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PSB *et al*

Partnership for Structural Biology Newsletter

Scientific highlights

Bunyavirus polymerase structure illuminates viral genome reading

Bunyaviruses constitute one of the largest families among segmented negative stranded RNA viruses (sNSV). It includes human viral pathogens such as La Crosse (LACV), and shares the same strategies for the genome transcription and replication as other better known viruses such as Influenza (Orthomixovirus) and Lassa (Arenavirus). All these viruses have single stranded genomic RNA that is divided into several fragments and coated by viral nucleoproteins (1), which are assembled with their polymerase to form competent circular RNA structures. This assembly, called the ribonucleoprotein (RNP), is the functional unit of replication and transcription. Transcription is carried out by RNPs through a unique mechanism known as 'Cap Snatching", where 5'-CAP labeled cellular mRNAs are cleaved by a specific enzymatic domain located in the polymerase (2), and subsequently used for priming the viral mRNA synthesis. These functional similarities contrast with the sequence divergence shown between these viral polymerases. For example the Influenza virus polymerase is a heterotrimer (3) instead of a large single polymerase, also called the L protein, utilized by Arenavirus and Bunyavirus.

The structure of the LACV Orthobunyavirus polymerase (4) revealed by both protein crystallography (utilizing the EEF facility, the HTX facility and the high intensity ESRF beam lines), and cryo-electron microscopy (using the IBS and EMBL EM facilities) reveals a striking overall similarity between Bunyavirus and the recently reported structure of the Influenza polymerase (3). The structure reveals several domains extending from the universally conserved polymerase core, building up a large and complex protein fold, where four access channels converge in a central polymerase reaction chamber (Figure 1). The internal cavity distribution allows for modeling of the RNA traffic inside the polymerase, illustrating how such polymerases work in the context of the circular RNPs, and how the viral RNA is maintained as a single strand after duplication.

The RNA, to be read for transcription and genome replication, enters and exits from the polymerase through neighboring entry and exit channels, allowing for minimal disruption of the circular RNP. The existence of two exit channels, one for the template RNA and the other for nascent RNA, allows the dissociation of the RNA duplex produced inside the cavity into single stranded RNA.

The crystal structure of the L protein in complex with the viral RNAs shows how the 3' and 5' RNA ends are bound in separate binding sites, and illustrates how the circularity of the RNP assembly is achieved. The high specificity for the self-viral RNA recognition is mediated by extensive sequence specific protein-RNA contacts into positively charged clefts outside the catalytic site and close to the template entrance channel. The binding of the 5'-vRNA end has an allosteric effect on the active site that activates the polymerase.

Based on these, and previous studies, we propose a structure based model of replication that might be shared by all sNSV and other related viruses such as Rabies or Ebola that replicate in the RNP context (4).

J. Reguera and P. Gerlach (EMBL)

[1] Reguera J, Malet H, Weber F, Cusack S. (2013) Proc. Natl. Acad. Sci. U S A., 110, 7246-7251.

[2] Reguera J, Weber F, Cusack S. (2010) PLoS Pathog., 9, e1001101

[3] Pflug A, Guilligay D, Reich S, Cusack S. (2014) Nature, 516, 355-360.

[4] Gerlach P, Malet H, Cusack S, Reguera J. (2015) Cell, 161, 1267-1279.

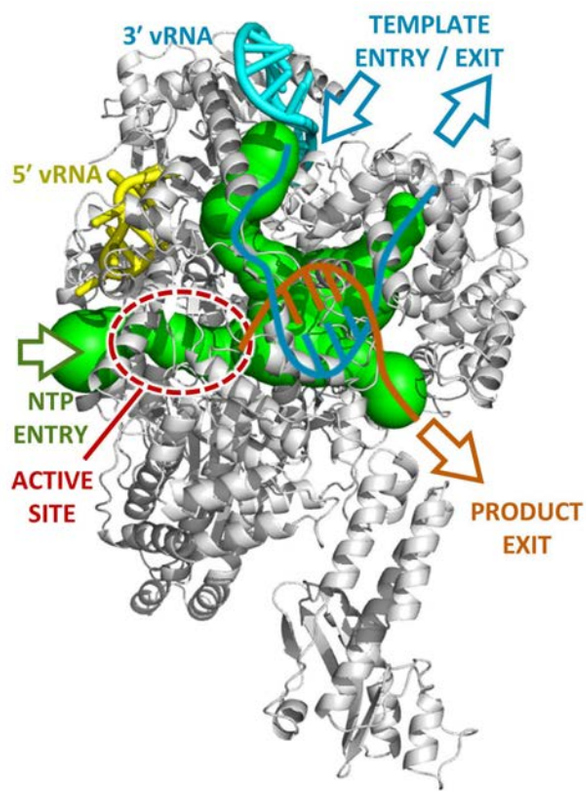

Figure 1: Model of the elongation state showing the trajectories of template RNA (cyan), product RNA (orange) and NTPs through the polymerase tunnels (green). The 3' and 5' ends of the RNA are shown as well as the position of the active site. After a short template-product duplex, which is accommodated in the interior cavity, each strand exits separately along different tunnels. The template exits back to the front of the polymerase, where it can re-integrate into the RNP while the nascent strand exits to the rear, where product processing occurs.

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A new approach and software to generalize serial protein crystallography at synchrotrons and XFELs

The advent of X-ray free electron lasers (XFELs) has driven the emergence of serial protein crystallography, whereby data are collected from myriads of randomly-oriented crystals at RT, rather than from a single crystal oscillated in the X-ray beam, at 100 K. Compellingly, the collection of serial data allows a more detailed and realistic description of structural protein dynamic and radiation damage at RT. Furthermore, it allows macromolecular structure determination from highly radiation sensitive micro- and/or nano-crystal, owing to the fact that each crystal is exposed only once. Alas, this data collection strategy is not largely widespread, especially in France, and this, for the following two reasons. Firstly, a large amount of crystalline sample is generally required to collect a full serial dataset, discouraging structural biologists working on difficult proteins. Secondly, know-how is lacking, notably in terms of data sorting and processing.

In the featured article [1], IBS and ESRF-ID13 researchers report on a new data collection strategy based on the raster-scanning of a protein crystal slurry sandwiched between two solid supports, and which only requires ~200-600 nl of settled crystals (Figure 1). They also present the first version of a new pre-processing software, *NanoPeakCell*, able to sort, background-correct and Bragg-peak-search data produced by nearly all X-ray detectors used at synchrotrons (MARCCD, ADXV, PILATUS, EIGER, etc) and XFELs (MPCCD, CSPAD). The software and its graphical user interface are coded in Python, allowing painless installation and usage on various architectures (Linux, OS-X, etc). *NanoPeakCell* (Figure 2) is currently being deployed at the ESRF, LCLS and SACLA, where it will hopefully allow users to more comprehensively sort, correct and process their serial data.

