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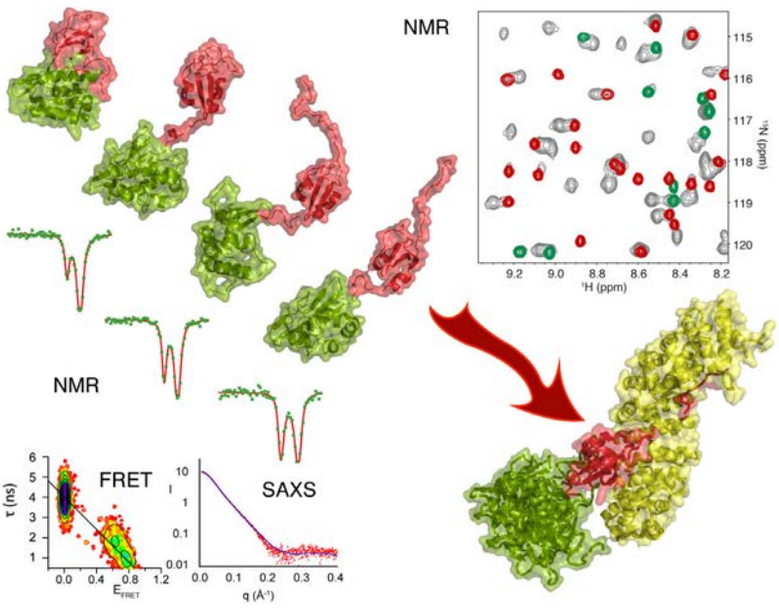
PSB *et al*

Partnership for Structural Biology Newsletter

Scientific highlights

Large Scale Conformational Dynamics Control the Function of H5N1 Influenza Polymerase



The structure of the 627-NLS domain (green and red) of the PB2 domain of H5N1 viral polymerase opens and closes at a rate of around 100s^{-1} at room temperature. Both forms can be studied simultaneously by NMR spectroscopy (top right). The open form interacts with Importin α (yellow) allowing transport of this part of the polymerase into the nucleus of the cell. These results were derived from a combination of NMR (top right and left), single molecule FRET (bottom left), and small angle X-ray and neutron scattering (bottom). © Martin Blackledge

A unique combination of complementary physical techniques has been used to reveal the molecular function of a protein essential for replication of H5N1 influenza virus [1]. A sub-domain of the viral polymerase undergoes large-scale structural reorganization to enable an essential part of the polymerase to enter the nucleus of the infected cell, where the viral genome is replicated. This study, published in the Journal of the American Chemical Society, illustrates how the flexibility of a protein allows it to adapt its function, facilitating infection of the host.

The polymerase allows the virus to reproduce copies of its genomic material in the infected cell, and thereby produce new viruses. It is known that adaption of the influenza virus occurs through mutations in the viral polymerase, in particular in the C-terminal domain 627-NLS of the PB2 polymerase protein. This two-domain protein is required for import of the viral polymerase into the nucleus, by binding to importin α.

The protein has been crystallized, in isolation [2] and in the context of the entire polymerase [3], but the crystallized conformation appeared incapable of binding to importin α, due to a strong steric clash between importin α and 627-NLS. The molecular basis of this essential interaction therefore remained mysterious [4].

Combining nuclear magnetic resonance spectroscopy (NMR), small angle scattering (SAS) and single molecule Förster resonance energy transfer (sm-FRET), researchers at the IBS, UVHCI, EMBL and ILL revealed that in solution the protein exhibits a far more complex behaviour. In fact the crystalline conformation indeed exists in solution, but this conformation exchanges, around 100 times per second, with another form of the protein, in which the two domains, attached by a flexible linker, dislocate and can move quite freely relative to each other.

Crucially, the 'open' form of the protein indeed interacts with importin α – via a highly dynamic interaction - and it is this conformational equilibrium that allows for PB2 to enter the nucleus. NMR exchange spectroscopy shows that the rate of exchange between 'open' and 'closed' forms of the protein, and their populations, are highly temperature dependent, and it seems possible that this thermodynamic equilibrium between closed and open conformations plays a role as a molecular thermostat, controlling the efficiency of viral replication in the different species where the virus needs to evolve as a function of the temperature of the host environment.

This study again highlights the remarkable efficiency of viruses to exploit conformational flexibility to extend their functional diversity with limited genetic material. The same two-domain protein has at least two distinct functions: the 'closed' form is necessary for viral replication within the polymerase once the protein enters the nucleus, but the 'open' form is necessary for import into the nucleus.

More generally, inter-domain dynamics play crucial roles in a multitude of molecular recognition, transport and signaling processes. These complex dynamic modes cannot be understood from static structures of either the entire protein or individual domains. The study demonstrates the importance of solution-state structural biology to accurately describe the relationship between structure, dynamics, thermodynamics and biological function.

D. Hart (UVHCI/IBS) and M. Blackledge (IBS)

[1] E. Delaforge *et al.* (2015). J. Am. Chem. Soc. DOI: 10.1021/jacs.5b07765, in press.
[2] F. Tarendeau *et al.* (2007) Nat. Struct. Mol. Biol., 14, 229-233.
[3] A. Pflug, D. Guilligay, S. Reich, S. Cusack (2014). Nature, 516, 355-360.
[4] S. Boivin, D.J. Hart (2011) J. Biol. Chem., 286, 10439-10448.

CONTENTS

Altered dynamics observed in an intrinsically disordered protein

Intrinsically disordered proteins (IDPs) are a class of proteins that fulfill a wide range of biological processes. The first IDP to be identified was casein in the 1950s. The casein proteins are members of the paralogous group of secreted calcium phosphate binding proteins (SCPP) which includes bone, tooth, milk and salivary proteins. In milk, the major function of casein proteins is to prevent the precipitation of calcium phosphate. This is achieved by sequestration of calcium phosphate into calcium phosphate nanoclusters. These nanoclusters contain a core of calcium phosphate surrounded by a protein shell. Because the caseins are disordered they can readily adjust their shapes to fit onto the irregular surfaces and even bend over the edges of the core and thus surround them. Recently it has been shown that osteopontin, another SCPP, can similarly sequester calcium phosphate. Unlike casein protein osteopontin is present in all biofluids where it has been sought, including saliva, blood and urine, it has been shown that at biological concentrations this protein can stabilise biofluids in a similar manner [1].

