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PSB *et al*

Partnership for Structural Biology Newsletter

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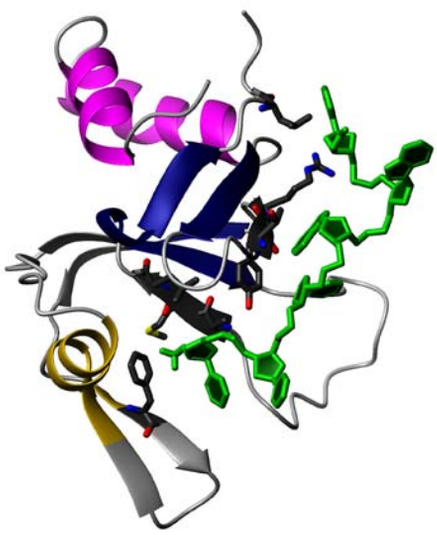
Getting a grip on small RNAs. Molecular insight into recognition of the stabilizing 2'-O-methyl modification of small RNAs by a conserved RNA-binding module.

Approximately 50% of mammalian genomes are composed of transposons, which are parasitic genetic elements capable of moving about in the genome. Thankfully, they are kept silent by genomic DNA methylation. Focusing on mouse as a model system, the Pillai lab at the EMBL-Grenoble is interested in understanding how small non-coding RNAs participate in transposon silencing.



Schematic representation of Miwi PAZ recognizing a piRNA.. The piRNA mimic (in green) carries a 2'-O-methyl mark at the 3' end.

The group studies a special class of ~30 nucleotide (nt) small RNAs called the Piwi-interacting RNAs (piRNAs) which are abundantly expressed in mouse testis. Millions of individual piRNA sequences exist (compared to a few hundred microRNAs), many of which derive from transposons. These piRNAs are believed to guide the DNA methylation machinery to transposon-rich genomic loci to mediate silencing.

The central core of silencing complexes is an Argonaute protein, which in the case of piRNAs is called Piwi. Argonautes bind small RNAs, and structural studies have illuminated our understanding of how the conserved PIWI and PAZ domains recognize the 5' phosphate and 3' hydroxyl (OH) groups, respectively.

One defining feature of piRNAs in all animals is the presence of an additional 2'-O-methyl modification on the 3' terminal nucleotide. In a collaborative effort with the Carlomagno's group at EMBL-Heidelberg, the molecular interactions involved in recognition of the 3' end methyl mark by the Piwi PAZ domain are now revealed.

The solution structure of Miwi (mouse Piwi) PAZ with an 8-mer piRNA mimic determined in the NMR group at EMBL-Heidelberg reveals a hydrophobic cavity made of conserved amino acid residues, into which the RNA 3' end is inserted. The cavity is formed by a central β-barrel and a α-helix/β-hairpin motif. Within this cavity, the long hydrophobic side-chain of a methionine reaches out from the back of the β-barrel to contact the methyl group. The overall features of the Miwi PAZ are similar to that described for PAZ from siRNA-binding Argonautes. Nevertheless, subtle structural differences reveal flexibility in the basic architecture of the PAZ domain to allow acceptance of RNA substrates with different 3' ends.

Accumulating evidence indicates no role for piRNA methylation in specifying their entry into Piwi proteins. In fact, piRNA methylation is now believed to be a post-loading event that protects the 3' end from nucleases. Our structure captures the state when the modified 3' end is locked into the Piwi PAZ, affording stability to the small RNA. Current efforts are focused on understanding the molecular details of piRNA methylation in the context of Piwi proteins and hand-over of the methylated 3' end to the PAZ domain.

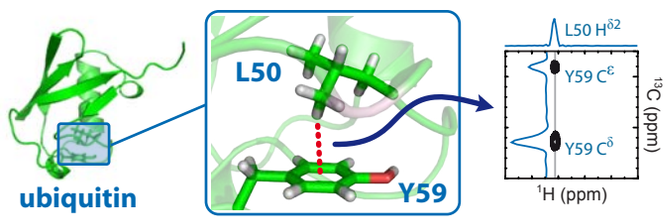
Ramesh Pillai (EMBL-Grenoble) & Teresa Carlomagno (EMBL-Heidelberg).

Simon B., Kirkpatrick J.P., Eckhardt S., Reuter M., Rocha E.A., Andrade-Navarro M.A., Sehr P., Pillai R.S. & Carlomagno T. (2011) Structure, January 13.

Studying weak non-covalent interactions in proteins

The three-dimensional (3D) structure and function of a protein is dependent on a multitude of different types of covalent and non-covalent interactions. The primary amino acid sequence of a protein defines its basic chemical structure and the arrangement of covalent bonds. What is not directly reported in this representation are the types of non-covalent interactions present, where they are located and how they contribute to either the tertiary/quaternary structure or biological function. A large number of classes of non-covalent interactions exist in nature ranging from the relatively strong hydrogen bonds seen in protein secondary structure to van der Waals interactions between apolar side chains in the hydrophobic cores of proteins. Despite being considerably weaker than their covalent counterparts, the cumulative contribution of non-covalent interactions across a protein is considerable.

