF)

[1] F. Cipriani, M. Röwer, C. Landret, U. Zander, F. Felisaz, & J. A. Márquez (2016) Acta Crystallogr. D. Struct. Biol. 68, 1393-1399.
[2] U. Zander, G. Hoffmann, I. Cornaciu, J.P. Marquette, G. Papp et al. (2016) Acta Crystallogr. D. Struct. Biol. 72, 454-466.
[3] O. Svensson, S. Monaco, A. N. Popov,, D. Nurizzo, & M. W. Bowler (2015), Acta Crystallogr. D. Struct. Biol. 71, 1757-1767.



The CrystalDirect™ to beam setup on ID30B at the ESRF.

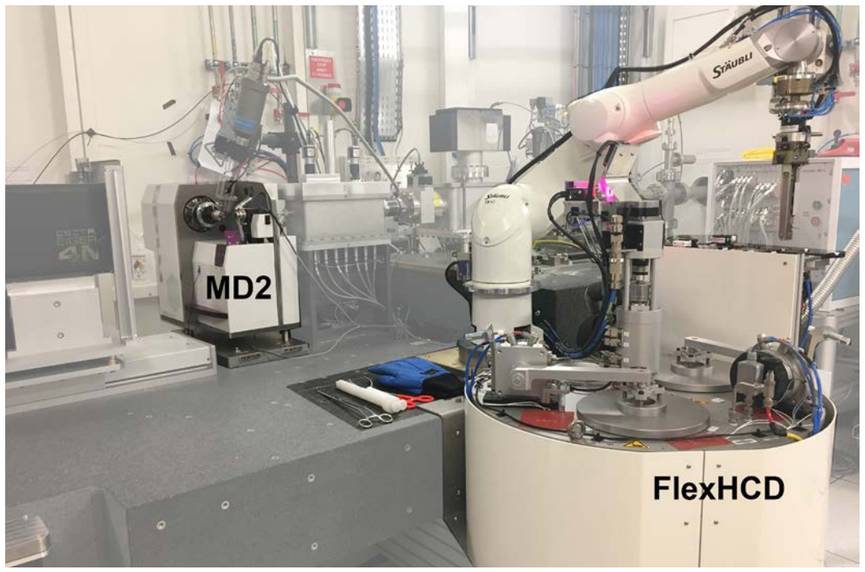
News from the Joint Structural Biology Group beamlines

In the last six months, systematic improvements on the EMBL-ESRF Joint Structural Biology Group (JSBG) beamlines have continued. All the user operated macromolecular crystallography (MX) beamlines are now equipped with new generation FlexHCD sample changers developed by the EMBL Grenoble Instrumentation team and ESRF structural biology group. These new, fast and reliable sample changers can accommodate samples stored in both EMBL/ESRF or Unipuck formats (with the exceptions that MASSIF-1 can only accept EMBL/ESRF type pucks and ID23-2 can only accept Unipuck format). The FlexHCDs are under continuous improvement and a new double gripper compatible for Unipucks formats with a sample unload/load transfer time of <12s was recently installed and commissioned on ID30B. This new development will soon be available on the other MX beamlines.

Other beamline improvements include:

- The deployment of the new Extended user interface for ISPyB (Exi, http://exi.esrf.fr/) for MX and bioSAXS. PSB users are encouraged to migrate to this new version. Improvements include speed of page loading and better feedback on experimental results, including auto processing and phasing (both *ab initio* and molecular replacement). Users are also welcome to submit feedback on possible improvements.
- The MD2 micro-diffractometers on MASSIF-3 and ID29 will both soon be upgraded to allow continuous mesh scanning as implemented on ID30B.
- The staff of the BM29 bioSAXS beamline are continuously improving data analysis software routines, online HPLC protocols, and testing the integration of new 3D printed sample exposure pods manufactured by the ESRF sample environment group.
- A Crystal Direct™ (CD) harvester was integrated into the experimental setup of ID30B to evaluate the potential of coupling crystal harvesting to data collection (see article by Sinoir *et al.* on page 6).
- A general method for *de novo* phasing of membrane proteins has been developed, see article by Menlikov *et al.* on page 3 for further details.
- Lastly, the upgrade of ID23-2 to provide a high intensity (~1.2 x 10^{12} phs/s) in the original beam size of ~6 µm in diameter and in a reduced beam size of ~1.5 µm in diameter is in the last stages of commissioning and will be open for users from July 2017. **Please note that only samples stored in Unipuck format will be supported by the FlexHCD on ID23-2** due to the rotation axis orientation on the new diffractometer.