It is thought that in biomineralisation an increased sampling of conformational space is vital to allow sequestration of calcium phosphate prior to precipitate formation. In this work an international collaboration consisting of scientists from the ILL, Lund University and the University of Glasgow have studied the structure and dynamics of osteopontin from bovine milk. Benefitting in part from the biophysical platform at the PSB, osteopontin was determined to be intrinsically disordered. SAXS experiments, performed at the I911-SAXS beamline at the MAX lab in Lund, provided an ensemble of transient structures that osteopontin can sample in solution ranging from compact to highly extended. The dynamics of the protein were measured by incoherent neutron scattering on IN16 at the ILL. This technique provides information about protein dynamics on the angstrom and nanosecond resolution. It was shown that osteopontin is able to sample large areas of conformational space; upon nanocluster formation this ability is significantly reduced [2].

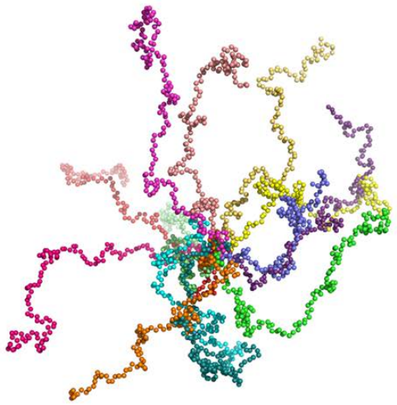
The results suggest that a high flexibility is required for peptides to sequester calcium phosphate, facilitating the so called “fly-cast mechanism” and allowing for tight packing on the surface of a calcium phosphate nanocluster. The results show that the characteristics of IDPs can alter depending upon their binding state. In the case of osteopontin this alteration is not of a structural nature but rather a dynamical one, suggesting that care has to be taken when the solution behavior of IDPs is related to their behavior in an *in vivo* crowded environment, often in the presence of many interaction partners. This research provides the ground for future in-solution studies using state-of-the-art third generation neutron backscattering instruments such as the newly available IN16B at the ILL [3].

S. Lenton (ILL)

[1] Holt, C., Lenton, S., Nylander, T., Sørensen, E. S., & Teixeira, S. C. (2014). Mineralisation of soft and hard tissues and the stability of biofluids. Journal of structural biology, 185(3), 383-396.

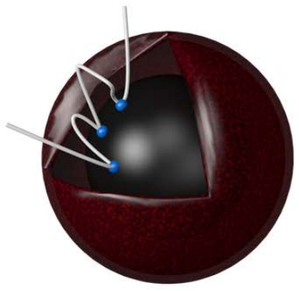
[2] Lenton, S., Seydel, T., Nylander, T., Holt, C., Härtlein, M., Teixeira, S., & Zaccai, G. (2015). Dynamic footprint of sequestration in the molecular fluctuations of osteopontin. Journal of The Royal Society Interface, 12(110), 20150506.

[3] Grimaldo, M. *et al.* (2015). High-resolution neutron spectroscopy on protein solution samples. In *EPJ Web of Conferences* (Vol. 83, p. 02005). EDP Sciences.



Sample	<k’> (Nm^{-1})
OPN	0.088
CPN	0.133

Δ<k’> = 0.045 Nm^{-1}



Osteopontin is a highly flexible IDP that becomes more rigid upon nanocluster formation. **Left**: Several ensemble structures that represent the disordered structure of osteopontin in solution. **Right**: The formed calcium phosphate nanoclusters, containing a core of amorphous calcium phosphate surrounded by the protein shell. The mean force constants obtained by neutron scattering are shown in the table.

A beamline that runs experiments by itself: protein crystallography goes fully automatic

Considerable human effort is dedicated to evaluating protein crystals at synchrotron sources in order to find the few crystals that diffract well or to find the one crystal that contains a potential new drug candidate. Much of this work is repetitive and the time spent could be better invested in the interpretation of results. MASSIF-1 is a unique facility for the high throughput, fully automatic characterisation and data collection of crystals and is run as a collaboration between the EMBL and ESRF [1]. The combination of robotic sample handling (RoboDiff) and advanced software protocols [2] has provided a new tool to structural biologists. The beamline is not designed to replace user visits to the synchrotron but rather to do the hard work of screening crystals or collecting data sets through the night, freeing researchers to spend time on more challenging data collection problems and study the underlying biology.

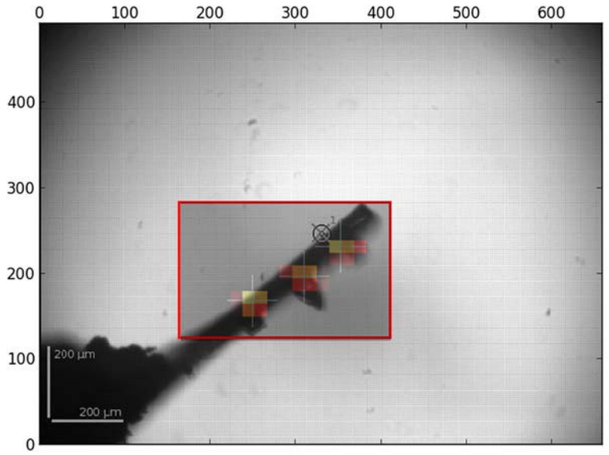


Figure 1: Sample mounts are analysed and an area chosen to scan through the X-ray beam (red box). Analysis of the diffraction images obtained yields a heat map defining the location and diffraction quality of crystals. Optimum points are then selected for data collection (crosses), in this case, three separate crystals.

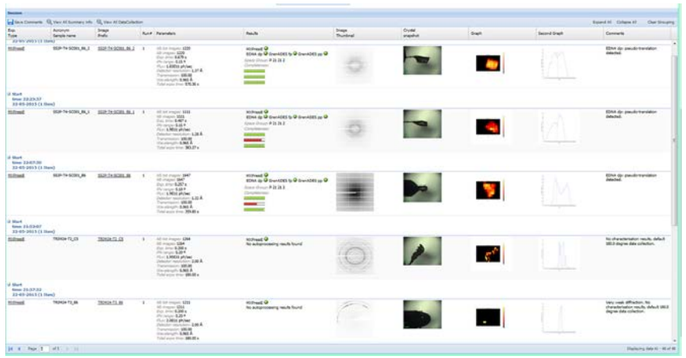


Figure 2: A screenshot showing the display in ISPyB of results, such as diffraction maps and auto-processing, for a series of samples processed at MASSIF-1. Comments are automatically written (far right column) to inform users on various stages of the process such as ‘weak diffraction’, default 180° data collection’ etc.