Researchers at the IBS and the University of Ottawa, Canada, have used NMR spectroscopy to study XH/π interactions, a class of weak non-covalent hydrogen bond-like interaction commonly found in structured proteins. In an XH/π interaction the acceptor is



a chemical group containing a delocalised system of electrons (e.g. aromatic rings, peptide bonds, etc) while the donor group consists of a hydrogen atom covalently bonded to a heavy atom. The strength of an XH/π interaction is estimated to be between 1 and 4 kcal.mol^{-1} and is therefore comparable to classical hydrogen bonds.

The IBS group was interested in characterizing a class of XH/π interactions in which the donor is a methyl group (Me/π). Such interactions are often located in the hydrophobic cores of proteins. Their experimental approach was based on a fundamental principle of NMR spectroscopy: that it is possible to transfer nuclear magnetization between two covalently-bonded nuclei via a phenomenon called scalar coupling. It had been thought that such inter-nuclear interactions were limited to covalently bonds nuclei, but research from the 1990s demonstrated that scalar couplings also exist between nuclei involved in hydrogen bonds. The group at the IBS was able to show that similar NMR interactions exist between nuclei involved in Me/π interactions. This discovery allowed them to devise NMR experiments that can directly detect individual Me/π interactions in proteins and identify the participating groups (see Figure).

In their study they utilized a new isotope-labelling strategy developed at the IBS. Highly perdeuterated recombinant protein samples were prepared in which selective methyl groups remained protonated. Using a protein prepared this way it was possible to detect NMR signals in each instance where a methyl group was located on top of an aromatic ring. In a series of experiments, Me/π interactions were detected between Leu or Val methyl donor groups and Tyr, Phe or Trp aromatic acceptor groups.

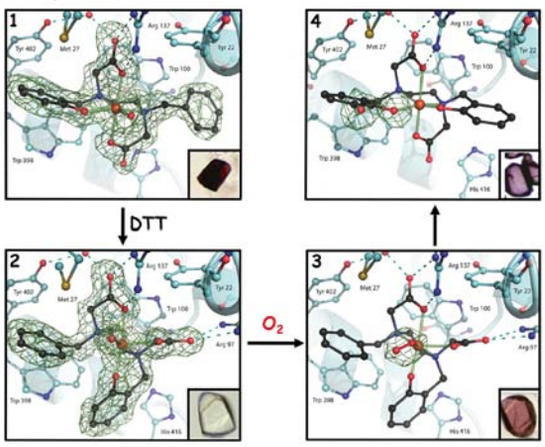
Are the results of this study just an interesting spectroscopic quirk or do the Me/π interactions discovered have some biological importance? One protein used in the study was ubiquitin, which is involved in the cellular protein degradation system. Using NMR spectroscopy it was possible to detect a number of Me/π interactions in ubiquitin, including one between Leu-50 and Tyr-59 (see Figure). The 3D structure of ubiquitin shows these residues adopt a classical XH/π geometry and that they reside in a functionally important region of the protein. This pair of residues is well-conserved across the family of ubiquitin-like proteins at both the sequence and structural levels which suggests this XH/π interaction has a strong functional or structural role.

Michael Plevin (IBS).

Plevin M.J, Bryce D.L. & Boisbouvier J. (2010) Nature Chemistry. Jun 2(6):466

How to decipher reactions catalyzed by an inorganic complex using a protein matrix?

X-ray crystallography is the method of choice for the elucidation of chemical reaction mechanisms through the determination of the three-dimensional structure of reaction intermediates. However in inorganic chemistry, trapping intermediates is quite difficult because each of them needs to be stabilized and crystallized separately. Consequently the chances of observing a full catalytic cycle are quite low especially if the reaction is too fast. Conversely, protein X-ray crystallography has been amply used in the field of structural enzymology to characterize chemical reaction mechanisms. These studies take advantage of the fact that, generally, enzymes remain active in the solid state thanks to the high solvent content of its crystals, which also allows the limited diffusion of substrates to the active site. Therefore intermediates can be trapped by flash-cooling the crystals at different times after reactant addition. In the field of dioxygen activation by iron-containing enzyme, this methodology was successful, illustrated for instance by the determination of the major reaction steps of a catechol dioxygenase.



Crystal structures and omit Electron Density maps of NikA-bound iron complex at different aromatic hydroxylation stages. 1: The complex binding site. 2: The structure of the reduced NikA/1 hybrid. 3: The diatomic oxo intermediate prior hydroxylation. The electron density peak was modeled as a peroxide. 4: The structure of the doubly hydroxylated iron complex-NikA species. The two electron density peaks were modeled as two oxygen atoms. The corresponding crystals are depicted in each frame.

Scientists from the IBS and the LCBM (CEA-Grenoble) combined model synthetic chemistry and protein X-ray crystallography to unravel the catalytic cycle of an aromatic dihydroxylation by a complex mimicking the active site of iron monooxygenases. The target arene was incorporated into the iron complex that was bound to the crystallized *E. coli* periplasmic nickel-binding protein NikA. In this manner, the hydroxylated species is generated in an intramolecular reaction.

