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PSB *et al*

Partnership for Structural Biology Newsletter

Scientific highlights

Zooming into the heart of a killer

Variola virus (family: *Poxviridae,* genus*: Orthopoxvirus*), was responsible for smallpox, the most lethal and devastating viral infection in history. After an intense campaign of vaccination led by the World Health Organization, smallpox was declared eradicated in 1979. With the arrest of the vaccination program, a high proportion of the world population are no longer immunised. In this context, variola virus, if used as a biological weapon, could represent a serious threat to the civilian population since no specific antiviral treatment is yet available against orthopoxvirus infections.

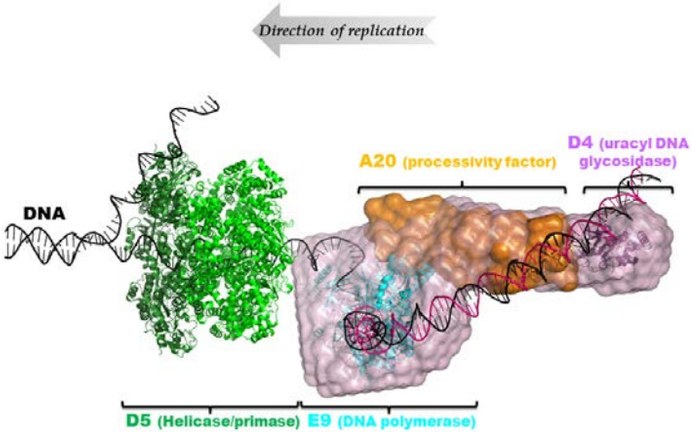


Figure 1: Model of the vaccinia virus replication fork. The envelope of the polymerase holoenzyme (E9-A20-D4) obtained by SAXS is shown. The hexameric helicase-primase (D5) shown as cartoon has been modeled using related protein domains.

Since the terrorist attacks of September 2001 the French Armed Forces Biomedical Research Institute (IRBA), which is also the National Reference Centre (CNR) for orthopoxviruses has made important efforts to develop new countermeasures against variola virus.

One line of research carried out as a collaboration between the IRBA (Institut de Recherche Biomédicale des Armées) and the "Integrated biology of persistent viruses" group at the UVHCI focuses on the structural study of essential proteins involved in poxvirus DNA synthesis. Ultimately this work will facilitate the design of new drugs targeting the DNA replication machinery, the heart of the virus, which consists of D5, a helicase-primase, and the DNA polymerase holoenzyme comprising the catalytic subunit E9, the uracil-DNA glycosylase (UNG) D4 and A20, a protein with no known catalytic activity (Figure 1).

The work presented here was performed on the proteins from vaccinia virus (the model system for orthopoxviruses) which are more than 90% identical to their variola virus counterparts. We have set up the expression of D5, D4, A20 and E9 in the insect cell-baculovirus system. After protein purification, small-angle X-ray scattering (SAXS) data collected at the ESRF yielded the envelopes of E9, of the A20-D4 heterodimer and of the E9-A20-D4 complex (Figure 1, [1]). This first structural information revealed the elongated shape of the DNA polymerase holoenzyme with a 150 Å distance between the polymerase active site of E9 and the DNA-binding site of D4. This suggests that at least 50 base-pairs of DNA would fit between the two sites [1]. Electron microscopy carried out in collaboration with the IBS EM-platform also showed the hexameric organization of the helicase-primase D5 (Figure 1). We continue to push forward to high-resolution structures of the replication complex. A first success is the crystal structure of D4 bound to the first 50 residues of A20 ($A20_{1\text{-}50}$) which are necessary and sufficient for its interaction with D4 (Figure 2, [2]). The structure shows that complex formation does not interfere with the UNG activity and DNA-binding property of D4 as these functions are located on different sides of the protein. Our structure solved at 1.85 Å reveals the contact surface engaged in the D4/A20 interaction in particular the important role of the A20 Trp43 involved in stacking interactions with Arg167 and Pro173 of D4. We further showed that point mutations of these residues disturb D4-$A20_{1\text{-}50}$ complex formation and reduce significantly its thermal stability [2]. An ongoing collaboration with X. Morelli's group (CNRS UMR 7258, INSERM U 1068, Marseille) aims at the structure-based drug design of compounds binding at the D4/A20 interface and interfering with complex assembly.

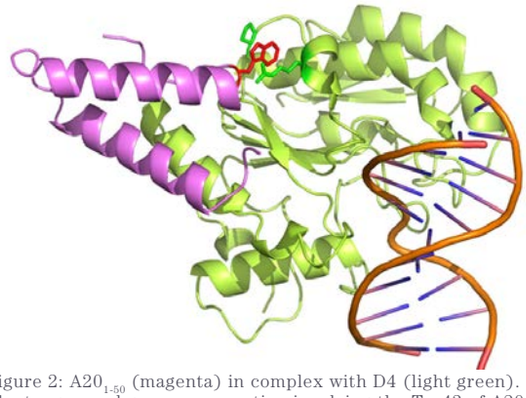


Figure 2: $A20_{1\text{-}50}$ (magenta) in complex with D4 (light green). The tongue and groove connection involving the Trp43 of A20, the Arg167 and Pro173 of D4 which forms part of the interface is shown in red and green, respectively. Using the structure of human UNG, the putative position of the DNA (orange) could be modeled.