M. Bowler[1], M. Brennich[1], D. de Sanctis[2], D. Flot[2], G. Leonard[2], A. McCarthy[1], S. Monaco[2], C. Mueller-Dieckmann[2], M. Nanao[2], D. Nurizzo[2], P. Pernot[2], A. Popov[2], M. Tully[2], D. von Stetten[2], U. Zander[1] ([1]EMBL; [2]ESRF)



MASSIF-3 experimental setup highlighting the MD2 diffractometer (from BM14) and FlexHCD sample changer.

Events

FluoDay 2017

The third edition of the FluoDay was organized at IBS this year on June 20th and benefited from financial support from GRAL, UGA and the partner institutes (IBS, IAB and CEA). This meeting brought together 85 experts and users of fluorescence microscopy from the different research institutes in the Grenoble area (GIN, IAB, UGA, LIPHY, CEA, IBS, EMBL, ILL and ESRF). The day started with a presentation of the different microscopy facilities available in Grenoble and was followed by a round table discussion that provided the user community with the opportunity to ask questions and receive advice and tips from the experts. After the lunch break, six scientific talks illustrated the know-how of microscopists in the Grenoble area and the great added value of fluorescence imaging. The audience was stunned by all the beautiful microscopy images and animations. This one-day meeting ended with a second round table during which the experts and

Group photo (credit J. Timmins)

representatives of the different institutes discussed future developments of the microscopy infrastructure in Grenoble, including its integration into the France BioImaging network and strategies to share and disseminate their expertise within the user community.

J. Timmins (IBS)

Neutrons in Structural Biology (NISB 2017)

The Neutrons in Structural Biology meeting took place at the ILL (7th-9th June). This biannual meeting focuses on highlighting recent scientific neutron results in structural biology as well as considering the future of the field. As in all areas of structural biology nowadays, there is a very strong emphasis on interdisciplinarity with neutrons occupying a crucial role alongside the other major techniques (X-rays, NMR, EM…), which was very well illustrated throughout the meeting.

93 participants gathered for this edition that was particularly international and dynamic, with cutting edge scientific talks highlighting the uniqueness of the type of information that can be obtained with neutrons.
The meeting ended with a round table discussion with a panel composed of high profile neutron experts as well as the ILL science director. The discussions put strong emphasis on just how far the field has come over the years. Neutrons have become an increasingly powerful and sophisticated tool particularly through unique access to non-selective and selective deuteration. The community highlighted the

need to keep this momentum going in particular through the upgrades of the various instruments. NISB2017 was a very successful meeting also thanks to the financial support of ILL, ESS, ISIS and Mitigen.

E. Mossou (ILL)

The PSB Student Day

The annual PSB Student Day took place in the Chadwick Amphitheatre on the 3rd of April, with participation from PhD students and interns from across the four institutes. The keynote speaker this year was the newly appointed EMBL group leader, Wojtek Galej, giving a presentation of his success story so far in studying the structure of the spliceosome and future plans to continue studying this system here, in Grenoble. First year students presented clips introducing themselves and their projects during the morning session, whilst second and third years presented posters on their results so far during the poster session, over lunch. Throughout the day we had presentations from: Alycia Yee (ILL), Paul Sauer (EMBL), Marco Nedeljkovik (IBS), Silvia Achilli (IBS) and Igor Melnikov (ESRF) each giving a talk on the interesting results they have obtained during their PhD projects.
The PSB Student Day was concluded with the annual prize giving with the clip prize going to Joanna Wandzik (EMBL) and the poster prize being awarded to Laura Lemel (IBS). On behalf of the PSB student committee, I would like to thank Florent Bernaudat for his guidance and help in organising a successful and enjoyable event.

The students of the PSB gathered together for the PSB Student Day.

If you would like to volunteer to help organise next year's event, please get in touch with Florent (florent.bernaudat@esrf.fr).

J. Channell (ILL) on behalf of organising committee:
T. Uroda (EMBL), R. Bouverot (ESRF), L. Lemel and C. Rousset (IBS), J. Channell (ILL) and F. Bernaudat (PSB Coordinator).

