CISB

et al.

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Filming a protein at work!

Most of the research done on protein structure is based on studies in a "resting state". However, conformational changes are at the basis of functional activity, so the investigation of proteins in movement is extremely important. The "kinetic crystallography" team at the IBS has recently produced a movie of an enzyme at work.

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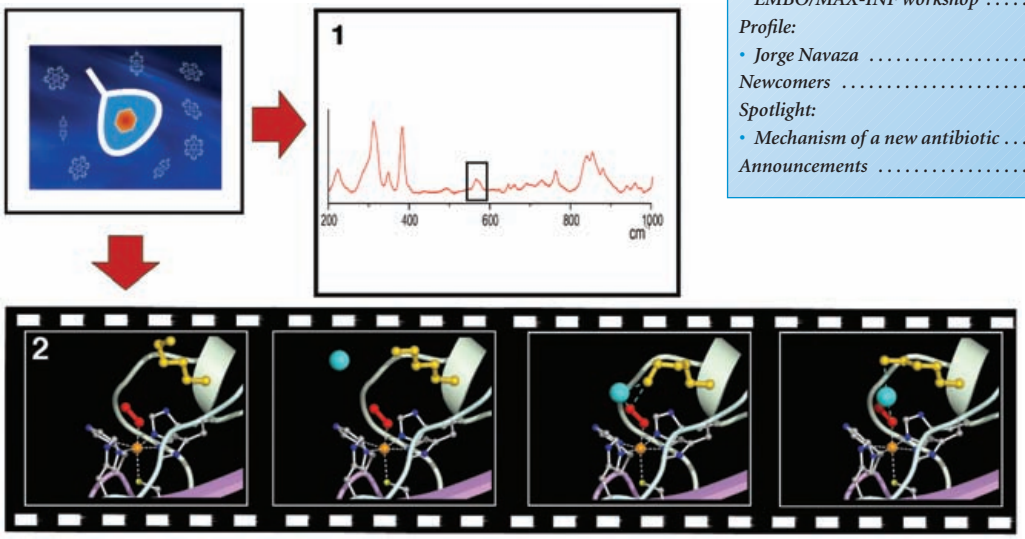

Kinetic crystallography of superoxide reductase: The Raman spectrum of the frozen soaked crystal reveals a band at 567 cm^{-1} (1, black box), consistent with the formation of "end-on" iron-peroxide bonds. Crystallographic data allowed to produce a tentative film of the reaction pathway (2). On this film, the substrate is shown in red and the iron atom in orange. The key lysine (yellow) catches a water molecule (blue) and imports it in the active site, close to the substrate. A corresponding movie is available at www.sciencemag.org/content/vol316/issue5823/images/data/449/DC1/1138885s1.mov. (Figure courtesy of G. Katona)

The "kinetic crystallography" team of the IBS, in collaboration with the ESRF and the iRTSV (Institut de Recherches en Technologies et Sciences pour le Vivant, CEA-Grenoble), has produced a movie of an enzyme executing its biological function. The achievement of this research is two-fold: first, the results on the enzyme studied, superoxide reductase (SOR), contribute to the precise understanding of how this enzyme works. Second, a new methodology combining kinetic crystallography and Raman spectroscopy has been developed to record structural snapshots of proteins in action. The combination of crystallography and "in crystallo" Raman spectroscopy is expected to be useful for many researchers pursuing structural studies with mechanistic perspectives.

Superoxide reductase is an iron containing metalloenzyme that eliminates the "superoxide radical" $O_2^{\cdot-}$, a cytotoxic by-product of oxygen metabolism. In humans, about 2% of the oxygen used to breathe is transformed into this superoxide radical, instead of water. The amount of $O_2^{\cdot-}$ produced is increased in people affected by neuro-degenerative diseases such as Alzheimer's and contributes to a worsening of these illnesses. Scientists are therefore looking for drugs to eliminate this toxic compound.

Although SOR is present uniquely in some microaerophilic bacteria and archae, it carries out a simpler reaction than its counterpart in humans, superoxide dismutase. Understanding the chemical tricks used by superoxide reductase is not only of fundamental importance in the field of iron biochemistry, but could also open up new possibilities for developing future drugs or biomimetic compounds.

A stroke of luck allowed us to produce the film of SOR in action: in a single crystal, three intermediate states were trapped at once by freezing the sample at an appropriate moment after the reaction was triggered. This resulted from

(see page 2)

the fact that, in the crystal asymmetric unit, several nominally identical active sites are exposed to slightly different packing forces, creating slight differences between them. In order to make sure that the proper intermediate states were trapped, the technique of *in crystallo* Raman spectroscopy was developed at the "Cryobench" laboratory (ESRF-IBS, CISB platform). First, the crystal was fished out under the microscope, using a small nylon loop, and immediately transferred into a solution containing the substrate, in order to trigger the reaction. After a few minutes, the sample was "trapped" by flash cooling in liquid nitrogen and analysed by *in crystallo* Raman spectroscopy. The spectra provided strong evidence that the trapped states were biologically relevant, showing features also found in the solution state. Furthermore, the spectra were not modified by a moderate exposure to synchrotron X-rays, known to potentially damage metal sites. Crystallography could thus be safely used at ESRF to determine the high resolution three dimensional structures of SOR in these different iron-peroxide intermediate states (see figure).

Several hypotheses for the mechanism of SOR activity have been inferred from biochemical studies in the past. The binding mode of the superoxide radical, the role of some key amino-acid residues, and the direct participation of a water molecule in catalysis were anticipated. Our direct visualization of these events allows a better understanding of how they fit together to actually do the job of transforming the superoxide radical into the reaction product, hydrogen peroxide. We found that a key lysine residue moves around at the surface of the enzyme to grab a water molecule from the surroundings and brings it into the enzyme active site, at a very strategic point where this water molecule may donate a proton to the substrate (see figure). Many puzzling questions remain, however, which will be addressed in future experiments. For example, when this lysine is mutated out, the enzyme still functions, although at a slower rate: thus the mechanism suggested by our study might correspond to only one of several possible reaction pathways.

