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PSB et al

Partnership for Structural Biology Newsletter

Scientific highlights

The structure of the oligomerization domain of SEPALLATA3

Figure 1: Schematic of SEPALLATA3 bound to DNA with sunflowers, poppy and Arabidopsis flowers in the background. The ability of SEP3 to form different protein complexes and bind DNA at multiple sites results in the formation of different floral organs. The protein is shown as a surface cartoon in dark blue, light blue, dark green and light green. DNA is colored pink and orange and shown as a surface. Flower images taken from 19th century botanical prints.

Flowering plants, or angiosperms, have colonized the vast majority of terrestrial habitats, even though they are a relatively recent evolutionary event. How flowering plants came to dominate the terrestrial landscape is due to an evolutionary leap in reproductive fitness- the development of the flower (Figure 1). Angiosperms unite the male and female organs in one structure, the flower, and enclose the seed within an ovary, unlike in their sister phylum, the gymnosperms, or non-flowering plants, where the male and female organs form separate structures and the seeds are not enclosed. The evolution of flowers has been the subject of speculation from the time of Charles Darwin who termed the dominant rise and diversification of flowering plants as "an abominable mystery" due to the lack of a smooth transition from non-flowering to flowering plants in the fossil record. With the sequencing of multiple genomes from basal angiosperms, gymnosperms and higher flowering plants, certain gene families have been identified which play a central role in the development of the flower. Our team is focused on the genes and proteins that orchestrate flower development and understanding the molecular mechanisms at play in their complex functions.

MADS transcription factors are key players in many different developmental processes in the plant, most notably flowering and floral organ development. SEPALLATA3 (SEP3) is a MADS TF implicated in the development of all floral organs. SEP3 is able to form different dimeric and tetrameric complexes with other MADS TFs and acts as a "hub" for higher order complex formation which is critical for its in vivo activity. We recently solved the structure of the oligomerization domain of SEP3 at atomic resolution and directly demonstrated DNA-protein binding and looping using single molecule AFM studies (Figure 2, [1]). These results characterize the atomic and molecular level determinants for dimer and tetramer formation and provide the first structural information for any plant MADS TF. This work provides the structural foundation to probe the molecular level mechanisms responsible for in *planta* function of the MADS TF family.

Taken together, these results will enable us to understand how the MADS TFs chose their in vivo partners and how these complexes lead to the formation of the flower. As a long-term goal, these studies will enable us to tune the function of the MADS TFs in the plant and alter different reproductive pathways and structures in a predictable manner.

C. Zubieta (ESRF; New affliation: iRTSV/PCV, CEA).

[1] Puranik S., Acajjaoui S., Conn S., Costa L., *et al.* (2014) *Plant Cell*, 26(9), 3603-15.

Figure 2: Model of SEP3 homotetramer and SEP3-DNA binding. (left) The composite model of SEP3 MIK domains using the structure of SRF residues 141- 227 (PDB 1SRS). The DNA binding site is located at the distal extremes of the tetramer based on the SEP3$^{75\text{-}178}$ (I and K-domain) structure determined in Puranik, *et al.* The SEP3 structure is displayed as a surface colored by monomer and the SRF DNA-binding domain as a cartoon with protein in light and dark gray and DNA in orange and blue. (right) AFM image of SEP3 bound to a 1kb DNA with two SEP3 binding sites. The inset indicates DNA looping by the SEP3 tetramer.

CONTENTS

Do bacteria have an immune system?

Bacteria, and notably the species that cause disease, are often the subject of studies that aim at deciphering their potential for causing infection or the strategies they employ to resist antibiotic treatment. Bacterial defense mechanisms, however, are still poorly studied, largely because to date it was unclear if bacteria carried anything that resembled an 'immune system'. Recently, Wong & Dessen structurally and functionally characterized an alpha-macroglobulin (A2M) from the human pathogen *Salmonella enterica* [1]. A2Ms are large, complex molecules that are present in human plasma and whose role is to 'trap' proteases, often secreted by invading organisms, and thus they play key roles in the immune system.

Until recently, A2Ms were believed to exist only in eukaryotic species, such as humans. However, A2M-like proteins were identified in a number of pathogenically invasive bacteria, as well as in species that colonize plants, fish, and insects [2], suggesting that bacteria could also require 'protection' from proteases secreted by the target host or by other organisms competing for nutrients or space.

It turns out that the structure of the first bacterial A2M, made possible by employment of the HTX screening platform and the use of ESRF beamlines, reveals a multi-domain, flexible molecule that resembles proteins from the eukaryotic immune system. It contains regions such as a 'bait', to attract the proteases that it intends to 'trap', and a 'lock', to keep the trapping machinery hidden within the structure prior to the trapping step (Figure 1). *S. enterica* A2M also traps target molecules using a mechanism that is highly reminiscent of the one employed by human A2M, suggesting that, in the bacterial cell, it could have similar protective functions as its human counterpart. These discoveries suggest that bacteria have a rudimentary immune system that not only mimics initial steps of the human one but that could be very important to protect pathogens during the infection and colonization processes.