At the core of the beamline is a fully automatic system to mount, locate, centre to the optimal diffraction volume, characterise and collect data, if possible, from multiple cryocooled crystals [3]. The system operates at about the same speed as a human operator, taking an average of 6 minutes per sample, but performing far more than is possible with a user present, without getting tired. In the first step, the sample mount is analysed in order to determine the area to be scanned (Figure 1). A fast X-ray based routine is then launched to locate crystals and centre them to the beam systematically at the position of highest diffraction signal. The routine is often able to locate crystals more effectively than the human eye, either as they are mounted in opaque medium or a large excess of liquid causes lens effects. In many cases, diffraction data have been obtained when centring a crystal was not possible manually. Once centred, the samples are characterised and important parameters, such as flux, beam size and crystal volume, are automatically taken into account, ensuring calculation of optimal data collection strategies. Optimised data sets are then collected and automatically processed before the next sample is mounted. The X-ray centring routine developed for MASSIF-1 has been exported to all ESRF MX beamlines and is currently the most frequently used workflow by users present at the beamlines.

Automation necessarily involves standardisation; however, in order for an automatic data collection service to be successful for a range of projects, considerable flexibility in data collection parameters must be allowed. This has been implemented by allowing user preferences (such as type of data collection, resolution required, beam size etc.) to be entered at the sample level in the beamline data base ISPyB that also displays the results for each sample (Figure 2). An online booking system adds to the flexibility: rather than the rigid system in place on other beamlines, users can book time on the MASSIF-1 calendar with very short notice and samples then enter a queuing system.

Over 15,000 samples have now been processed with no human intervention. The latest developments now include options to allow the collection of data sets from multiple crystals on the same sample support (Figure 1). The new level of automation should decrease project lifecycles and, in partnership with development being made in the automatic mounting of crystals at the EMBL, a fully automatic pipeline from protein to structure can now be envisioned.

M. Bowler and D. Nurizzo (EMBL, ESRF)

[1] M. W. Bowler *et al.* J. Synch. Rad. 22, 1540-1547 (2015).
[2] S. Brockhauser *et al.* Acta Cryst. D 68, 975-984 (2012).
[3] O. Svensson *et al.* Acta Cryst. D 71, 1757-1767 (2015).

MeshAndCollect: A New Synchrotron Serial Crystallography Method

Serial Crystallography is a protein crystallography method where partial data sets are collected from many, sometimes hundreds or thousands, different crystals. Although this principle can be traced back to the very beginnings of protein crystallography, this method is today mostly known in context of the X-Ray Free Electron Lasers (XFEL). The data collection from small crystals at XFELs, for instance by injecting them in a liquid stream that intersects the beam, is called Serial Femtosecond Crystallography (SFX). However, as for many projects microcrystals are available in crystallization trials and the growth of big crystals is the last challenging optimization step before a diffraction experiment, there is demand for serial crystallography at synchrotrons as well.

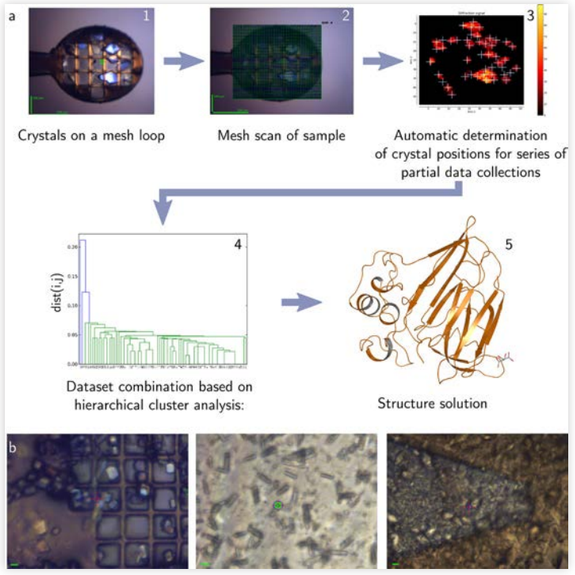


Figure 1: a) Outline of the multi crystal data collection method. Crystals are mounted on a standard sample holder that is oriented perpendicular to the X-ray beam (1+2). After a low dose mesh scan points of interest on the target are determined automatically and represented in a heat map (3). At each point data are collected over a small rotation range, usually 10°. By hierarchical cluster analysis datasets are selected for merging based on isomorphism for the structure solution (4+5). **b)** Beamline camera pictures of typical crystal samples where the MeshAndCollect method was successfully applied. The green scale corresponds with a length of 10µm.

The first Synchrotron Serial Crystallography (SSX) experiments made use of the highly standardized sample environment of modern MX beamlines: many microcrystals (1µm - 5µm in size) were loaded on a standard sample mount that could be moved continuously through the X-ray beam while data were collected on the fly. Processing data that has been collected by such a "mesh scan" style is challenging: only very few diffraction images per crystal are collected and it has to be determined a posteriori which diffraction frames belong to the same pattern.

With MeshAndCollect we developed a SSX method that allows protein crystallographers to collect data in a different way: similar as above, microcrystals are mounted on a suitable sample holder and rastered in a low-dose X-ray mesh scan. The collected images are inspected automatically by the software DOZOR that can identify protein diffraction patterns and well diffracting positions on the sample holder are ranked according to their score. Data wedges of 10° are collected at each determined position. These partial data collections are performed like for single crystals by rotation over the Omega axis of the goniometer and thus they can be integrated using standard programs such as XDS. To generate a complete dataset the partial data sets obtained are inspected by the program ccCluster: a correlation coefficient is calculated for each pairing of datasets based on the similarity of their structure factors and represented in an interactive dendrogram that allows the user to select the best possible combination. The merged data can be used for subsequent structure solution methods such as Molecular Replacement or SAD (Figure 1).

In the paper presented [1] the MeshAndCollect method is described in detail and a set of test cases is shown: the membrane protein bacteriorhodopsin of which data from crystals of about 5µm grown in lipidic cubic phase could be successfully collected (Figure 2), also crystals of thaumatin that diffracted to atomic resolution, crystals of monoclinic lysozyme to demonstrate the method for low symmetry cases, Thermolysin as a test case for high resolution SAD phasing on the Zn-edge and the novel protein maelstrom as an example for low resolution Se-Met SAD phasing.

MeshAndCollect is available as a workflow in MXCube to users on all MX beamlines of the ESRF.

U. Zander (ESRF)

[1] U. Zander *et al.* Acta Cryst. D 71, 2328-43 (2015).

