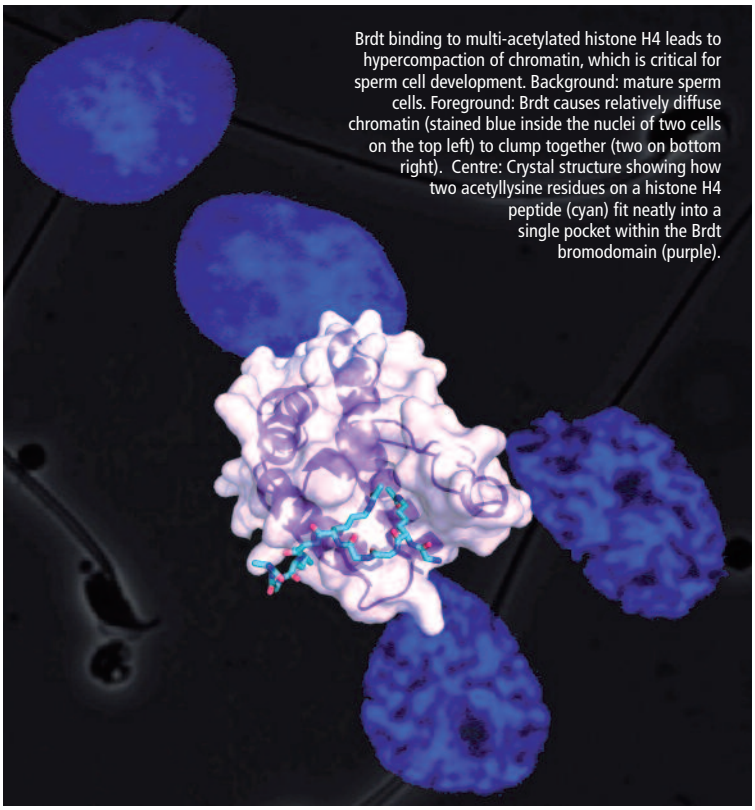
PSB et al.
Partnership for Structural Biology Newsletter
N°6
December 2009

A new way to read the histone code

A collaboration between the EMBL, the IBS and the Institut Albert Bonniot sheds new light on how covalently modified chromatin is recognized



Brdt binding to multi-acetylated histone H4 leads to hypercompaction of chromatin, which is critical for sperm cell development. Background: mature sperm cells. Foreground: Brdt causes relatively diffuse chromatin (stained blue inside the nuclei of two cells on the top left) to clump together (two on bottom right). Centre: Crystal structure showing how two acetyllysine residues on a histone H4 peptide (cyan) fit neatly into a single pocket within the Brdt bromodomain (purple).

For the average person, the term 'genetics' is closely linked to the idea of heredity. Most people would agree, for example, that genetics is the reason you look more like your parents than like your neighbour or your cat. In more sophisticated terms, related individuals share heritable traits like hair or eye colour because the DNA sequence of genes coding for those traits is conserved between them. But heredity is not only about genetics.

'Epigenetics' refers to heritable traits that are not transmitted through the DNA sequence. Over the past decade, epigenetics has attracted increasing attention as it has become clear that epigenetic factors are implicated in cancer and other diseases. Two molecular mechanisms are behind most epigenetic phenomena: the methylation of DNA and the post-translational modification of histones. Histones are basic proteins whose main role is to associate with DNA to form nucleosomes, which in turn are packaged into higher-order chromatin structures. Histones are marked with different chemical tags – acetyl, methyl and phosphoryl groups, for instance – that act as a 'code' to direct changes in chromatin structure and function. Different chromatin-associated proteins bind to these tags through modular, tag-specific domains. Examples include bromodomains and chromodomains, which recognize acetylated and methylated lysines, respectively. Until recently, it was generally believed that a single such domain could recognize only one tag at a time.

Surprisingly, a single bromodomain was found to bind both marks simultaneously

Recent work, however, led to the discovery of a different recognition mode, thanks to a collaboration involving the IBS, the EMBL in Heidelberg and Grenoble, and the Institut Albert Bonniot. The discovery was made while studying a protein called Brdt, which specifically recognizes histone H4 bearing two acetylation marks. Brdt has two bromodomains, and consequently these were expected to recognize one acetylation mark each. Surprisingly, however, a single bromodomain was found to bind both marks simultaneously. A crystal structure showed that this cooperative binding is due to an extra-wide ligand binding pocket on the surface of the bromodomain. This

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has widespread implications, as many other bromodomain-containing proteins also have such a pocket. Critical to the success of this project was the role played by an ESRF beamline. Crystals initially seemed unusable because they were internally highly disordered, but exposure to X-rays grazing the edges of a crystal revealed a clean diffraction pattern. Using the microfocused X-ray beam at ID23-2, enough data could be collected from a single crystal to solve the structure.

Brdt is implicated in sperm cell development, and mutations in the bromodomain that mediates cooperative binding cause male infertility. Thus, apart from advancing our basic understanding of how the histone code is deciphered, this work sheds light on the molecular mechanisms that underlie a significant human disease.