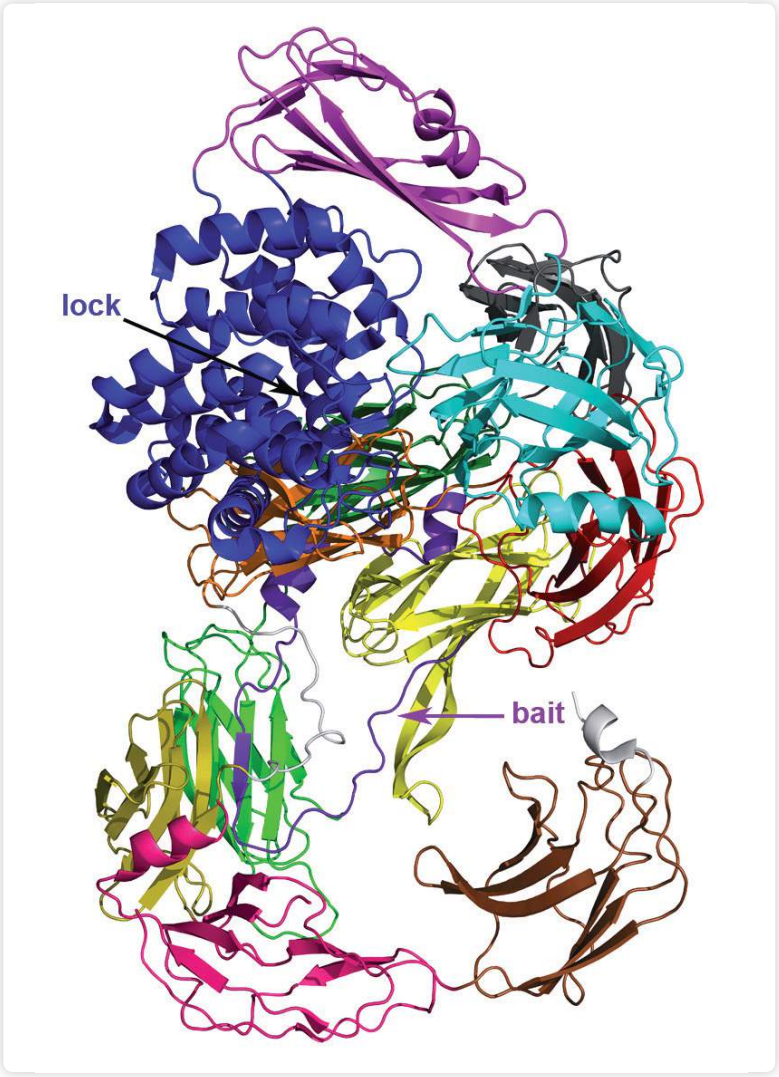


Figure 1: The structure of the first bacterial alpha-macroglobulin reveals a multi-domain, flexible protein that harbors an exposed 'bait' region (purple loop region) as well as a hidden 'lock' that protects the trapping mechanism from hydrolysis by solvent in the absence of the target.

A. Dessen (IBS)

[1] S.G. Wong and A. Dessen (2014). Nature Communications. 5, 4917.
[2] Budd A, Blandin S, Levashina E, Gibson TJ (2004). Genome Biol. 5: R38.

Exquisite monitoring of very low-dose X-ray induced bond breakage unravels the enzymatic mechanism of urate oxidase

The enzyme urate oxidase (UOX, also known as uricase or rasburicase) is a tetrameric enzyme that catalyzes the dioxygen-mediated degradation of uric acid (UA), a breakdown product of purine nucleotides, to 5-hydroxyisourate, which is then further metabolized to the easily secretable allantoin. UOX is present in almost all mammals, except humans and a few other primates, where uric acid is not degraded but is secreted directly, leading to much higher uric acid levels in human blood compared to most animals.

UOX is one of the few cofactor-free oxidases. Albeit several structures of cofactor-free oxidase are already known, their reaction mechanism remains unclear. In particular, no X-ray structures are available so far for any reaction intermediates. The most accepted reaction mechanism involves the peroxidation of the substrate and the subsequent release of a hydrogen peroxide molecule. Therefore, by the law of mass action, increasing the concentration of hydrogen peroxide leads to a partial reversal of the reaction, and thus to an accumulation of the peroxo intermediate. When UOX crystals previously treated with peroxide are diffracted with a low X-ray dose (tens of kGy), the electron density of the uric acid substrate (MUA) in the active site is different from standard data collections at higher X-ray dose (hundreds of kGy or several MGy): in low-dose datasets recorded at 100 K, the uric acid substrate is modified with a two-atom group (Figure 1, left), while in higher-dose datasets, a diatomic molecule is found instead as a detached density above the plane of the substrate radical (Figure 1, right, [1]).

Interestingly, in crystallo Raman spectroscopy helped to clearly identify the exact character of the observed reaction partners [1]. With a newly developed online-Raman motorized setup that is available on ID29, it was possible to easily record Raman spectra from the same protein crystal, interleaved with the collection of X-ray diffraction data. The Raman spectra remained almost exactly the same with increasing X-ray dose, except for a tiny Raman band at 605 cm^{-1} that decreased (Figure 1, centre). The ability of monitoring this small band highlights the quality of the experimental setup.

For the peroxo intermediate, QM/MM calculations predict a vibrational mode at that frequency, which involves the bond between UA and the peroxide group. Consequently, this vibrational band does not exist for the configuration that is seen at higher X-ray doses, where this moiety is cleaved off. Therefore, X-ray dependent decrease of this Raman band is consistent with the observed X-ray-induced breaking of this band described above. Use of isotopically labeled peroxide ($H_2{}^{18}O_2$) experimentally proved that this Raman band is indeed due to this vibration mode, as it is down-shifted by 9 cm^{-1} upon use of the ^{18}O-labeled peroxide [1].

Altogether, this shows for the first time that the UOX mechanism proceeds via a C5-(hydro)-peroxide intermediate (5-PMUA). Additionally, the observed decay exhibits biexponential behaviour, which shows that there is not only X-ray induced bond breaking, but also a competing X-ray induced bond repair, a phenomenon that has been reported before for X-ray induced disulfide bond breakage [1].

A. David von Stetten (ESRF), B. Daniele de Sanctis (ESRF), C. Antoine Royant (IBS/ESRF)

[1] Bui, S., von Stetten, D., Jambrina, P. G., Prangé, T., Colloc'h, N., de Sanctis, D., Royant, A., Rosta, E., Steiner, R. A. (2014) *Angew. Chem. Int. Ed.*, 53, 13710-13714