The protein-bound arene-containing iron complex was able to activate dioxygen in the presence of a reductant, which led to the formation of a catechol as a sole product. Structure determination and Resonance Raman spectroscopic measurements performed on flash-cooled crystals were used to characterize four intermediates and the end product of O2-mediated aromatic hydroxylation (diffraction data were collected on the ESRF MX and FIP beamlines and Resonance Raman spectra were collected at the "Cryobench" (ESRF, IBS)). This study revealed that the hydroxylation is carried out by an iron peroxo-generated hydroxyl radical species.
Our *in situ* determination of a catalytic cycle illustrates that a protein scaffold can control reactions smoothly enabling the structural observation of transient species, without interfering with the nature of the reaction. Solution studies are unlikely to provide such a degree of detail. Consequently, our approach may have a general application in the study of challenging chemical reaction intermediates using protein crystallography.

Christine Cavazza (IBS).
Cavazza C., Bochot C., Rousselot-Pailley P., Carpentier P., Cherrier M.V., Martin L., Marchi-Delapierre C., Fontecilla-Camps J.C. & Ménage S. (2010) Nature Chemistry Dec 2(12):1069

Neutrons and food

Neutron and food experts recently met during a conference in Sydney to discuss how best to use neutrons to cater for novel food and food-related materials. Despite major developments in neutron research its use in Food Sciences remains underexplored. There is arguably an innate bias of neutron experts towards broader scientific questions of a more "academic" nature, in detriment of specific goal-oriented developments of interest. As a non-destructive deeply penetrating probe to materials on wide length scales, neutrons can provide valuable information to food experts who in turn are aware of the growing demand of neutron beamtime in structural biology. Even with planned and new neutron sources coming up worldwide, "cooking with neutrons" will require a focus on the questions where experimental data is most needed. This can only be done through direct interaction with and advice from food experts: devoting a meeting to the subject of Neutrons and Food was definitely a step in the right direction.

The cruise dinner at the "Neutrons and Food" conference in Sydney.

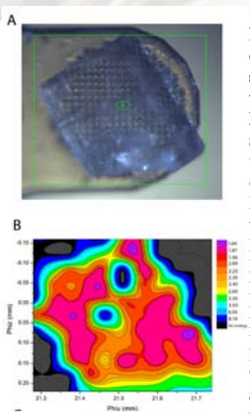
In a follow-up article of the Australian Cosmos Science journal [1] on the presentation from an ILL Scientist at the Sydney conference, the importance of neutron crystallography was highlighted. The mystery of the sweet taste mechanism was used to illustrate how such complex systems cannot be tackled without a detailed study on all structural characteristics of the molecules involved, including those often assumed - as opposed to experimentally determined - such as protonation states. Such parameters can determine how critical properties related to texture, taste and stability of food ingredients manifest under the wide range of conditions required for transport and processing. Deuterium labelling for these studies was done at the ILL/EMBL Deuteration Laboratory [2]: as in other areas, isotope labelling is clearly an essential tool in neutron and NMR studies of food, as the Australian National Deuteration initiative also bears witness. Better and healthier food is a major challenge which cannot be met without an integrated approach that does not stop at a lonely description of structure. Dynamics and interactions with solvent are also crucial aspects, particularly where a large fraction of water is directly associated with the systems involved: drying/re-hydration and freezing methods are of major importance for storage of food, during which biological processes are typically reduced. The role of the water and its implication on the evolution of the quality of the food were also investigated.
Structural and dynamical properties of proteins in frozen, dried, and hydrated states differ. Research in this area is essential, particularly with the added demands of sustainability. In collaboration with the Institut Agroalimentaire de Dijon, CNR/IOM Italian scientists at the ILL investigated the importance of structure and dynamics of protein and water content of β-lactoglobulin (β-LG) milk protein powders. The team studied a freeze dry process that does not impact on β-LG structure and dynamics. When the milk protein powder was fully re-hydrated or dissolved in solution all the inferred experimental parameters were found immutate. Protein powders with a monolayer of water and with freezable water were also investigated and it was shown that only the presence of freezable water allows the inset of dynamical transition at 220K which is the signature of protein large amplitude motions. The observed phenomena have been found to be correlated to water relaxation phenomena occurring at ~180K [3]. Water dynamics is the driving force governing protein motions and conformations. Food storage and quality evolution can be controlled in absence of freezable water.

Daniela Russo (CNR-IOM c/o ILL) & Susana Teixeira (ILL & Keele University)
[1] http://www.cosmosmagazine.com/news/3853/the-structure-sweetness [2] Teixeira et al. (2010). Acta D66, 1139-1143.
[3] Loupiac. C et al. (2010) Bioph. J. Submitted

Diffraction cartography: enhanced reality in macromolecular crystallography.