Carlo Petosa (IBS)

Morinière et al., Nature, 2009. 61(7264):664-8

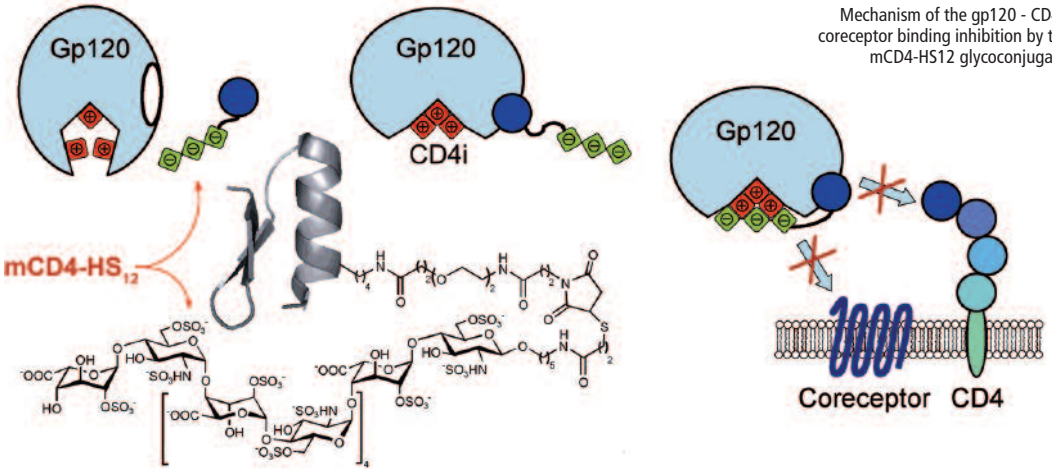
Stopping HIV: A new glycoconjugate opens the envelope to better block it

HIV entry begins when the viral envelope glycoprotein gp120 binds to its primary receptor CD4. This first event induces a conformational rearrangement that enables gp120 to recognize a coreceptor also expressed at the cell surface, usually CCR5 or CXCR4. gp120 is thus key for virus entry. However it exhibits unusual features that make it a difficult target: its most exposed domains are highly variable while its conserved structures are either covered by glycan, cryptic (CD4 induced coreceptor binding site) or located in partially occluded cavities (CD4 binding domain).

HIV is also known to bind to heparan sulfate (HS), a cell surface polysaccharide. Binding to HS is believed to immobilize the virus on the host cell surface prior to its interaction with CD4, thereby enhancing the local concentration of the virions. Although a variable loop region of gp120 was known to be implicated in this interaction, we recently found that the coreceptor binding site also interacts with HS but, consistently with its cryptic nature, only after CD4 engagement [1].

Thus, with the aim of blocking this site by HS we designed a glycoconjugate, comprising a CD4 moiety covalently linked to a HS oligosaccharide. For that purpose we synthesized a CD4-mimetic peptide (mCD4) containing a single lysine residue, allowing saccharide attachment at a position suitable for the HS to reach the co receptor site while the peptide was bound to gp120 (see figure). A conjugating strategy was developed to covalently attach a HS dodecasaccharide (HS12, chemically produced) to the peptide.

Kinetic analysis of the binding supports a mechanism where the mCD4 binds first to gp120 and opens the coreceptor site for subsequent binding by HS12, resulting in an overall very strong affinity (3-10 nM). In competition assays, mCD4-HS12 simultaneously inhibited binding of gp120 to each of CD4, HS and an antibody used as a coreceptor surrogate. Finally, in cell culture, replication of R5-, X4- and dual tropic HIV strains was inhibited by very low nanomolar amount of mCD4-HS12.



Mechanism of the gp120 - CD4 - coreceptor binding inhibition by the mCD4-HS12 glycoconjugate.

This compound, which has thus the unique ability to target two critical and highly conserved structures of the viral envelope glycoprotein, is conceptually distinct from any other existing ones and importantly inhibits the replication of HIV strains independently of coreceptor usage. Blocking viral entry is a promising approach in the fight against HIV and *in vivo* work will be needed to evaluate whether this kind of molecule can be further developed for clinical applications. The concept of linking HS to mimetic peptides might be applicable to the many other biological systems that rely on simultaneous recognition of both HS and a specific receptor at the cell surface.

Hugues Lortat-Jacob (IBS)

1. Crublet et al. 2008 J. Biol. Chem. 283, 15193–15200

2. Baleux et al 2009, Nat. Chem. Biol. 5, 743-748

Training at the PSB

6th EMBO/MAX-INF2 workshop

The 6th EMBO/MAX-INF2 workshop on Structure Determination in Macromolecular Crystallography was held at the ESRF from 15th-19th June. The course, which is directed at young scientists, illustrated theoretical and practical aspects of macromolecular crystal structure solution using synchrotron radiation. A total of 21 students from 18 different countries participated.

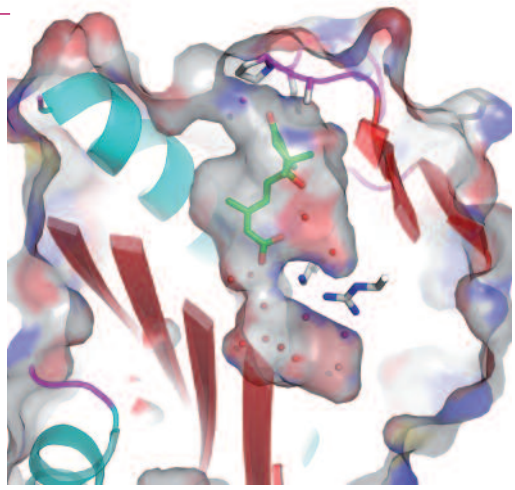
Lectures and software demonstrations were given by 14 invited speakers while practical sessions on the beamlines were overseen by several 'tutors' from the ESRF, EMBL Grenoble outstation and the IBS. During the workshop the students collected both test and 'real' data on one of BM14, ID14-4, ID23-1, ID29 and BM30 end stations, and a series of roaming tutorials allowed them to deepen their knowledge in the use of a wide variety of data collection, processing and analysis techniques as well as in the latest versions of structure solution software.

As usual the workshop was a success and resulted in the elucidation of first crystal structures for several proteins!

