JULY 2013 - NUMBER 11

10TH PSB et al ANNIVERSARY

Partnership for Structural Biology Newsletter

2013 marks the 10th anniversary of the Partnership in Structural Biology and there is a lot to celebrate. Our newsletter first came out in April 2006 as a two page list of announcements circulated by email. Its success soon brought a longer printed version and by the end of 2006 you could find the Number 1 paper-copy on coffee tables around the EPN campus, for students, staff and visitors to enjoy. The PSB has come a long way since!

This issue of the PSB Newsletter offers a 'special', extended 'News from the Platforms' section, in which you will find a full list of the available platforms within the PSB and a description of some of the new developments. We hope you enjoy reading it and will be looking forward to report on future PSB challenges and achievements.

The Editorial Team.

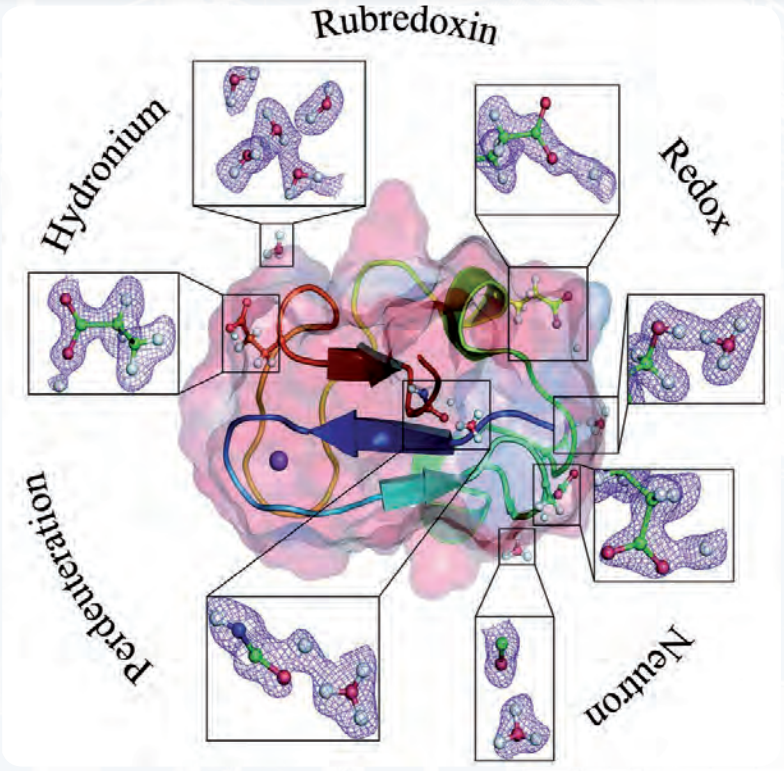
© Emmanuelle Bensaude

A. Watts, C. Carlile and E. Pebay-Peyroula enjoying cake and champagne for the celebration of the PSB's 10th anniversary.

SCIENTIFIC HIGHLIGHTS

Neutron crystallography sheds light on redox changes in Pyrococcus furiosus Rubredoxin

Rubredoxins are small monomeric non-heme mononuclear iron proteins found in prokaryotes and some eukaryotes. The [FeS_4] cluster provides electron transfer capabilities with redox partner proteins such as ferritin, rubredoxin oxidoreductase, rubrerythrin or superoxide reductase [1]. Macromolecular deuteration greatly enhances the visualisation of hydrogen-based species by neutron crystallography. While the power of X-ray crystallography is well known to structural biologists, they are a number of key limitations. Firstly, it is difficult to reliably image hydrogen atoms, which are often crucial to biomolecular structure and interactions. Secondly radiation damage issues may restrict the scope of structural studies, particularly in the case of metalloprotein systems where the X-ray beam itself may cause oxidation and compromise structural studies of (for example) a redox change.



The overall oxidised Fe-form structure of *Pf* Rubredoxin showing the corresponding hydronium ions and protonated carboxylic acids in the structure (enlargements).

Both issues are central to the recent work on rubredoxin by Cuypers *et al*[2] as part of a collaboration linking ILL's Life Sciences group with colleagues at the ESRF and Keele University in the UK. The D19 diffractometer was used to study the reduced and oxidised forms of the protein at near-atomic resolution[2]. The results show the presence of numerous hydronium (H_3O^+) ions within the protein, and illustrate protonation shifts following the change between the Fe^{2+} and Fe^{3+} forms of the protein. Four hydronium ions are identified in both oxidised and reduced forms of the structure: three are located close to the protein main chain and one forms part of a hydrogen bonded network with ordered solvent. One of the hydronium ions is located near the main chain amide group of Leu51 and appears to be involved in a redox-driven tauto-

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meric shift between amino and imino forms of the residue. The three other hydroniums are bound in essentially the same places in both the reduced and the oxidised forms of the protein. In two of these there is evidence of water-hydronium equilibria that may be important in charge transfer. In the oxidised form, Asp15 and Glu47 are seen to be protonated, while Asp13 is not protonated. In contrast, in the reduced form, Asp13 is protonated and Asp15 and Glu47 are not protonated.

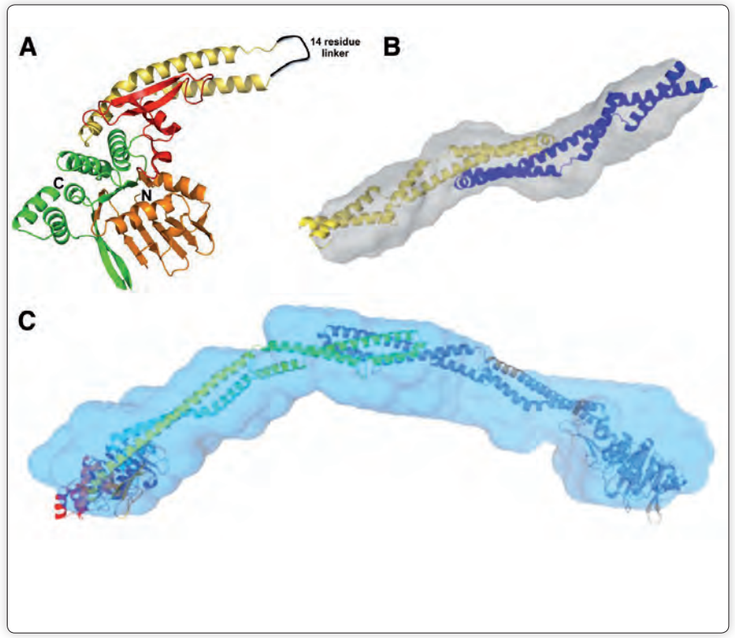
These observations suggest that hydronium ions and their interaction with the surrounding solvent play a key role in the protonation shifts and charge transfer processes associated with the change between oxidised and reduced forms of the protein. While H_3O^+ ions have been identified in chemical systems and in one protein, this is the first time they have been found in a redox protein where they are likely to be implicated in charge transfer. What we have here is a remarkable set of results that not only demonstrates the unique power of high-resolution monochromatic neutron crystallography and also the use of deuterated proteins to probe protein structures to image these vital details of protein structure, but also the opening of a totally new area of protein science that is directly relevant to critical aspects of biological function.