International workshop on advanced isotopic labelling methods for integrated structural biology

From 6th to 9th March, the second edition of the AILM2017 workshop was held at the IBS and the ESRF. This meeting brought together 160 researchers, nearly half of which were international visitors, including researchers from the USA, Canada, India, Australia and Japan. Two plenary lectures, 27 invited lectures and 20 lectures selected from submitted abstracts were programmed. A total of 49 oral presentations and some 60 posters covered the latest advances in isotopic labeling and their applications to the study of complex biological systems by NMR, neutron and mass spectroscopy. The participation to the workshop lectures was free for EPN campus employees and more than 50 researchers from EMBL, ESRF, IBS and ILL attended the presentations. The AILM2017 workshop, co-organized by IBS, IBPC, IGBMC and York University, was supported by GRAL, Biochemical Society, Instruct, Frisbi, CEA, CNRS, and University of Grenoble Alps. Thanks to the support of these sponsors and numerous exhibitors, the organizers were able to offer free accommodation to all young researchers who requested it.
16 young trainees were selected to pursue from 10th to 17th March with an advanced practical training in isotopic labeling and had the opportunity to be trained in advanced techniques using their own constructions. The next edition of the AILM workshop is scheduled for March 2019.

J. Boisbouvier (IBS)

PSB Spotlights

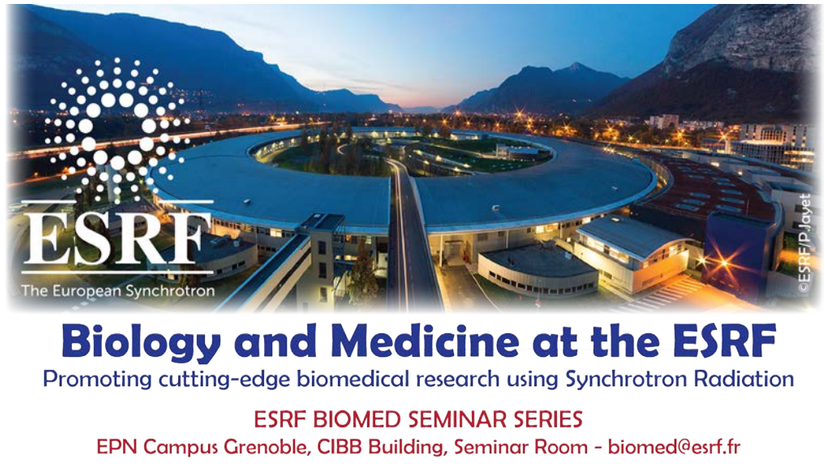
The PSB has launched a new initiative in 2017, entitled "PSB Spotlights". These one-day scientific meetings comprise introductory talks and practicals focused on a specific technique, methodology or resource, and Spotlight events aim to inform local students and researchers on how to exploit these techniques to advance their research projects.
The first meeting “PSB Spotlight on NMR spectroscopy” took place on 16th February 2017 at the IBS and included presentations on NMR applications by Bernhard Brutscher, Adrien Favier, Beate Bersch, Jean-Pierre Simorre, Cédric Laguri Jérôme Boisbouvier, Paul Schanda and Malene Ringkjøbing-Jensen (all from the IBS). The morning talks were attended by over 60 persons and in the afternoon a selection of 21 participants took part in a practical session on the NMR platform.
“PSB Spotlight on crystal & EM structures” was the second event organized on 28th April 2017. The speakers in the morning session, Matthew Conroy (Protein Data Bank in Europe – PDBe), Ardan Patwardhan (EMDataBank – EMDB), Gerard Bricogne and Clemens Vonrhein (both from Global Phasing) provided the audience of the Chadwick amphitheatre (~60 persons) with tricks and tools for exploiting data and databases. A practical session was also organized in the afternoon in the new 3D graphics room of the CIBB, to advise participants on how to find and evaluate structure data in the PDB and EMDB archives.
A third “PSB Spotlight on Molecular Dynamics” 2017 is currently under preparation for autumn 2017 and more information will be sent out soon.

Speakers of the PSB Spotlight on crystal and EM structures. From top left to bottom right: M. Conroy, A. Patwardhan, G. Bricogne and C. Vonrhein.