The large multi-component complexes and membrane proteins now routinely studied in Structural Biology tend to produce either very small crystals or crystals that can be extremely heterogeneous in their diffraction properties. The increasing availability of micro-focused X-ray beams coupled with experimental environments optimised for MX has allowed the design of advanced sample evaluation (mesh scans) and data collection (helical oscillations) protocols. Here we present two examples of how mesh scans can be used in the most challenging projects: searching, within a sample-containing loop, for crystals of maximum dimension 10 µm using an X-ray beam 5 µm diameter and selecting the most ordered regions of relatively large crystals using an X-ray beam 30 µm diameter.
For many projects, particularly those initially producing microcrystals, the rapid identification of crystallisation conditions yielding protein crystals is a considerable advantage. However, locating microcrystals within a large loop is problematic. Lens effects frequently make it difficult to locate small, thin crystals with visible light and the lack of explicit cryoprotection often means that the resulting glass in which microcrystals are contained is opaque. In such cases, the most convenient way to identify the both the position and composition of crystals is to use

X-ray diffraction coupled with a mesh scan capability. An interface has been implemented within the beamline control GUI MXCuBE [1] based on developments made on beamline ID13. In the sample display area of the GUI, the limits of a grid are drawn and a step size for sampling the grid chosen. Each position in the grid is associated with a specific coordinate (expressed in the position of the motors phiz (vertical translation of the goniomter axis) and phiy (horizontal translation)) so that the position and diffraction qualities of a previously invisible crystal are recorded.
In many of the most challenging projects it has proved essential, rather than advantageous, to be able to collect partial diffraction data sets from multiple positions within a crystal and to combine these to produce a complete data set. [2]. However, intra-crystal variation in diffraction quality means that it is not always obvious, a priori, from which regions of a crystal, partial data sets should be collected. To identify which regions of a crystal will produce the best data, we have developed an automated process combining mesh scans and on-line data analysis with an intuitive presentation of data that systematically defines the most ordered part of a crystal. We call this process diffraction cartography. The results both map diffraction quality (as determined by EDNA [3]) to different areas of crystal being studied and define the shape and location of the crystal. The attached figure shows a crystal of bovine F_1-ATPase (a large complex, 20 nm in the largest dimension), diffraction cartography reveals the large variation in diffraction quality across the face of the crystal. The results can be projected onto the optical view of the crystals clearly defining crystal shape and position as well as the best crystal volumes for data collection.



Diffraction cartography of a single crystal of bovine mitochondrial F1-ATPase. A mesh scan was carried over the entire area of the crystal (green box).
B. Diffraction images were processed with EDNA and the 'ranking resolution' (blue lowest, mauve highest) plotted against the goniometer axis motor positions phiy and phiz. Contour lines mark areas of equal diffraction quality.

Matthew Bowler (ESRF)

Bowler M.W., Guijarro M., Petitdemange S., Baker I., Svensson O., Burghammer M., Mueller-Dieckmann C., Gordon E.J., Flot D., McSweeney S.M. & Leonard G.A. (2010) Acta Cryst. D Aug. 66(8):855

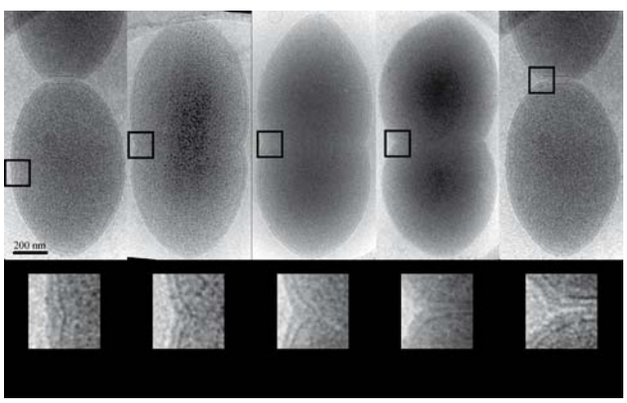
References

[1] J. Gabadinho, *et al.* J. Synch. Rad. 17, 700-707 (2010). [2] T. Warne, *et al.* Nature 454, 486-491 (2008). [3] M.F. Incardona, *et al*, J. Synch. Rad. 16, 872-879 (2009).

News from the platforms

The new POLARA

A new 300kV cryo-electron microscope arrived at the IBS in February 2010. The purchase of this microscope was a joint PSB effort and IBS, UVHCI and EMBL have all contributed to securing funding for the POLARA that cost over 2M€. After some laborious assembly and extensive testing, the FEI TECNAI POLARA microscope is today fully operational. First the microscope will be dedicated to PSB in-house projects, then it will also be opened to external users for suitable projects.



Different steps of *Streptococcus pneumoniae* division imaged by cryo-electron microscopy (A-M. Di Guilmi & G. Schoehn).

Why did the PSB invest in such an expensive microscope?
The answers are found in the specifications of the microscope. Due to its ultra-stability, 3D structures of biological objects such as e.g. icosahedral viruses have been solved to near-atomic resolution using the POLARA. The POLARA also offers great convenience and efficiency in data collection. Samples can be preserved for days at liquid nitrogen temperature with no apparent contamination which significantly extends the data collection time on a good sample. The (4K by 4K) pixels CCD camera on the microscope gives users the choice to record digital images directly. Automatic data collection will also be possible in the future. In addition, the microscope is equipped with an energy filter that increases the signal-to-noise ratio by removing inelastically scattered electrons and allows elemental analyses by energy loss electron spectroscopy. Last but not least, the POLARA is the only microscope designed and dedicated to cryo-electron tomography in Grenoble. Tomography makes possible the study of unique objects like membrane viruses, cellular organelles, such as mitochondria and chloroplasts, as well as small cells and cell sections. It also allows *ab initio* 3D models of proteins to be determined for higher resolution refinement using single particle analysis. In summary, the new microscope increases the range of objects we can study and the resolution accessible by electron microscopy at the PSB.