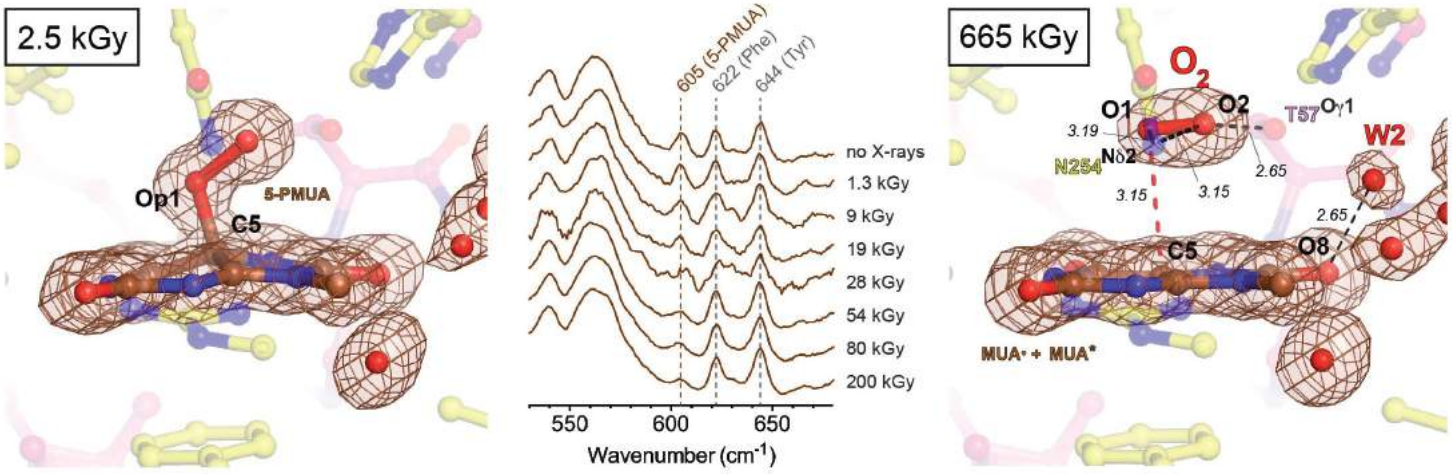
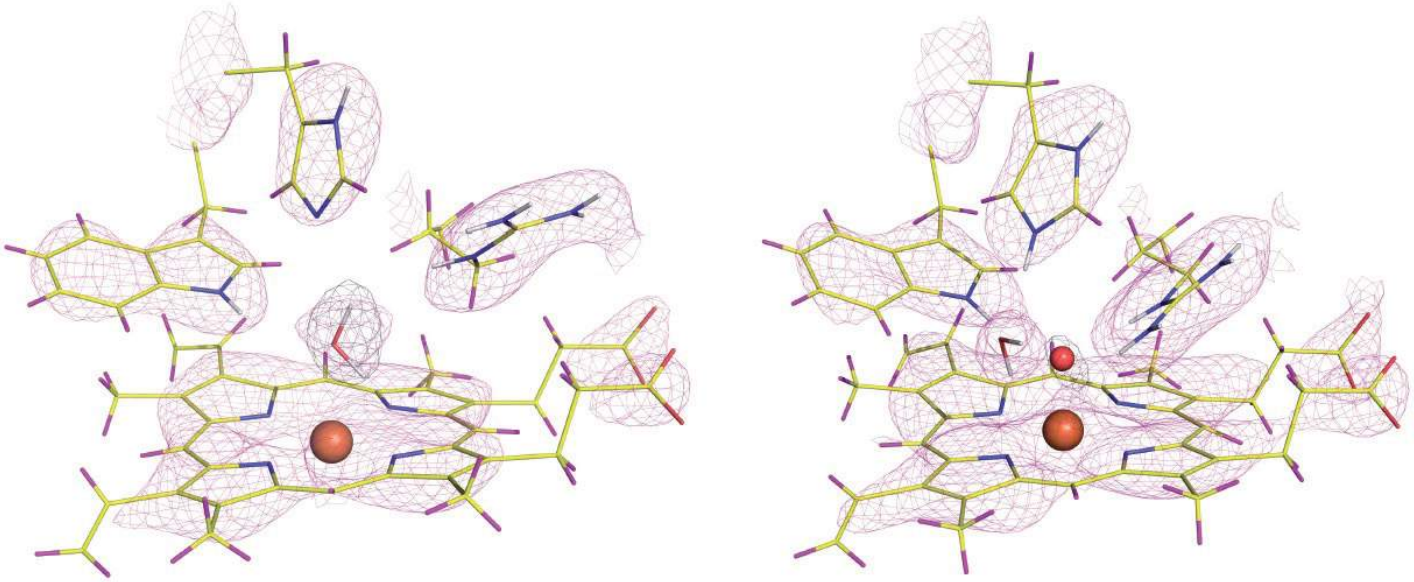


Figure 1: (left) Snapshot of UOX:5-PMUA at low X-ray dose. *2mFo-DFc* electron density contoured at 1σ level is shown in brown for the organic moieties and solvent molecules in close proximity. (centre) In crystallo Raman spectroscopy shows a dose-dependent decrease of the 605 cm 5-PMUA "fingerprint band". (right) At X-ray high-dose, C5-Op1 rupture leads dioxygen to be trapped above the ensuing planar moiety.

Neutron cryo-crystallography sheds light on heme peroxidases reaction pathway

Heme peroxidases are a family of catalytic iron-containing proteins found in nearly all living organisms. These enzymes catalyze the H_2O_2-dependent oxidation of a substrate, thereby removing this potentially hazardous molecule from the cell. Heme peroxidases share a common reaction mechanism that involves the presence of two intermediate species, known as Compounds I and II. Reduction of Compound I by one electron equivalent yields the closely related Compound II intermediate and structural characterization of the active site in the transient Compound I is of particular interest. The protonation state of the iron bound oxygen ligand has become a key question in the study of heme enzymes. In particular, attention has been focused on whether the ferryl can be formulated as Fe(IV)=O or Fe(IV)-OH.



Neutron scattering density maps of Cytochrome c Peroxidase in the resting state (left) and of transient Compound I (right). $2F_o$-F_c neutron map shown in magenta. F_o-F neutron difference map shown in black.

The methodologies that were traditionally employed to address this question appeared to be inadequate. Early approaches to the problem used resonance Raman methods to examine the iron – oxygen bond as an indirect reporter on the oxygen protonation state. However the photolability of Compound I during laser excitation is well documented and results in ambiguous experimental findings. More recently, X-ray crystallography was employed with the purpose of inferring the ligand protonation state from the study of the iron-oxygen distance. However, the catalytic centre in these proteins is particularly sensitive to radiation damage effects and X-ray determined Fe-O distances are now considered unreliable.

For these reasons we adopted a different approach, described in a recent publication [1]. Due to the lack of radiation damage effects and its capability of localizing deuterons, neutron crystallography is an excellent tool for the study of hydrogen related biochemistry. Neutron crystallography data collection from cryo-trapped reaction intermediates is a unique tool for probing reaction mechanisms, but presents a number of challenges, in particular the need to flash cool relatively large crystals. We determined the neutron structure of the heme enzyme Cytochrome *c* Peroxidase (C*c*P) in the resting state, using the quasi-Laue diffractometer LADI III, at the ILL. The data were collected at room temperature on a D_2O-soaked single crystal. We have also determined the neutron structure of C*c*P Compound I at 100 K: a deuterium exchanged C*c*P single crystal was reacted to form Compound I and subsequently cryo-cooled at 100 K. Monochromatic neutron data were collected at the BioDiff instrument at FRM II.