N. Coquelle (IBS), M. Burghammer (ESRF) and J.-P. Colletier (IBS)

[1] Raster-scanning serial protein crystallography using micro- and nano- focused synchrotron beams. N. Coquelle, A. S. Brewster, U. Kapp, A. Shilova, *et al.* (2015). Acta Crystallogr D, 71, 1184-96.

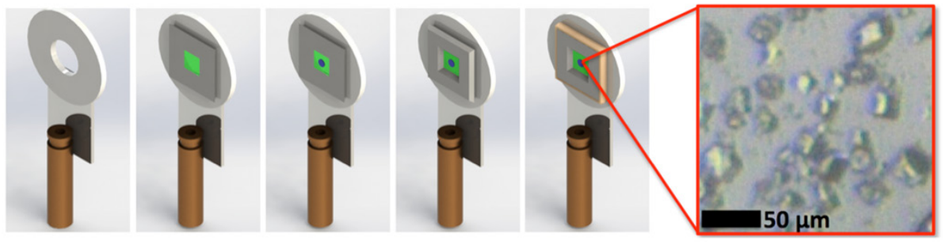


Figure 1: Sample presentation to the X-ray beam. (left to right) (i) A brass pin and a washer are glued onto a glass piece. (ii) The concave surface of the first Si3N4 wafer is glued onto the washer. (iii) 500 nl of crystalline slurry is then gently deposited onto the membrane. (iv) The second Si3N4 wafer is sandwiched over the first. (v) The sandwich is sealed with Araldite® resin in order to avoid drying of the material. Following this step, the brass pin is introduced into a magnetic crystal mount. The far-right panel circled in red shows a close-up view of the lysozyme crystals in a Si3N4 sandwich.

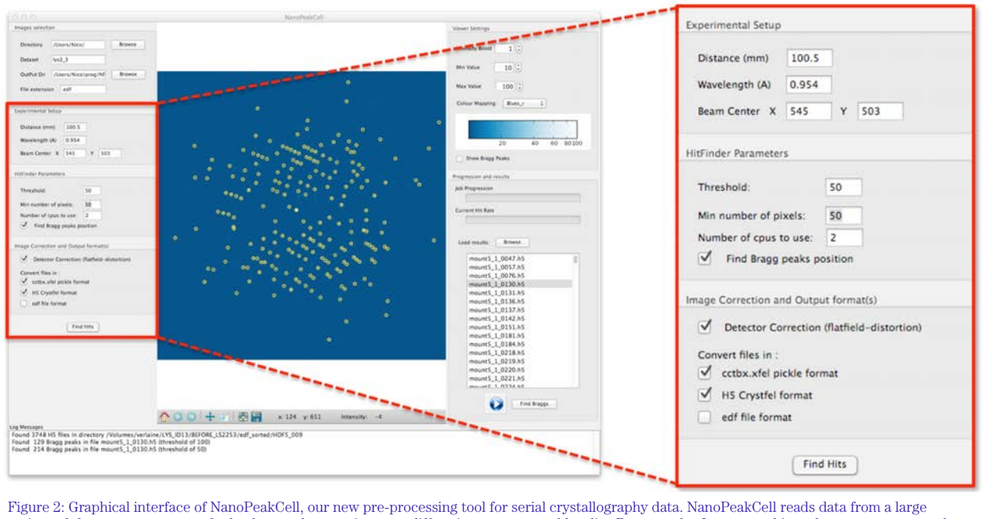


Figure 2: Graphical interface of NanoPeakCell, our new pre-processing tool for serial crystallography data. NanoPeakCell reads data from a large variety of formats, can correct for background-scattering, sort diffraction patterns and localize Bragg peaks. It uses, to this end, as many cores as the user can make available. These pre-processing steps ease further data processing with CrystFEL or cctbx.xfel (for the indexing and integration steps).

A major structural determinant of the motility and infectivity processes of the human pathogen *Mycoplasma genitalium*

Mycoplasma genitalium is a motile, self-replicating human pathogen that is responsible for several sexually transmitted diseases associated with non-gonoccocal, non-chlamidial urethritis in man and inflammatory reproductive tract syndromes in women. *M. genitalium* cells are asymmetric due to the presence of a complex cytoskeleton localized at one of the poles of the cells that is composed by a very well defined structure as observed in cryo-Electron Microscopy studies. This cytoskeleton constitutes the Terminal Organelle (TO) which is responsible for adhesion to host cells and solid surfaces, cell division and cell motility being, therefore, directly related to infectivity and pathogenic processes. Despite its importance very little is known about the functioning of mycoplasmas motility machinery, which is unique among prokaryotes. This lack of information together with the increasing necessity to find new antibiotics or multivalent vaccines against this microorganism led the authors of this work to study the proteins that compose the TOs of *M. genitalium*.

In *M. genitalium* up to 12 proteins have been localized in the TO. Among these, at least four are components of the TO wheel complex which is one of the ultra-structures that compose the TO and connects it to the cell body and to the cell membrane. The direct interaction between the TO wheel complex proteins MG200 and MG491, with a K_D in the 80 nM range, was identified and characterized by Biacore assays performed at the SPR technical platform of the Grenoble Partnership for Structural Biology. MG200 is a multi-domain protein which contains an Enriched in Aromatics and Glycine Rich box (EAGRbox) that is conserved and exclusively found in proteins of the TOs of motile mycoplasmas and that has been described as a protein-protein interaction domain [1]. Given this, its interaction with MG491 was investigated by NMR which allowed determining the residues from the EAGRbox which participate in complex formation (Figure 1). The interaction of this domain with a small peptide from the C-terminus of MG491 was also assayed by NMR which revealed that only the residues from this segment participate in the interaction.

The biophysical and structural information, combined with the crystal structure of the MG200 EAGRbox, resulted in a model of the MG200-MG491 interaction that affects cell motility and cell morphology in a very specific way, as determined by the *in vivo* study of the MG491ΔCt deletion mutant (Figure 2). Therefore, the interaction between the TO wheel complex proteins MG200 and MG491 revealed to be critical to the stability of the TO, suggesting that it could affect mycoplasma infective capabilitites.

B. Calisto (ESRF)

[1] B. Calisto, A. Broto, Martinelli, L., *et al.* (2012). *Molecular Microbiology*, 86, 382-393;
[2] L. Martinelli, D. Lalli, L. García-Morales, M. Ratera, E. Querol, J. Piñol, I. Fita, B. Calisto (2015). *The Journal of Biological Chemistry*, 290, 1699-1711.