Gergely Katona and Dominique Bourgeois (IBS)

Katona et al. (2007) Science *316, 449-453*

The ESRF Science and Technology Programme 2008-2017

Can you spot 10 differences between these two pictures? The ESRF before (left) and after (right) the extensions of the ESRF Experimental Hall. Four extensions will be made allowing sixteen beamlines to be extended to between 105m and 140m in length and new space for support and infrastructure.

The ESRF is planning a major Upgrade of its facilities to meet the challenges of science over the coming ten to twenty years. The ESRF Science and Technology Programme 2008-2017 is a ten-year plan to update and enhance the ESRF beamlines and infrastructure in five "highlight" areas identified by the ESRF Science Advisory Committee and the Council:

- Nanoscience and nanotechnology
- Pump and probe experiments and time-resolved science
- Science at extreme conditions
- Structural and functional biology and soft matter
- X-ray imaging

The Upgrade will push back the boundaries of synchrotron radiation-based science with new beamlines (up to 16) targeted at the requirements of forthcoming science. Major programmes are also planned to develop the necessary enabling technology, engineering and infrastructure, for example detectors, nano-science compatible apparatus (e.g. high precision diffractometers, sample location and manipulation), online data analysis, new buildings and extreme conditions of pressure, temperature and magnetic field. Much of this will be carried out in collaboration with expert partners and other synchrotrons: the requirements for the new technologies are often common amongst light sources.

Much of the ESRF Upgrade is based upon the use of nano-sized X-ray beams to probe matter at unprecedented spatial resolution using minute samples, including biological material. Longer beamlines (up to 140 m, double the current normal length) will allow nano-sized X-ray beams to be generated more effectively and with more space around the sample location, permitting equipment (detectors, cooling, sample visualisation, etc.) to be arranged around the sample. Sixteen of the ESRF's beamlines will be able to be long after the construction of extensions to the current Experimental Hall (see figure): 21,000m^2 of new space will be created (equal to an extra one-third of the current ESRF usable area). This new space will be extremely valuable, allowing new infrastructure and support facilities and space for partnerships and collaborations to grow.

The Upgrade has been in the pipeline for several years. Over the last twelve months and at the request of the ESRF Council, a detailed scientific and technical report has been in preparation (known as the "Purple Book" – www.esrf.fr/AboutUs/Upgrade/purple-book/), which was published in October of this year. It describes the new science to be made possible by the Upgrade together with a set of Conceptual Design Reports setting out the ideas for forty beamlines: more than the ESRF can handle! Some of the reports are aimed at refurbishing current beamlines, others at wholly new beamlines (for example a beamline dedicated to the imaging of biological assemblies using coherent X-ray diffraction). The user communities and the Science Advisory Committee of the ESRF will help guide the choice and priorities for the beamline projects to be carried out. This work started at the Upgrade Information and Discussion meeting which took place on 24th October, where over 450 participants discussed, debated and gave their feedback on the Science and Technology Programme.

The European Strategy Forum for Research Infrastructures (ESFRI) Roadmap identifying large-scale projects core to the European Research Area includes the ESRF Upgrade Programme, and also the 20/20 upgrade of the ESRF's sister institute,

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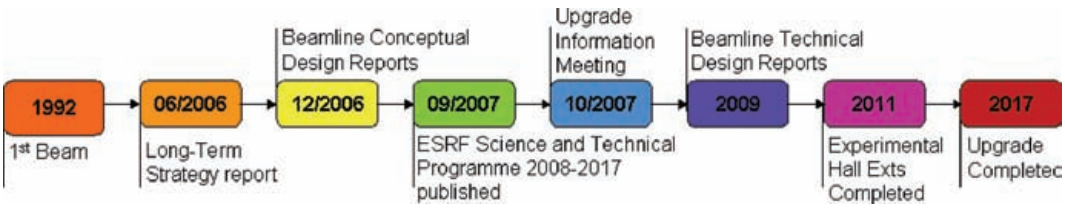
the Institut Laue-Langevin, both located on the same site in Grenoble as the EMBL Grenoble outstation. An application for specific FP7 funds for the Roadmap projects has been well received by the EC and will catalyse the ESRF Upgrade Programme into an initiated project approved by the ESRF member countries. Parts of the Upgrade can start in 2008, and it is expected to start in earnest in 2009 (see Timeline, right).

Ed Mitchell (ESRF)



Timeline for the Upgrade

The ESRF Upgrade: How will it affect structural biologists?

Synchrotron light has become essential in probing the 3D structure of macromolecules and looking at life in action. A host of synchrotron radiation-based techniques exist covering a vast range of length scales from macromolecular crystallography and extended X-ray absorption fine structure spectroscopy (atomic scale), small-angle X-ray scattering and X-ray photon correlation spectroscopy (molecular scale) to micro-X-ray fluorescence (micron scale work) and X-ray imaging over the cellular to the whole organ scale, to mention but a few. With structural biology and imaging as two of the core Upgrade areas, the use of the ESRF beamlines for biology will become stronger with, importantly, enhanced cross-disciplinarity between areas traditionally not overlapping. Synchrotron light will become an even more potent tool for biologists.