Christoph Müller-Dieckmann (ESRF)

The switch that activates the defence against drought in plants

Detail of the plant hormone Abscisic acid (centre) bound to its cellular receptor PYR1



The crystal structure of the receptor for the plant hormone Abscisic Acid illuminates one of the key steps in the mechanisms of defence of the plants against adverse environmental conditions and opens the door to the synthesis of chemicals to improve the tolerance of cultivated plants to drought and cold

The plant hormone Abscisic Acid (ABA) plays a key role in activating the defence mechanisms against adverse environmental conditions, like drought or cold. During dry periods, ABA levels in the plant rise up to 40-fold, triggering a specific signalling cascade that will ultimately result in the activation of metabolic responses to adapt to the loss of water. It's importance in plant development, but also it's obvious biotechnological potential has made of the ABA signalling pathway one of the most intensively studied in plants.

The ABA pathway requires the activity of two types of proteins, the protein phosphatases 2C (PP2Cs) and the Serine-Threonine protein kinases, of the SnRK family. In the absence of ABA, the kinases are held in an inactive state, by the PP2Cs, however in the presence of ABA the PP2Cs become inactive, releasing the SnRKs and leading to the transcriptional activation of stress-responsive genes. Since ABA neither binds nor inhibits the activity of PP2Cs directly there had to be a receptor that conveyed the signal. The search for the ABA receptors has been long and intense, with false starts and some highly disputed candidates… until this year!

In May 2009, a number of research groups reported the discovery of a family of proteins named PYR/PYL/RCAR, able to bind ABA and inhibit the activity of the PP2Cs. The crystal structure of the ABA receptor PYR1 has now been obtained by our group and shows how the hormone is bound in a large cavity inside the PYR1 protein. The loops surrounding the entry to this cavity act as "gates", closing over the hormone once it is inside. Interestingly amino acids in these same loops are also important for the interaction between PYR1 and the phosphatases, suggesting that binding of the hormone to the receptor and the closure of the gating loops generates an optimal surface for binding of the PP2Cs.

This work has been the result of a collaboration between the group of Dr. Pedro Luis Rodriguez of the Instituto de Biologia Molecular de Plantas (Valencia, Spain) and our group. The group of Dr. Rodriguez participated in the initial discovery of the receptors and wanted to pursue structural studies. The EU-funded PCUBE program (http://www.p-cube.eu, see accompanying article) allowed this group to benefit from access to the high throughput crystallization screening services offered by the HTX lab, which was the beginning of a successful collaboration between the two groups. Access to the MX beamlines at the ESRF, the recently inaugurated BioSAXS station and the biophysical characterization capabilities of the CISB, were also critical.

This work represents the definitive confirmation of the PYR/PYL/RCAR protein family as ABA receptors, but also provides insights into the basic mechanisms of sensing and signalling. Moreover, the structure of the hormone-receptor complex paves the way for the design of small molecules able to bind to the ABA receptors and activate the stress signalling pathway. These molecules should be easier to synthesise and more stable than ABA itself and could potentially be used to improve the tolerance of crops to drought and other type of environmental stress.

José A. Marquez
(EMBL–UVHCI)

J. Santiago et al., 2009, Nature (in press) doi: 10.1038/nature08591

Announcements

PSCM 2009 – Workshop on Scattering and Complementary Techniques. ILL Chadwick Amphitheatre, 16-18 December. The ESRF and the ILL are setting up a Partnership for Soft Condensed Matter (PSCM; see PSB Newsletter Issue 2). The aim of this workshop is to gather potential Associate Partners and discuss the scientific agenda of the partnership as well as possibilities for external funding.

MaM2010 - Molecules and Membranes: The shape of things to come. ILL, 20 January

A symposium to honour Roland May and Peter Timmins' lifelong contributions to SANS and structural biology, covering important results obtained on multicomponent biological structures such as membrane proteins and their assemblies, large interacting nucleic acid-protein and protein-protein complexes, and ongoing methodological developments.

Flipper 2010 - International Workshop on Single-Crystal Diffraction with Polarised Neutrons ILL, 26-30 January

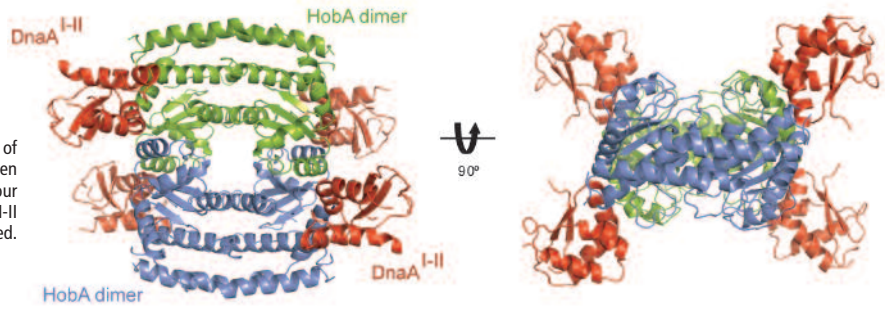
A workshop with emphasis on scientific applications, exotic magnetism, and the promise of new applications at both reactor and spallation sources, aiming to bridge the gap between excellent technique-oriented biennial PNCMI conferences and the broader triennial Sagamore Conference on Spin, Charge and Momentum Density. One session will be in honour of the long and fruitful career of Francis Tasset.

MX School: Getting the most from the ESRF MX beamlines. 8-11 February 2010.

The school will be held on three days either side of the ESRF'S 2010 Users Meeting and is designed to introduce the art of collecting good diffraction data at synchrotron-based MX facilities, familiarise the participants with the tools available at the beamline to allow correct pre- and post-data collection decisions to be taken and present the various ancillary techniques and equipment accessible at the ESRF's MX beamlines. The number of participants will be limited to 20 with all local costs (registration fees, accommodation, subsistence) being met by the ESRF. The deadline for applications is 13 December (midnight).