M. Cuypers (Keele University, UK & ILL, France).

[1] Weinberg *et al.*, J Bacteriol. (2004), 23, p 7888–7895.
[2] Cuypers *et al.*, Angewandte Chemie Int. Ed. (2013), 52, p 1022–1025.

RecN: the tethering protein

The genetic code of each living organism is encoded in its DNA. The prime objective for every life form is to deliver its genetic material, intact and unchanged, to the next generation, despite constant assaults from both endogenous and environmental sources on the DNA. The average human cell suffers over 10,000 DNA lesions per day. If left unrepaired, damaged DNA generates mutations, replication errors, persistent DNA damage and genomic instability that can ultimately threaten cell or organism viability, but can also lead to disease and cancer. Cells have therefore developed a number of mechanisms to repair such damages and thus maintain and/or restore the integrity of their genetic material. The extreme radiation-resistant bacterium *Deinococcus radiodurans*, has been shown to be more efficient than other organisms in doing such a job. It can survive in very harsh environmental conditions withstanding the presence of high doses of ultra-violet or ionising radiation or the absence of water [1]. Among the factors contributing to *D. radiodurans'* outstanding resistance to DNA damaging agents is its highly efficient DNA repair machinery that has been the focus of our research over the past few years.



A. Crystal structure of the globular 'head' domain of RecN. B. Crystal structure of the dimeric coiled-coil region of RecN superimposed on the envelope derived from the SAXS data collected on this construct in solution. C. Quasi-atomic structure of intact RecN reconstituted from the individual crystal structures and the SAXS data collected on the full-length protein.

In particular we have been studying the mechanism of repair of lesions occurring on both strands of the DNA, called double strand breaks (DSB), which represent the most lethal type of DNA damage. The repair of DSBs involves many proteins and the aim of our work is to improve our understanding of this complex process leading from DSB recognition to RecA-dependent repair [2].

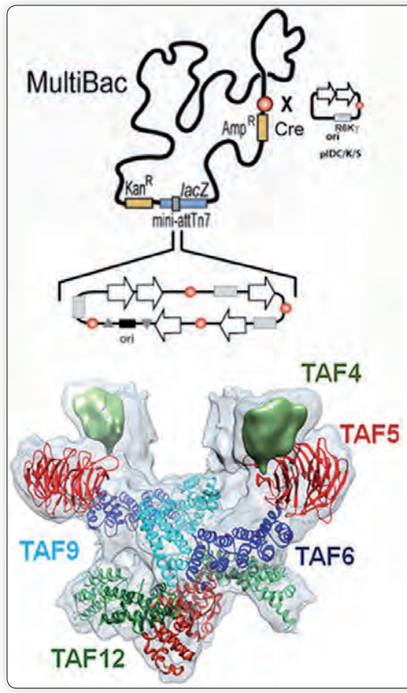
Recent results have revealed the importance of a particular protein, called RecN (Recombinase N), in bridging together different extremities of DNA molecules [3]. RecN is a member of the Structural Maintenance of Chromosome (SMC) family and was predicted to form a globular ATPase domain comprising its N- and C-terminal regions, connected to a "coiled-coil" domain, consisting of two long anti-parallel α-helices. During the course of his PhD at ESRF, Simone Pellegrino performed an in-depth functional and structural characterization of RecN using various techniques available within the PSB, such as Xray crystallography, small-angle X-ray scattering, dynamic and static light scattering. We determined three high-resolution crystal structures of overlapping domains of RecN and together with solution scattering data obtained for the intact protein, we were able to reconstitute a quasi-atomic structure of RecN, providing us with a detailed understanding of its overall shape and architecture (Figure 1). This structure corresponding to the first structure of an intact SMC-like protein represents a major result in the field of DNA repair. Additional enzymatic assays were carried out in this study to investigate the role of ATP binding and hydrolysis in this process and to explore the proposed DNA tethering activity of RecN. These data led us to propose a model for the mode of action of RecN in the early steps of DSB repair.

S. Pellegrino & J. Timmins (ESRF/IBS)

[1] Cox M. and Battista J. R. (2005), Nature Reviews Microbiology, Vol. 3, pp. 882-892;
[2] Bentchikou E, *et al.* (2010), PLoS Genetics, Vol. 6;
[3] Pellegrino S[1,3] *et al.* (2012), Structure, 20, pp. 2076-2089

Architecture of essential human transcription factor revealed

The PSB research groups of Imre Berger and Christiane Schaffitzel at the EMBL Grenoble and the research groups of Patrick Schultz and Laszlo Tora at the IGBMC in Strasbourg have, for the first time, described in molecular detail the architecture of the central scaffold of TFIID [1]. This complex was previously identified in cell nuclei and is thought to represent the functional human TFIID core, onto which the remaining subunits assemble to give rise to the complete holo-TFIID complex. By applying innovative methods for recombinant protein production, developed and implemented at the EMBL, the scientists coaxed insect cell cultures which they infected with a custom designed baculovirus to produce their TFIID complex in the quality and quantity required for detailed studies. Thus, the study, recently published in Nature, opens new perspectives in the study of transcription and of the structure and mechanism of other large multi-protein assemblies involved in gene regulation.