A few highlights of what the Upgrade could provide for structural biologists are outlined below:

1. A "village" of regrouped macromolecular crystallography beamlines with a dedicated sample screening facility at its core and a set of specialised beamlines to which the best crystals will be sent after screening. Of course the ideal location for these beamlines would be the ESRF sectors close to the Carl-Ivar Brändén Building housing the PSB and UVHCI!
2. A new beamline for the imaging of biological assemblies from cells to organelles to macromolecular assemblies using coherent diffraction imaging.
3. An updated small-angle X-ray scattering beamline able to detect the ultra-small-angle range to look at protein complexes and tissues, for example.
4. An X-ray imaging platform covering a multitude of techniques, including a new scanning fluorescence and imaging beamline using a nano-sized X-ray beam able to raster scan across biological materials to pick out the location of metals (for example across a cell).
5. Steady-state and time-resolved X-ray emission and absorption spectroscopy will be used by a beamline to probe, for example, the state of metals in metalloproteins. Time resolutions on the order of 100ps will be possible to follow reactions in macromolecules.
6. Nano- and microbeam crystallography for looking at the structure of biological materials, including the use of scanning SAXS/WAXS.

Training at the CISB

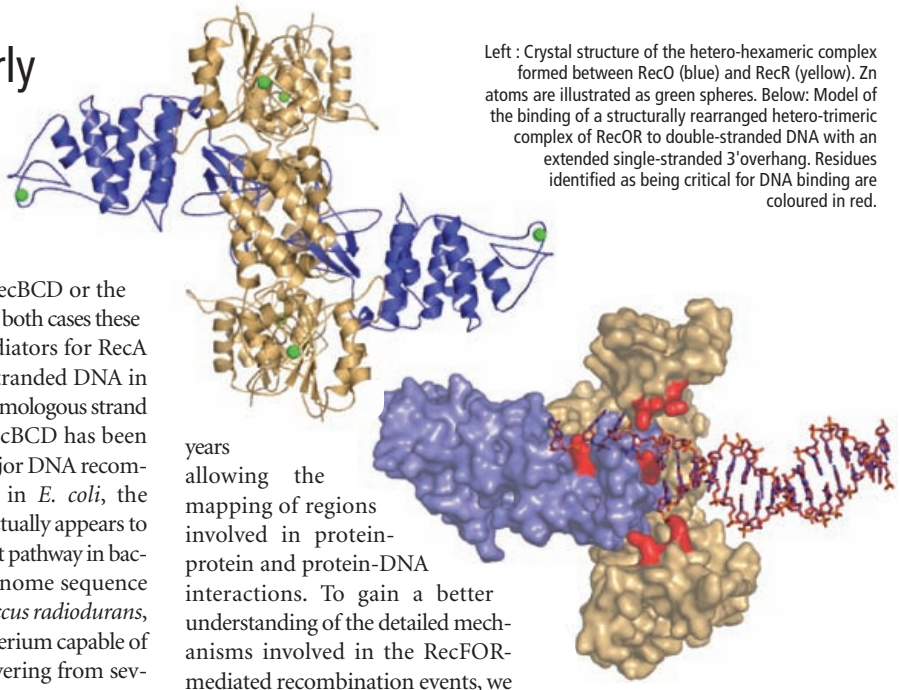
LIFE stops in Grenoble

29 students from the Life Science & Technology Programme in the Netherlands visited the PSB research facilities on June 6-8. The programme, a Bachelors and Masters degree established between the University of Leiden and the Delft University of Technology, combines the expertise of both – biological research and technological inventiveness, respectively – to educate the future generation of Dutch molecular technologists and prepare them for careers in industry and academia. Life Science & Technology has its own study association, LIFE, which promotes activities like lectures, workshops and, of course, study trips like this one. Each year LIFE's study trip gives around 30 students the opportunity to visit world-leading research facilities in a different European country and interact with their scientists. This year was the turn of France, and Grenoble was one of their destinations. Ingeborg Te Groen (ILL), Jose A. Marquez (EMBL), Rob Ruigrok (UVHCI), Klaartje Houben (IBS) and Annette da Silva (ESRF) each prepared a programme for the students, which gave them the opportunity to visit the unique installations of the PSB, some of which they might use during their professional career. They were also presented with an overview of our research activities and had the opportunity to discuss topics with the scientists.

"We soon realised Grenoble was unique, with so many large institutes on one site, offering us the opportunity to hear about many different studies. I found it interesting to see how the researchers from the different institutes work together; such cooperation is important and inspiring," said Mia Urem, President of the LIFE study trip organising committee. "Many students will have followed a number of courses that at least touched upon the techniques used in Grenoble, though some were new to us. I found it very useful to see the techniques we learned about being put into practice, to see everything one can achieve." For more information on LIFE and the Life Science & Technology Programme, visit www.leidendelft.nl.

José A. Márquez (EMBL)

Structural insights into the early steps of homologous recombination in bacteria



Left : Crystal structure of the hetero-hexameric complex formed between RecO (blue) and RecR (yellow). Zn atoms are illustrated as green spheres. Below: Model of the binding of a structurally rearranged hetero-trimeric complex of RecOR to double-stranded DNA with an extended single-stranded 3'overhang. Residues identified as being critical for DNA binding are coloured in red.

DNA damage is a common occurrence that compromises the functional integrity of our genome. Well over 10,000 DNA damages are estimated to occur daily in every human cell. The causative agents of these damages are mainly free radicals, which are normally produced as natural by-products of food metabolism. If damaged DNA is left unrepaired, it generates mutations, replication errors, persistent DNA damage and genomic instability, which ultimately is associated with cancer and aging. The mechanisms of DNA repair at a molecular level are largely unknown and a better understanding of the detailed mechanisms and principles underlying damage recognition in prokaryotes is an essential step towards obtaining a complete overview of the more complex human DNA repair systems. In prokaryotes, four repair pathways exist (homologous recombination, base-excision repair, nucleotide-excision repair and mismatch repair), all of which are essential for viability.