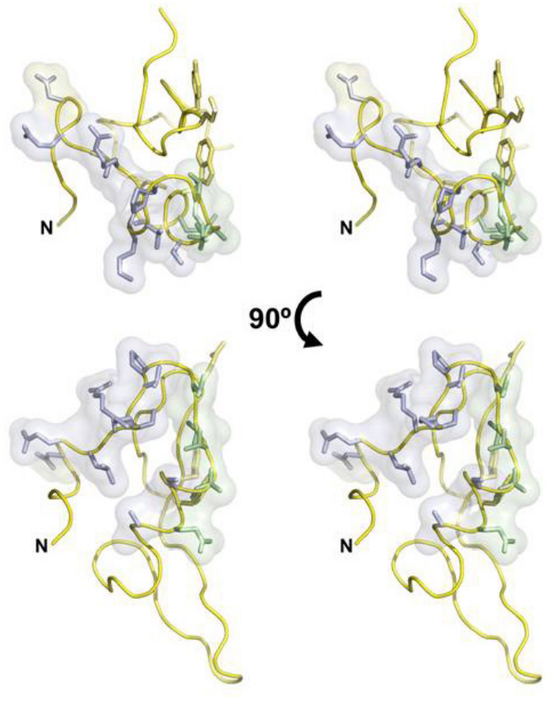


Figure 1: Binding surface of MG200 EAGR box with MG491. Stereo view representations of the MG200 EAGR box with the residues participating only in the interaction with MG491 depicted in blue sticks. Residues depicted in yellow sticks participate in the formation of the crystallographic dimer, whereas residues depicted in green participate both in the interaction with MG491 and in dimer formation.

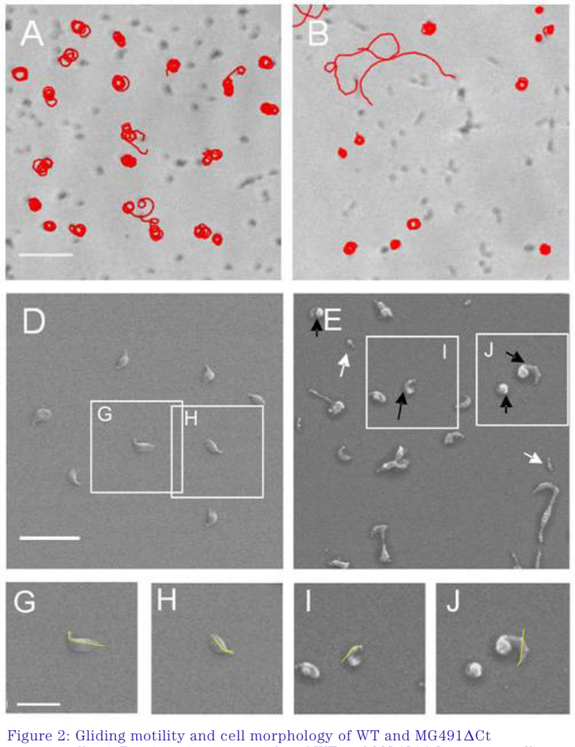


Figure 2: Gliding motility and cell morphology of WT and MG491ΔCt mutant cells. A-B, microcinematographs of WT and MG491ΔCt mutant cells, respectively. Bar, 5 µm. D, E and G-J, scanning electron micrographs of WT and MG491ΔCt mutant cells (D and insets G and H are from WT cells; E and insets I and J are from MG491ΔCt mutant cells. Yellow lines indicate the TO-body axis angle. White and black arrows point to minute cells and bipartite cell bodies, respectively, detected in MG491ΔCt mutant cells. Bars, 2 µm in D and 1 µm in G.

Insights into the functioning of a light-driven sodium pump

Microbial rhodopsins are a large family of membrane proteins that use the light energy to perform a variety of functions. While photosensors, light-driven proton and anion pumps, and light-gated channels have been known for some time, the first light-driven sodium pump from this family has only recently been discovered [1].

Using the automated crystallization and crystal imaging systems available at the IBS membrane protein crystallization platform, and applying the *in meso* crystallization approach, we were able to crystallize the protein in two functionally distinct forms: monomeric blue and pentameric red [2]. Despite the generally small size of the crystals, thanks to the availability of the high-brilliance microfocus beamlines ID23-1 and ID29 at ESRF, the diffraction data could be collected up to the resolution of 1.45 Å for the first form, and 2.2 and 2.8 Å for the second. Complementary optical characterization of the crystals was conducted at the ESRF cryobench laboratory ID29S.

The structures revealed that the light-driven sodium pump KR2 is composed of seven transmembrane helices, A to G, connected by three intracellular and three extracellular loops, and a short N-terminal α-helix capping the inside of the protein. The protein is able to form symmetric pentamers (Figure 1). Inside it, an ion translocation pathway lined with polar and ionizable residues is readily observed. The pathway consists of three major cavities: the ion uptake cavity, the cavity around the retinal's Schiff base, and the ion release cavity. All these are notably unusual. The ion uptake cavity has an unexpectedly large opening to the surface. The retinal-binding pocket is very flexible, assuming different conformations in the five protomers of the pentamer. Finally, the ion release cavity is capped by a unique N-terminal α-helix, not observed in other microbial rhodopsins. The structures have also revealed the position of the bound sodium ion (Figure 2).

The role of the novel features of the ion uptake and ion release cavities was not immediately apparent. Designed mutations revealed that the N-terminal α-helix, and the cluster of charged amino acids that it is part of, serves mostly as a stabilizing structural element. What was more interesting, however, is that the ion uptake cavity turned out to be the protein's selectivity filter. Mutation of the glycine residue at the side of the cavity, G263F, imparted the sodium pump with the ability

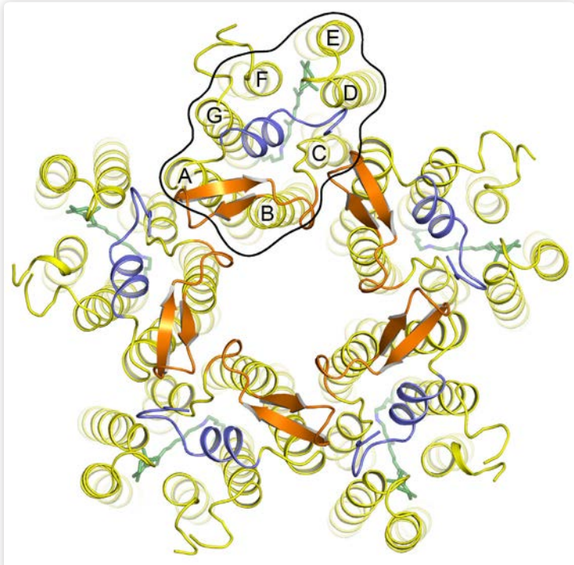


Figure 1. Overall architecture of KR2 pentamer. The α-helices of one of the protomers are labeled A-G. The novel N-terminal α-helix preceding the helix A is shown in blue, the B-C loop is shown in orange, and the retinal is shown in green.

to pump potassium. These results are particularly interesting for the field of optogenetics, where incorporation of microbial rhodopsins into neurons allowed precise control of neural impulses and thus opened the possibility to study neural circuits in living tissues and even animals. Still, some particular research problems required active light-driven sodium or potassium transporters, which were not known before. Consequently, the new data fills this gap, expands the spectrum of the available optogenetic tools and allows design of new ones with desired particular properties.