MultiBac. Technology to produce eukaryotic multiprotein complexes for structural and functional analysis.
CREDIT: Imre Berger.

Structure of the human TFIID core complex. Two copies each of the different subunits of the complex (TAF4 (green), TAF5 (red), TAF6 (blue), TAF9 (light blue) and TAF12 (light green) form a symmetrical structure.
The density determined by cryoelectronmicroscopy is shown as a grey mesh.
CREDIT: Gabor Papai.

By controlling the transcription of DNA into messenger RNA, TFIID forms the cornerstone of the machinery that controls gene expression in our cells. Despite its crucial role, very little was known about its architecture. TFIID is present at very low levels in cells, and is a very large protein complex of 20 subunits. This combination largely prevented previous attempts to decipher its structure and function in molecular detail. Even the most advanced methods for recombinant protein production met their limits when trying to produce its various subunits in the right proportions.

The solution to this bottleneck came from studying the strategy certain viruses, such as Coronaviruses, use when they replicate: they produce very long protein chains that are then divided into individual proteins. Mimicking this technique led to highly abundant and correctly assembled complexes of the core scaffold of TFIID (comprising 10 subunits), which could be purified and analysed at high resolution by combining electron microscopy and data from X-ray crystallography. The MultiBac technology used in this study is one of the central PSB platforms and can be accessed by all PSB members.

This ground-breaking analysis reveals the inner workings of the core-complex of human TFIID in unprecedented detail. It shows that some of its subunits adopt a defined structure, whereas other parts appear to adopt intricate, extended geometries winding like worms through the complex, holding it together. The overall architecture of the complex is symmetric; however, the authors describe how it becomes asymmetric once it binds to other subunits to finally form the complete TFIID complex. This work is the result of many years of intense effort and opens the way for further studies on the architecture of the entire human TFIID complex, and other large multi-protein assemblies involved in gene regulation.

C. Bieniossek and I. Berger (EMBL)

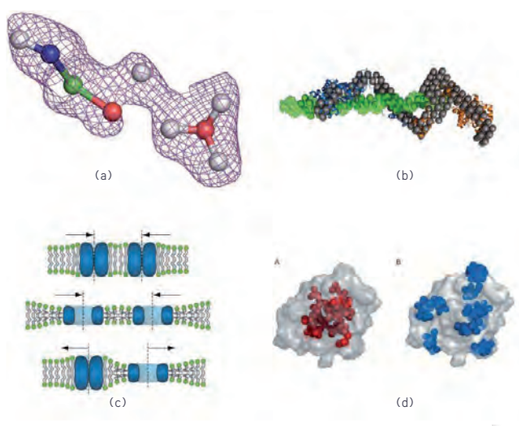
C. Bieniossek et al., Nature. (2013), Jan 31; 493 (7434) p.699-702

Platform	Location	Contact person
Analytical Ultra Centrifugation & PAOL	IBS	Aline Le Roy / Christine Ebel
Biophysics Platform	UVHCI	Marc Jamin
Cell free expression	IBS	Lionel Imbert / Jérôme Boisbouvier
Cell imaging	IBS	Rose-Laure Indorato / Françoise Lacroix / Jean-Philippe Kleman
Cryobench	IBS/ESRF	Antoine Royant
Deuteration lab	ILL	Martine Moulin / Michael Härtlein
Electron microscopy	UVHCI/IBS	Guy Schoehn
ESPRIT	EMBL	Philippe Mas / Darren Hart
Eukaryotic expression facility	EMBL	Fred Garzoni / Imre Berger
FIP-BM30 beamline	IBS/ESRF	Jean-Luc Ferrer
H/D exchange MS	IBS	Eric Forest
High-field NMR	IBS	Adrien Favier / Bernhard Brutscher
HT crystallization	EMBL	Josan Marquez
HT Membrane Protein Crystallization	IBS	Ekaterina Round / Valentin Gordeliy
Isotope labelling	IBS	Isabel Ayala / Jérôme Boisbouvier
Mass Spectrometry	IBS	Luca Signor / Elisabetta Boeri
MX beamlines	ESRF	Sean McSweeney
Neutron Diffraction beamlines	ILL	Matthew Blakeley / Susana Teixeira / Trevor Forsyth
NMR Quality Control	IBS	Adrien Favier / Eric Condamine
Protein Sequencing & Amino acid analysis	IBS	Jean-Pierre Andrieu
Robiomol	IBS	Marjolaine Noirclerc / Thierry Vernet
SANS/SAXS	ILL/ESRF	Anne Martel / Petra Pernot / Adam Round
Surface Plasmon Resonance	IBS	Isabelle Bally / Nicole Thielens

Deuteration Laboratory (D-Lab)

The Deuteration Laboratory is based in ILL's Life Sciences group. It was established with strong support from the UK EPSRC (Engineering and Physical Sciences Research Council) on behalf of the ILL and its user communities. The purpose of the platform is to enhance the quality and scope of neutron scattering studies in the Life Sciences through the provision of suitably deuterated biological macromolecules. The initiative has had very clear impact on biological science carried out using neutron instrumentation at the ILL and throughout Europe.

For neutron crystallographic work, it allows the production of samples for which the impact of hydrogen incoherent scattering is effectively eliminated and from which reliable information on protonation states, hydroxyl groups, water molecules, and hydronium ions can be obtained; such information is of direct relevance for an understanding of protein structure/function, ligand interactions, protein-nucleic acid interactions, and protein-protein interactions. In small-angle solution scattering studies, deuteration of specific parts of macromolecular structures is very effective in the study of multi-component systems – particularly for the many that do not crystallise. In addition macromolecular labelling is extremely powerful in neutron reflection experiments where there may be interest in modelling specific parts of a membrane system.



Applications of macromolecular deuteration: (a) neutron crystallography - perdeuterated protein structure showing protonation states and hydronium ions [1]; (b) SANS/contrast variation - work carried out on deuterium-labelled mariner transposase – allowing protein and nucleic acid components to be clearly distinguished [2]; (c) neutron reflection - the mechanosensitive protein MscL, involved in osmoregulation [3]; (d) dynamics - amino acid specific deuteration applied to distinguish between external and internal dynamics of colbindin [4].