Structure of a DnaA/HobA complex from the human pathogen *H. pylori*

Insight into bacterial DNA replication and potential for a new drug target



Views of the dimer of dimers of HobA (green and blue), bound to four subunits of DnaAI-II coloured in red.

The human pathogen *Helicobacter pylori* is the primary causative agent for peptic ulcer disease and stomach cancer. This bacterium is probably the most widespread infective agent amongst humans as it colonises the stomach of almost half of the world's population. The understanding of biochemical mechanisms essential for the survival of *H. pylori* is a major challenge for the design of new drugs which can target the bacteria, thereby preventing stomach cancer development.

DNA replication is the most vital cellular process required for survival in all organisms. DNA replication in all bacteria begins when the protein DnaA binds to specific DNA sequences within the origin of replication of the chromosome called oriC. This is followed by a complex process involving several proteins, which eventually leads to the formation of a replication fork, resulting in the bidirectional replication of DNA. DnaA is an AAA+ ATPase consisting of four domains (DnaAI to IV). The function of DnaAIII (ATPase) and DnaAIV have been identified, while those of DnaAI-II are less clear. In *Escherichia coli* the functions of these domains are regulated by a number of proteins that directly bind DnaA. Until recently, much less was known about *H. pylori* DnaA and DNA replication in general. For instance, none of the known bacterial DnaA regulators could be identified in *H. pylori* genomes. Recently, HobA, a protein unique to *H. pylori*, was found to bind DnaAI-II. In addition, HobA enhances the binding of DnaA onto the oriC and is absolutely essential for the survival of *H. pylori*. A couple of years ago, we solved the structure of HobA and unexpectedly discovered that it was in fact a structural homologue of DiaA, a DnaA regulator from E. coli, known to ensure the timing of replication during the cell cycle.

We have now solved the crystal structure of the DnaAI-II in complex with HobA using data collected at the ESRF beamlines. The structure reveals that the four DnaAI-II monomers bind to one HobA tetramer via DnaAI and that most of DnaAII is unstructured (see Figure). The resulting HobA4(DnaAI-II)4 stoichiometry was further confirmed in solution by using Isothermal Titration Calorimetry in the PSB facility. The self-oligomerisation of DnaAI-II is a very important step for replication initiation in E. coli. However, we found that DnaAI-II from *H. pylori* exceptionally, cannot oligomerise by itself. Our structure of HobA4(DnaAI-II)4 thus suggested that HobA could serve as a scaffold onto which the oligomerisation of DnaA can occur. Together with our collaborators, M-F Noirot-Gros (INRA, France) and A. Zawilak-Pawlik (IIET, Wroclaw, Poland) we mutated residues in HobA to disrupt the complex of HobA with the full length DnaA *in vitro* and *in vivo*. Introduction of these HobA mutants into *H. pylori* chromosome results in a severe phenotype, in which the bacteria reject the mutations and revert to the wild type, suggesting that HobA/DnaA interaction is essential for the bacteria. Our work also shows that the residues of HobA involved in DnaA binding are remarkably conserved in DiaA. Thus, the oligomeric arrangement of DnaA mediated by these regulators is also likely to be relevant for DNA replication regulation in *E. coli* and other bacteria. Although the exact function of HobA regulators remains to be discovered, the study we present will greatly facilitate the design of new experiments to understand the details of the replication fork formation. Moreover, because the DnaA-HobA interaction is essential for the survival of *H. pylori*, the structure of this complex can be used to design drugs to disrupt DNA replication and combat *H. pylori* infections.

Ganesh Natrajan and Laurent Terradot (ESRF)

Natrajan, G. et al. PNAS (in press)

PSB Science Day on Cellular Imaging in Grenoble

On November 23, the Albert Bonniot Institut (IAB) and the PSB organised a joint Science Day on Cellular Imaging in the ILL's Chadwick Amphitheatre. *In vivo* studies being an obvious follow-up to structural studies, it seemed timely for the PSB to estimate the today's current Grenoble capacities in cellular imaging and to bring closer the large *in vivo* imaging community of the city and the PSB structural biologists.

As introduction, Michel Robert Nicoud (IAB), a major figure of the development of microscopy in Grenoble for almost twenty years, gave a short and rich overview of the different microscopy and imaging techniques used today all over the city, the east site of Grenoble focusing more on *in vivo* approaches while the west site focuses mainly on *in vitro* studies.

The first session was thus dedicated to introduce the developments and the current research fields held at the IAB, the Grenoble Institute for Neurosciences, the Laboratory of Physical Spectrometry and the Laboratory for Medical Engineering. After the coffee break, the technologies developed within the 'Polygone Scientifique' were highlighted, such as the PSB electron microscopy platform (IBS), the X-ray tomography applied to biological samples (ESRF) and the imaging of protein in motion using fluorescent probes (iRTSV-CEA).

This PSB Science Day was attended by scientists working at both the molecular and cellular level. We hope that all the excellent presentations and discussions we had, will promote tighter interactions and fruitful collaborations. The organisers would like to thank all the speakers for their enthusiastic participation and their help in making this day such a successful event.

Dominique Housset and Laurence Serre

P-CUBE opens three PSB platforms to European scientists

A prerequisite for any type of structural biology research is the availability of sufficient amounts of purified macromolecular sample. Over the last decade, various structural genomics initiatives worldwide have attempted to tackle this problem and a number of high-throughput cloning, expression, purification and crystallisation methodologies have been established in specialist laboratories. Although locally accessible (here, some are PSB platforms), access to the wider scientific community has been limited.