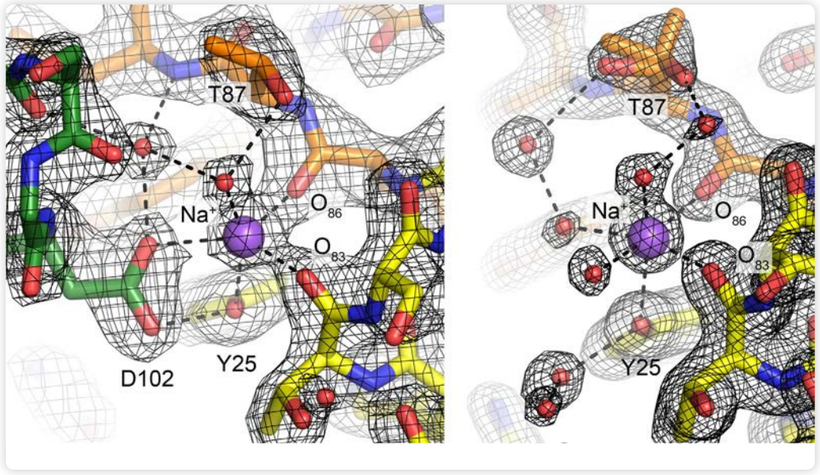


Figure 2. Details of the sodium ion interactions at the interface in the pentameric structure of KR2 (left; one protomer is shown in green, and the other in yellow and orange), and the monomeric structure (right). The sodium is shown in purple and water molecule oxygens are shown in red. $2F_o$-F_c electron density maps (black) are contoured at the level of 1.5σ.

I. Gushchin and V. Gordeliy (IBS)

[1] Inoue K, Ono H, Abe-Yoshizumi R, Yoshizawa S, Ito H, Kogure K, *et al.* A light-driven sodium ion pump in marine bacteria. Nat Commun. 2013;4: 1678.
[2] Gushchin I, Shevchenko V, Polovinkin V, Kovalev K, Alekseev A, Round E, *et al.* Crystal structure of a light-driven sodium pump. Nat Struct Mol Biol. 2015; 22, 390-395.

Dancing water molecules activate proteins

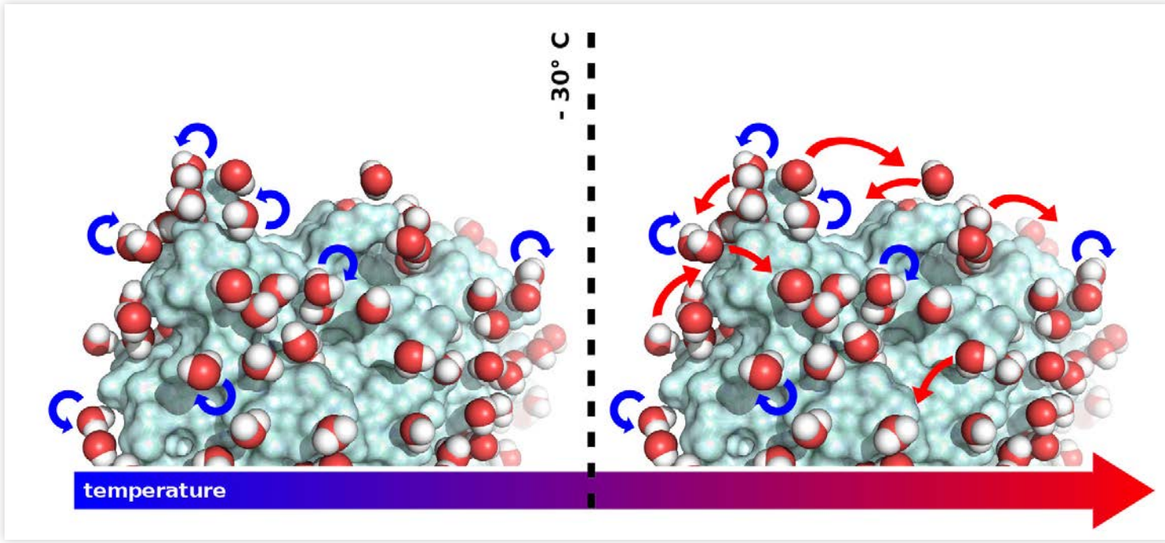
Water is often called the *matrix of life* because its presence is essential to all known living organisms. At the molecular level, proteins need to be covered by water in order to be biologically active. This so-called hydration water is generally acknowledged to enable a protein to undergo the internal motions that are so fundamental for its capacity to fulfill a specific biological function. Yet, the molecular mechanism behind water's importance for functional protein dynamics has remained elusive.

An international team of researchers from the Institut de Biologie Structurale, the Institut Laue-Langevin, the Forschungszentrum Jülich, the University of California Irvine, the Australian Institute of Science and Technology Organisation, the Max Planck Institute Mülheim and the University of Perugia has shed light on the molecular mechanism behind the importance of water for functional protein dynamics. The team has been able to characterize water motions on the protein surface at different temperatures, thereby highlighting the key dynamical features in water that correlate with protein activity. A combination of protein deuteration, neutron scattering and molecular dynamics simulations was employed during the study. The use of deuterated proteins allowed the researchers to focus in neutron scattering experiments solely on water motions on the protein surface.

When "visualising" water movements on the protein surface (see figure), the team discovered that the molecules rotate around their own axes at temperatures below -30°C; temperatures at which proteins are inactive. At -30°C however, whilst continuing to rotate, the water molecules also start to undergo translational diffusion. This is the temperature at which proteins start to be biologically active and the researchers suggest that the capacity of water molecules to "dance" on the surface of proteins enables the dynamics they need to function.