Studies of protein dynamics also make extensive use of deuteration. Access to the platform is rapid and involves an initial feasibility assessment followed by electronic peer review.

M. Haertlein (ILL) and T. Forsyth (ILL/Keele University)
http://www.ill.eu/deuteration

[1] M.G. Cuypers *et al*, *Angew. Chem.* 52, 1022-1025 (2013)
[2] M.G. Cuypers *et al*, Nuc. Acids Res. 41 (3), 2020-2033 (2013)
[3] S. Grage *et al*, Biophys J. 100(5),1252-60 (2011)
[4] K. Wood *et al*, *Angew.Chem.Int. Ed.* 52, 665-66 (2013)

Hydrogen/deuterium exchange mass spectrometry fully automated at the IBS

Hydrogen/deuterium exchange mass spectrometry (HDX MS) is being used to study the dynamics and interactions of proteins. It enables a rather fine identification (515 amino acids) of regions of (soluble or membrane) proteins involved in conformational changes or interactions with partners.

CTC autosampler in the back of the Agilent ESI-TOF mass spectrometer

The technique, developed and used at the IBS for many years, has been fully automated recently, which makes it now available as a platform open to public research groups or industrial laboratories for short or medium term studies. The approach involves incubation in a deuterated buffer of the protein in different forms or in presence and absence of its partner, to exchange some amide hydrogens with deuterium. This labile labeling with D atoms is trapped by decreasing pH and temperature and the labeled protein is very quickly proteolysed. The mass of each labeled peptide is then measured using electrospray MS.

When studying conformational changes, if a specific peptide from the protein under different forms shows different masses, it indicates differences of deuterium incorporation and thus local conformational changes.

When working on interface identification (e.g. epitope mapping) a mass decrease for a peptide from the protein between the free and the complexed form indicates that the region of this peptide is implicated in the interface with the partner.

A study requires some hundreds of µg of pure homogeneous protein at a minimum concentration of 5 µM and lasts from some weeks to some months. Membrane proteins can be studied if they are soluble in DDM. Other nonionic detergents (Triton X100, polyoxyethylenebased, PEG, PPG) can be also envisionned.

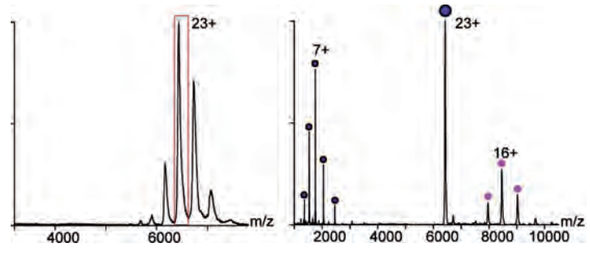
After discussion with Eric Forest (eric.forest@ibs.fr), PSB members may access to this platform while answering to a call for proposals (ANR, EU, etc) and/or with a specific person implied in the study.

E. Forest (IBS)

Mehmood, S., Domene, C., Forest, E. & Jault, J.M. (2012). Proc. Natl. Acad. Sci. USA 109, 1083210836.
www.ibs.fr/platforms/otherfacilitiesdevelopments/hydrogendeuteriumexchangemass/?lang=e

Native mass spectrometry: a new tool to study intact protein complexes

In December 2012 a custom-modified instrument was installed at the IBS to perform native mass spectrometry (MS) experiments. This is an electrospray triple quadrupole-time-of-flight mass spectrometer (ESI-Q-TOF Ultima, Waters) and able to transmit non-covalently bound protein complexes with masses up to 2.5 MDa. It allows one to determine the mass of intact protein complexes, their precise stoichiometry (an aspect of a protein assembly inaccessible by other techniques), the interactions between subunits, the position of subunits within the complex (core and peripheral subunits) and the strength of the interactions [1-3]. By doing tandem MS experiments, protein complexes are dissociated under controlled conditions to confirm their stoichiometry (see figure) and the position of subunits within the assembly. To do that, the instrument was custom-modified by the Dutch company MS Vision. After a successful installation, the ESI-Q-TOF is fully operational and its performance in terms of sensitivity and resolution are excellent. Among the few French laborato-



To confirm the stoichiometry of an assembly, tandem MS experiments are carried out. This means that a specific ion population (with a charge state 23+, in this case) is accelerated into a collision cell filled with an inert gas (e.g. Argon). By collision, the ion translational energy is converted into internal energy, generating dissociation products which are analysed by the TOF. In the depicted case a 12mer is dissociated into 1mers and 11mers.

ries with such instrument, the IBS mass spectrometer is an outstanding equipment because MS Vision has improved the instrumental performance over the years.

Regarding the advantages of native MS to study inter-subunit interactions, by this technique -unlike other approaches- one directly monitors the interactions between subunits, without introducing tags. Native MS has “unlimited” mass range (from 20 Da to 2.5 MDa), high sensitivity (i.e. 20-30 ul of sample with a concentration 10-30 uM are normally needed), high accuracy and selectivity (i.e. several species with different masses can be simultaneously analysed). The main limit of native MS is its intolerance to salts. Protein samples have to undergo buffer exchange into ammonium acetate prior to native MS analysis.

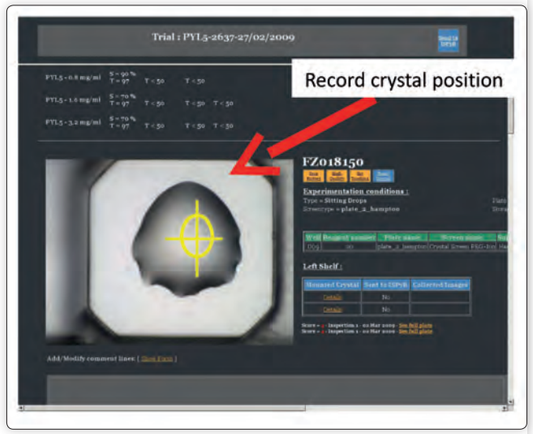
E. Boeri Erba and L. Signor
http://www.ibs.fr/platforms/protein-characterization/mass-spectrometry/
elisabetta.boeri-erba@ibs.fr