In order to provide transnational access (TNA) to these advanced protein production methods internationally, the P-CUBE "infrastructures" project has been funded as part of the EU Seventh Framework Program. The project is coordinated by the University of Zurich and involves facilities in Zurich, Oxford, and at the EMBL in Grenoble, Heidelberg and Hamburg. Zurich offers access to the DARPin technology developed by Andreas Plückthun for assisting protein crystallisation, especially of membrane proteins, through in vitro selection of binders in a similar manner to using Fabs. The Oxford Protein Production Facility provides access to high throughput cloning and expression testing in E. coli, and transient mammalian cell expression. The EMBL in Hamburg offers high throughput crystallisation similar to that already existing in the PSB, with Heidelberg providing materials, training and microscopes for fluorescence labelling of proteins and in-cell measurements via its Protein Expression & Purification and Advanced Light Microscopy core facilities. In the PSB, the ESPRIT platform, the HTX Lab and Eukaryotic Expression Facility (EEF) are P-CUBE access facilities open to scientists working in European research institutes.

P-CUBE applications are reviewed for eligibility by an independent TNA review board comprising a panel of scientists from outside the P-CUBE project. Approval of the project releases all the necessary funding to cover consumables, travel and accommodation for a visiting scientist. Projects are judged based on scientific importance and technical feasibility. Also, as "TNA" implies, they must involve access (visits or sending of samples) by scientists from EU member states outside that in which the facility is located. However, since EMBL is an international organisation, French laboratories are able to apply for access to the three platforms in Grenoble.

So what does this mean for the PSB? Firstly, there will be new faces around, both P-CUBE-funded staff and visiting scientists. Secondly, PSB research projects are all eligible to apply for fully-funded access to the facilities in Oxford, Zurich, Hamburg and Heidelberg. Thirdly, your collaborators outside the PSB can use the three PSB-based platforms and have their visit or experiments paid for. A notable example of this was a group from a research institute in Bratislava who began submitting purified protein samples by courier to the PSB HTX Lab, for which no similar facility exists in Slovakia. Crystallisation conditions were identified that ultimately led to a successful structure determination at the ESRF.

To find out what is on offer from the P-CUBE project, visit the website (www.p-cube.eu), register to set up an account and submit your (brief) project proposal. Projects are reviewed rapidly and very significant levels of funding are available.

Darren Hart

Improvement in diffraction properties of macromolecular crystals by controlled dehydration

The online humidity control device HC1b

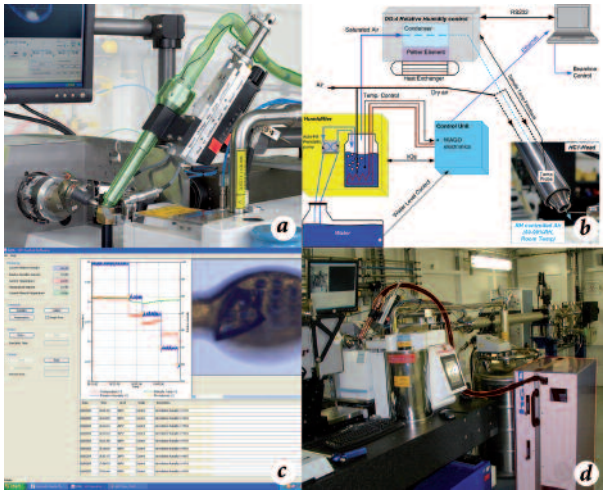
The weakly diffracting nature of protein crystals often leads to data of insufficient quality to answer the biological question being asked, either due to a failure to solve the structure or to other factors, such as insufficient resolution. A number of methods exist to improve macromolecular crystals but dehydration is often very effective. Despite many descriptions of successful dehydration experiments and the availability of mounting systems dedicated to the controlled dehydration of protein crystals, the technique remains little used as it can be difficult to reproduce and problematic to implement. In order to standardise this technique for the macromolecular crystallography user community the EMBL and ESRF developed a humidity control device (HC1) (Sanchez-Weatherby *et al*, 2009). Based on a modified cryostream nozzle, it produces an air stream at the sample position with precise control of the relative humidity (RH) between 50 and 99%. The device is fully compatible with the standard experimental environment at the ESRF and coupled with the time resolution available at a synchrotron beamline, dehydration experiments become a practical way to improve the diffraction properties of some protein crystals.

The dehydration of protein crystals can have many effects: the decrease in the length of one or more unit cell axes is often accompanied with an increase in resolution, a decrease in the mosaic spread and improvement in the profile of Bragg peaks. Of course, in many cases, dehydration can have a detrimental effect on crystals.

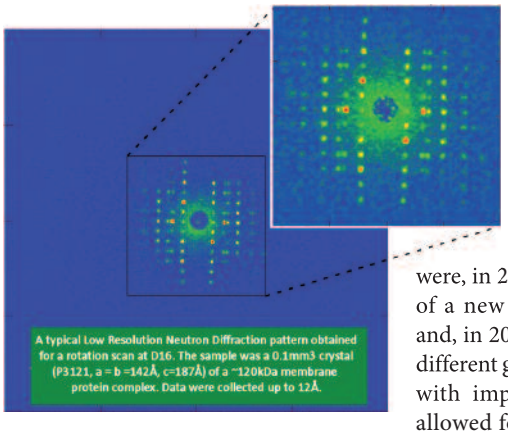
Some successes from the first users are the Chromatin remodelling complex (an improvement in resolution limit from 8 Å to 2.9 Å) and the Na^+-translocating NADH: quinone oxidoreductase (NQR) from *Vibrio cholerae* (an improvement in resolution limit from 6 Å to 4 Å). Crystals of plant photosystem I have also been observed to undergo a transition upon dehydration with a significant improvement in diffraction limit from around 6 Å to 4 Å. The highest resolution achieved for this 530 kDa integral membrane protein is 3.4 Å. As more examples are found, it is hoped that general rules on dehydration protocols can be developed.