Homologous recombination, in addition to its fundamental role in genetic diversification of bacterial genomes, plays a key role in the repair of a variety of DNA lesions, including the lethal double-strand breaks. In *E. coli*, the initiation of homologous recombination can be carried out by either the RecBCD or the RecFOR proteins; in both cases these proteins act as mediators for RecA binding to single-stranded DNA in order to allow for homologous strand invasion. While RecBCD has been shown to be the major DNA recombination pathway in *E. coli*, the RecFOR pathway actually appears to be the more frequent pathway in bacterial genomes. Genome sequence analysis of *Deinococcus radiodurans*, an outstanding bacterium capable of repairing and recovering from several hundred DNA double-strand breaks in its genome, has revealed that the genes encoding RecB and RecC proteins are missing, while all the genes encoding members of the RecFOR pathway (RecQ, RecJ, RecO, RecF and RecR) are present. Inhibition of the RecFOR pathway in *D. radiodurans* increases the sensitivity of *D. radiodurans* cells to gamma-radiation, suggesting that RecFOR is indeed essential for repair of double-strand breaks.

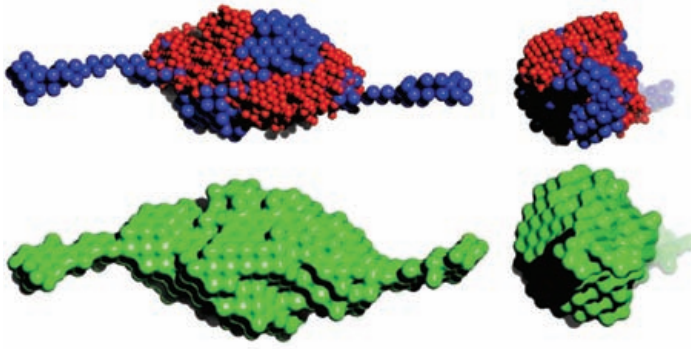
An increasing amount of structural information is becoming available for these proteins, which together with the biochemical and genetic data will no doubt improve our current understanding of the initial steps in homologous recombination. The individual crystal structures of RecF, RecO and RecR from *D. radiodurans* have been solved over the past three years allowing the mapping of regions involved in protein-protein and protein-DNA interactions. To gain a better understanding of the detailed mechanisms involved in the RecFOR-mediated recombination events, we have now determined the crystal structure of the RecOR complex from *D. radiodurans* at 3.8 Å resolution. The complex consists of a hetero-hexamer of two RecO molecules and four RecR molecules in which the RecO molecules are positioned on either side of the tetrameric ring of RecR, obstructing access to the interior of the ring. This structural data served as a framework for designing mutants to investigate the ability of RecOR to bind DNA. This study revealed that two regions of the complex, one on RecO and another on RecR are critical for DNA binding. Our mutagenesis and biochemical analyses taken together with the 3D arrangement of the RecOR hetero-hexameric complex strongly indicate that RecOR most likely undergoes both local conformational changes and a larger architectural reorganisation upon binding to sites of double-strand break repair.

This study has shed light on the importance of studying protein complexes as opposed to the individual proteins. Many of the features described for the single proteins were no longer observed within the context of the complex. Protein-protein and protein-DNA interactions are key to regulating many cellular processes. A better understanding of the processes regulating complex formation between the various Rec partners will now certainly help in establishing the detailed sequence of events leading to RecA-dependent homologous recombination.

Joanna Timmins (ESRF)

Timmins et. al. (2007), EMBO J., *26, 3260-327*

A SANS study of the DNA Methyltransferase M.AdhI



Two views of the low-resolution ab initio model for M.AdhI (M_2S_2), derived from contrast variation SANS experiments. In green, for comparison, the model obtained for the whole unlabelled complex.

DNA methylation has been reported to be essential for bacterial virulence, and it has been suggested that DNA adenine methyltransferases (Dam) could be potential targets for both vaccines and antimicrobials. Drugs that block Dam could slow down bacterial growth.

In prokaryotes, DNA methylation plays a role in a number of essential processes in the cell life. The distinction of self and non-self DNA, for example, is associated with R-M (restriction-modification) systems which function as defence mechanisms against infection of bacteria by bacteriophages. DNA Methyltransferases (Mtases) could not only be interesting drug targets as they may also be useful in the development of non-radioactive DNA probes. Being nucleotide-sequence-specific, DNA Mtases can also provide excellent model systems for studies on protein-DNA interactions.

The Mtase from *Aeromonas hydrophila* (see page 5)

(M.AdhI) recognises the self-complementary sequence $GACN_5GTC$ and methylates the DNA, thus protecting it from cleavage by the endonuclease. M.AdhI is a 170kDa tetramer formed by two S and two M subunits (responsible for Specificity and Methylation, respectively) that can be expressed separetely and reconstituted *in vitro* without loss of methylation activity. Although crystal structures were determined for related M and S subunits, the overall shape and subunit organisation of the Mtase was unknown.

The first structure of a type I Mtase was now determined by SANS using differential deuteration and contrast variation techniques. Hydrogenated M.AdhI and partially deuterated (perdeuterated S subunits) M.AdhI were expressed and purified at the Deuteration Laboratory. The hydrogenated sample allowed for the radius of gyration (R_g) and the longest dimension of the M.AdhI to be determined. For the M.AdhI with perdeuterated S subunits, neutron scattering data were collected (D22 diffractometer at the ILL) at 40% and 100% ^2H_2O, allowing for the contrast-matching of the M and S subunits respectively. The S and M subunits of M.AdhI are 25 kDa and 60 kDa, respectively, and have been well characterised both biochemically and biophysically. *Ab initio* shape determination was performed for the data at different contrasts: the shape of the M.AdhI complex was determined and the M and S subunits were assigned (see figure).