[1] Levy ED, Boeri Erba E, *et al.* Assembly reflects evolution of protein complexes. Nature. 2008;453(7199):1262-5
[2] Boeri Erba E, *et al.* Quantifying protein-protein interactions within noncovalent complexes using electrospray ionization mass spectrometry. Anal Chem. 2011;83(24):9251-9
[3] Sharon M, *et al.* Symmetrical modularity of the COP9 signalosome complex suggests its multifunctionality. Structure. 2009;17(1):31-40

Towards the integration of crystallization and x-ray data collection

Lots of news at the HTX lab since the last edition of the PSB newsletter. First, new staff: Sonia Rodriguez, Vincent Mariaule and Guillaume Hoffmann have joined and are currently running the HTX screening service. As always, they will be happy to help you with any question you may have. We have also incorporated a new microfluidic crystallization system and a new robot for the preparation of crystal optimization grids. These two systems can help you to optimize the quality of your crystals after an initial screening and are available to all the members of the PSB with training being provided by the HTX lab staff. Details are available through our web pages https://embl.fr/htxlab.

In addition to this you may have noticed that some of your experiments are now set on a new type of crystallization plate. This is part of a project run in collaboration between the HTX lab and the EMBL instrumentation team whose aim is to fill the gap between automated crystallization and automated data collection by developing a new method for automated crystal harvesting that we named CrystalDirect [1]. In the CrystalDirect plates crystals grow on a very thin film that is already compatible with X-ray data collection, rather than on the traditional hard plastic support. Hence, instead of “fishing” the crystals out of the plate, an often delicate and manpower-intensive operation, it suffices to excise the film piece on which the crystal sits and attach it to a pin for data collection. This simplifies enormously the process of crystal harvesting enabling its full automation. Indeed an automatic crystal harvesting robot prototype is currently in operation at the facility. We believe this new technology may contribute significantly to the advancement of difficulty projects in structural biology, which often require the analysis of large numbers of crystals. However, it will also benefit local and remote users of the HTX facility, by allowing to decrease the delay between crystal identification and measurement with X-rays. Interfaces to implement remote operation of the crystal harvesting system are currently being developed in the Crystallization Information Management System (CRIMS) and industrial production of the CrystalDirect plates is under way.



Crystallization Information Management System (CRIMS) crystal pointing tool for integrated in-plate x-ray measurements.

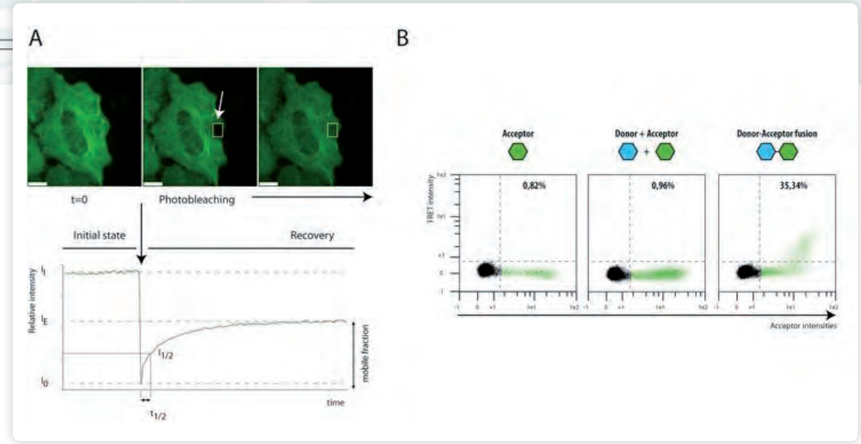
One additional advantage of the CrystalDirect plates is that they are very well suited for inplate X-ray diffraction experiments, since contrary to standard plates, their very thin films produce extremely low background. A plate goniometer able to expose CrystalDirect plates to X-rays has been developed at the BM14 beam line and is now being used for the rapid evaluation of crystal hits identified at the HTX lab. For this purpose a crystal pointing tool has been developed in CRIMS (see picture). This tool allows users of the HTX facility to identify and record the position of crystals that will be later measured at BM14. These developments will not only help scientists at the PSB, but also many other European researchers that access to the services offered by the facility through the E.C. funded Biostruct-X program. If you are interested please do not hesitate to contact us at htx@embl.fr or check our web pages

J. A. Marquez (EMBL)
[1] F. Cipriani, *et al. Acta Cryst.* (2012). **D68**, 1393-1399.
http://www.biostruct-x.eu/

Cell-imaging

The Cell-Imaging platform aims at providing PSB users with state of the art cell biology tools to visualize, measure, and analyze a broad range of fluorescent proteins or probes in fixed and living cells.

Fluorescently tagged proteins are of major interest in studying protein localization, dynamic behavior and physiological interactions in living cells. We recently installed new instruments to complement our live-imaging epifluorescence microscope that is already available to users since 2008.



A, FRAP analysis of GFP-tubulin dynamics expressed in Hela cells. The mobile fraction represents the percentage of GFP-tubulin that actually relocalizes in the living cell and accounts for the physiological dynamics of cytoskeletal tubulin. B. FRET signal measured in transfected HEK293 cells expressing controls (acceptor only or donor + acceptor) or donor-acceptor fusion proteins. Each dot represents a single cell (up to 30000 cells/sec can be analysed). Dot plots of the FRET signal correlation with acceptor fluorescence clearly highlight the FRET positive cells (measured percentage of FRET positive versus Acceptor positive cells [green dots] is shown).

