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 [FeS₄] cluster provides electron transfer capabilities with redox partner proteins such as ferritin, rubredoxin oxidoreductase, ruberythrin or superoxide reductase. 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.

Both issues are central to the recent work on rubredoxin by Cuypers *et al* 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. The results show the presence of numerous hydronium (H₃O⁺) ions within the protein, and illustrate protonation shifts following the change between the Fe²⁺ and Fe³⁺ 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-

The overall oxidised Fe-form structure of *Pf* Rubredoxin showing the corresponding hydronium ions and protonated carboxylic acids in the structure (enlargements).
Recent results have revealed the importance of a particular protein, called RecN (Recombinase N), in bridging together double strand breaks (DSBs), which represent the most lethal type of DNA damage. The repair of DSBs involves many proteins and the aim of our research over the past few years has been to understand the repair machinery that has been the focus of our studies.

We were able to reconstitute a quasi-atomic structure of RecN, providing us with a detailed understanding of its overall shape and function. Using X-ray 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, were able to place various crucial 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.

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 and 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 withstandings the presence of high doses of ultra-violet or ionising radiation or the absence of water [1]. Among the factors contributing to radiation resistance is the presence of high densities of hydronium ions and their interaction with the surrounding solvent may be important in the process of charge transfer. In the oxidised form, Asp13 is protonated and Asp15 and Glu47 are not protonated. 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 H2O 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 changes 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.

1 M. Cuypers ( Keele University, UK & ILL, France).
6 B. Bieniossek and I. Berger (EMBL)}
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 (5-15 amino acids) of regions of (soluble or membrane) proteins involved in conformational changes or interactions with partners.

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 proteins in different forms or in presence and absence of its partner, to exchange some hydrogen 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 hundreds of µg of pure homogenous 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, polyoxyethylene-based, PEG, PPO) can be also envisioned.

After discussion with Éric 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)


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 laboratory.
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 orientation 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://embli.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 CrystalDirect1). 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. Instead, instead of “fishing” the crystals out of the plate, an automatic and multipurpose ionotropic 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 systems are currently being developed in the Crystallization Information Management System (CRIMS) and industrial production of the CrystalDirect plates is under way.

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 film 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 BioStruc-X program. If you are interested please do not hesitate to contact us at ht@embli.fr or check our web pages.

J. A. Marquez (EMBL)


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 systems) the challenge is to solubilize the MP/MPC using detergents while maintaining its functionality and integrity. Optimisation 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 automatized. This small-scale process compares the efficiency of detergents for the solubilization of the membranes containing the tagged MP/MPC and constant 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).

J. A. Marquez (EMBL)

As a given detergent efficient for solubilization is not necessarily compatible with 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 performed 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

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 GPC/MAX was installed on BM29 (see figure).

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. Perriot (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 ESRF project. This system, launched by the GE Healthcare company in 2010, has technical characteristics that enable it with high performance and versatility, allowing:

- high quality kinetics over a broad range, from fastest rate constants (3 x 10^4 M/s 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 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 microinjection 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. Thielemans (IBS)
http://www.ibs.fr/labs-frmures/purification-et-caracterisation-de/spr-technologie-biacore/

N. Thielemans (IBS)
http://www.ibs.fr/labs-frmures/purification-et-caracterisation-de/spr-technologie-biacore/

Events

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

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 Pechay-Peyroula, IBS director, gave the opening presentation and retrae 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 Brandén 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 outpost, 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 multi-disciplinary 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
like super-resolution microscopes 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 structural genomics era started in Grenoble and the PSB becoming a leading centre for Structural Cell Biology in Europe. 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

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 Ringkøbing-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), Hakam Niyazi (ILL), Anette Von Loefelholz (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 Srinarayana 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 tutorial in the afternoon. A total of 60 people from various PSB groups took part and were provided with user manuals. The course was much appreciated and we hope to organize another one in the future.

D. Paume and M. Nanao (EMBL)

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

The biannual EMBO Practical Course on Small Angle Scattering (SAXS) from proteins in solution took place on the EPN campus between the 6th and 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 P. Gabel (IBS and ILL), P. Pernot (ESRF), A. Remond (EMBL), M. Zamani (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

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. Romers (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 sainte-Croix (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 Parry (CEA), Hugues Nury (IBS).

More details:
http://houches.sji.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 Jérome BARUCHIEL (ESRF), Sylvain IBOHIC (Inserm U936).

More details:
http://www.esrf.fr/events/conferences/IBS/IBC15/

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D. Paume and M. Nanao (EMBL)
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.

**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 Halg 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 Halg 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)