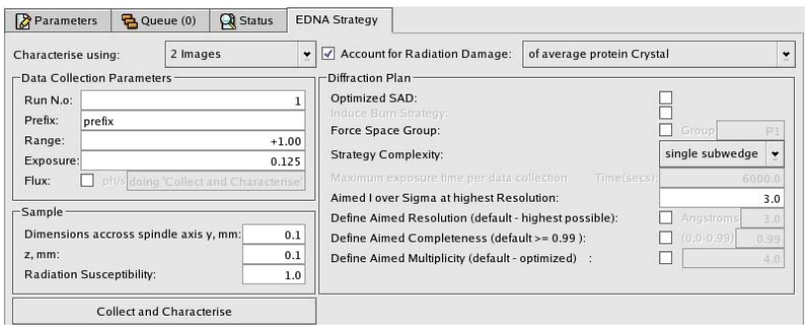
Which samples did this microscope look at so far?
A wide range of samples including viruses, bacteria, mitochondria, small complexes in the range of 200kDa, ESCRT complexes and ribosomes have been studied with the POLARA so far. Both contrast and resolution met our expectations. All users have been delighted by the stability of the microscope ("rock stable" quoting Kevin Knoops from EMBL referring to the microscope). This microscope is a powerful tool that will help to answer important questions in biology. We are all well aware how fortunate we are to have this wonderful microscope in Grenoble and are enjoying using it.

Wai-Li Ling (IBS) & Guy Schoehn (UVHCI).

The Inaugurating ceremony of the New Microscope will be held on the 11 February 2011 at the IBS.

EDNA – The BEST way to collect data

EDNA is a pan-European, collaborative on-line data analysis project that has developed a framework for plug-in based applications. EDNA was initially developed for preliminary on-line analysis in macromolecular crystallography (MX) experiments but today its remit includes BioSAXS experiments, tomographic reconstruction and more complete structure determination pipelines.



In MX experiments EDNA replaces, the DNA characterisation and data collection strategy calculation software which had been running on the ESRF MX beamlines for many years. EDNA streamlines this process using RADDOSE to calculate accurate estimates of crystal lifetime in the X-ray beam and BEST to produce optimised data collection strategies. A particular feature of EDNA is that it can produce strategies consisting of multiple wedges– where each wedge may have different experimental parameters. This provides the opportunity to collect complete and high quality data in a highly efficient manner.

At the ESRF, EDNA is accessed through the beamline control GUI mxCuBE. The user selects how many reference images are to be collected and inputs the maximum and minimum dimensions of the crystal in the beam. Using the composition of an 'average protein crystal' in RADDOSE calculations, a data collection strategy will be proposed and written directly to the 'Queue' sub-tab of mxCuBE. Beamline flux and beam size are measured in real time and are passed to EDNA for use in calculations. Other parameters can also be defined, such as multiplicity, I/σ(I) in the outer resolution shell and data collection strategy complexity (i.e. how many different wedges).

All characterisation information is stored and accessible in ISpyB and is also available in the 'EDNA Log' tab of mxCuBE . In the near future a 'burn' feature will be implemented. Here, a crystal - or region of a crystal - can be sacrificed so that its radiation tolerance can be measured directly and subsequently used for other positions within the crystal or for other crystals of the same type. EDNA will also be modified to produce 'helical' data collection strategies, where the fact that the crystal is translated during data collection is taken into account.

EDNA has been in use at the ESRF for nearly a year with excellent feedback from users.

A version of EDNA has been developed especially for use with the new Pilatus 6M detector on ID29. Longer term developments will see the implementation of a data collection strategies taking advantage of mini-kappa goniometers and EDNA controlling automatic data reduction on the ESRF MX beamlines. A BioSAXS pipeline is also being developed in collaboration with Diamond Light Source and EMBL Hamburg. This will help automate data reduction and ab initio model calculation in such experiments.

To find out more about the EDNA crystal characterization option in mxCuBE please see the ESRF webpages: http://go.esrf.eu/EDNA

Elspeth Gordon (ESRF)

Training & Education at the PSB

Training course tutored by Postdocs

A training course entitled "How to use the PyMOL* software", has been organized on the 13th July 2010 in the computing/training course room of the ESRF. Thirteen participants from the different PSB institutions, mainly PhD students, attended the session tutored by Cyril Dian (IBS), Babu Manjasetty (EMBL) and MariaRosa Quintero (IBS) all three currently postdocs at the PSB.

The aim of the course was to provide a basic understanding on how the program works and how to use it to prepare high quality figures for posters and publications. During a full afternoon, participants could learn the basic commands to run PyMOL and get familiar with the software from a few examples that were prepared in advance for them by the three tutors.

This course initiated by the PSB student committee led to a productive and successful experience for both students and trainers.

The handout and all the example files can be found at on the PSB website (http://www.psb-grenoble.eu/spip.php?rubrique46).