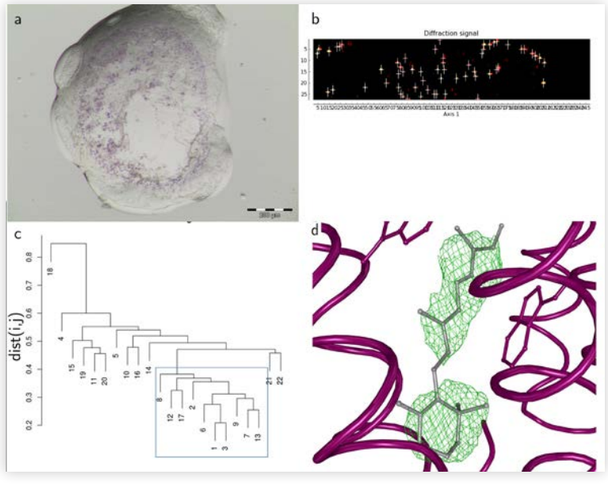


Figure 2: Mesh and Collect of Bacteriorhodopsin. a) LCP crystallization drop with purple bacteriorhodopsin crystals used in this experiment, crystal size ca. 5µm. **b)** Heat map representation of the low-dose mesh scan. The white crosses mark the positions that were used for subsequent data collections with a rotation range of 10°. **c)** Hierarchical cluster analysis of the data sets collected. The blue rectangle marks 10 partial data sets that were merged to obtain the final data set. **d)** OMIT difference density (mFobs DFcalc, αcalc) map at the end of the refinement procedure (contoured at 2.0 r.m.s.) for a retinal molecule.

How is Nonsense-mediated mRNA Decay activated?

Nonsense-mediated mRNA Decay (NMD) is a conserved mechanism involved in messenger RNA (mRNA) quality control in eukaryotic cells. NMD detects and degrades mRNAs containing a premature termination codon (PTC) to avoid the production of truncated proteins that can be detrimental for the cell. Several pathologies have been related to either failure of NMD to recognize faulty mRNA or to degradation of mRNA that encodes a truncated, but functional protein, which is crucial for proper cellular function. The NMD pathway is highly regulated, fine-tuned and involves multiple macromolecular complexes. A poorly understood cascade of events involving mRNA, ribosome and a number of proteins results in the recruitment of nucleases that degrade the faulty mRNA. To add a level of complexity, redundant pathways have evolved ensuring NMD is a robust, but very difficult system to study in humans. Importantly, a single event is necessary and sufficient to achieve NMD in humans: UPF1 phosphorylation by the SMG-1 kinase. SMG-1 is a phosphatidylinositol kinase-like kinase associated with SMG-9 to form an active complex or to the SMG-8/SMG-9 complex to form a complex of reduced activity.

One NMD model proposed based on immunoprecipitation experiments is that the recruitment of four proteins (SMG-1, UPF1 and the release factors 1 and 3a) by the ribosome stalled at a PTC triggers UPF1 phoshorylation by SMG-1 kinase [1]. Next, NMD factors UPF2 and UPF3 are recruited, leading to a decay-inducing complex. We studied SMG-1-8-9 and SMG-1-8-9-UPF1 complexes by cryo-electron microscopy using the IBS and EMBL EM facilities. The EM structures revealed important conformational changes in SMG-1 upon UPF1 binding [2]. Computational sorting of the cryo-EM data revealed different stages of UPF1 binding to SMG-1-8-9, highlighting a concerted movement of the SMG-1 C-terminal insertion domain, SMG-8 and SMG-9 when UPF1 enters the active site of SMG-1 kinase (Figure 1).

We corroborated our structural findings by kinase activity measurements, which confirmed the importance of the hitherto uncharacterized SMG-1 C-terminal insertion domain for the control of UPF1 phosphorylation. We found that SMG-8 is regulating SMG-1 kinase activity via interactions with the SMG-1 C-terminal insertion domain. Indeed, the presence of SMG-8 leads to specific phosphorylation of two sites in UPF1, consistent with previous data indicating that two phosphorylation sites in UPF1 are sufficient for NMD activation. Moreover, we found that UPF2 competes with SMG-1 for interaction with UPF1 [2, 3] and thus enables the release of phosphorylated UPF1 from SMG-1-8-9 complexes (Figure 2). UPF2 could therefore play an important role in the recruitment of phosphorylated UPF1 to the 3'RNP to nucleate the formation of mRNA decay complexes.

In summary, we provide evidence for a network of SMG-8, SMG-9 and the SMG-1 C-terminal insertion domain interactions that regulate UPF1 substrate recruitment and phosphorylation, a key step in human NMD activation [2].

A. Deniaud and C. Schaffitzel (EMBL, iRTSV/LCBM, CEA Grenoble)

[1] I. Kashima, A. Yamashita, N. Izumi, N. Kataoka *et al.* (2006). Genes Dev., 20, 355-67.
[2] A. Deniaud, M. Karuppasamy, T. Bock, S. Masiulis *et al.* (2015). Nucleic Acids Res., 43, 7600-11.
[3] M. Clerici*, A. Deniaud*, V. Boehm, NH. Gehring *et al.*, (2014). Nucleic Acids Res., 42, 2673-86.

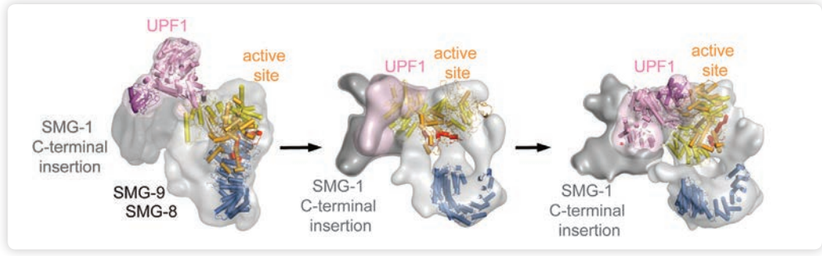


Figure 1: Cryo-EM structures of the dynamic SMG-1-8-9-UPF1 complex. Three states of the complex were identified by computational sorting of the cryo-EM data. The suggested order of UPF1 binding to SMG-1-8-9 and entry into the kinase active site (orange, labelled as active site) is shown from left to right: UPF1 first binds the C-terminal insertion, leading to conformational changes in the SMG-1-8-9 complex that allows UPF1 to reach the kinase active site where it is tightly bound between the SMG-1 C-terminal insertion and the active site (right). Pink: UPF1; yellow-orange-red: SMG-1 kinase domain, blue: SMG-1 HEAT repeats, dark grey transparent density: SMG-1 C-terminal insertion domain.