Y. Fichou, G. Schiro and M. Weik (IBS)

[1] Schiro G, Fichou Y, Gallat F-X *et al.* (2015) Translational diffusion of hydration water correlates with functional motions in folded and intrinsically disordered proteins. *Nature communications* **6**: 6490



Water translational diffusion appearing above -30 °C enables protein large amplitude motions, which are essential for protein activity.

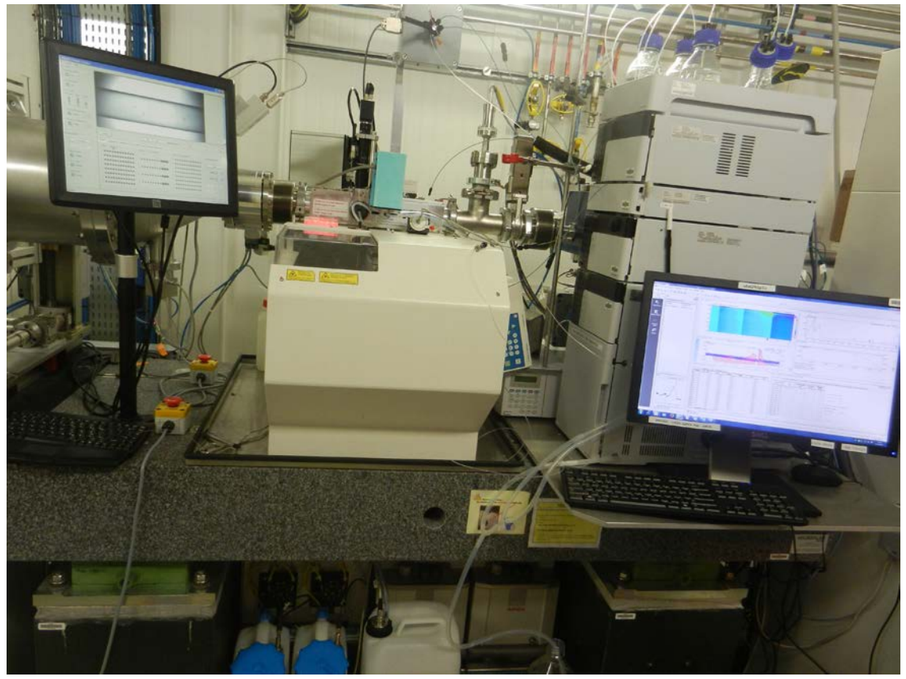
News from the platforms

New SEC on-line set-up at BM29 Bio-SAXS beamline (SAXS-SANS platform)

Small-angle X-ray scattering (SAXS) can provide accurate structural information and low-resolution shapes of macromolecules in solution. Often, it is not possible to obtain a stable monodispersed sample of the protein of interest required for a successful SAXS experiment, in particular for multi-subunit protein complexes. In these circumstances, size exclusion chromatography (SEC) coupled with SAXS can facilitate the separation of monodispersed protein from a polydispersed sample for a sufficient amount of time to collect useful SAXS data.

The commissioning of the new SEC system in-situ at the BM29 beamline, to be used in parallel with the sample changer robot, is under way and the device will be available for external users from June 2015. The new system (Shimadzu) replaces the old one (Malvern) used and has improved characteristics: auto sampler allowing sample loading without loses (smallest sample quantity needed 10 µL), buffer gradient possible (up to 4 components) and conductivity measurement is included. The figure shows the actual SEC set-up (white boxes on the right part of the photograph behind the display) installed on the experimental marble table in the BM29 experimental hutch. A particular attention was made to keep as short as possible the tubing between the column end and the quartz capillary where samples are exposed to X-rays to minimise dilution. The switch between two modes (robot and SEC) is quick and fully automated.

P. Pernot (ESRF)

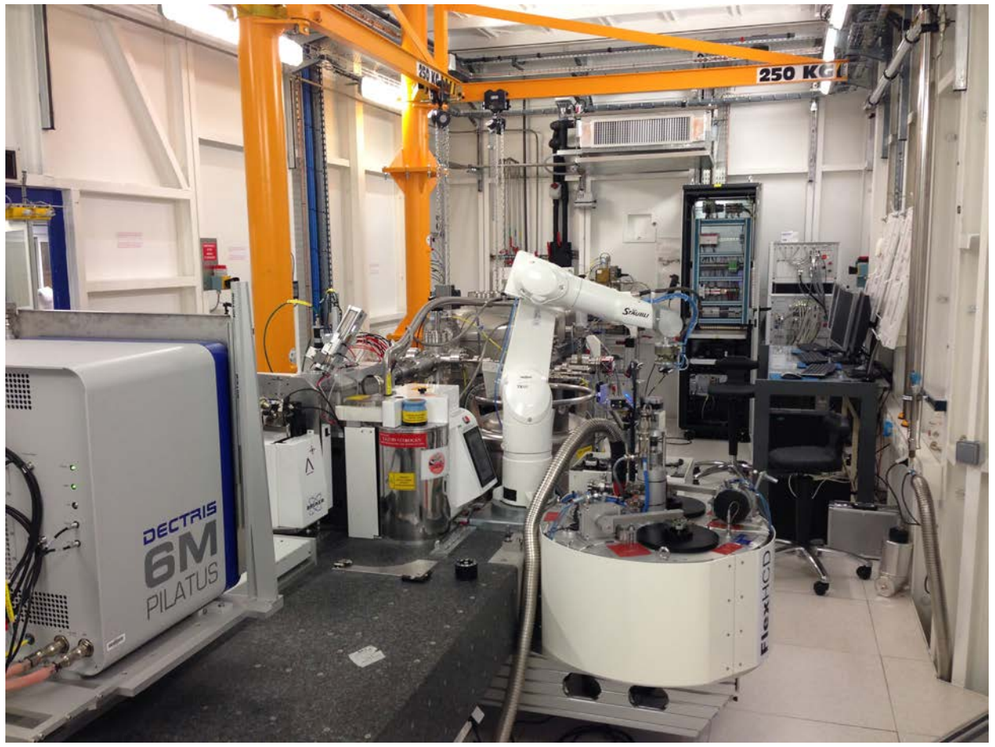


Sample environment at the ESRF BM29 Bio-SAXS beamline: sample changer robot in the center of the photograph with the display on its left showing capillary where liquid samples are exposed to X-rays and the SEC on-line set-up (white boxes on the right).