The structures showed that the distal histidine residue in the active site is neutral in the resting state but doubly protonated in Compound I, which was unexpected. The iron axial ligand in Compound I is an oxygen atom, and it is non-protonated. Our observations indicated that the widely assumed role of the distal histidine in Compound I formation needed to be reassessed and we proposed alternative possible mechanisms for O-O bond cleavage [1].This work shows the feasibility of using neutron cryo-crystallography for the clarification of reaction mechanisms in enzymatic pathways.

C.M. Casadei and M.P. Blakeley (ILL)

[1] C.M. Casadei *et al.* (2014). *Science*, 345, 193-197.

The heart of influenza virus at atomic resolution

The influenza virus is a major threat to mankind. According to the World Health Organization, seasonal outbreaks result in about 250,000 to 500,000 deaths annually. Pandemics, caused by evolution of totally new viruses, for instance by recombination of avian and human strains, are potentially very serious, with the 1918 'Spanish flu' causing several million casualties. Fortunately, the last pandemic influenza virus, the 2009 'swine flu', was much less virulent but still infected a large fraction of the global population. However, the outbreak dramatically demonstrated our helplessness to combat the virus efficiently. Available drugs mainly target virus entry and release, and are already insufficient since the virus has evolved and become insensitive. Seasonal vaccinations provide immunity to only some of the presently circulating viruses, but viruses frequently mutate and eventually escape the given immunity entirely.

Pivotal to the influenza virus' 'life' cycle is the virally encoded heterotrimeric polymerase-complex composed of the subunits PA, PB1 and PB2, with PB1 constituting the actual RNA-dependent RNA-polymerase (RdRp). It both replicates the viral single-stranded RNA genome and transcribes mRNAs encoding for viral proteins that pack and wrap the viral genome copies to form new infectious virus particles. The RdRp is highly conserved among different virus serotypes and quasi-species, providing an attractive drug target.

Stephen Cusack and co-workers from EMBL-Grenoble have solved the crystal structure of the 260 kDa polymerase complex for both influenza A and B [1, 2]. The structures open the door for a new era of structure-based drug development of RdRp-targeting anti-influenza agents. They also provide important insights into the mechanism of this elaborate molecular machine, including the binding of the RNA promotor and the cap-snatching mechanism. Overall the polymerases of influenza A and B are very alike; they carry a conserved core RdRp (protein PB1), that is closely intertwined with the PA and PB2 proteins (Figure 1). One major difference between the polymerase structures of influenza A and B is the positioning of the PB2 cap-binding domain (Figure 2). It turns out that this conformational change reveals the structural basis for an important function of the polymerase complex, so-called 'cap-snatching'. Cap-snatching is a mechanism by which the polymerase binds to the cap structure on the 5' end of the host messenger RNA, cleaves the mRNA 10-15 nucleotides downstream from the cap, and then uses the resulting fragment as a primer to initiate the transcription of viral mRNA. It was previously unclear how the polymerase accomplished this multi-step task. The new structures reveal a substantial rigid body rotation of the cap-binding domain that directs the bound host mRNA first towards the endonuclease domain and subsequent relocation to the active site of the RdRp (Figure 2).

During the work full advantage was taken of multiple PSB platforms; the 'Eukaryotic Expression Facility' (EEF) for recombinant protein production in insect cells, the 'High Throughput Crystallization Facility' (HTX), and ultimately the powerful beamlines of the ESRF for the collection of weak diffraction data.

A. Pflug and S. Reich (EMBL)

[1] Pflug A, Guilligay D, Reich S, Cusack S. (2014) Nature, 516, 355–360
[2] Reich S, Guilligay D, Pflug A, Malet H, Berger I, Crépin T, Hart D, Lunardi T, Nanao M, Ruigrok RW, Cusack S. (2014) Nature, 516, 361–366

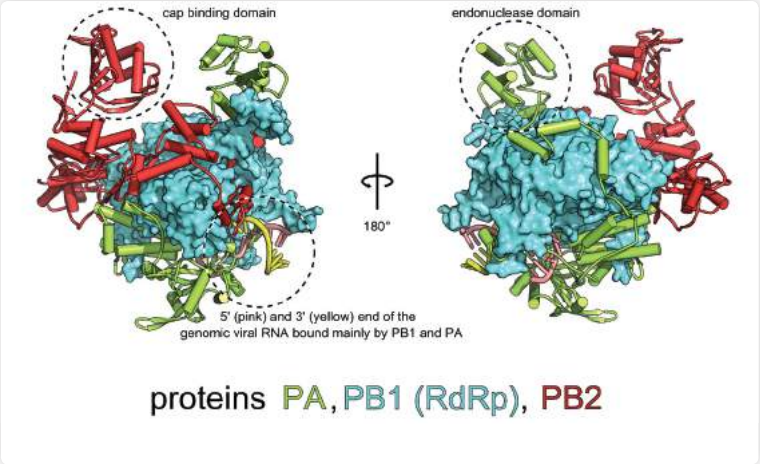


Figure 1. Overall architecture of the influenza polymerase complex. The RNA-dependent RNA-polymerase (RdRp) forms the core of the molecule, wrapped by the proteins PA and PB2. The figure was created using the coordinates of the influenza A polymerase structure 4WSB.

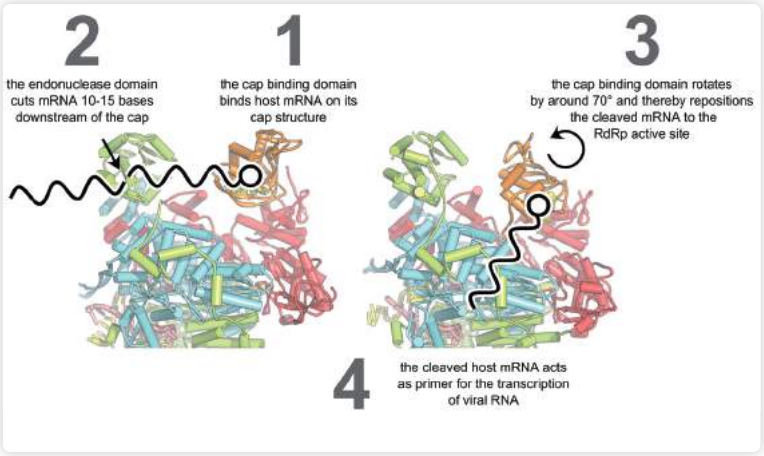


Figure 2. Model for initiation of transcription within the polymerase complex, deduced from the crystal structures of the polymerases of influenza A (left, structure 4WSB) and B (right, structure 4WSA). The structures depict two different orientations of the cap-binding domain, compatible with endonuclease activity and priming of the transcription respectively. Coloring of the proteins is identical to Figure 1 with the cap-binding domain highlighted in orange.