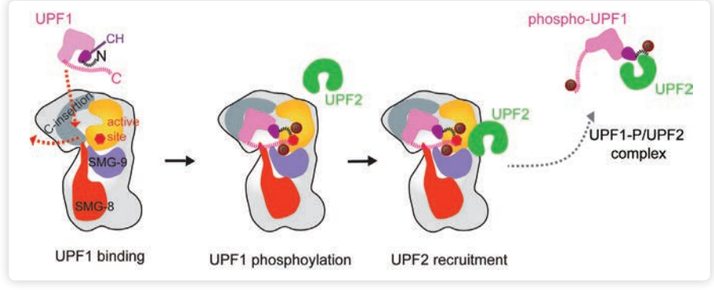


Figure 2: Model for the NMD activating step: UPF1 is recruited by the SMG-1 C-terminal insertion domain to the SMG-1-8-9 complex that opens to allow UPF1 to reach the kinase active site. UPF1 is phosphorylated and the subsequent recruitment of UPF2 allows phosphorylated UPF1 to be released and recruited to mRNA decay complexes involving UPF2.

News from the platforms

Two new Atomic Force Microscopes dedicated to imaging, pulling, and indenting biological samples

A new Atomic Force Microscopy (AFM) team has recently joined the Methods and Electron Microscopy Group at the IBS. AFM belongs to the scanning probe microscopy family where the "sensor" is a very thin tip located beneath a micro-scale cantilever. By controlling the size, shape, and chemistry of the sensor, AFM is able to image, pull, and push on single molecules or cells both in air and in liquid environment. Two AFM instruments are available at the IBS: a Dimension 3100 from Digital instruments (now Bruker AXS) and a multimode 8 from Veeco (now Bruker AXS) both equipped with a Nanoscope V controller. The former AFM allows imaging of large sample (several cm) since the moving scanner holds the cantilever above the scanning area whereas the latter AFM uses a fixed cantilever and a moving sample stage. In practice, the Dimension 3100 microscope is dedicated to dynamic force spectroscopy or nanobiomechanical experiments, while the Multimode microscope is used for high-resolution imaging, although it is also capable of performing force spectroscopy and nanobiomechanical measurements. The Multimode microscope is equipped with the latest off-resonance imaging mode (Peak-Force Tapping) allowing "softer" imaging of fragile samples. There is a certain complementarity between these new AFM instruments at IBS and those available at the Surface Science Lab, ESRF. Indeed, whereas SSL possesses physics-oriented own-made AFM as well as a "high-speed" AFM machine, they complement the more biological-oriented AFM machines present at IBS. **Contact: jlpellequer@cea.fr**

Current AFM set-up at IBS: the Dimension 3100 with the fixed sample stage is located at the back of the room whereas the moving stage multimode is located at the front.

J.-L. Pellequer and J. -M. Teulon (CEA-IBS)

News from the Joint Structural Biology Group beam lines

The official end of the ESRF Phase I upgrade was celebrated with a ceremony on December 4th, 2015. With this, the upgrade of the ID14 beam lines on BM29 and ID30, also known as UPBL10, is complete. During the last 7 years, four brand new, state-of-the-art end stations have been constructed. The first to take up full users' operation (since June 2012) was the bioSAXS facility located on the BM29. This beam line is equipped with an automatic sample changer and an online HPLC system, and complemented with an automated data analysis pipeline. The second and third beam lines opened to the public users were MASSIF-1 (ID30A-1) and MASSIF-3 (ID30A-3), the first a completely automated macromolecular crystallography beam line which accepts samples FedEx'ed directly to the beam line, where diffraction data are collected based on a protocol chosen by the users prior to data collection (see scientific highlight). MASSIF-3 combines a small beam (15 µm in diameter) with an extremely high flux (>2x10^{13} ph/s), allowing ultra-fast data collection, even at room temperature. This will be especially useful when the latest generation of fast read-out detectors, such as the Eiger 4M, will be installed (early 2016). Last but not least, ID30B was officially inaugurated in November 2015, but had already accepted users from June. This energy-tunable beam line is equipped with a versatile sample changer (FlexHCD) able to accept samples in both SPINE standard pucks and Unipucks as well as being able to mount crystallization plates for in situ diffraction experiments.

With the end of Phase I of the upgrade programme of the ESRF, Phase II has been launched, called EBS (Extremely Brilliant Source). During this phase, the complete storage ring will be rebuilt to reduce the horizontal beam size and divergence, resulting in increased beam brilliance and a decreased beam size. This storage ring improvement requires a shutdown period of 18 months, starting from December 2018.

A. McCarthy (EMBL) and C. Mueller-Dieckmann (ESRF)

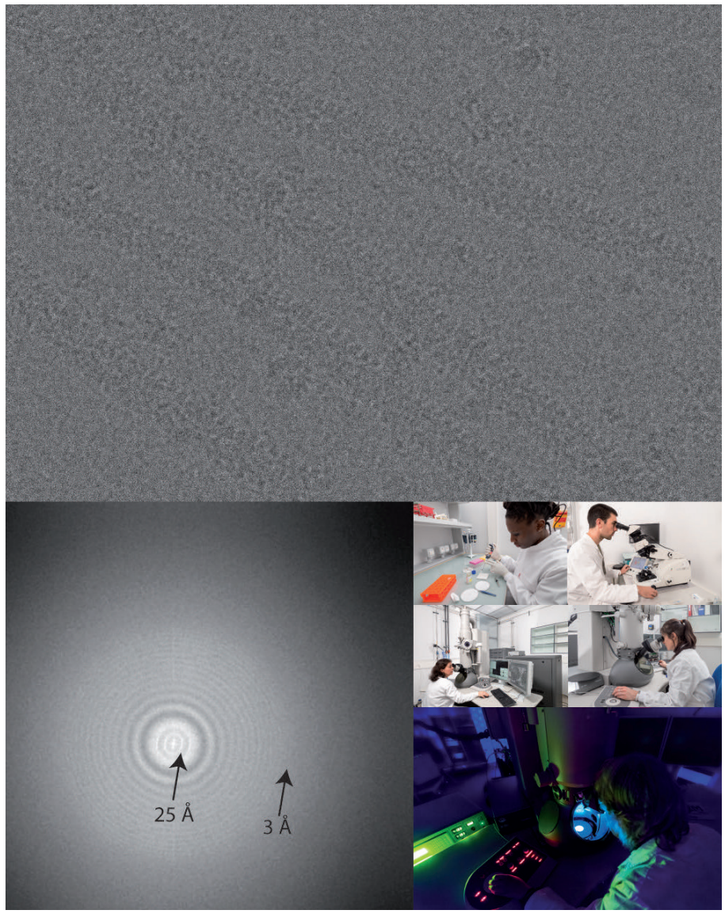
News from the Electron Microscopy Platform

Recently, the "EM revolution" has been the subject of several articles in various high-profile journals [1, 2]. Our EM platform is definitely in sync with the technical developments at the heart of this revolution. Indeed, thanks to financial contributions from FRISBI/FEDER and EMBL, we have upgraded the camera on the Polara microscope to a direct electron detector, the breakthrough that helps bring about the "revolution".