ID30B – A new high intense, tunable and variable focus MX beamline

ID30B, located on the second branch of the canted ID30 setup, is the last macromolecular crystallography beamline to be constructed as part of the UPBL10 upgrade project at the ESRF. ID30B is operated by the ESRF/EMBL Joint Structural Biology Group and will soon commence user operation. ID30B is equipped with a micro diffractometer (MD2S), a SC3 sample changer and a PILATUS 6M-F single photon counting pixel detector to maximize the potential of the high photon flux available (see figure). Its tunable undulator configuration allows the energy of the beam to be varied from 6–20 keV, while the beam size at the sample position can be changed between 20–200 µm. Commissioning of an EMBL/ESRF-developed versatile sample changer in combination with a high capacity Dewar is ongoing. User access to this configuration, which will allow users to send samples or come to the ESRF with samples mounted either in SPINE standard pucks or in unipucks, will be available from September 2015.

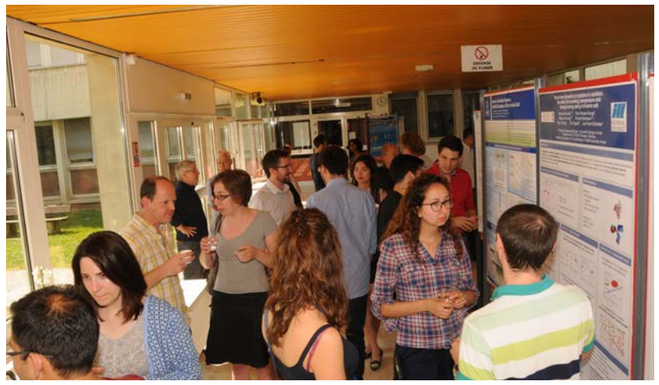
A. McCarthy (EMBL) and C. Mueller-Dieckmann (ESRF)



View of the ID30B experimental setup, including the future FlexHCD sample changer that is currently under development.

Events

EPN PhD student day



The first EPN PhD student day took place at the CIBB on the afternoon of 8th June 2015. Through this event the EPN management wished to create a common strategic vision, provide the students with information and to get a feedback from the students on their working experience on the EPN campus.

The meeting started by a clip session during which 20 third-year PhD students in biology and soft matter, from all of the institutes of the EPN campus, presented their work through 2-min clips. Next, C. Bieniossek, a former EPN alumnus now working in Roche (Basel, Switzerland), was invited to give a presentation to share his experience and give advice to students wishing to work in industry after their PhD.
Then followed a presentation by T. David (GIANT, Grenoble) who provided the students with information and advice on European grant applications (ERC, Marie Curie fellowships,...) and also described the help and support that GIANT can provide to the local scientists.

Many group leaders and Directors attended the meeting and fruitful discussions with the students took place during the poster session and especially during a round table on the "Scientific life on the EPN campus" where several ideas for improved interactions have emerged.
The day ended with a PSB Get Together.
The organisers would like to thank all the participants who took part in the meeting.

F. Bernaudat (PSB Coordiantor)

40th anniversary of the EMBL-Grenoble outstation

Andrew Miller (EMBL: 1975-1980 and ESRF: 1986-1991) and Stephen Cusack (1977-now) together at the EMBL-Grenoble 40th anniversary celebrations.

The EMBL Grenoble outstation celebrated its 40th anniversary on the 4-5th of June.
An informal afternoon of talks outlined the evolution and scientific contribution from the outstation's foundation in 1975 to the present day. Andrew Miller, the founding head of the outstation (and later the first director of research at the ESRF) gave an illuminating insight on the first pioneering years and the groundbreaking research he and his colleagues (David Hulmes and Stephen White) carried out at the ILL during this time. This was followed by how the introduction of molecular biology techniques in the 80s, the construction of the ESRF in the 90s and the foundation of the PSB in 2002 impacted on the outstation's development over the last 40 years.

There was champagne and a cake cutting ceremony later in the afternoon and the celebrations were continued with a dinner, fireworks and dancing at the Château de la Commanderie. The following day included lab reunions and a tour of the current facilities.

It was fantastic to see so many former colleagues return to reminisce and celebrate their contribution to the success of the EMBL-Grenoble outstation over the last 40 years.

A. McCarthy (EMBL)

INSTRUCT-FRISBI-PSB Workshop, Molecular interactions: the complementarity between biophysical methods

Measuring and quantifying molecular interactions are increasing demands in the biological community in response to the explosion of information coming from the application of high-throughput methods to various problems.

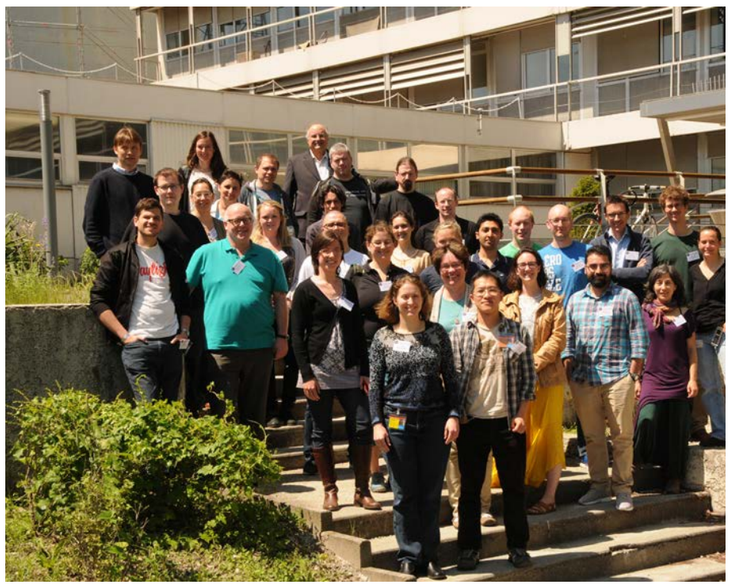
A workshop on the study of molecular interactions was organized on the EPN campus from 1st to 5th June 2015.

The aim of the course was to provide theoretical, practical and data analysis training in five modern methods, for which state-of-art instruments are present in the PSB platform on the EPN campus, for monitoring and quantifying molecular interactions – SEC-MALLS, analytical ultracentrifugation, surface plasmon resonance, isothermal titration calorimetry and microscale thermophoresis. Emphasis was put on the complementarity of these different approaches with structural methods in the field of macromolecular biology, as illustrated in the general lectures by F. Fieschi (IBS), J. Kadlec (EMBL/IBS), T. Forsyth (ILL) and A. Imberty (CERMAV, Grenoble University campus), and discussed in round tables. Combined analysis of datasets coming from different methods was presented by P. Schuck and J. Zhao (NIH, USA).