News from the platforms

News from the MX Beamlines

In the last few months, Structural Biology facilities available at the ESRF have been extended by the opening of two new end stations located on the ID30A slot: MASSIF-1 and MASSIF-3.

MASSIF-1, in users operation since September 2014, is a highly intense (~3 x 10^{12} ph/sec in a 50 x 50 µm^2 beam) beamline equipped with an ESRF-developed RoboDiff sample changer/diffractometer, a high capacity dewar (HCD) holding up to 240 SPINE standard sample holders and a fast Pilatus3 2M detector. What is new is the way this end-station is operated: Users simply send their samples to the ESRF, define via ISPyB what kind of experiment(s) they would like to have performed on any given sample (ranging from simple diffraction tests to strategy-based diffraction data collection with subsequent data processing) in a completely, hands-off, automatic mode. All results, including processed data, are made available to users through ISPyB.

MASSIF-3 has been operational since December 2014 and is a highly intense (~2 x 10^{13} ph/sec) micro-focus (~15 µm in diameter) beamline, ideal for performing experiments on small crystals or at different points on the same crystal. Currently equipped with a MD2M minidiffractometer, a SC3 sample changer and a Mar225 CCD detector, the final experimental set-up of MASSIF-3 will comprise a RoboDiff sample changer/diffractometer, a HCD and, currently being commissioned, a Dectris Eiger 4M (up to 750 Hz frame rate) pixel detector (Figure 1). This new, high-frame-rate detector makes this beamline ideal for room temperature experiments or even time-resolved experiments.
In the near future (first quarter of 2015), ID30B, which replaces the recently closed ID14-4 beamline, will also come on stream. In its final configuration this independent energy-tunable beamline will be equipped with an MD2S diffractometer (compatible with *in-situ* diffraction experiments in crystallization plates), a HCD and a fast Pilatus3 6M (up to 100 Hz frame rate) detector. Beam size at the sample position will be variable between 20 and 200 µm in diameter with a flux of roughly 10^{13} ph/sec.

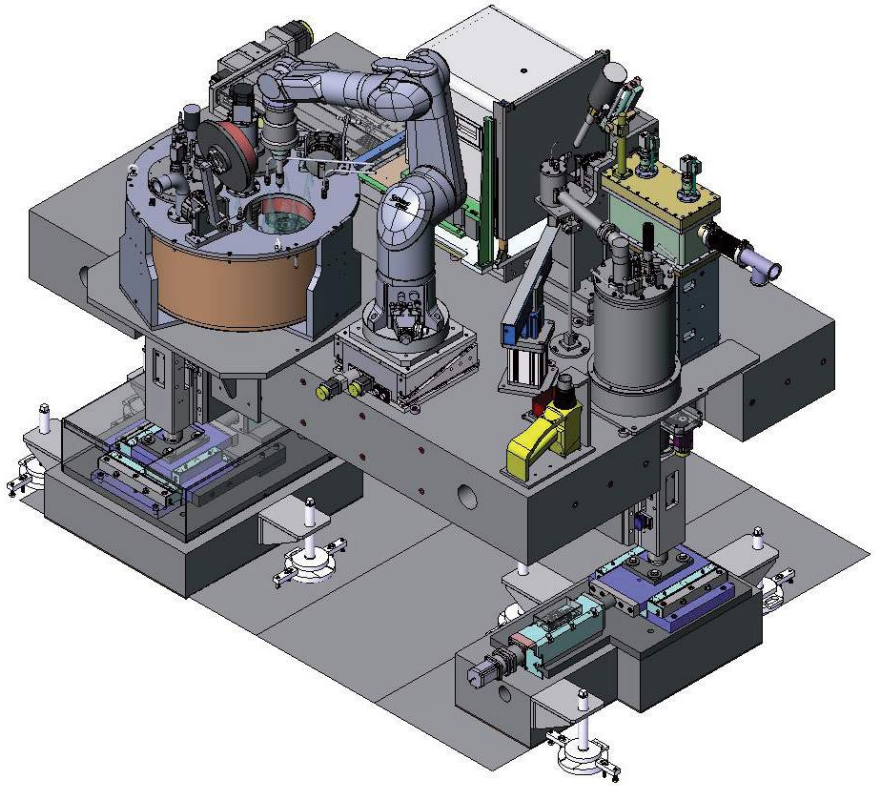


Figure 1: Set up of the experimental hutch in MASSIF-3.

Of the remaining end-stations, the mirrors of the Kirkpatrick-Baez focusing element of ID23-2 will be replaced in January 2015. The main effect of this will be to increase the flux density of the beam line's 7 x 5 µm^2 focal spot by a factor of at least 2.5 making the beam line even more suitable for experiments with microcrystals.

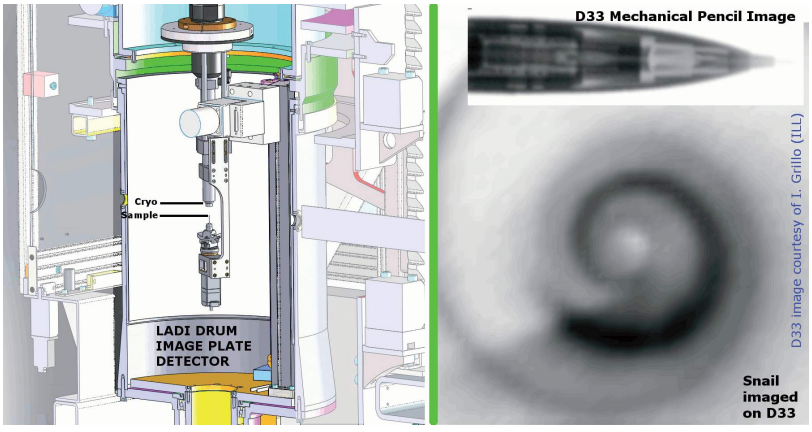
C. Mueller-Dieckmann (ESRF)

News from the ILL instruments

With this first 2015 issue of the newsletter and the ILL reactor cycles restarting in May, recent instrument developments promise new and improved applications of neutrons to Structural Biology.