It allows us to:
- acquire tens of sub-frames with very short exposure time (instead of one image with a long exposure time), which are then aligned to compensate for drift and averaged to achieve a high contrast drift-free image.
- detect and count every electron that hits the scintillator bypassing noise arising from photo-to-electron conversion as in CCD cameras.
- take advantage of the super-resolution mode that interpolates a 5 x 5 µm^2 physical pixel to four 2.5 x 2.5 µm^2 virtual pixels (which means that we have a nearly 8k x 8k camera).

Altogether the new setup (Polara plus K2 summit direct electron detector) in combination with the new image analysis software have made near-atomic resolution on biological macromolecular assemblies possible (current, but still decreasing, in house limit is 4Å).
In addition, the ISBG EM platform is equipped with two other electron microscopes: a 120 kV T12 for quality control negative staining and cellular EM at room temperature and a 200 kV F20 cryo-microscope for tomography and for optimizing sample preparation before data collection on the Polara.

The platform is also at the final stage of making room temperature and cryo-cellular EM available (most of the techniques are already in place).
To know more about the different possibilities offered by the platform (in terms of sample requirements, delay, training, access, tariffs, collaboration possibilities, etc…) please contact us at ibs-plateforme-em.contact@ibs.fr or come directly to see us at the IBS.



Background: field of view obtained in cryo-EM with the new direct electron detector (Measles virus nucleocapsid).
Lower left: power spectrum of the EM image showing Thon rings down to 3Å resolution.
Lower right: EM platform members and instruments. EM Platform images © CEA , D. Morel

The IBS/ISBG EM platform members

[1] Bai XC, McMullan G, Scheres SH. (2015). How cryo-EM is revolutionizing structural biology. Trends Biochem Sci. 2015 Jan;40(1):49-57.

[2] Callaway E. (2015). The revolution will not be crystallized: a new method sweeps through structural biology. Nature. 2015 Sep 10;525(7568):172-4.

Events

3-day course: Structural biology of membrane proteins: from expression, to crystallization and serial crystallography

In the frame of Nanomem, 2 teams from IBS and ESRF both members of this Marie-Curie ITN network, organized a 3-day-course addressed to the PhD students of the European network and also to the students and young researchers of the site.
The course took place from the 7th to the 9th of December. The first day was devoted to the presentation of several expression systems followed by a discussion, and to purification. The 2nd day happened on ID13 where students could experience serial crystallography using microcrystals of thaumatin, GLIC, and ubiquitin. Students also visited the EMBL crystallization platform and discovered the new developments for automatic crystal harvesting. On the 3rd day, the importance of the stability of membrane proteins for functional and structural studies was highlighted and several strategies to enhance stability were presented. A presentation of the new possibilities offered by electron microscopy with a visit to the microscopes ended the session. One of the speakers, scientist in a biotech company also nicely showed in his presentation how research activities are possible in private companies. Up to 12 students followed the course including 6 from the Nanomem network.

M. Burghammer (ESRF), H. Nury, E. Pebay-Peyroula and S. Ravaud (IBS)

MXIS 2015: Practical course on in-plate crystallography

The MXIS training course was dedicated to *in situ* protein crystallography. The 2015 session, lasting 3-days from 17th to 19th November, included full lectures at the IBS (day 1) and practical sessions at the ESRF on beamlines FIP-BM30A and BM14 (day 2 to day 3). 13 participants from 9 different countries attended this workshop.

MXIS-2015 was the opportunity for participants to test the large number of possibilities offered by *in situ* crystallography and associated techniques, such as ligand dry-coating, crystallization, *in situ* diffraction for crystal screening and structure determination. The participants were given the opportunity to practice on their own samples.

This workshop was organized by IBS (Grenoble), EMBL-Grenoble and CBS (Montpellier). MXIS-2015 was made possible thanks to financial support from IBS, EMBL, and the GRAL and FRISBI programs.
http://workshops.ibs.fr/MXIS-2015

J.-L. Ferrer (IBS)

Fête de la Science

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From 3rd to 11th October the annual science festival "Fête de la Science" opened its doors for exciting science encounters with the public. Naturally, the EPN Science Campus was there to inform the public about our institutes and excellent research results.
At the Parvis des Sciences, next to MINATEC, the institutes of the EPN Science campus shared a big stand where we proposed crystallography and diffraction experiments, as well as outstanding presentations about proteins, neutrons and neutrinos, over two days.
On Friday 9th October, six classes of students from the region's high schools discovered the principles of spectroscopy and electromagnetic radiation during hands-on sessions organised by the ESRF.
On Saturday 10th October, the ESRF, ILL, EMBL and IBS invited the public to discover the activities that take place on the EPN science campus, at the same time as demonstrating the complementarity of the institutes and the importance of geographical proximity.

These activities attracted more than 3500 people over the two days. We would like to thank all the volunteers and persons of the EPN Science Campus involved in the organization of those two days: Alain, Bob, Chantal, Charles, Damien, Dominique, Ennio, Estelle, Florent, Françoise, Giovanna, Halina, Lucie, Mariam, Monica, Montserrat, Nicolas, Odile, Olivier, Romain, Sylvain, Thomas, Ulli, Victor, Virginie, Yannick, and all those who helped us. They all did a great job before the event and during the event, for the greatest delight of the visitors.

Y. Lacaze (ESRF)

Second symposium "Signaling Through Chromatin"

The second symposium on Signaling Through Chromatin, organized by the Chromatin Club of Grenoble, took place on 22-24th September 2015 at the Maison Minatec. With over 170 registered participants and an outstanding line-up of international speakers, this event was a great opportunity to find out about some of the most exciting research in the chromatin field this year.
The meeting consisted of 6 sessions with over 20 talks covering latest advances in chromatin dynamics as well as DNA- and RNA-based processes. Several short talks were selected from the submitted abstracts with preference for students and post-docs. This event was only possible thanks to support from local research institutes as well as several sponsors, in particular Epigenesys and Active Motif. This symposium once again confirmed that the Grenoble/Lyon area has a large and dynamic scientific community with interests in chromatin, epigenetics and gene expression research. Given the very positive feedback form both the speakers and participants, the activities of the Chromatin Club (www.epigenetics.fr), including the next symposium scheduled for autumn 2017, will continue increasing the visibility and providing new networking opportunities for the local community within the European chromatin/epigenetics research field.