F. Bernaudat (PSB coordinator)

Seminar series in Biology and Medicine at the ESRF: promoting cutting-edge biomedical research using Synchrotron Radiation

Being a new initiative from the ESRF to foster transversal topics, the main objective is to promote cutting-edge interdisciplinary research and strengthen collaborations with the scientific community in Grenoble interested in applying synchrotron-based techniques in their biomedical research.
To this end, we organize seminar series that combine biomedical themes and synchrotron methodologies with presentations and round table discussions in an informal atmosphere. Quarterly series are dedicated to a particular topic, organized as monthly seminars of 1h duration (i.e. 3 seminars/topic) on Fridays from 10h to 11h in the CIBB seminar room. Both ESRF and external speakers are encouraged to participate. The seminars are scheduled and advertised in advance for organizational purposes. For updated information, please visit our website at: http://www.esrf.fr/home/events/Seminars/biomed-seminar-series.html
We very much welcome any suggestion, please feel free to contact us at biomed@esrf.fr. Interested people can join the discussions by sending an email to biomed-subscribe@esrf.fr. Looking forward to seeing you in the seminars!



M. Soler López (ESRF)

PROFILE

Anja Winter

I obtained my MSc in Biochemistry from Freie Universität Berlin, Germany, in 2004, focussing on proteins, biophysics and crystallography. My Masters project was to monitor peptide-HLA interactions and peptide dissociation kinetics of two HLA-B27 subtypes by fluorescence depolarization techniques. I then went on to pursue a PhD at the University of Edinburgh, UK, on a Darwin Trust scholarship. My main PhD topic was very challenging and centred on structural biology of prohibitins, and I was successful in refolding the protein complex for structural studies. My side project was to determine the molecular structure of Annexin B1. Both projects firmly established my preference for working with proteins and elucidating their structure-function relationship using biophysical techniques.

After a brief stay in Brisbane, Australia, I took up a postdoctoral position with Prof. Sir Tom Blundell at the University of Cambridge in 2008. During this post I became interested in fragment-based drug discovery, which is still an emerging field when targeting protein-protein interactions. I was part of an international FP7 project investigating HGF/SF and MET in metastasis and how to target their interaction, including the development of mouse models, medicinal chemistry approaches and antibody therapy. I developed skills in crystallography, biophysical techniques (CD, fluorescence, DSF, SPR), biochemical and cell-based assays as well as compound design, molecular docking and comparative modelling. After completing a postdoctoral position in Prof. Richard Bayliss’ lab at the University of Leicester investigating the molecular make-up of separases, I was delighted to start a lectureship position at Keele University which is a joint appointment with ILL, Grenoble, France.

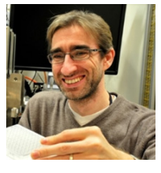
Research undertaken in my lab is based on work I did during my PhD. My lab investigates the molecular make-up of prohibitins and studies their interaction with viral envelope proteins, such as Chikungunya virus, dengue serotype 2 virus and white spot syndrome virus. My aim is to study these protein-protein interactions at a molecular level utilising neutron scattering and X-ray scattering as well as biophysical methods offered through the PSB platform. Outcomes from these studies could be used to inform a drug discovery campaign aimed at finding novel anti-viral therapies. Other areas for my future research are prohibitins’ involvement in mitochondrial homeostasis, cell signalling, tumour development and obesity.

The joint position with Keele University and ILL gives me the opportunity to interact with many scientists and experts from different fields, which I find both exciting and stimulating. I am looking forward to collaborating on different aspects of my research with scientists based at the Grenoble campus, particularly with instrument groups using SANS and neutron reflection techniques. Part of the work I am now engaged in will depend crucially on SANS and SAXS and is being carried out in collaboration with Dr Sylvain Prévost, who is responsible for the D11 instrument at the ILL. Equally, I will be happy to contribute my expertise to the capabilities of ILL's user programme - particularly in its Life Sciences Group.