The overall shape of the enzyme in solution shows a compact structure, approximately 100Å x 60Å x 50Å, comprising the two S subunits and the core domains of the two M subunits. However, the outer regions of the M subunits extend the longest dimension of the Mtase to 190 Å. It is proposed that these extended regions of the M subunits in type I Mtases are flexible and collapse around the DNA to form a more globular structure in the MTase-DNA complex, consistent with the large conformational change deduced from SAXS for M.*Eco*R124I, another type I MTase. It would also offer an explanation for the large DNAse I footprint, indicating that ~23 bp (80 Å) of the DNA are almost completely enclosed in the DNA-protein complex.

This study illustrates well the potential of SANS and contrast variation techniques in studies of selectively labeled biological samples for which the structural assembly of subunits cannot be determined by other methods, albeit being essential for a better understanding of the different mechanisms of action of Mtases and of the molecular evolution of DNA recognition.

Susana Teixeira (ILL)

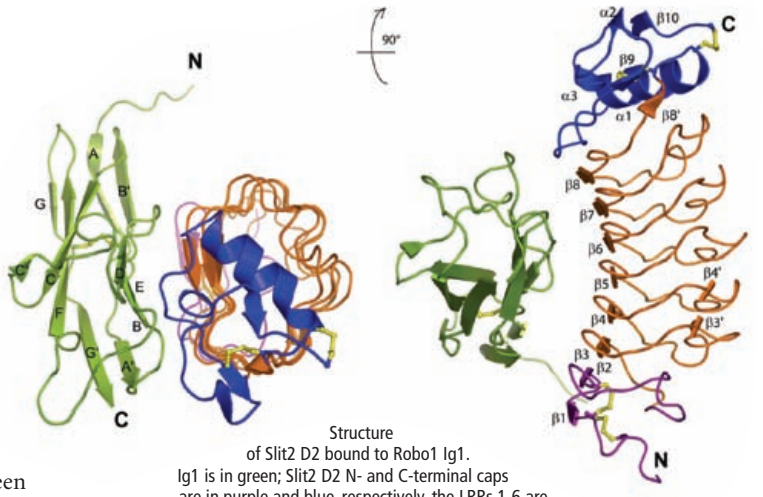
Callow et al. (2007). J. Mol. Biol. *369, 177-185*

Structural insights into the Slit-Robo complex

Bilaterally symmetric nervous systems, such as those found in vertebrates, possess a special midline structure that establishes a partition between the mirror image halves. In order to connect and coordinate both sides, a sunset of axons has to cross this midline. These are called the commissural axons and developing commissural axons navigate through the embryo by processing a number of different signals in their immediate environment. Slit and Roundabout (Robo) provide a key ligand-receptor interaction for this process during neuronal development, especially at the midline of the central nervous system of vertebrates and invertebrates. More recently, Slit and Robo have been implicated in heart morphogenesis, angiogenesis and tumour metastasis.

Slits are large, multi-domain, leucine-rich repeat (LRR) containing proteins, while the Robo (Roundabout) receptors belong to the immunoglobulin (Ig) superfamily of transmembrane signalling molecules. The Slit-Robo interaction is known to be mediated by the second LRR domain of Slit and the two N-terminal Ig domains of Robo, but the molecular details of this interaction and how it induces signalling remain unclear. In collaboration with the high throughput eukaryotic expression unit in Utrecht University we have successfully expressed, in milligram quantity, each of the four LRR domains from mammalian Slit2. Some of this technology has been transferred here, allowing us to express a construct containing the first two Ig domains from Robo1 (Robo1 Ig1-2). Using the EMBL Grenoble high throughput crystallisation facility and the macromolecular crystallography beamlines at the ESRF we were able to determine the crystal structures of the second LRR domain of human Slit2 (Slit2 D2), the first two Ig domains of its receptor Robo1 (Ig1-2) and the minimal complex between these proteins (Slit2 D2-Robo1 Ig1).

The Robo1 Ig1-2 domains together form an elongated structure, belong to the I1 set of the immunoglobulin superfamily, and are similar in structure to other neural cell adhesion molecules (NCAMs) involved in cell-cell or cell-matrix adhesion that elicit intracellular signalling. The Robo1 Ig1 domain was shown to be the primary binding site for Slit2 D2 binding using surface plasmon resonance experiments carried out on the BIAcore 3000 instrument at the IBS. Slit2 D2 binds with its concave surface to the side of Ig1 (see figure) with electrostatic and hydrophobic contact regions mediated by residues which are conserved in other family members.



Structure of Slit2 D2 bound to Robo1 Ig1. Ig1 is in green; Slit2 D2 N- and C-terminal caps are in purple and blue, respectively, the LRRs 1-6 are in orange and the disulphide bridges are in yellow.

This is the first structure of a complex between an Ig CAM domain and an LRR domain, two of the most widely found and important protein-protein interaction domains in Nature. These structures provide the first molecular insight into Slit-Robo complex formation and the unique interaction region provides an attractive target for the rational design of specific Slit-Robo signalling inhibitors. Such inhibitors would have applications in anti-angiogenic therapy or in blocking Slit-Robo mediated cancer cell metastasis.

While these new structures provide vital new information on the exact molecular interactions between Slit and Robo there are still many unanswered questions, such as how an extracellular signal is transmitted into intracellular signalling. In order to resolve some of these basic questions we will continue our structural and functional research on these important developmental proteins.