J. Kadlec (IBS)

International AFMBioMed summer school in Life Sciences and Medicine

The 7th AFMBioMed (www.afmbiomed.org) summer school took place in Grenoble from August 24-28th, 2015. Twenty-six students and young researchers originating from 13 different countries, including Europe and North and Central America, participated to this edition. All the seven French participants were geographically located in Grenoble. Fifteen trainers were actively involved either in providing 1h30 long morning courses and/or participating to afternoon hands-on sessions. (http://www.afmbiomed.org/Data/Sites/1/grenoble-2015/agenda2015_v1.pdf).

The main goal of the summer school is to bridge the gap between biologists, who are among the most numerous users of AFM in biology, and physicists, who were among the first scientists to build and run AFM instruments. The summer school is oriented exclusively towards

biological samples and participants could learn standard practice in AFM set-up and sample preparation from leading scientists in this field. Every important aspect of modern AFM usage was tested: high-resolution imaging, high-speed imaging (SSL, ESRF), protein pulling and unfolding, cell nanomechanics, and plant tissue indentation. The school was co-sponsored by a Grant from the Labex Gral and by the generous help of Bruker AXS, which provided three additional AFM instruments, as well as one engineer, for the whole week. Local organization included the precious help from the Surface Science Lab at the ESRF.

J.-L. Pellequer (IBS), J. -M. Teulon (IBS) and L. Costa (ESRF)

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PROFILE

Pascal Poignard

I recently moved to Grenoble on a joint position between the Department of Virology at the University Hospital and the Institut de Biologie Structurale (IBS).

My main interests are HIV, antibodies and vaccines.

Following medical studies at the University of Tours, I went on to do an internship in Internal Medicine and Infectious Diseases at the University of Marseille. It is at this time that I became involved in science and research, completing a Master in Immunology before moving to Denver, Colorado to work on T cell responses in Toxoplasmosis. Following my interest in science, I then finished my clinical studies and embarked on a Ph.D. program in Immunology at the Centre d'immunologie de Marseille-Luminy (CIML). There, I started working on HIV and antibodies, studying viral entry and the mechanisms by which antibodies can block viral infectivity. In 1996, I left Marseille to start a post-doc at the Scripps Research Institute in La Jolla, California. Three years later, I got promoted to Assistant Professor and started my own laboratory, focusing on the use of a humanized mouse model of HIV infection to study antibodies and viral entry blocking therapeutic strategies.

In 2004, I was invited to join the University of Marseille as a Professor of Immunology and a group leader at the CIML, where I pursued work with the humanized mouse model of HIV infection and on antibody neutralization of HIV. In 2008, I had the opportunity to be part of the creation of the Neutralizing Antibody Center (NAC) funded by International AIDS Vaccine Initiative (IAVI) within The Scripps Research Institute, a center entirely dedicated to the development of an antibody-based HIV vaccine. Vaccines are one of the most cost-effective medical treatments in modern civilization and are responsible for saving millions of lives. Ultimately, an effective HIV vaccine is the best guarantee to put an end to the AIDS pandemic. Yet the development of an effective HIV vaccine has been challenging. At the NAC, I focused on the isolation of broadly neutralizing antibodies, which had become possible thanks to recent technical advances in the field, allowing for large scale high throughput screening of donors for broad neutralization, and for human monoclonal antibody cloning. The discovery of the first generation of novel broadly neutralizing antibodies in 2009 started a revival of the field of antibody-based HIV vaccine research. The following years have seen the isolation of a number of broadly neutralizing antibodies of remarkable potency that have shown prophylactic and therapeutic activities in animal models. These antibodies are key to rational vaccine design and may lead to new therapeutic strategies for HIV.

As no vaccine candidate has yet demonstrated an ability to elicit broadly neutralizing antibodies, together with my group we decided to further focus on understanding how such antibody responses eventually develop in a subset of HIV-infected donors. The goal is to gain knowledge from natural infection for the further design of effective immunogens and vaccine strategies.

In Grenoble, my team will pursue this work, investigating broadly neutralizing antibody responses in HIV-infected patients and tracing the path by which some individuals develop antibodies targeting highly conserved neutralization epitopes on the viral envelope glycoproteins. The characterization of the developmental pathway of broad antibody lineages in interplay with HIV will provide critical information for vaccine design.

P. Poignard (IBS/CHU)

ANNOUNCEMENTS

iNEXT: A new European infrastructure for NMR, EM and X-ray crystallography for translational research.

iNEXT is a consortium, funded by the Horizon2020 Program of the European Commission, of which the goal is to offer European researchers access to a wide range of advanced structural biology technologies, including X-ray, NMR spectroscopy, Electron Microscopy and Biophysics. The network, which is coordinated by Rolf Boelens (Utrecht University) and Anastassis Perrakis (NKI, Amsterdam; Deputy coord.) brings together 15 partners and will offer access to 3 synchrotron sites, 6 NMR centers, 5 EM facilities, 2 biophysics facilities and several Network and Training centers across Europe. A major focus of the iNEXT project is in promoting translational research in biomedicine and biotechnology by facilitating access to scientists with or without previous experience in structural biology. The network is also unique in that it offers for the first time access to high-end transmission electron microscopy facilities and to biophysics facilities to study macromolecular interactions in cells and *in vivo*.

Three PSB member institutes, the EMBL, the ESRF and the IBS play key roles in the iNEXT consortium, which will benefit both the PSB and the users of our X-ray and NMR infrastructures at European scale. The ESRF and the EMBL will cooperate towards the integration of novel technologies for automated crystal processing and X-ray data collection to support large scale ligand screening pipelines which should both contribute to the removal of critical bottlenecks during the early stages of structure-guided drug design and facilitate access to structural biology for new communities. The IBS will contribute within the RALF-NMR network with access to advanced NMR instruments to study structure and dynamics by NMR.

More information at: www.inext-eu.org

World class Science and Innovation with Neutrons in Europe 2020 (SINE2020)

SINE2020 is a neutron project of the European Commission's Horizon 2020 which officially started through a kick-off meeting that took place in Copenhagen on 16th October 2015. This new project is a consortium of the main European facilities and academic partners (covering a total of 13 countries) conducting neutron research and is coordinated by the ILL. SINE2020 has two objectives: preparing Europe for the unique opportunities at the European Spallation Source (ESS) in 2020 and developing the innovation potential of neutron large scale facilities.

Within SINE2020, a postdoctoral fellow will be recruited in the ILL's Life Sciences Group to work on the development of protein crystallogenesis methods for the production of large crystals suitable for neutron protein crystallography (NMX) and the project will involve interaction with partners/collaborators at FRM-II, Jülich, the European Spallation neutron Source (ESS) in Lund, and scientists from GlaxoSmithKline, Douglas Instruments, CFEL (DESY, Hamburg) and ISIS (RAL).