MariaRosa Quintero (IBS)

**PyMOL has been developed by Warren L. DeLano and is a user-sponsored molecular visualization system on an open-source foundation.*

PDBRoadshow stopped by the PSB

The PDB roadshow took place on September 27 and 28, 2010 at the ESRF training course room (MTBF) and was an opportunity for 28 PSB scientists to discover both basic and advanced tools developed by Protein Data Bank Europe (DBe). The morning session was dedicated to data deposition and annotations while the afternoon session focused on more specialized tools such as PISA (quaternary structure and interface analysis), PDBeFold (Structural alignment and analysis) and PDBeMotif (search for similar motif, active site, etc...). After a presentation of these tools by Glen van Ginkel and Martyn Symmons from EBI-Hinxton (UK), we tested these tools on our favourite proteins, thanks to the computers installed in the MTBF room. We missed time to test all the possibilities of this very elaborated set of services but it was enough to realize how they could truly help us for structural analysis.

Dominique Housset (IBS)

PSB gets involved in the ERASMUS-MONDUS MASTER program

The proposal entitled "master in BioHealth Computing" presented by the Pharmacy and Medical School of the Joseph-Fourier University (Grenoble) has been selected by the Erasmus Mundus Master program in 2010. The successful consortium composed of five European universities (Torino, Barcelona, Maastricht, Cluj-Napoca and Grenoble) and associated partners from the private sector proposes a multidisciplinary program integrating clinical research, environmental animal health, molecular biotechnology and modelling computational mathematics. It aims to develop new tools and train future experts in the field of predictive medicine based on fundamental research data. Similar integrated masters training courses already exist in the USA but not in Europe. This one year MSc programme targets students who have already completed one year MSc course or obtained an equivalent degree in clinical research, environmental and animal health, molecular biotechnology or computational mathematics. The Master course is structured in two semesters and prepared at two different Partner Universities, each of them awarding ECTS credits (30:30 ECTS).

PSB Contact: Emmanuel Drouet (UVHCI)

Partners of the consortium:
Academics: Université Joseph-Fourier Grenoble, Universitat de Barcelona, Università degli Studi di Torino, Universitatea de Stiinte Agricole si Medicina Veterinara Cluj-Napoca, Universiteit Maastricht.

Private sector partners: Floralis (Grenoble), Parc Cientific de Barcelona (Barcelona), BioIndustry Park del Canavese (Torino) and BioPark (Archamps-Genève Technopole), Centre Hospitalier Universitaire (Grenoble-La Tronche), Clinic Hospital (Barcelona) and Teaching Hospital (Cluj-Napoca), Lyon BioPôle, Rovaltain PôleEcotox, Sanofi-Pasteur, BioMérieux, Mérial and SoBioS.

Opinion

Being a Postdoc at the PSB

The post-doctoral researcher is a somewhat vague description for a vast group of Ph.D. holders who are actively involved in research but do not hold a permanent position. Post-docs come with previous research experience, which enables them to devise and direct their own research, supervise less experienced scientists and in some cases seek their own funding.

Often motivated by a never-ending sense of curiosity, the post-doctoral scientist has chosen to pursue a career in basic research: "There are so many questions to be answered.., new discoveries to be made and new challenges to be accomplished," says Maxime Cuypers (ILL). After the completion of a Ph.D. degree, a post-doctoral position is an attractive choice for the young investigator interested in a career in science "as you can really focus on research," says Leandro Estrozi (EMBL). But never far from the back of the mind of a post-doc is the prospect of obtaining a permanent position, a challenging undertaking which requires a strong track record in research and, of course, good publications. The post-doctoral period also offers an opportunity to reflect: What defines a good post-doctoral stage? Is it determined by the number of publications or whether it helps you secure a permanent position? What is the best way to progress your research? What defines good research or a good scientist? "We have to ask ourselves what kind of researcher we would like to be," considers Simon Trowitzsch (EMBL).

In many cases post-doctoral training is not carried out in the same institute as the Ph.D. thesis. As with any other job, moving cities or countries presents as many challenges as it does benefits. Working abroad can be a great experience with the chance to be exposed to different societies and cultures, but it can also be stressful on a personal level particularly for families or long-distance relationships. According to post-docs at the PSB, living and working in Grenoble is a rewarding experience and, while the city itself is not a bustling metropolis, there is a lively social environment on offer. Many post-docs speak highly of living so close to the mountains and having prime access to all the associated activities. The French language does appear to prove a challenge for most foreign post-docs. Speaking French is not necessarily needed to work within the PSB scientific community but it is nonetheless encouraged and free lessons are available to foreign scientists.

Being a post-doctoral fellow at the PSB has many advantages as this international site provides a unique environment with excellent facilities and scientific expertise. "Here the richness of competences is great and people are very motivated," explains Sylvie Chenavas (UVHCI). Despite a shared interest in Structural Biology, there are many differences between institutes that comprise the PSB. For post-docs, the mix of national and European research centres can mean variations in salary, duration of contracts, medical care and benefits. There are also important distinctions in the career paths available.

The future is an open question for many post-docs. At the PSB, most post-docs say that they would like to stay in research. However, "there are not positions for all post-docs to stay in academia and some will have to find alternatives," points out Andrés Palencia (EMBL). Perhaps the PSB could offer career advice, scientific writing or other professional training courses to help guide these young scientists towards a rewarding future. "We don't really know what to do after completing our post-doc position and more information on career opportunity would be useful," says Meike Stelter (ESRF).