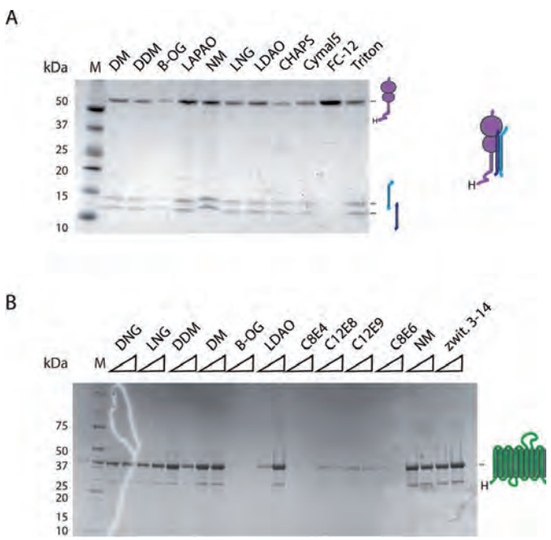
Our new spinning disk confocal microscope (Andor and Olympus) is fully optimized to analyze fluorescent proteins dynamics with reduced phototoxicity [1]. It is equipped with a 6 lasers acousto-optic tunable excitation filter covering a broad range of excitation wavelengths from violet (405 nm) to far red (640 nm), compatible with most fluorescent proteins or probes. A photoactivation/photobleaching device coupled with 2 EMCCD for simultaneous acquisition permit among other applications, real-time interaction analysis using Fluorescence Resonance Energy Transfer (FRET) after acceptor photobleaching, or dynamic relocalization studies (FRAP) in living cells (see figure, panel A). Furthermore, the system provides temperature and CO_2 controls for long-term experiments (48h, 72h or more).

We also installed a new automated flow cytometer (Miltenyi). Flow cytometry is a powerful technique to analyze entire cell populations, and obtain a statistical snapshot of labeling correlations within thousands of cells in a few minutes. Non-labeled cells are also distinguishable by the scattering intensities that define cell size and complexity. Cytometry is broadly used, for instance, for cell-cycle analysis (cell-cycle progression or checkpoint controls), or to assay cell-death and apoptosis states.
It is also suitable for the analysis of prokaryotic cells. The cytometer is equipped with 3 solid-state lasers coupled with 10 photomultipliers acquisition channels to cover the entire range of the fluorescent proteins and probes. Moreover, it is compatible with FRET measurements between a broad range of donor and acceptor probes as illustrated (panel B). The instruments will be located in a L2 environment within the new IBS building.

JP. Kleman (IBS), F. Lacroix (IBS) and RL. Revel-Goyet (IBS)
http://www.ibs.fr/plates-formes/autres-instruments-et/imagerie-cellulaire/ icroscopy,” *Adv Biochem Eng Biot,* 95, 57–75.
[1] Graf, R., Rietdorf, J. and Zimmerman, T. Advances in Biochemical Engineering/Biotechnology 95: 57-75 (2005).

News from the RoBioMol Platform

The RoBioMol platform [1], hosted by the Pneumococcus Group of the IBS, offers high throughput molecular biology processes. RoBioMol has recently set up a new service dealing with **membrane proteins** or **membrane protein complexes** (MP/MPC). Once a given recombinant MP/MPC has been expressed in the membranes of *E. coli* (or alternative expression system) the challenge is to solubilize the MP/MPC using detergents while maintaining its functionality and integrity. Optimization of the solubilization conditions requires the **screening of different detergents**. These time-consuming steps are now being performed on a 96 well plate format using one of the RoBioMol automates. This small-scale process compares the efficiency of detergents for the solubilization of the membranes containing the tagged MP/MPC and concomitant purification by affinity chromatography column. The solubilized and purified MP/MPC are analyzed by SDS-PAGE allowing the simultaneous assessment of the efficiency of MP/MPC solubilization, its ability to be purified and, in the case of complexes, the maintenance of protein interactions (see fig. A).



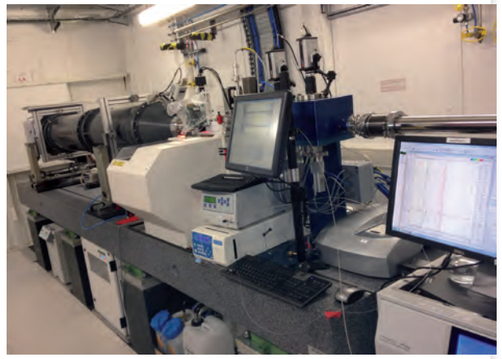
Exemple of detergent screening for the solubilization and purification of a ternary membrane protein complex (A) or for detergent exchange of a membrane protein (B). A. Purified membranes from E. coli cells over-expressing three bitopic membrane proteins were solubilized with different detergents. Only one protein is His-tagged, After Ni-NTA affinity chromatography, the elution fractions were analyzed by Coomassie-stained SDS-PAGE. **B.** A His-tagged multitopic membrane protein solubilized by a detergent was bound to an affinity resin in a multi-well plate. Each well was put into contact with buffers containing a different detergent. The eluted fractions were visualized by Coomassie-stained SDS-PAGE.

As a given detergent efficient for solubilization is not necessarily compatible wit functional or structural studies, this automated process can also be used to test **detergent exchange** during the purification step. (see fig. B). SDS-PAGE analysis performed by the platform and, when appropriate, functional and structural analyses perform by the user, allows for the identification of the most appropriate detergent. The RoBioMol platform is providing a set of standard detergents and buffer conditions to which the platform users are invited to complement with detergents and buffer conditions of their choice. A comprehensive detailed report is delivered for each project. In summary, the RoBioMol platform efficiently circumvents the time-consuming and cumbersome manual steps of screening detergents for the preparation of native recombinant MP/MPC enabling functional and structural studies.

M. Noirclerc-Savoye, V. Lantez, A.-M. Villard and T. Vernet
[1] http://www.ibs.fr/platforms/gene-cloning/robiomol/

BioSAXS Online SEC, improving sample quality

The ESRF BioSAXS beamline (BM29) sample throughput is higher than ever, using the automatic sample changer. However, not every project can be solved by measuring more conditions. Many samples have a tendency to form mixtures of oligomeric states, some are functional and others random aggregations. Many users routinely purify their samples before data collection to maximize sample and data quality. Unfortunately if the oligomerisation process is fast this step is ineffective. In order to measure samples which are dynamic the purification must be directly prior to the SAXS measurement i.e. online. It is to this end that a Malvern GPCMax was installed on BM29 (see figure).



Malvern GPC-MAX (incorporating UV, refractive index RALS and LALS detection modules) in place on BM29 next to the BioSXAS sample changer.