Matthew Bowler (ESRF)

Sanchez-Weatherby J. et al. Acta Cryst. D65, 1237-1246



Right: the HC1b Humidity Control Device. a; The HC1b head mounted on a standard ESRF MX-Beamline. All standard elements are in place, including an open MiniKappa. Red tape shows the vertical movement required in the cryo-mount to accommodate the new head. b; Schematic of the HC1b design. c; The HC1b GUI, a step gradient and a crystal being conditioned are shown. d, The device installed in the experimental hutch of BM14.



D16 merges with DB21: going EASY!

Two instruments are currently merging at the ILL to produce a versatile redesigned instrument with extended applications: D16, a small momentum transfer diffractometer, and DB21, a low resolution neutron crystallography diffractometer.

D16 evolved from the first biological membrane diffractometers built in Brookhaven and Harwell in the early seventies. It was originally designed to study structures of ~5nm periodicity diffracting to a resolution of a fraction of 1nm. In the last decade the instrument has undergone several major upgrades accompanying new designs and positions on the neutron guides. A few highlights were, in 2002, the installation of a new 3He PSD detector and, in 2007, the moving to a different guide hall (in ILL22) with improved design that allowed for much easier and rapid wavelength changes (between 4.7 Å and 5.6 Å). Finally, in 2009 the original design was replaced altogether and modifications were introduced to accommodate a new detector (MILAND), which has been commissioned and will be installed during the 2009-2010 winter shutdown of the ILL reactor cycle.

Since 2006, a high-resolution SANS setup is used routinely in experiments requiring the 1% wavelength band width and the high angular range and resolution of the instrument. In terms of Q-space and resolution, D16 nicely filled the gap between small angle instruments and classical diffractometers, remaining unequalled in versatility for the study of a wide range of systems in biology, physics and physical chemistry. These include large unit-cell lamellar organisations such as membranes or clays, two-dimensional membrane and surface lattice structures, colloidal structures and magnetic systems, and now crystals of large biological systems (see figure). The sample environment includes controlled temperature and humidity chambers, furnaces, magnets, cryomagnets, high pressure cryostats, high pressure cells and an automatically positioned, temperature controlled horizontal sample changer.

After the winter shutdown of the ILL reactor, D16 will also be available for crystallographic studies of large biological complexes, bridging the high resolution studies (already done at the ILL on the LADI-3 and D19 instruments) to studies of much larger structures. Even relatively large crystals (typically 0.01mm^3) of complex biological assemblies tend to inherently diffract to low resolution, but with neutrons extra phase information can be extracted through the use of contrast variation. Neutron crystallographic data at 10Å or lower can be used to study viruses, consisting of protein and nucleic acid, or protein-detergent complexes of solubilised membrane proteins.

Proposals for DB21 should therefore be submitted to D16 at the next ILL proposal round (deadline 15 February 2010). D16 has also joined the EASY access system, open all year round (see http://club.ill.eu/cvDocs/EASY_Guidelines.pdf). EASY is really good news for unstable samples or preliminary measurements. If you're new to neutrons, D16 scientists are available to help you plan experiments (teixeira@ill.eu or deme@ill.eu).

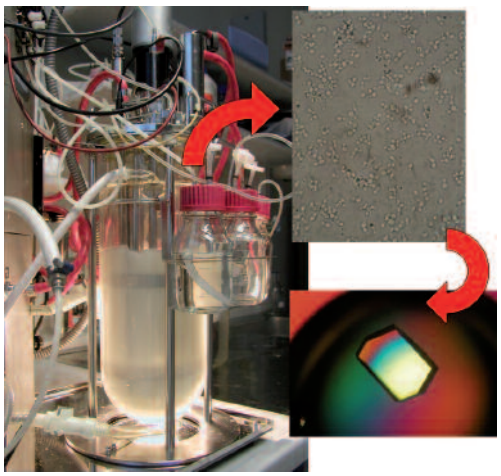
Susana Teixeira (Keele University and ILL) and Bruno Demé (ILL)

(http://www.ill.eu/d16)

Pichia pastoris: a newcomer at the D-Lab

Pichia pastoris yeast now joins the bacterial production team at the ILL-EMBL Deuteration Lab (www.ill.eu/deuteration), opening new opportunities for the production of deuterated recombinant proteins.

To date, the production of deuterated samples mostly involved obtaining high cell density cultures of recombinant *E. coli* using deuterated minimal medium or other specific labeling schemes depending on the purpose of the subsequent experiment (photosynthetic bacteria are also used when applicable but not routinely). *E. coli* may however produce misfolded, inactive or insoluble eukaryotic proteins, adding important constraints to obtaining isomorphous pure samples.



From cradle to crystal: adaptation of *P. pastoris* cells to perdeuterated medium, expression using automated fermentor systems, and large crystals of perdeuterated lysozyme.

In these cases, *P. pastoris* is a valuable alternative as an expression system: it is able to grow on a simple minimal medium (adequate for isotope labeling), it has a high growth rate and a strong inducible promoter for recombinant protein induction. Pichia is also capable of many post-translational modifications performed by higher eukaryotic cells, such as proteolytic processing, disulfide bond formation, and glycosylation. Furthermore, the existing D-Lab fermenter facilities are fully equipped to optimise very high cell density cultures of *P. pastoris* (over 150g/L wet cell weight or OD600>300), essential for the expression of large amounts of protein required for a complete range of structural studies, particularly those involving neutron diffraction.