The PSB macromolecular crystallography community will be happy to hear the LADI instrument now benefits from significant improvements in signal-to-noise ratio, due to alignment and shielding optimisation. Such developments directly impact on the crystal volume required, so that increasingly smaller samples can be used (cf. 0.05mm^3, smallest used prior to recent developments). Enhanced design and optics also bring promising applications to crystals with larger unit cells. Improved flexibility in wavelength-band selection means that larger molecules will be tackled, thus continuing to stretch the boundaries of neutron crystallography at this world-leading instrument. Finally LADI is now equipped with a Cobra cryo-cooling system, critical for the study of transient states (see highlight on page 4 of this issue), crystals unstable at room temperature, and improved X-ray complementary data.
The D16 high-resolution diffractometer is fully operational in its new position in the ILL22 guide-hall. At this new position and with new graphite monochromator crystals, the instrument now counts with an impressive 10 fold increase in neutron flux and an added longer wavelength option (now 4.5Å, 5.7Å and 8Å). Such developments strongly benefit the study of thin films in reflection geometry as well as powders and liquids in diffraction mode, in an intermediate q-range, overlapping with SANS and atomic resolution diffractometers. For a highlight on possible applications see for example how D16 contributed to new insights on how living things capture energy from the sun [1].

The small angle scattering ILL triad of instruments brings very good news for solutions studies. D11 benefits from new detector electronics with an improved spatial resolution, made available by new digital processing. A bidimensional multiwire proportional gas chamber (MWPC) detects neutrons through a reaction with ^{3}He nuclei, giving rise to a “charge cloud” in the detector. Each event is converted to an electronic signal, amplified and mapped to where a neutron impacts the detector. The new electronics allow a finer-grained 2D image than the physical grid defined by the MWPC wires, as well as reduced dead times. The end result is faster, better data.



2015 also welcomes the return of D22 to the user program, now with improved collimation mechanics for enhanced alignment stability. Backscattering contaminations from the detector tubes' supports have been suppressed, improving the signal-to-noise ratio. In terms of sample environment, *in-situ* dialysis now offers new experimental possibilities.

D33 reached its final state in February 2013 with the installation of 4 front detector panels. In addition to a standard monochromatic mode of operation, it offers a time of flight mode to cover an enhanced dynamic q-range in one instrument setting. D33 is well adapted to work with low concentration bulky biological samples, to follow kinetics in soft matter samples with acquisition times ~50 ms, or to investigate flux line lattices in very tiny supra-conductor samples. In addition to classical SANS measurements, the first imaging tests with a spatial resolution of 200 µm have shown the strength of neutrons to reveal the inner structure of composite materials (see image).

Finally there are also good news for the studies of bio-membranes and protein interactions. A standardisation of sample environment, not only for different reflectometry instruments but also across facilities, resulted in a new sample environment for solid-liquid studies (available for example on D17 or FIGARO). While further developments are on-going, the new design significantly improves a number of aspects of particular relevance to biological samples. Namely it reduces the amount of sample required (~10 times less), makes sample alignment faster and improves temperature control (faster and accurate to 0.1°C; cf. ±3°C before).

The developments summarised here are by no means a full account of the many exciting opportunities the ILL has to offer the PSB. Readers, and potential new neutron users in particular, are strongly encouraged to visit the suggested links or contact the author for advice as required.

S. Teixeira (Keele University / ILL)

[1] B. Demé, C. Cataye, M. A. Block, E. Maréchal and J. Jouhet, FASEB J. 28 (2014) 3373-3383

Suggested instrument webpages (contact details for instrument scientists included within):

LADI: http://www.ill.eu/ladi-iii **D16:** http://www.ill.eu/d16
D17: http://www.ill.eu/d17 **D19:** http://www.ill.eu/d19
D11: http://www.ill.eu/d11 **D22:** http://www.ill.eu/d22
D33: http://www.ill.eu/d33 **FIGARO:** http://www.ill.eu/figaro

Events

PSB student day

On the 28th of January 2015, the seventh edition of the PSB student day was opened with a seminar by group leader Marco Marcia who recently joined the EMBL Grenoble Outstation. Throughout the day, the Chadwick Amphitheater has seen second and third year PhD students: Ziad Ibrahim (IBS), Ashley Jordan (ILL), Alice Tissot (UVHCI), Benedicte Lafumat (ESRF) and Piotr Gerlach (EMBL) giving talks about their research projects. During two clip sessions, 11 first year PhD students introduced themselves and their work.

A large audience attended the event and took the opportunity to discuss around poster sessions during the lunch and coffee breaks in a friendly atmosphere.
The afternoon session was opened by a presentation from Alexandrine Sadoul (GIANT/CEA), highlighting the various activities which exist for pre and post-doctoral students within the GIANT campus and Céline Guillouet (Labex GRAL) presented the combined Labex GRAL/GEM (Grenoble Ecole de Management) program dedicated to PhD Students.
The day's proceedings were drawn to a close with the usual prize ceremony for the Best Poster and Best Clip. Best Poster prize was awarded to Mariam El Khatib (IBS) and Best Clip prize went to Maria Lukarska (EMBL), who also treated the audience with an improvised piano concert during the afternoon break.
On behalf of the PSB student committee, I would like to sincerely thank our PSB scientific coordinator, Florent Bernaudat, for his precious help.
Congratulations to all the participants who took part and we look forward to seeing you again next year!

A. Jordan (ILL), on behalf of the PSB student committee.

PSB platform exposé 2015

In the last 10 years, the technical platforms of the PSB have grown in number and complexity from the 6 that were installed at the outset to the 23 that are currently available. These platforms are in constant evolution and to give an update to the local community, the 2nd PSB platform exposé was organized on 20th January in the Chadwick amphitheatre.

The meeting was very informative and started with a session of 5-minute clips during which the platform scientists presented the latest developments and access mode of their platforms (presentations are available on the PSB website*). In the context of increasing relationships between the PSB and the GRAL community (Grenoble Alliance for Integrated Structural & Cell Biology, constituted of iRTSV, IBS and UVHCI), the scientists of the iRSTV were invited to attend the meeting and two iRSTV platforms, the "MÖSSBAUER" analytical platform and the "CMBA: Screening for Bioactive Molecules" platform, were also presented. The meeting then continued with a poster session that enabled further discussions on the possibilities offered by the platforms, and snacks and drinks were served (the poster session was organized by the PSB Get Together committee) in order to create a friendly atmosphere.