Twenty participants were selected for the practical and advanced data analysis sessions from 9 European countries. All agreed that the course will definitively help them to define and perform the appropriate experiments for the purpose of their particular project. The course tutors and organizers were C. Ebel (IBS), A. Le Roy (IBS), M. Jamin (UVHCI), N. Thielens (IBS), J.-B. Reiser (IBS), A. Palencia (ISBG), F. Bernaudat (PSB) and C. Birck (IGBMC Strasbourg). In addition to INSRUCT and FRISBI, the labex GRAL supported the workshop.

C. Ebel (IBS)

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



The biannual EMBO Practical Course on Small Angle Scattering (SAS) from proteins in solution took place on the EPN campus between the 18th and the 22nd of May 2015. It welcomed an international range of 23 participants from academic as well as industrial research institutes.

The course included lectures, tutorials and practicals at ILL and ESRF beamlines, and aimed to help the participants make the most out of future neutron and X-ray SAS experiments. Highlights were the keynote lectures by Dr. Dmitri Svergun (EMBL Hamburg) and Prof. Jill Trewhella (University of Sydney), which were open to all PSB members.

This event was organized by F. Gabel (IBS and ILL), P. Pernot (ESRF), A. Round (EMBL), M. Jamin (UVHCI) and A. Martel (ILL), with the highly appreciated support of L. Tellier (ILL), S. Claisse (ILL) and all of the invited speakers.

More information can be found here:
http://events.embo.org/15-saxs/

F. Gabel (IBS and ILL)

NMX@ESS 2015

A new facility, the European Spallation Source (ESS), for the use of neutrons began its construction in Lund, Sweden. This source will include among others a diffractometer for crystallography of biological macromolecules (NMX diffractometer), whose commissioning is planned around 2023. The expected neutron flux and reduced beam size should allow a real revival of neutron crystallography, raising much of the current limitations, particularly in terms of crystal size and available beam time.

To present this future equipment and collect feed-back, the NMX@ESS meeting was organized the 23-24th March 2015 at the IBS. The programme included an introduction to the future NMX station, the presentation of results obtained with neutrons, and on the methodology and instrumentation for neutron crystallography, followed by round tables. Each of the participants was also asked to give a short presentation describing their interest in structural biology and neutron diffraction.

This meeting was supported by the material and financial support of the IBS (Grenoble; www.ibs.fr), the LLB (Saclay, www-llb.cea.fr), the ILL (Grenoble, www.ill.eu), the iRTSV (Grenoble, irtsv.cea.fr/dsv/irtsv) and the AFC (www.afc.asso.fr).

J.-L. Ferrer (IBS/Synchrotron Group)

Advanced Isotopic Labelling Methods for Integrated Structural Biology

AILM2015 was held at IBS between 2nd and 5th February 2015. The overarching objective of the conference was to bring together researchers with a shared interest in the production and exploitation of isotopically labelled protein and nucleic acid samples for structural and biophysical investigations. This field is applicable to a range of biophysicists and structural biologists, including those specialising in mass spectrometry, neutron diffraction and solution and solid-state NMR spectroscopy.

The organising committee invited renowned experts in these fields from Europe, Asia and North America. The main focus focus of the meeting was the practical aspects of isotopic labelling and consequently all invited speakers were asked to include thoughts and experiences on sample preparation in their presentations. In addition, there were 20 abstracts selected for oral presentations and another 39 poster presentations.
In total we welcomed over 120 people to Grenoble, including a large number of PhD students and post-doctoral researchers.

AILM2015 was the first international conference to focus on the practical aspects of isotopic labelling. Feedback was extremely positive with respondents overwhelmingly supporting a proposal to run the conference again in the future.

More details and full program is available online:
http://ailm2015.jimdo.com/

J. Boisbouvier (IBS)

25th ESRF Users' Meeting, 9-11th February 2015.

The 25th annual ESRF Users' Meeting was held on the EPN Campus from Monday 9th to Wednesday 11th February 2015 using a new format in which the traditional Plenary Session was sandwiched between two days of tutorials and User Dedicated Microsymposia (UDMs).

For those ESRF Users of whom the main interest is Structural Biology there were two main events: A meeting of ESRF BAG Responsibles and a user-dedicated micro symposium (UDM4) "Structural Biology at the ESRF: More than crystallography". Both events were organised by Marina Mapelli (ESRF Users Organisation), Gordon Leonard, Fabienne Mengoni and Deborah Davison. The BAG Meeting involved a series of presentations from beam-line staff, visits to the group's beam-lines and a round table discussion. UDM4, held in the IBS2 Seminar Room, attracted more than 95 registered participants for a programme comprising talks by three keynote speakers (Hugues Nury, IBS; Célia Romao, ITQB, Lisbon, Portugal; Roberto Steiner, King's College, London, UK.) and 6 talks chosen from submitted abstracts.

While many crystal structures of outstanding interest were presented, all speakers made it clear that information from complementary techniques helps give a much clearer picture of the biological question being asked and that much more use of these will be made in the future.

Feedback, both formal & informal, suggests that both meetings were appreciated by participants. Moreover, it was clear that the new format of the Users' Meeting – including a 'non-scientific', but very well attended Monday night Wine and Cheese event – was also a big hit. Many thanks to IBS for making their seminar room available for UDM4, to all participants and speakers, and to Fabienne and Deborah for making sure everything ran smoothly.

G. Leonard (ESRF)

PROFILE

Winfried Weissenhorn

What brought you to EMBL Grenoble, and how did your time here get your career started?

After my postdoctoral training at Harvard I wanted to return to Europe. At the time independent group leader positions were quite rare in Europe and the position of group leader at the EMBL Grenoble Outstation was a unique opportunity. EMBL Grenoble was and is still an excellent environment to set up a research group. EMBL provides sufficient funds to start a small group, work on your own projects and publish independently to acquire international reputation.

What difficulties did you encounter in setting-up your own research team? What advice would you give young scientists in such a situation nowadays?

Of course in the beginning, when you become responsible you tend to question yourself constantly whether everything moves in the right direction and whether your research is competitive. Competition can be fierce and sometimes you win and sometimes you lose. That of course produces some stress at times, but in the end you learn to cope with it. Because science is driven by competition and young scientist should not be afraid of, but follow their instincts. Most importantly in setting up a research group are your co-workers, because you can have great ideas, but you can't do everything yourself. I was lucky and had the chance to work with very gifted and enthusiastic PhD students and post docs when I started the lab.

What did you do at EMBL Grenoble, highlighting in particular the achievements / contributions you made that you find significant when looking back?