A. Winter (ILL/ Keele University)

Newcomers

Michael Hons joined the EMBL Grenoble outstation in April 2017 as a Staff Scientist for Cryo-Electron Microscopy. He will be one of three scientists (together with one each from the ESRF and IBS) to jointly run the new EM facility at the ESRF. Michael received his PhD at the University of Göttingen in Germany and worked at the Max Planck Institute for Biophysical Chemistry in Structural Dynamics department of Holger Stark on several single particle EM projects.
Contact: mhons@embl



Mark Tully joined the structural biology group at the ESRF in March 2017 and is the beamline scientist for the BioSAXS beamline, BM29 at the ESRF. After his bachelor’s degree, Mark initially worked in industry for 5 years helping to take a fibrin sealant from the bench to clinical trials, before returning to academia, obtaining his PhD in Biochemistry from Liverpool University. Mark continued as a postdoc at Liverpool before moving to Oxford for a postdoc at Diamond Synchrotron working on the BioSAXS beamline, B21 under Rob Rambo. Here he developed the SEC-SAXS platform for the beamline while he worked on several other projects including radiation damage in SAXS, protein glycosylation and on heparin interactions in the extracellular matrix.
Contact: mark.tully@esrf.fr

DATES FOR YOUR DIARY

6th to 7th July 2017 - Cryo-EM Symposium on the EPN campus
A symposium comprising plenary talks and sessions from leaders in the field of 3D cryo-electron microscopy (EM) will be held at the ESRF auditorium on July 6th and 7th, 2017. The aim of this conference is to promote the exciting opportunities in structural biology opened by the advances in cryo-EM and also to introduce the newly established cryo-EM platform on the EPN campus to the user community. Since the event is full, the symposium will be broadcasted via the ESRF YouTube Live Streaming. Stay tuned at http://www.esrf.eu/cryo-em.fr for further details!

August 21st to 25th 2017 - 9th AFMBioMed Summer School at IBS
The School offers an introduction to Atomic Force Microscopy in Life Sciences and Medicine for PhD students, post-docs, scientists, core facility technicians and engineers. Lectures in the morning are complemented by hands-on experiments in the afternoon usually supervised by the lecturers. There are about 20 places for students doing hands-on experiments. Participants are encouraged to send a CV and a motivation letter to the organizers. AFM beginners are welcome. This school is co-organized by the Pasteur Institute at Lille and ILL. Registration deadline: June 15th, 2017.
More information here: http://www.afmbiomed.org/grenoble-2017.aspx
Contact: Jean-Luc Pellequer

September 11th to 15th 2017 – EMBO Practical Course Small angle neutron and X-ray scattering from proteins in solution
This EMBO Practical Course will take place on the EPN campus and will cover the use of small angle scattering (SAS) of both neutrons and X-rays for the determination of the structures of biological macromolecules. Particular attention will be paid to sample preparation and the analysis and interpretation of SAS data in a biological context. The aim of this course is to enable the participants to maximise the information gained from the SAS technique in their future experiments.
More information here: http://meetings.embo.org/event/17-small-angle-scattering

November 10th 2017 – "PSB Spotlight on Molecular Dynamics"
A third "PSB Spotlight" is currently under preparation for autumn 2017 that will focus on Molecular Dynamics. More information will be sent out soon.

ANNOUNCEMENTS

Prestigious grant for imaging single molecules

Human Frontier Science Program (HFSP) supports intercontinental collaboration involving PSB partners in ILL

An international team of researchers, including Trevor Forsyth (ILL), has been awarded a research grant by the Human Frontier Science Program (HFSP) to develop a novel method for imaging individual biomolecules with atomic resolution. The team will receive a total of 1.35 million US-Dollars over a period of three years. The partners include Henry Chapman from Hamburg/DESY (Germany), Ned Seeman from New York University (USA), Rick Millane from the University of Canterbury (New Zealand). The involvement of ILL's Life Sciences Group forms a strongly complementary team with backgrounds in neutron and XFEL diffraction (single crystals and fibres), as well as data processing and novel sample preparation capabilities.
For more information check: http://www.hfsp.org

Focus on structural biology in ESRFnews

The latest issue of the ESRFnews magazine (July 2017, No. 76) has a focus on structural biology that includes reports on BioSAXS and microcrystallography at the ESRF and features articles on DNA repair, diabetes linked proteins, and an ESRF-EMBL protein pipeline for industry.

For more information check: http://www.esrf.eu/UsersAndScience/Publications/Newsletter

Contacts
Editors: Andrew McCarthy (EMBL), Estelle Mossou (ILL) and Montserrat Soler Lopez (ESRF)
Student representative: Jennifer Channell (ILL)
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.