P. pastoris also has the added advantage that heterologous proteins can either be expressed intracellularly or secreted into the medium. Since *P. pastoris* secretes only low levels of endogenous protein, the secreted recombinant protein comprises the vast majority of the total protein in the culture supernatant. Secretion thus serves as a major first step in purification, separating the foreign protein from the bulk of cellular proteins.

Over the last year the D-Lab have developed optimised protocols for the production of deuterated biomolecules in *P. pastoris* using fully deuterated media, with very successful results for several model proteins (human serum albumin, beta-galactosidase, hen egg lysozyme – see image) while others are currently being produced, proving the feasibility of the use of this system for the expression of labeled proteins to be used in neutron, NMR or other studies. This extends the capability of the D-lab to welcome a wider range of studies for users from the PSB and elsewhere.

J.B. Artero and V. Laux (ILL)

Profile

Sine Larsen

ESRF Research Director 2003-2009

interviewed by Joanna Timmins (ESRF)

In June 2009, Sine Larsen relinquishes her post as director of research at ESRF to return to her laboratory in Copenhagen. Joanna Timmins caught up with her before she left to hear about some of her Grenoble memories and achievements.

How did you find the ESRF when you arrived in 2003, and how had it changed when you left in 2009?

The scenario of European synchrotrons was undergoing dramatic changes when I arrived in 2003. Until 2003 the ESRF had been the only third generation synchrotron facility in Europe, but the construction of several new National facilities in Switzerland (SLS) , UK (Diamond), France (Soleil), Germany (Petra III) and Spain(ALBA) was clearly going to have an impact on the role the ESRF should have in the future. Therefore we started working on the role the ESRF should have in the future immediately after I arrived. First, developing the Long Term Strategy for the ESRF that in 2006 materialized into the ESRF's Science and Technology Upgrade Programme which I was happy to see in place by the time I left in 2009.

What has been the impact of the PSB on the ESRF and on European Science in general over the last six years?

The creation of the PSB was one of the things that made me very excited about the job at the ESRF. It represented a unique assembly of expertise in structural biology which included the two world leading synchrotron (ESRF) and neutron (ILL) sources. The PSB gave a great stimulation and enhancement of the in-house research program for the macromolecular crystallography (now structural biology) group at the ESRF. On the European scene PSB has been an inspiration that has led to the creation of similar laboratories at other synchrotron facilities in Europe.

The PSB has been a real success. Could you comment on the difficulties encountered in establishing and maintaining the high standards of the PSB from an administrator's point of view?

It is the common research interests that united the four PSB partners but on the organisational level the four partners are very different. The ILL and ESRF are user facilities which prime goal is to serve European scientists, and have to make sure that the PSB also matched the interests of the shareholders of the two facilities, 19 European countries in the case of the ESRF. Making the PSB partners understand and appreciate the differences between them has been and will remain a major challenge for the successful function of the PSB.

How do you see the future of the PSB within the context of the ESRF Upgrade?

Nobody will now question the success of the PSB, and the collaborations and interactions of the partners are an essential and important component of the development of the MASSIF beamline project. The new IBS building on the international site will also be an important strengthening of the PSB. For the ESRF, the PSB has served as a model for other scientific partnerships that will be part of the Upgrade, e.g. the Partnership for Soft Condensed Matter.

What are your best and worst memories from your time at the ESRF?

I have many good memories from my time at the ESRF, among those related to the PSB is the groundbreaking for the CIBB in June 2004 (see photo) and the inauguration of the building 19 months later in January 2006. The scientific case for the PSB was so convincing that it was a very bad experience for me when the project to construct the joint laboratory building was not approved at the first Council meeting I attended. This still remains as the worst memory from my six years in Grenoble.

Training at the PSB

Combining EM and X-ray crystallography

From October 18 to 24, 2009, the Partnership for Structural Biology organised an EMBO course on "Combining the data from Electron Microscopy and Crystallography to solve the three dimensional structures of large biological assemblies".

Twenty students were selected out of fifty applicants coming from Europe and as far as New Zealand and Singapore, most of them having a strong background in electron microscopy or protein crystallography. Teaching was in the hands of prestigious software developers internationally recognised in this field.

Participants and teachers first met on the evening of October 18 around an informal buffet held at the Hotel "Suisse et Bordeaux". Each day was dedicated to the description of a specific software package and to its practical use. The morning session held in the CIBB seminar room focused on general aspects, and the entire afternoon was reserved to practical sessions organised in the ESRF training room. Willy Wriggers (NYC) and Tom Goddard (UCSF) introduced the most recent developments of SITUS and CHIMERA though a course mixing practical and theoretical aspect. Jorge Navaza (IBS, Grenoble) and Alwyn Jones (Uppsala, Sweden) gave a combined course to introduce UROX and O programs. Stefano Trapani (CBS, Montpellier) and Fred Vellieux (IBS, Grenoble) focused their course on the use of electron microscopy models in molecular replacement and phase extension.

Along the entire week, after dinner held at the ILL-EMBL-ESRF canteen, participants and teachers carried on discussions in the Hotel's seminar room. During these evening sessions, volunteers had the opportunity to introduce and discuss their own research project and received advice from the audience. Every day from 9:30 am to 11:30 pm, the course has been very intense in scientific and friendly interactions.

The organisers would like to thank all persons within the Partnership who helped to make this course such a successful event.

Leandro Estrozi (EMBL), Jorge Navaza (IBS), Laurence Serre (ESRF and PSB coordinator), Fred Vellieux (IBS).