Our group made important contributions in the field of structural virology notably on Ebola virus assembly and the conformational flexibility of its matrix protein. Secondly the collaboration with the Ruigrok group produced the first structure of a viral negative strand RNA virus nucleoprotein in complex with RNA, which revealed the molecular details of how these proteins polymerize with their genome into nucleocapsids. During our work on virus assembly we moved away from viral proteins to cellular budding factors that are recruited by enveloped viruses such as HIV-1 and Ebola to complete budding. We published the first crystal structure of an ESCRT-III protein, which laid the ground work to understand how these proteins assemble on membranes and exert membrane remodelling processes during virus budding, multivesicular body biogenesis and cytokinesis.

After leaving EMBL, you became Professor at Grenoble University and a Group Leader at UVHCI in Grenoble, in what way did this move contribute to your career development?

Moving to Grenoble University and running a research group at UVHCI allowed me to continue my research in an excellent scientific environment. This was certainly much easier than setting up a new group and research lab elsewhere from scratch.

How difficult is it to combine research and teaching?

I think it is not too difficult although the teaching load at a French University is immense in the beginning when you have to set up all courses from scratch. But if you are able to organize it well and your research group functions well you learn to cope with it. Maybe some of my co-workers were pleased not to see me all the time in the lab. Although I teach much less nowadays, it was a very good experience and I recommend that every scientist should be involved in some teaching at the University level.

What did you do at UVHCI/IBS, highlighting in particular achievements / contributions you have made there?

At UVHCI we continued to work on budding to understand how ESCRT-III polymerizes and is regulated. We were the first to show

that most ESCRT-III proteins form filaments that can be loose or organized in tight helical tubular structures, which can also coil up to form end caps or dome-like structures in vitro. I think it is widely accepted that such filaments assemble within the neck of a budding virus or vesicle and at the midbody. Based on our work we proposed that the coiling up of these filaments into dome-like structures can induce sufficient membrane constriction thereby supporting membrane fission. Although this is a compelling model, much work lies ahead of us to confirm it. We also published the first structure of the innate immune factor tetherin that physically retains HIV-1 at the plasma membrane once budding is completed in the absence of a viral antagonist. Close to my heart is still my post doc project on gp41 and we succeeded to obtain a more complete structure of the ectodomain that contains the epitopes for broadly neutralizing antibodies. However, we still don't know how we can induce broadly neutralizing anti-gp41 antibodies, although many patients generate such antibodies during the course of infection. Evidently this would be important for HIV-1 vaccine development. Since January 2015 we are at the IBS and we continue to work on these projects.

How do you see your role as the director of a French research Institute?

Most importantly I must help to maintain and to improve the research conditions so that every scientist can excel. At the same time, because funds are limited the IBS needs to focus to stay at the forefront. Although our main research will remain basic science, we can improve on applied or translational research aspects. Furthermore, training of PhD students and post docs will be an ongoing challenge to prepare young scientists for future employments within academia as well as industry.

What are the strong points of IBS? In what way do you think the scientific output of the IBS and its positioning on a national and international scale can be improved in the years to come?

A strong point of the IBS are its highly skilled staff. Several IBS scientists are at the forefront of state of the art methods development and others use new technologies successfully in their research. My predecessor Eva Pebay-Peyroula and our partners from the PSB have set up the infrastructure that will allow us to move to more integrated structural biology, which in my view should be an important future focus of the IBS. We should also make an effort to concentrate our research on a limited number of areas to increase visibility. However, in the end it's the excellence judged by the scientific community, which counts.

What is your vision for an on-campus collaboration between IBS and EMBL Grenoble? In what way would this differ from the current PSB agreement, which additionally involves ESRF and ILL?

You are right we already have very good collaborations with our international partners within the PSB and can still improve them. The EMBL is special due to our common mission of performing excellent basic science. This will be the basis for a closer link with EMBL through the extension of bilateral infrastructure projects on electron microscopy for example, which already exist, and common collaborative scientific projects.

What advice do you have for PSB scientists?

Follow your instincts, make use of the great opportunities!

How has the PSB helped your career?

The PSB and its platforms that are accessible for everybody have been extremely important in planning my research. To have this all on the same campus is an invaluable asset for the site, because collaboration works best when it is next door.

J. Timmins (IBS) & F. Bernaudat (PSB)

ANNOUNCEMENTS

Stephen Cusack, head of the EMBL-Grenoble outstation
Fellow of the Royal Society and recipient of the first Ivano Bertini award.

Congratulations to Stephen who was recently elected to the Fellowship of the Royal Society, the national academy of science in the UK, which was founded in the 1660s. Stephen also became the first recipient of the Instruct Ivano Bertini Award, presented during the second Instruct biennial meeting in Florence. These honors recognize the seminal contributions Stephen has made to X-ray crystallography, particularly his structural insights into protein-RNA complexes and proteins involved in host-pathogen responses.
"It's a great honour to be elected FRS and to receive this Award," says Stephen. "Thanks to all my co-workers and collaborators who have made doing science such fun over the years and allowed us to produce exciting results on challenging projects."

AFMBioMed Summer School 2015
The next AFMBioMed Summer School will be organized in Grenoble from 24th-29th August 2015. The School offers an introduction to Atomic Force Microscopy in Life Sciences and Medicine. Lectures in the morning are complemented by hands-on experiments in the afternoon. Participants are encouraged to send a CV and a motivation letter to the organizers. Local organizer: J.-L. Pellequer (IBS). For more information, please visit the website:
http://www.afmbiomed.org/grenoble-2015.aspx

"Signaling Through Chromatin" symposium
The "Signaling Through Chromatin" symposium will take place at Minatec from 22nd-24th September 2015. The goal of this meeting is to highlight new concepts and technologies emerging in the field of chromatin biology and to foster stimulating and creative discussions around topics covering chromatin dynamics, DNA- and RNA-based processes. Local organizer : J. Kadlec (IBS). For more information and registration, please visit the website :
http://www.epigenesys.eu/en/in-the-news/other-events/eventdetail/324/-/signaling-through-chromatin

NEWCOMERS

Andrés Palencia has joined the Integrated Structural Biology Grenoble unit (UMS 3518; CNRS-CEA-UJF-EMBL) as Head of the Biophysical Platform. He will provide scientific support and training to users, both local and external, the latter through national FRISBI and European Instruct projects, and he will work to increase the visibility of the platform. Previously, Andrés worked at EMBL since 2008, first as a Post-doctoral fellow and then as a Research Scientist.
For more information, contact Andrés at:
palencia@embl.fr

Jan Kadlec joined the IBS in May 2015. Following his postdoctoral work at the University of Oxford, Jan was a staff scientist at EMBL. Jan is now setting up his ATIP/AVENIR funded team at IBS with the major focus on structural and functional analysis of epigenetic regulatory complexes.

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The Partnership for Structural Biology (PSB) is a collaboration between a number of prestigious European and French scientific laboratories in Grenoble 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.