On a scientific level, collaborations between the different PSB institutes are numerous and interactions at the post-doctoral level should continue to be encouraged. "It would be productive to enhance interactions between post-docs," explains Cyril Dian (IBS). The existing seminars and journal clubs are an appreciated starting point, but additional activities with more focus on the post-doc community would also be welcome. For example, social events designed to reinforce the link between the different institutes would be beneficial, as more relaxed situations often favour easier communication. All of these activities could be organised by a post-doc association that covers the component institutes of the PSB. An association which could voice the needs of these fellows and organise activities has been suggested by several current post-docs who believe they could benefit from it. How such and association would be organised and by who seem like questions worth thinking about.

MariaRosa Quintero (Postdoc, IBS)

Visits

Industrialists visit the EPN Science Campus

French Local and regional competitiveness clusters, the so-called Poles de Competivites, have been created to boost synergy between science and industry. Ecobiz Filiere Chimie Environnement and Axelera are two of the local Poles, focused on the Chemistry and Environmental sectors for Isère and the Rhone-Alpes region respectively. In 2010 both ILL and ESRF joined these two Poles bringing an opportunity for over 40 industrialists to visit the EPN Science Campus on the 18th January 2011, to discover the facilities and opportunities for industrial R&D. The visitors were impressed by the potential available and expressed their wish to follow this first step with most promising contacts to create the new synergies. There bring a very positive impact to the science done at the PSB, enlarging the scope for collaborations with complementary areas and approaches.

ESRF & ILL Business Development Offices

Spotlight

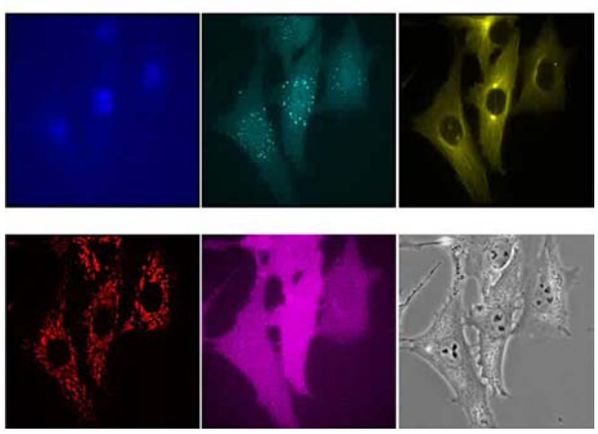
Light It Up: In collaboration with Paul Scherrer Institute, PSB Scientists develop highly efficient method for multigene transfer in mammalian cells.

A key method in contemporary biology involves the integration of foreign genetic material into cells. This can serve many purposes. Structural biologists clone genes into expression plasmids which they then introduce into *E.coli* cells, for producing proteins they then analyze by EM, NMR or X-ray crystallography. Expressing proteins in this prokaryotic organism is now common practice in molecular biology laboratories world-wide.
Introducing foreign genes into mammalian cells, in contrast, is still more of a challenge to date, especially if many genes are to be introduced simultaneously. Genetic engineering of mammalian cells with transgenes, however, is an essential need in contemporary biology. Reprogramming of somatic cells into stem cells by coexpressing an array of specific transcription factors is a prominent example.

Efficient simultaneous monitoring of many parameters in living cells with fluorescent-protein sensors is an essential prerequisite for cell biology experiments, for example if multi-component pathways are to be followed precisely. Achieving robust results in such experiments has been a considerable challenge, requiring specialist knowledge and highly trained personnel. Existing approaches were hampered by many impediments, rendering it virtually impossible to generate mammalian cell populations in which every cell simultaneously expresses all desired genes. This could now change with a new technology jointly developed by scientists at the EMBL-Grenoble and the Paul Scherrer Institute (PSI) in Villigen, Switzerland.

Some time ago, Imre Berger from the EMBL faced a similar challenge when attempting to produce large multiprotein complexes for structural studies. His team used the baculovirus/insect cell system for producing such specimens. Complexes with many protein subunits are encoded by many genes. How to ensure that the baculoviruses deliver each gene in a way that all proteins belonging to a complex are expressed at the proper levels in each and every cell in the culture used for protein expression? Imre addressed this challenge by creating the MultiBac system. MultiBac is based on rapidly engineering a single recombinant baculovirus containing all relevant genes of choice, which is then used to infect a cell culture for protein complex production. The MultiBac system is currently used by many scientists at the PSB's Eukaryotic Expression Facility (EEF).

In a recent publication in Nature Communications, Imre Berger and Philipp Berger from the PSI now teamed up to adapt this technology concept to introducing multiple transgenes into mammalian cells. The resulting technology, called MultiLabel, overcomes previous impediments by using a single multigene plasmid which is rapidly built from custom-designed, tiny progenitor DNA molecules by a method which the scientists termed "tandem recombineering" (TR). In the article, the scientists demonstrate highly efficient co-



expression of currently five genes in a transfected cell. MultiLabel also enables the generation of stable multigene expresser cell lines due to specific elements that can be readily introduced by the TR method. The viability of the transfected cells is not adversely affected, as they show the expected behaviour for example when stimulated with growth factors.