Andrew McCarthy (EMBL)

Morlot et al. (2007), Proc. Natl. Acad. Sci. USA, *104, 14923-14928*

News from the platforms

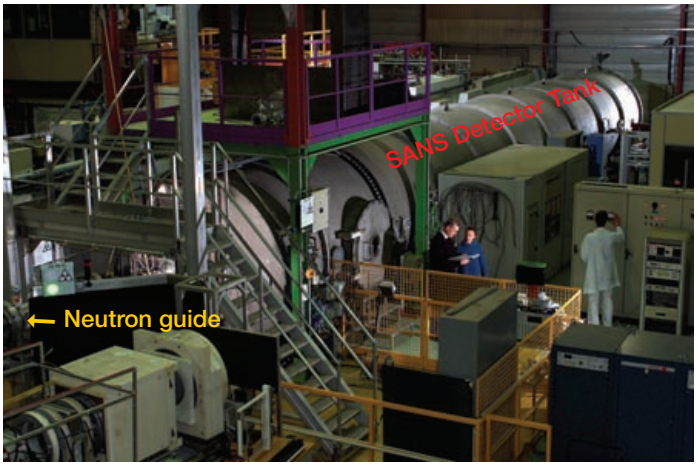
Small Angle Scattering – A New PSB Platform

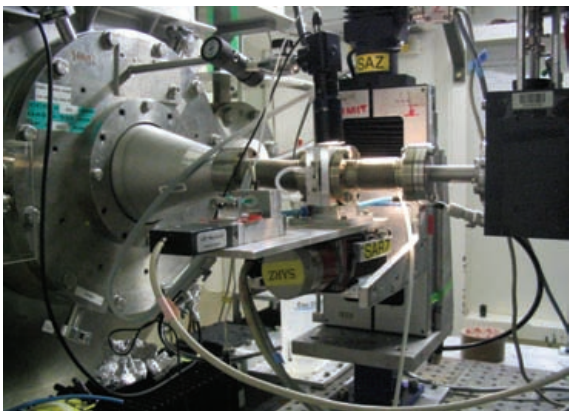
The new Small Angle Scattering platform of the Partnership for Structural Biology (PSB) is now available. The platform brings together the small angle X-ray scattering facilities (SAXS) of the ESRF and the small angle neutron scattering (SANS) facilities of the ILL. Access to the facilities is via the normal peer review procedures of the two institutes. The platform aims, in addition to the provision of instruments, to bring together the software resources and expertise necessary for efficient use of the facilities. In the medium term it is planned to enable joint proposals to the review committees allowing SANS and SAXS experiments to be carried out more or less simultaneously on the two facilities.

Currently the ILL has two instruments for SANS, D11 and D22. Both are well equipped for experiments in structural biology with automatic sample changers. D22 operates a BAG system for biological experiments that allows flexibility in scheduling as well as rapid access for test experiments. Both instruments are heavily overbooked for experiments across a wide range of science and the ILL is therefore planning the construction of a third SANS instrument (D33) to reduce this load. The ILL/EMBL Deuteration laboratory within the PSB is available to users to optimise sample preparation within the framework of the ILL User programe. X-ray small angle scattering at the ESRF is well established, but it is clear that the full potential of this technique has not yet been fully exploited in the study of biological samples. The high brilliance beamline (ID02) is among the best suited for this type of experiments, but it is also a beamline that is in high demand from the wider ESRF user community. The requests for beam time exceed the available time by a factor of 4-5. The ESRF is therefore planning the construction of a dedicated biological SAXS line at ID14-3. Equipment has already been purchased for this beamline and it is planned to receive its first users in September 2008. The EMBL will also participate in the beamline construction and operation through funding of a beamline scientist position.

Scientists from each of the PSB's member organisations have participated in the setting up of the platform and are available to share their expertise with new CISB users. For more information on SANS contact Phil Callow (callow@ill.fr) and for SAXS contact Petra Pernod (rejma@esrf.fr).

Peter Timmins (ILL)





Two Small Angle Scattering beamlines: the D22 instrument (SANS) at the ILL and the ID2 beamline (SAXS) at the ESRF.

Training at the CISB

New structures solved at EMBO/MAX-INF workshop

The fifth EMBO/MAX-INF workshop on "Exploiting Anomalous Scattering in Macromolecular Structure Determination" was held at the ESRF from June 18 to 22. The course, which was directed at young scientists, illustrated both theoretical and practical aspects of macromolecular crystal structure solution using synchrotron radiation. A special session was reserved for the discussion of the automation of this process.

A total of 23 students, 19 invited speakers/tutors and several tutors from the ESRF worked with both test and "real" data – the latter collected by participants during the course on ESRF beamlines BM14, ID23-1, ID29 and BM30 – to deepen their knowledge in the use of a wide variety of data collection techniques and the data analysis and structure solution software currently available.

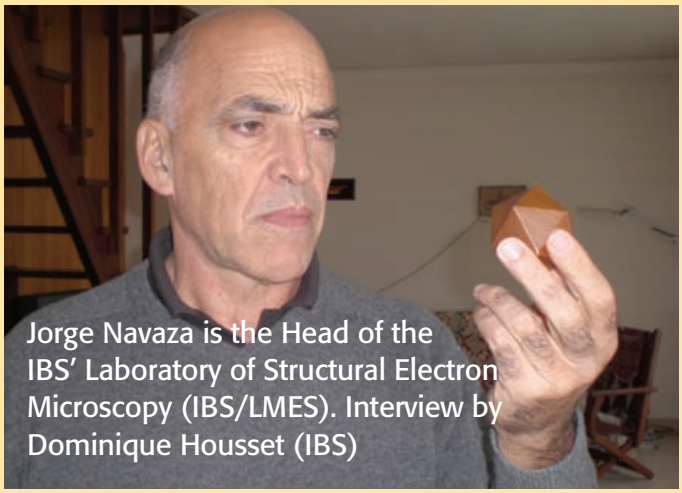
Eight new protein crystal structures were solved by students during the course. The lectures were open to the public and their content can be found on the ESRF website: www.esrf.fr/events/conferences/ past-conferences-and-workshops/ embo2007/Proceedings.