More information at: www.sine2020.eu

Prizes

Malene Ringkjøbing Jensen (IBS) is the recipient of a **2015 CNRS Bronze medal** for her work on intrinsically disordered proteins, a class of proteins that remain functional despite the lack of a well-defined three-dimensional structure.
Hugues Nury (IBS) was awarded the **2015 Claude Paoletti Prize** for his work on the structural characterization of pentameric transmembrane neurotransmitter receptors.
Giorgio Schirò (IBS) was awarded the **Seventh Erwin Felix Lewy Bertaut Prize** of the European Crystallographic Association (ECA) and European Neutron Scattering Association (ENSA) for his scientific contribution in the innovative field of protein dynamics.
Andrea Dessen (IBS) was awarded the **2015 Charles-Louis de Saulses de Freycinet Prize** for her remarkable work on the development of new strategies to fight bacterial infections.

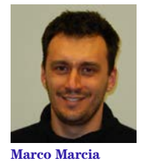
Andrea Dessen

Hugues Nury

Giorgio Schirò

Malene Ringkjøbing

Pauline Macheboeuf



Marco Marcia

ANRJCJC laureates

Malene Ringkjøbing Jensen, IBS. Project title: NMR studies of the role of intrinsic disorder in mitogen-activated protein kinase cell signalling pathways. Project duration : 3 years.
Pauline Macheboeuf, IBS. Project title: Structural basis of viperin-induced changes in lipid synthesis upon virus infection. Project duration : 3.5 years.
Marco Marcia, EMBL. Project title: Principles of molecular recognition between Polycomb protein SCML2A and human long non-coding RNAs. Project duration : 3 years.

ERC Consolidator Grant laureate

Irina Gutsche, **IBS**. Project title: Catching in action a novel bacterial chaperone for respiratory complexes. Project duration : 5 years.

Irina Gutsche

IBS-UVHCI fusion As of 1st January 2016 the French groups of the UVHCI have joined the IBS, but will stay at the CIBB. The arrival of the new groups will strengthen IBS research activities in host-pathogen interaction, which is further supported by the new research group directed by Pascal Poignard (see Profile p. 10).

DATES FOR YOUR DIARY

8th - 10th February 2016 - ESRF User meeting

The annual ESRF User Meeting 2016 will take place on the EPN Campus (ESRF Auditorium) and will cover the launch of the ESRF-EBS, a strategic project to completely construct and commission a new "Extremely Brilliant Source". Three Microsymposia with tutorials will be organised on: Nanoscience, X-ray diffraction and coherence; dynamics of complex systems; and the future of (time resolved) room temperature protein X-ray crystallography. The Plenary Session will be held on Tuesday 9th February.
The ESRF user community is warmly invited to participate through the submission of abstracts and posters and by meeting together onsite for discussion, interaction and networking. More information at http://www.esrf.fr/UM2016

29th February – 4th March 2016 – Tutorial in Macromolecular Crystallography

This year, the Macromolecular Crystallography tutorial will take place again as a one-week compact course from 29th February to 4th March 2016 and will be given in English. Fundamental aspects of crystallography will be treated in theoretical sessions (mix of lecture and problem solving) and 3 practical sessions of 2h each will treat case studies including data collection on a synchrotron beamline. The tutorial is aimed primarily at Université Grenoble Alpes graduate students and PSB students, who will be given priority for registration. The tutorial will also be open to post-docs and the staff of the PSB partners. The tutorial will take place in the CIBB seminar room on the EPN campus.
Registration (e-mail to wim.burmeister@ujf-grenoble.fr) is open and limited to 25 participants.

7th March 2016 - PSB student day

The eighth edition of the PSB student day will take place on 7th March in the IBS seminar room. All PhD students will present their work either through scientific talks, clip sessions or the poster session. The program will be posted soon on the PSB website. Everyone is welcome and a large buffet will be served for lunch.

14th - 15th March 2016 - GRAL 48h

Within the framework of the Labex GRAL, the second edition of the GRAL 48h seminar will take place in Autrans on 14-15 March 2016. This meeting is open to all GRAL scientists (iRTSV, IBS, UVHCI/EMBL and their Grenoble partners) and will consist of short talks and poster presentations on the major research themes of the Labex, as well as keynote lectures by three invited speakers, Gisou Van der Goot (École Polytechnique Fédérale de Lausanne), Nolwenn Jouvenet (Institut Pasteur, Paris) and Michael Hothorn (University of Geneva).

For further information and the meeting's program check: http://www.labex-gral.fr/event/gral-48h-march-14th-and-15th-autrans

5th - 6th April 2016 - PSB Scientific Advisory Board review

The PSB Scientific Advisory Board (SAB) review will take place on 5-6 April 2016. The SAB is an international committee composed of eminent scientists in the field of structural biology and the current members are: Anthony Watts (Chair, Oxford U., UK), Jean Cavarelli (Institut de Génétique et de Biologie Moléculaire et Cellulaire, France), Helen Saibil (Birkbeck College, UK), Michael Sattler (Helmholtz Zentrum München, Germany), Gunter Schneider (Karolinska Institute, Sweden), Titia Sixma (Netherlands Cancer Institute, The Netherlands), Vladimír Sklenář (Mazarik U., Czech Republic) and Henning Stahlberg (Biozentrum - U. of Basel, Switzerland).

21st - 27th May 2016 - EMBO Practical Course on Structural characterization of macromolecular complexes

This course will take place in Grenoble on the EPN Campus (ILL Auditorium). It aims to teach how to expedite structural biology projects involving macromolecular complexes by combining multiple experimental approaches. In particular, the course will cover the techniques used to produce, purify, reconstitute and characterize multi-subunit protein and protein/nucleic acid complexes for structural analysis. The full course program consists of 18 h of lectures plus 18 h of practical work and is primarily intended for advanced PhD students and early-stage postdocs.
The number of participants is limited to 20. More information at http://www.embo.org/events

Newcomers

Caroline Mas joined the Integrated Structural Biology Grenoble Unit (ISBG, UMS 3518, CNRS-CEA-UJF-EMBL) as Manager of the CIBB Biophysical Platform. She will provide scientific support and training to users. Caroline obtained a PhD in biochemistry from the University of Montreal and subsequently carried out a postdoctoral work at the University of Queensland. Since 2013, Caroline worked at the UVHCI and IBS as a research scientist. Contact : cmas@embl.fr

Contacts
Editors: Florent Bernaudat (PSB), Andrew McCarthy (EMBL), Estelle Mossou (ILL) and Montserrat Soler Lopez (ESRF)
Student representative: Alice Tissot (IBS)
Editor-in-chief: Joanna Timmins (IBS)
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 which has received support from the EU FP6 programme. 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, notably host-pathogen interactions.