Although the Malvern system is still in its commissioning friendly users are already collecting data using it. The hardware integration stage is now complete with the addition of a valve to facilitate automated switching between static and SEC acquisition and also to protect the exposure unit in case of capillary leaks, (divert the liquid flow and avoid flooding) making it safe for users to work independently. Combining the results from the Malvern biophysical characterization in the analysis and interpretation of the SAXS data is now our major focus.

We hope the additional information gathered will soon be integrated into the processing pipeline (EDNA framework) with all results being logged in the BioSAXS extension to the ISPyB database.

A. Round (EMBL)
P. Pernot (ESRF)

The SPR platform (Biacore technology)

The Biacore technology uses the optical phenomenon of surface plasmon resonance (SPR) for real-time detection and monitoring of biomolecular binding events without labelling of the interactants. The SPR platform provides a novel apparatus, the Biacore T200, financed by the FRISBI project. This system, launched by the GE Healthcare company in 2010, has technical characteristics that endow it with high performance and versatility, allowing:

- high quality kinetics over a broad range, from fastest rate constants (3×10^9 $M^{-1}s^{-1}$ for proteins) to slowest dissociation rate constants (1 s^{-1})
- simple kinetic analysis using single-cycle kinetics, eliminating the need for surface regeneration between injections of different sample concentrations
- detection of binding of very low molecular weight compounds (< 100 Da), because of its high sensitivity
- deriving transition state thermodynamics from the kinetic rate constants measured at several temperatures (between 4 and 45 °C).

The Biacore T200 is a versatile apparatus, allowing use of up to 4 different running buffers within a single experiment to investigate the influence (pH, salts, etc.) on the interaction. An integrated buffer degasser prevents the formation of air bubbles at elevated temperatures. This apparatus supports the use of microtitration plates (96 and 384 wells) and vials.
The booking procedure of the Biacore T200 is identical to that of the other two instruments of the SPR platform (Biacore 3000), as indicated on the platform Website. The three machines are presently located in the IBS building (room 6336).

N. Thielens (IBS)
http://www.ibs.fr/plates-formes/purification-et-caracterisation-de/spr-technologie-biacore/

Events

Celebrating ten years of the PSB From Structural Genomics to Integrated Structural Biology 2002 – 2012

© Serge Claisse

Past and present members of the PSB gathered on Tuesday June 4th along with invited dignitaries, industry representatives and senior representatives of all the partner institutes for an eventful day that included seminars from eminent scientists, poster sessions from PSB post-docs and students, a musical performance by PSB scientists and a barbecue party.

Eva Pebay-Peyroula, IBS director, gave the opening presentation and retraced the early days of the PSB and the important dates leading to the signing in summer 2002 of the Memorandum of Understanding of the PSB by EMBL, ESRF, ILL and by the supporting research organisations (CEA-CNRS-UJF) of the IBS. The Carl-Ivar Brändén building that hosts groups from all PSB member institutes was inaugurated in 2006, and in 2007 the EMBL and the IVMS merged to form the UVHCI in 2009 that is now a member of the PSB. In October 2013, the PSB will be further consolidated when the IBS moves into its new premises on the EPN campus. At present, the PSB represents over 300 active scientists including 60 post-docs and 70 PhD students. The PSB is a unique centre in Europe, offering access to numerous technical platforms and large instruments for the local, national and European scientific community. The PSB provides an ideal scientific environment for the development of Integrated Structural Biology projects.

The day then proceeded with talks from two members of the PSB Scientific Advisory Board (SAB), Anthony Watts, current SAB chairman, and David Stuart, a long-standing SAB member, both from Oxford. They illustrated the role played by the PSB as a model for other Integrated Structural Biology centres, inspiring scientists throughout Europe and the world.

After lunch, Patrick Cramer, director of the Gene Center in Munich, who earned his PhD at the EMBL Grenoble outstation, summarized his extensive structural studies of RNA polymerases that enabled him to create an extraordinary movie of RNA polymerase II as it transcribes messenger RNA (Cheung & Cramer, Cell 2012). This movie is available online (http://www.cramer.genzentrum.lmu.de/assets/Lab-Cramer/Lab-Cramer-Publications/txnmovie.mov) and represents a wonderful tool for teaching. He then pursued his talk by illustrating the importance of using multidisciplinary approaches to study a given scientific problem and how powerfully structural work can be combined with functional studies and even genome wide computational and mathematical analyses.

The following talk was given by EMBL director general, Iain Mattaj. He reiterated the importance of having scientific centres, such as the PSB, that by grouping together different techniques in one place increase the efficiency of their researchers and allow them to tackle more challenging projects. He illustrated the power of new imaging approaches

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like super-resolution microscopies on his favourite subject, the nuclear pore complex. He also indicated that the next challenge would be the successful integration across institutions and/or synchrotrons in order to develop new tools and methodology to find solutions to long-standing problems.

The closing talk was given by Stephen Cusack, head of the EMBL Grenoble outstation, who discussed how the context of research, particularly in the field of Structural Biology, has changed over the past ten years. The PSB was conceived in the Structural genomics euphoria of the end of the 20th century, when there were many new technologies (particularly in automation) being developed and when there was strong European financial support. In the case of the PSB, this funding actually allowed scientists to tackle the 'high-hanging fruit', in contrast to the 'low-hanging fruit' targeted by the large-scale structural genomics projects worldwide. From the start, the PSB has had a very open organisation with a very light administration and S. Cusack emphasized the importance of maintaining such an organisation in the years to come, since these features have greatly contributed to the PSB's success. His talk ended with a discussion of the future prospects of the PSB and the challenges to face in the years to come. The talk concluded with a vision of Grenoble and the PSB becoming a leading centre for Structural Cell Biology in Europe.

Before the cakes and drinks, a final unexpected guest, joined us live from the US to send his best wishes: Barack Obama!

After this exciting day of seminars, the celebration continued all evening with cakes, Champagne, live music and a wonderful barbecue party!

J. Timmins (IBS)

The PSB Student Day 2013


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The fifth edition of the PSB student day was held on the 28th of January 2013, at the ILL Chadwick Amphitheatre and was a huge success with a large audience, ranging from students to senior scientists.
Malene Ringkjøbing Jensen (IBS) opened the event sharing her experiences from her graduation until her current position and gave the audience some wise advice to optimize their career.