Christoph Müller-Dieckmann (ESRF)

Profile

Jorge Navaza, IBS/LMES

Jorge Navaza joined the IBS last year to lead the Laboratoire de Microscopie Electronique Structurale. Pushing forward both methodological and experimental developments in strucutral electron microscopy (EM) is a real challenge for the EM PSB platform.



Jorge Navaza is the Head of the IBS' Laboratory of Structural Electron Microscopy (IBS/LMES). Interview by Dominique Housset (IBS)

How would you define your scientific role in the context of the structural biology carried out at the IBS and the PSB?

I like to introduce myself as a researcher in methods for solving molecular structures starting from the experimental data provided by crystallographers and electron microscopists. My activity, which somehow spans the fields of mathematical physics and applied mathematics, consists of formulating reconstruction problems, developing the software required by the formulations and defining strategies to conciliate theoretical formulations with experimental constraints. Since the software is the most visible part of this activity, scientists in the field are usually called "developers". My choice of problems to address are based on the needs in structural biology, the generality of the problem (other fields may benefit from similar formulations), the "savoir faire" and personal amusement.

Does the nature of the developments you are leading now in the field of EM differ significantly from the one you did in the past for macromolecular crystallography, such as the molecular replacement program AMoRe ?

The needs of crystallography (XR) and electron microscopy (EM) are quite different. The long-standing phase problem of crystallography has been experimentally and numerically solved, although new formulations are recurrently proposed. More recently, with the progress of structural genomics projects, there has been a great demand for automated techniques. As a consequence, many scientists successfuly use crystallographic tools with only little knowledge of the basics underlying the techniques. Most of the development required for automation involves, in general, bookkeeping rather than mathematical calculations. This kind of development is seldom seen as a true research activity. Notwithstanding, a user friendly and robust software is extremely appreciated by the scientific community.

In structural electron microscopy the situation is usually compared to that of crystallography a couple of decades ago. There are still basic problems to solve, strategies to define and new techniques to develop. Despite the effort to automate procedures most programs need a real user's expertise.

Does combining XR and EM data require specific needs?

There are also methodological problems related to the combination of data produced by crystallography and electron microscopy: the docking of XR atomic models into EM reconstructions and the use of EM reconstructions as models to solve the phase problem by molecular replacement.

Besides methodological advances, does the development of EM at IBS and at the PSB require new equipment?

Nowadays the challenge is to obtain high resolution EM reconstructions, which requires both effective instrumentation available at IBS-LMES and parallel processing computers, which need to be acquired. Microscopes at the IBS-LMES are used for characterisation of samples (a facility open to all PSB partners) and to obtain negative stained and cryo EM micrographs for single particle reconstructions. The development of tomography will be the next step at both the experimental and methodological level. The software developed at IBS-LMES is available free of charge at http://mem.ibs.fr.

What would you expect from structural biologists at the IBS and PSB?

Methodological developments benefit from feedback. The confrontation of the numerical techniques with different sorts of data leads to improvements or new developments. The idea is to encourage people to work with new ideas instead of doing always the same. The ideal situation is when a collaboration is established between users and developers. The different scientific partnerships in the Grenoble area should really contribute to the establishment of such stimulating collaborations, provided the partnerships surpass the state of « ce qui ne sert qu'à être défini », as Henri Poincaré used to say of some exotic mathematical structures.

Newcomers

Daniel Panne has joined **EMBL** as Group Leader. He comes from Harvard Medical School (Boston, USA), where he studied the structure of the interferon β enhanceosome. His group will focus on the study of large macromolecular assemblies implicated in the control of gene transcription.

Andrew McCarthy (**EMBL**) has been promoted to Team Leader and will replace Raimond Ravelli as head of the Synchrotron Crystallography group. His team will focus on the development of methods and new instruments for the EMBL-ESRF Joint Structural Biology Group beamlines at the ESRF. He will also continue his research on proteins acting as guidance cues in neuronal development and those involved in caffeine and chlorogenic acid biosynthesis in coffee.

Lawrence McIntosh, a Professor of Biochemistry and Chemistry at the University of British Columbia (Vancouver, Canada) has joined **EMBL** for one year as Visiting Scientist. When not off backcountry skiing, he applies NMR spectroscopy and other biophysical techniques to study the structural bases of transcriptional regulation, as well as the enzymatic mechanisms of carbohydrate synthesis and degradation.

Alexander Popov and **Matthew Bowler** joined the **ESRF** MX group as Beamline scientists on ID23-Eh1 and ID14-Eh2 respectively.

Two new teams led by **Jean-Michel Jault** and **Michel Vivaudou** have joined the **IBS** in 2007, both working on the structure and function of ABC transporters. Vivaudou's team also works on ion channels and brings new expertise in the area of electrophysiology (patch clamp technique), while Jault's team is also interested in the role and functioning mechanism of essential yet uncharted bacterial GTPases/ATPases.