A broad range of applications will benefit from this methodology. In the present study, fluorescent markers where used to genetically tag specific proteins to study cellular processes. Protein affinity purification tags could be introduced instead, thus benefitting structural biologists who wish to produce their protein complexes in mammalian cells as an expression host.

Imre Berger (EMBL-Grenoble) & Philip Berger (PSI-Villigen).
Kriz, A., Schmid, K., Baumgartner, N., Ziegler, U., Berger, I., Ballmer-Hofer, K. & Berger, P. Nature Communication (2010) November 1 (8), 120

Events

Visit of Venki Ramakrishnan


Photo: Serge Claisse (ILL)

On the 3rd September 2010, the ILL Chadwick amphitheatre was not big enough to welcome the audience coming from all over the city. This friday, the PSB had indeed the great honour to welcome Venki Ramakrishnan (MRC-LMB, Cambridge University) who is sharing with Ada Yonath (Weizmann Institute, Israel) and Tom Steitz (Yale University, USA) the 2009 Nobel Prize in Chemistry. Venki told us a splendid story about his research activity on structure/function of ribosomes from the early work in the eighties to the high resolution structure of the ribosomes complexed with endonuclease RelE and mRNA.
Before the seminar, PSB students and postdocs got the opportunity to exchange ideas about science and scientific life with Venki around a cup of coffee at the CIBB.

Laurence Serre (ESRF, PSB coordinator)

Portuguese Associate Membership of the PSB


Photo: Serge Claisse (ILL)

Portuguese scientists working at the PSB and PSB scientists working in collaboration with scientists in Portugal: Susana Teixeira, Maxime Cuypers, Ricardo Leal and Danielle de Sanctis (from left to right).

Portugal has been a Scientific Associate of the ESRF since 1998 and previously signed Associate Membership of the PSB in 2002. The Partnership will now welcome Portugal back on Associate Membership for another 5 years. While Prof. J. Sentieiro - President of the Portuguese Foundation for Science and Technology, recently elected to Vice-President of the European Science Foundation from January 2011 - was stranded at Lisbon airport by the many problems that marked the end of 2010 for European airports, Prof. Ana Damas (Institute for Molecular and Cell Biology, Porto) represented the Portuguese scientific community last December during her visit to the EPN campus. The tour of the PSB facilities included the MX synchrotron beamlines, the ESPRIT Protein expression and HTX Crystallisation platforms, as well as the ILL Deuteration Laboratory. A buffet lunch at the CIBB building provided an opportunity for Portuguese scientists working onsite to discuss their work and the impact of their contribution to the PSB scientific output with Prof. Damas.

Susana Teixeira (ILL and Keele University)

The ERC rewards two PSB young scientists

In 2010, the European Research Council (ERC) rewarded 37 projects in Life Science. Two PSB young team leaders appeared among the awarded scientists : Jerome Boisbouvier in the NMR group (IBS) for his project entitled « seeNanoLifeInAction » on real time studies of biological nanomachines in action by NMR and Ramesh Pillai (EMBL) for his project called « Pisilence » on Small RNA guided complexe machinery for epigenetic silencing.
Both received an ERC starting grant for young investigators. Congratulations boys !!!!!

Laurence Serre (ESRF, PSB coordinator)

Newcomers


Photo: Serge Claisse (ILL)

Elena Colas

Since the 1st October 2010, Elena Colas is the new CIBB secretary. She is employed by the Université Joseph Fourier and will share her time between the CIBB and the UVHCI. After a Degree in Latine and Slovakian literature in Slovakia, Elena moved to Canada for several years and came back to Europe in 2000. In 2003, she got employed at the Université Pierre Mendès-France in Grenoble. A few years later, she obtained a permanent position at the Université Paris-Est, Val-de-Marne in the international relation department. Finally, she came back to Grenoble and chose the CIBB. We all warmly welcome Elena!

Manuel Blanc has joined the Deuteration Laboratory as a joint ESRF/ILL/Keele University PhD student.

Viviana Cristiglio has joined the D16 neutron diffractometer team at the ILL.

Announcements

February 7th, 2011 -"Advanced Energy Materials Studied by Synchrotron X-ray and Neutron Techniques". This workshop organised by the ILL, ESRF and the partners of Giant covered hot topics and recent progress in the analysis by X-rays and neutrons of the properties of materials involved in fuel cells, hydrogen storage, photovoltaic, solid state lighting and thermoelectricity, among others.
February 7th–10th 2011 - The 2011 ESRF users' meeting will take place at the ESRF site and feature three associated workshops coupled with a plenary meeting devoted to recent advances and future directions in science at the facility.
February 18th 2011 - PSB Science Day on genome sequencing and evolution. Three prestigious speakers in the field of genomics will present their latest results – Peer Bork (EMBL,Heidelberg-Germany),Chris Tyler-Smith (Wellcome Trust Sanger Institute, Hinxton-UK) and Jean Weissenbach (Genopole, Evry-France).

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 of from the EU FP6 programme. The PSB is unique in combining world leading user facilities for synchrotron X-ray and neutron scattering with NMR, electron microscopy, molecular biology and high throughput techniques on a single site together with strong projects in a broad range of structural biology, notably host-pathogen interactions.

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