Spotlight

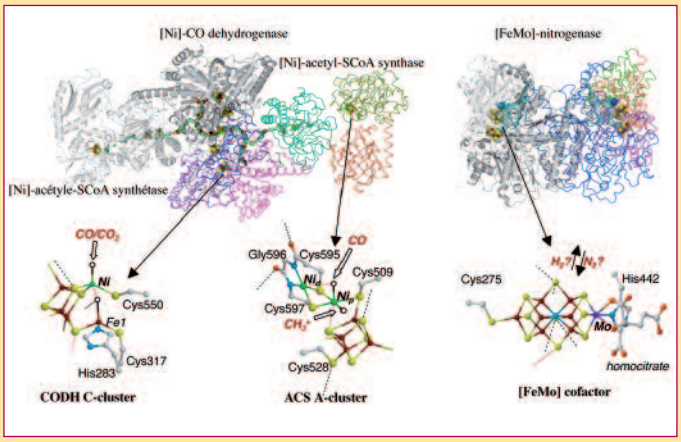
Structure-function relationships of anaerobic gas-processing metalloenzymes

Reactions involving H_2, N_2, CO, CO_2 and CH_4 are thought to have played a central role at the initial stages of life evolution. Indeed, during the initial period of Earth evolution, between 4.6 to 3.5 Ga ago, the atmosphere is thought to have been rich in these gases. The active sites of the enzymes that catalyze reactions involving H_2, N_2, CO and CO_2 are often reminiscent of FeS-containing mineral structures such as greigite, pyrrhotite, violarite and pyrite. This analogy has inspired the notion of primordial surface metabolism on transition metal sulphides which is the basis for the "iron-sulphur world" theory for the origin of life [1].

It has taken the combined efforts of protein crystallography, various spectroscopies, theoretical calculations and model chemistry to postulate plausible mechanisms for gas-based metabolism by contemporary microorganisms. Although the reactions are metal based, the protein component controls reactivity and the internal diffusion of substrate and product through binding and internal tunnels, respectively.

Conditions similar to those prevailing on the young Earth exist in the vicinity of contemporary hydrothermal vents, or black smokers, which are generated by plate tectonics at the bottom of the ocean. Thermophilic methanogenic Archaea can grow around these vents thanks to the oxidation of expelled H_2 and the reduction of CO_2 to CH_4. These microorganisms constitute the first component of a biological ensemble that culminate with the impressive tubeworms. Importantly, this shows that life can exist in the absence of solar energy, as it was required before the emergence of photosynthesis. The coupling of H_2 oxidation to carbon fixation was most likely carried out through the synthesis of acetyl-coenzyme A, a building block of anabolic processes, from two CO_2 molecules (see figure). Another central component of life, nitrogenase, that reduces N_2 to ammonia, probably started as a detoxifier, neutralizing the poisonous cyanogen gas $(CN)_2$ present in the early atmosphere [2]. Because these reactions took place in an anoxic environment, extant anaerobic H_2/CO oxidizers and CO_2 reducers are generally confined to contemporary anoxic settings, such as the bottom of lakes and oceans and the digestive track of animals. In all the gas-based reactions discussed in reference 3, catalysis appears to be metal-centred and the active site is buried in the protein.

Due to their complexity, the assembly of these metalloenzymes active sites and their insertion to the protein matrix generally requires a significant number of gene products. This is nicely illustrated by the examples of nitrogenase and [NiFe]-hydrogenase [4,5]. In an Insight Review we have recently published in Nature [3] we discuss the three known classes of hydrogenase ($H_2 \leftrightarrows H^- + H^+$), bi-functional CO dehydrogenase/acetyl-CoA synthase ($CO_2 + 2e^- + 2H^+ \leftrightarrows CO + H_2O$ and $CH_3–Co^{3+}$ FeS-Protein + CoenzymeA-S^- + CO $\leftrightarrows$ $CH_3C(O)$–S-CoenzymeA + Co+FeSP), methyl-coenzyme M reductase (CH_3–SCoenzymeM + CoenzymeBSH $\leftrightarrows$ CH_4 + CoenzymeMS–SCoenzymeB) and nitrogenase ($N_2 + 8H^+ + 8e^- +$ 16ATP $\leftrightarrows 2NH_3 + H_2 +$ 16ADP + 16P*i*). Although nickel is a very unusual metal in biology one of the hydrogenases, CO dehydrogenase, acetyl-CoA synthase and methyl-coenzyme M reductase contain this ion in their active sites. Also, cuboidal FeS clusters are present in five of these enzymes. These observations may be explained by the fact that the oceanic concentrations of transition metal ions such as Fe^{2+} and Ni^{2+} were relatively high at the early stages of life evolution. The complex gas-processing active sites pose a real challenge to synthetic chemists. Both bio-mimetic and bio-inspired models have been reported. A few examples are discussed in the review [3].



View of the active sites of some gas-processing metalloenzymes

Juan-Carlos Fontecilla-Camps (IBS)

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The Partnership for Structural Biology (PSB) is a collaboration between a number of prestigious European and French scientific laboratories in Grenoble which has received support from the EU FP6 programme. The PSB is unique in combining world leading user facilities for synchrotron X-ray and neutron scattering with NMR, electron microscopy, molecular biology and high throughput techniques on a single site together with strong projects in a broad range of structural biology, notably host-pathogen interactions.

Newcomers

Max Nanao has joined the **EMBL Grenoble** outstation as Staff Scientist. Previously, he was a senior scientist in the Structural Biology group of Exelixis, a biotech company in South San Francisco, California. During his time at Exelixis, he worked on a variety of cancer and metabolic drug development teams and developed software tools to increase the productivity of the SB group. He will be the beamline scientist for the microfocus beamline ID23-2.

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