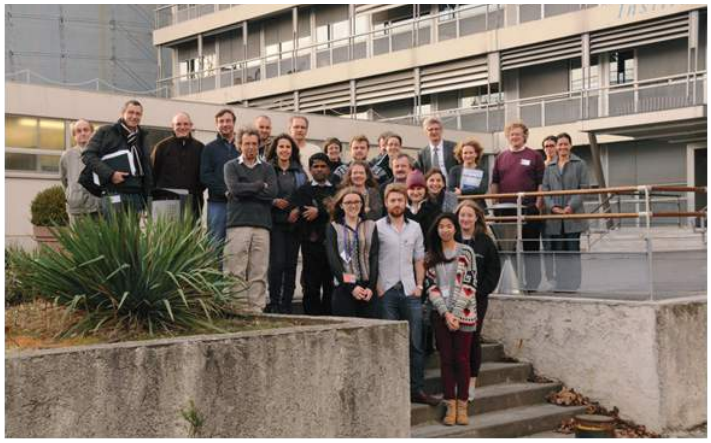
F. Bernaudat (PSB Coordinator)

* www.psb-grenoble.eu

2014 British Crystallographic Association - Biological Structures Group (BCA-BSG) Winter meeting

The 2014 BCA-BSG winter meeting, which ran from 15-17th December was, for the first time, held outside the UK, and hosted by the EPN campus thanks to its organisers: G. Leonard, E. Mitchell and T. Forsyth.

The exciting scientific programme of the meeting started with a focus on research infrastructures with the ESRF and ILL presented by H. Reichert and H. Schober respectively. After this session, attendees were given a guided tour of the facility of their choice (ILL, ESRF or PSB) and the evening was rounded-off with a cheese and wine reception. The next day began with a session on Future Opportunities for UK Structural Biology in Grenoble that included presentations by A. Harrison (Diamond Light Source) and C. Miles (BBSRC, UK) on the Strategic Significance of UK participation in Large Scale Infrastructure. This session ended with an interesting round table discussion, which pointed out, amongst other things, the need for personnel on the ground to support instrumentation developments. The meeting continued with numerous excellent scientific presentations (PSB members were well-represented amongst the speakers), many of which highlighted emerging techniques and the advantages of combining X-rays & neutrons in structural biology studies.



The BSG committee was extremely pleased with this edition of the meeting and warmly thanked the organisers for hosting it in Grenoble.

F. Bernaudat (PSB Coordinator)

Symposium on biomolecular dynamics, structure and NMR spectroscopy

This one-day meeting, which took place on December 4th 2014 at IBS, focused on recent developments of NMR spectroscopy and complementary biophysical techniques for the study of protein dynamics and structure. Eight international speakers from Denmark, Sweden, Germany, France and the US presented their recent research highlights. The increasing power of solid-state NMR in structural biology was illustrated by examples of structural studies on intact assembled virus particles, membrane proteins and entire ribosomes by solid-state NMR (Tatyana Polenova, Guido Pintacuda and Bernd Reif).

Furthermore, it was also shown how NMR spectroscopy can shed light on short-lived “excited” states of proteins, which are hardly visible by any other technique, and that even structures of such transient states can be obtained (Guillaume Bouvignies and Mikael Akke). Two presentations focused on photoactive proteins, attempting to understand their activation/deactivation from different viewpoints on a structural level (Frans Mulder and Dominique Bourgeois). Finally, Fabien Ferrage outlined the development of innovative NMR instrumentation to obtain a refined insight into biomolecular dynamics. Approximately 80 participants came to the event from 5 different countries.

P. Schanda (IBS)

Biomembranes and Membrane Proteins Workshop

The PSB Workshop on Biomembranes and Membrane Proteins organized by Christine Ebel, Valentin Gordeliy, Cecile Breyton, Christophe Moreau and Ekaterina Round from IBS took place on Friday 14th November 2014 at the ILL Chadwick Amphitheatre.

This event gathered together over 70 researchers, post-docs and PhD students to exchange information about scientific projects, methodology and instrumentation related to structural and functional studies of membranes and membrane proteins. The aim of the workshop was to provide information about PSB and local activities in order to develop synergy for membrane protein research on the EPN campus. The workshop was opened by J. Zaccai with a very brief history of membrane research at IBS and EPN campus. E. Bamberg (Director, Department of Biophysical Chemistry of Max-Planck Institute, Frankfurt, Germany) gave an outstanding lecture about microbial rhodopsins and optogenetics. Over 20 speakers from local organizations covered the research field of biomembranes and membrane proteins, including tools and instrumentation. The discussion at the end of the workshop on the needs for further development in membrane and membrane protein research benefited from the large and dynamic local scientific community.

The results of the workshop are highly positive and, with continuing support from the groups involved, we hope the suggestions made will be implemented shortly for the benefit of the membranes and membrane proteins scientific community working on the EPN campus.

E. Round (IBS)

GTBio 2014

Triennial meetings of GTBio from the Biology Group of the French Crystallographic Association (AFC) have been held since 1992. The last meeting took place on the EPN campus, from October 7th to October 10th, 2014. GTBio2014 was jointly organized by IBS and ESRF and welcomed 165 participants and 10 exhibitors. The local Organizing Committee comprised Stéphanie Ravaud, Beate Bersch, Guy Schoehn, Cécile Morlot, Eric Girard, Carlos Contreras-Martel, Jérôme Dupuy and Dominique Housset (IBS), Winfried Weissenhorn (UVHCI), Gordon Leonard (ESRF) and Annabelle Varrot (CERMAV).

The scientific program focused on different themes of structural biology, included 5 plenary lectures, 49 talks - many young investigators gave presentations of very high scientific quality - and 60 posters, and confirmed the strong tendency to combine techniques (integrated structural biology). The meeting also provided the opportunity to discuss the present and future of synchrotron and X-FEL facilities, and to present updates on the RéNaFoBiS educational network and other integrated structural Biology initiatives such as FRISBI and INSTRUCT. Based on feed-back received, participants enjoyed the meeting and the social events at the Grenoble Museum and Chateau de la Beaume. They were impressed by the many changes that have recently occurred on the EPN campus.

The GTBio2014 Organizing Committee.

Integrated structural and cell biology. From molecules to cells and organisms: Thinking out of the box

43 participants attended the 4-weeks summer school, which took place at Les Houches in the Chamonix valley in July 2014. The school aimed at preparing students and young researchers to new coming strategies also based on concepts in physics that open new ways of thinking biology.