Throughout the day, Emilie Poudevigne (UVHCI), Gaëlle Batot (ESRF), Hakan Niyazi (ILL), Ottilie Von Loeffelholz (EMBL), and Rémi Terrasse (IBS) gave talks discussing their most recent research achievements. Nineteen first year PhD students also introduced themselves and their thesis projects, often with humor, during a clip session.

The lunch and coffee breaks around posters (almost 30!) presented by the second and third year PhD students were a great opportunity to discuss with everyone in a friendly and stimulating atmosphere.

The closing seminar was given by a former PSB PhD student, Nicolas Martinelli, who now works in Patent counsel at the Nony Cabinet. At the closing ceremony Sriharsha Puranik (ESRF) was awarded the Best Clip prize and Francesca Coscia (IBS) was rewarded for the Best Poster. Congratulations to all the participants and we look forward to seeing you next year!

A. MONOD (UVHCI), on behalf of the PSB student committee

Pymol tutorial

The presentation of scientific results is an important skill for all researchers to acquire and most structural biology presentations require informative figures and movies to highlight specific details. PyMOL is a molecular graphics software suite freely available to academics, easy to use and has become the tool of choice for the generation of such figures and movies.

Since the tragic death of Warren Delano, the author of PyMOL, Schrödinger has taken over its development and maintenance. On the 22nd of February we took the opportunity to invite Thomas Holder (PyMOL developer) and Jason Vertrees (Principal Scientist) from Schrödinger for a workshop on PyMOL. First, there was a general presentation in the morning focusing on novel features and this was accompanied by two tutorials, a basic and advanced, in the afternoon. A total of 60 people from various PSB groups took part and were provided with users manuals. The course was much appreciated and we hope to organize another one in the future.

D. Panne and M. Nanao (EMBL)

Course on Small angle neutron and X-ray scattering from proteins in solution


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The biannual EMBO Practical Course on Small Angle Scattering (SAS) from proteins in solution took place on the EPN campus between the 6th and the 10th of May 2013. It welcomed an international range of 20 full and 5 partial participants from academic as well as industrial research institutes. The course included lectures, tutorials and practicals at ILL and ESRF beamlines, aimed to help the participants make the most out of future neutron and X-ray SAS experiments. This event was organized by F. Gabel (IBS and ILL), P. Pernot (ESRF), A. Round (EMBL), M. Jamin (UVHCI), S. McSweeney (ESRF) 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/13-SAXS/

EMBO Practical Course on Exploiting Anomalous Scattering in MX Structure determination

© Chantal Argoud

The 9th biennial EMBO course on anomalous scattering took place at the ESRF from the 10th to 14th of June 2013. Over 20 participants and 16 lectures from 18 countries took part, with several of the participants coming to the ESRF for the first time. The course consisted of introductory lectures on the principles of anomalous diffraction and expert tutorials on the latest methods and software developments used in data collection, data processing and experimental phasing. The lectures were open to all PSB members and we were pleased to see so many local people attending. The last two days were dedicated to beamline and software practicals, allowing the students to use the knowledge gained for their own projects. The course was organized by the EMBL and ESRF and was enjoyed by all. We would like to thank all the tutors for their time and especially C. Romero (ESRF) for organizational help. We also acknowledge financial support from EMBO, DECTRIS and ARINAX and look forward to a 10th edition in 2015.

D. de Sanctis (ESRF) and A. McCarthy (EMBL)

http://www.esrf.fr/events/conferences/EMBO2013.

ANNOUNCEMENTS

An Integrated Structural Cell Biology summer school, entitled **'From molecules to cells and organisms: Thinking out of the box'** will be organised in Les Houches in July 2014. The format will consist of ~40 long lectures and ~10 short lectures and tutorials. The school is open to graduate students, post-docs and junior academics. The course is organised by E. Pebay-Peyroula (IBS, Grenoble), Rob Ruigrok (UVHCI), François Parcy (CEA), Hugues Nury (IBS).

More details :
http://houches.ujf-grenoble.fr/

The Synchrotron X-ray Imaging for Biology course has the aim to provide an overview of the relevant X-ray imaging techniques available at a synchrotron for multiscale investigations in the biological field, through a combination of technical information, scientific presentations, tutorials, and practicals on beamlines. The course is organised by José BARUCHEL (ESRF), Sylvain BOHIC (Inserm U836).

More details:
http://www.esrf.fr/events/conferences/HSC/HSC15/

Newcomers

David Heß has been appointed as an Engineer at the Partnership in Soft Condensed Matter to work as responsible for the Chemistry and Soft Matter Laboratories. David obtained his PhD at the University of Munich before joining the ILL. Users can find / contact here: ILL20-216 office or hessd@ill.fr

Juliette Devos has been appointed as an Engineer to work alongside Martine Moulin and share responsibility in supporting the User Programme of the Deuteration Laboratory platform at the PSB. Juliette was a scientist at Haplogen, in Vienna, before joining the ILL.

Florent Bernaudat has just been appointed as the new PSB coordinator. He will take up his new duties beginning of July 2013.

Bauke Dijkstra is the ESRF's new director of research for chemistry and life sciences. After 40 years of working in structural biology and a full professor position at University of Groningen, Netherlands, he joined ESRF in September 2012. Dijkstra's research interests are in the field of enzyme catalytic mechanisms, protein engineering and biotechnology.

Audrey Spielmann has been appointed as the new EMBL Laboratory support officer in replacement of Annie Simon who will be retiring soon.

Giuseppe Zaccai will receive the 8th Walter Hälg Prize at a special ceremony during the International Conference on Neutron Scattering this July, for the long-term impact of his programme of research in neutron scattering science and techniques. The prize is awarded every 2 years since 1999, when the European Neutron Scattering Association decided to name it after the pioneer of reactor technology and neutron scattering in Switzerland. It was made possible thanks to a donation from the late Prof. Walter Hälg and is now sponsored by his wife. This year G. Zaccai joins a prestigious list of scientists: F. Mezei, J. Brown, R. Cowley, A.Furrer, H. Güdel, J. Penfold, D. Richter and G. Lander. Congratulations Joe!

S. Teixeira (ILL/Keele University)

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.

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