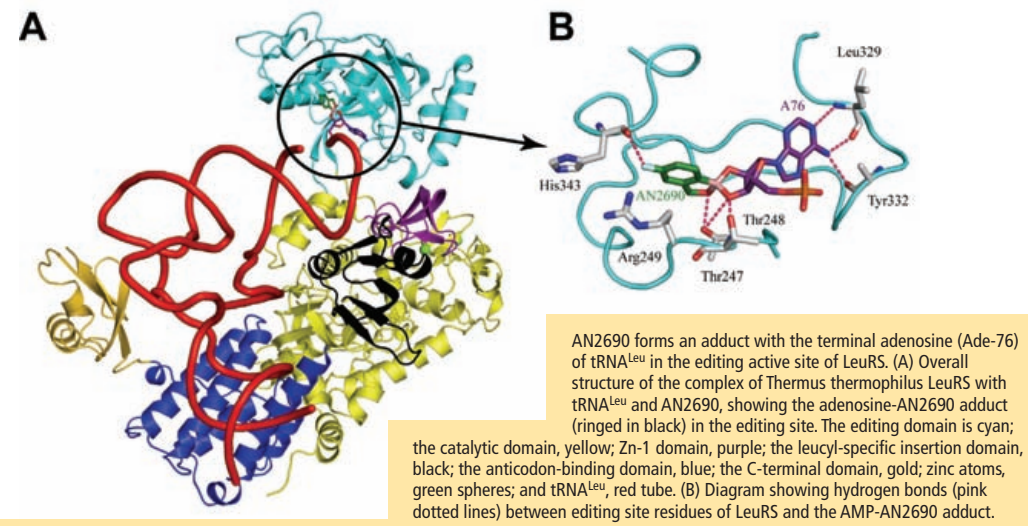
Spotlight

When fundamental studies on the fidelity of protein synthesis meet boron chemistry: mechanism of a new antibiotic

For many years Stephen Cusack's group has been studying aminoacyl-tRNA synthetases, essential housekeeping enzymes which specifically charge a tRNA with its cognate amino acid, thus providing aminoacyl-tRNA for protein synthesis on the ribosome. The specificity of an aminoacyl-tRNA synthetase both for cognate tRNA and amino acid is paramount to the fidelity of translation of the genetic code.

Recent studies by the group have focused on leucyl-tRNA synthetase, which is one of a subset of synthetases which cannot discriminate its cognate amino acid from similar hydrophobic amino acids (e.g. isoleucine) with sufficient accuracy in one step and has therefore acquired an additional proof-reading or editing activity to enhance specificity. Thus, in a separately folded domain attached to the main body of the enzyme, mischarged ile-tRNA^{leu} is hydrolysed, whereas the cognate leu-tRNA^{leu} is left untouched. Stephen's group earlier elucidated the structural basis for this process, known as post-transfer editing.

At a tRNA workshop in Bangalore, India in 2005, Stephen was approached by Dickon Alley from Anacor Pharmaceuticals Inc., Palo Alto, whose company had just identified the target of a newly discovered anti-fungal compound (denoted AN2690) as leucyl-tRNA synthetase. Furthermore, they had evidence from escape mutants that the likely binding site for the compound, which contains a critical boron atom, was the editing domain of the enzyme. However, it was very unclear how a compound that targeted the editing domain, which is not absolutely essential for cell viability, could be such an effective anti-fungal compound.



AN2690 forms an adduct with the terminal adenosine (Ade-76) of tRNA^{Leu} in the editing active site of LeuRS. (A) Overall structure of the complex of Thermus thermophilus LeuRS with tRNA^{Leu} and AN2690, showing the adenosine-AN2690 adduct (ringed in black) in the editing site. The editing domain is cyan; the catalytic domain, yellow; Zn-1 domain, purple; the leucyl-specific insertion domain, black; the anticodon-binding domain, blue; the C-terminal domain, gold; zinc atoms, green spheres; and tRNA^{Leu}, red tube. (B) Diagram showing hydrogen bonds (pink dotted lines) between editing site residues of LeuRS and the AMP-AN2690 adduct.

Within a few months, Stephen's group obtained a crystal structure at 3.5 Å resolution of leucyl-tRNA synthetase from the bacterium *Thermus thermophilus* complexed with AN2690 and tRNA^{leu}. This shows that AN2690 indeed binds in the editing site of the enzyme and makes a covalent adduct, via the boron atom, with the ribose of the terminal adenosine (A76) of tRNA^{leu}, thus trapping the tRNA on the enzyme (see figure). This prevents enzyme turnover, blocking protein synthesis and killing the fungal cell. It turns out that the same adduct can be formed with only AMP, mimicking the terminal adenosine of the tRNA and this led to a 2.0 Å structure of the inhibitory complex (figure). The unique boron-based mechanism of action underlying AN2690 was detailed in a recent issue of *Science* (detailed below).

Anacor reports promising results during phase II clinical trials of the compound as a treatment for chronic toe fungus, a common and unpleasant complaint (www.anacor.com/ pipeline/AN2690.htm).

However, the story does not end there. Now that it is known in great detail how AN2690 works, modified compounds are being designed that target dangerous bacterial pathogens, which we hope will help counter the problem of antibiotic resistance.

Stephen Cusack & Thibaut Crepin (EMBL); Anya Yaremchuk & Mikhail Tukalo (IBMG, Ukraine)

Rock, RL. et al., Science *316, 1759-1761 (2007).*

Announcements

The next PSB Science Day and Students Day will be on December 10, with a special morning session on NMR and selected presentations by PSB postgrads, a poster session and apéritif in the afternoon. A detailed program will be announced soon.

The second meeting of the CISB's Science Advisory Board was held in Grenoble on 18-19 June, opening with a visit to the CISB platforms in the IBS buildings followed by a poster session and apéritif. The following morning, an overview of progress since the last meeting was provided by Stephen Cusack and Sean McSweeney, followed by three scientific cases presented by Christoph Müller, Darren Hart and Dominique Bourgeois, all chosen to demonstrate the benefits of improved usage of the many platforms available within the CISB. The review panel were positive about the progress being made and offered further advice on how the platforms and the scientific environment could be improved. These recommendations will be followed up in the coming months.

Sean McSweeney (ESRF)

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The Centre for Integrated Structural Biology (CISB) 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 CISB 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.