The participants, 35 doctoral students and 8 post-docs, came from 14 different countries, mainly from Europe but also China, India, United States and Canada, with 21 different citizenships. Roughly half of the participants had been initially trained in biology, and half in physics. Based on 3 main biological topics, plant growth and flower development, interaction between viruses and host cells, membranes and transporters, the lectures illustrated various integrative approaches, and showed how concepts in physics and mathematics contribute to the understanding of biological processes from the molecular level to cells and organisms. The participants spontaneously organized late evening sessions on topics they had chosen themselves. Despite the bad weather, a few hiking and climbing sessions were also possible, and contributed to the extremely good atmosphere and the interactivity of the students during the lectures. A 4-weeks school is a unique opportunity for an interdisciplinary teaching.

The school was organized by E. Pebay-Peyroula and H. Nury (IBS), F. Parcy (iRTSV/PCV) and R. Ruigrok (UVHCI).

E. Pebay-Peyroula (IBS)

PSB discussion meeting on serial crystallography

A half-day PSB discussion meeting on serial crystallography at X-ray free electron lasers (XFEL) and synchrotron sources was held at the ILL on June 24th 2014.

Serial crystallography consists of collecting one or several diffraction patterns from a very large number of crystals and merging them into a single data set, from which the structure is determined.

Various researchers from all PSB member institutes, who have already successfully applied serial crystallography at the ESRF and at XFELs, reported their results at the meeting. Serial crystallography, at XFELs and potentially at synchrotrons, reduces X-ray radiation damage and can be carried out at room temperature, thus providing macromolecular structures at physiological temperatures. It also allows diffraction data to be collected from sub-micron-sized crystals and opens the door for time-resolved studies to be carried out down to the femtosecond time-scale at XFELs. Once the phase II upgrade of the ESRF is completed (2020) and the European XFEL opens its doors for users (2017), the structural biology community in general, and PSB members in particular, will fully benefit from ongoing hard- and software developments concerning serial crystallography.

At the half-day PSB meeting, it was decided to establish a PSB serial crystallography working group, whose roles will include helping PSB members in identifying possible serial crystallography projects and in preparing and carrying out experiments.

Jacques-Philippe Colletier (IBS), Stephen Cusack (EMBL), Gordon Leonard (ESRF), Andrew McCarthy (EMBL), Max Nanao (EMBL), Eva Pebay-Peyroula (IBS), Martin Weik (IBS)

PROFILE

Marco Marcia

I studied Pharmaceutical Chemistry at Bologna University (Italy) funded by a Collegio Superiore fellowship and was mentored by Prof. Giovanni Capranico.

I developed an interest in structural biology during my studies and a traineeship with Prof. Fritz Winkler and Dr. Xiaodan Li at the Paul Scherrer Institute (Switzerland).

I then moved to the Max Planck Institute of Biophysics and Frankfurt University (Germany) in 2006 for my PhD. In Frankfurt, I worked in the lab of Prof. Hartmut Michel. I solved the structure of sulfide:quinone oxidoreductase, a membrane protein and key regulator of sulfide homeostasis involved in detoxification, electron transfer and signaling processes.

I was awarded the Otto Hahn Medal of the Max Planck Society for my PhD. In 2010, I moved to Prof. Anna Pyle's lab at Yale University as a postdoctoral fellow and determined the structures of the group II intron at various stages of catalysis, contributing to clarifying the splicing mechanism. At Yale, I was also involved in the study of a novel but fundamental class of macromolecules, long non-coding RNAs (lncRNAs).

I started at the EMBL in September 2014 and lncRNAs will continue to be the focus of my research here in Grenoble, where I'm currently in the process of building up my research group. LncRNAs are transcripts of several hundreds to thousands of nucleotides in length. Some lncRNAs tightly interact with nuclear protein complexes and the resulting ribonucleoproteins (RNPs) play crucial roles in gene regulation and disease. However, little is known about how lncRNAs interact with their protein partners and their subsequent molecular mechanisms. The aim of my group is to characterize the structures of RNPs formed by lncRNAs to better understand their molecular functions.

Newcomers

Estelle Mossou has joined the D19 team (M. H. Lemee Cailleau and T. Forsyth) in August 2014 as co-responsible for the single crystal and fibre diffractometer D19 at the ILL. D19 is optimised for structural chemistry but is also very successful for high-resolution studies of small proteins.

Estelle obtained a PhD in biophysics from Keele University and subsequently carried out postdoctoral work in the ILL Life Sciences Group, namely the development of new techniques in protein crystallography, fibre diffraction, and solution scattering.
Contact: mossou@ill.fr

Linda Ponnet joined the *Unité Mixte de Service* "Integrated Structural Biology Grenoble" (ISBG) in November 2014, where she is responsible for the unit's administrative and financial aspects. This includes managing the ISBG budget, taking care of the billing for the IBS and UVHCI platforms and organising their instrument maintenance contracts. Her daily work involves interacting with both the platform scientists and the local headquarters of the CNRS.
Contact: linda.ponnet@isbg.fr

ANNOUNCEMENTS

2015 Edition of the Tutorial in Macromolecular Crystallography

This year the tutorial will take place again as a one-week compact course from 2nd to 6th March 2015. Fundamental aspects of crystallography will be treated in theoretical sessions (a mix of lecture and problem solving) and 3 practical sessions of 2 hours each. The tutorial will be given in English. The tutorial is aimed in the first place at graduate students from UJF, the PSB and the UVHCI who have a priority in registration. The tutorial counts for 3 ECTS credits needed for the "Ecole doctorale" of UJF. The tutorial is furthermore 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 by a simple mail to wpb@uvhci.fr is open and limited to 25 participants.

INSTRUCT-FRISBI-PSB workshop: Molecular Interactions – The Complementarity between biophysical methods

The workshop will take place on the EPN campus, Grenoble on June 1st to 5th 2015 and its goal is to provide theoretical, practical and data analysis training in various modern methods for monitoring and quantifying molecular interactions (SEC-MALLS, UAC, SPR, ITC...). The course will provide brief introduction to the biophysical concepts and theories, practicals to set experiments up on state-of-art instruments and introduction to data analysis methods.
The number of participants is limited to 20. Application deadline is fixed to March 8th, 2015.
For more information, please visit the workshop website:
http://workshops.ibs.fr/molecular-interactions
Contact: evt.workshop-interactions@ibs.fr

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.

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