CONTENTS

Altogether, our work gives new insights into the assembly of the vaccinia virus DNA replication fork and will promote the design of antivirals directed either against protein-protein interfaces or the active site of the polymerase complex. Such compounds would not only be precious in response to a malicious release of variola virus but could also be used to treat human poxvirus infections acquired from animal reservoirs (*i.e.* monkeypox virus and cowpox virus).

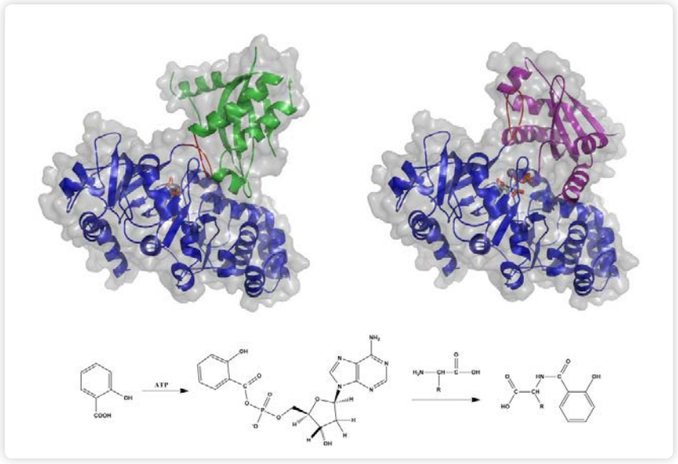
W.P. Burmeister[1] and F. Iseni[2] ([1]UVHCI / [2]IRBA)

[1] Sèle, C., Gabel, F., Gutsche, I., Ivanov, I., Burmeister, W.P., Iseni, F. & Tarbouriech, N. (2013). *J. Virol.* **87** : 1679-1689.

[2] Contesto-Richefeu, C., Tarbouriech, N., Brazzolotto, X., Betzi, S., Morelli, X., Burmeister, W. P., & Iseni, F. (2014). *PLoS Pathog.*, **10** : e1003978.

Probing enzymatic mechanisms through combined crystallography and bioSAXS

Plants use different hormones including salicylate, indole acetic acid and gibberelins to trigger diverse developmental processes, respond to external stresses and fight pathogens. Salicylic acid is a plant hormone that is critically important for defense response and often induced upon exposure to pathogenic bacteria. However, plants need to tightly control the levels of this highly active molecule. One way plants do this is to form less active amino acid conjugates of salicylate. The GH3.12 enzyme, a member of the GH3 hormone-modifying enzyme family, is able to bind salicylate, ATP and an amino acid such as glutamate or aspartate [1]. GH3.12 then performs two reactions- 1) the enzyme forms an activated acyl-adenylate intermediate and then 2) the enzyme catalyzes the addition of an amino acid, forming a stable hormone amino-acid conjugate. How a single enzyme is able to perform these two distinct reactions was unclear until a collaboration between researchers from the PSB partners - ESRF and EMBL Grenoble - and the University of Washington, USA was able to uncover the underlying mechanisms. Using a combination of protein crystallography and bioSAXS measurements, the different conformations of the protein were determined. High- resolution crystallography provided two different "snapshots" of the protein in two distinct conformations due to a rotation of the C-terminal domain [2]. This rotation was postulated to change the topology of the active site, allowing for the different reactions to occur. BioSAXS experiments were performed to monitor the conformation of the protein in solution in the presence of different substrates and products. By combining these two different types of data- high resolution static snapshots from protein crystallography and low resolution solution state data from bioSAXS, a detailed enzymatic mechanism was determined [3]. Based on the combined data, the GH3 enzymes are able to bind hormones and ATP in an open conformation and subsequently adopt a closed conformation that allows the formation of the highly reactive acyl-adenylate intermediate. This conformation likely protects the intermediate from catalytically non-productive hydrolysis and favors the second reaction- the formation of the hormone amino acid product. These studies provide the mechanism the entire GH3 family uses to perform hormone modification via amino acid conjugation. These reactions can now be manipulated and tuned in planta to favor different hormone modifications to increase pathogen resistance or alter fruit ripening, for example.



Structures of GH3.12 (top) and the two-step reaction catalyzed by the protein (below). Top left, GH3.12 in the closed conformation with the N-terminal domain colored blue and the C-terminal domain in green. Top right, GH3.12 in the open conformation with the N-terminal domain colored blue and the C-terminal domain colored purple. A flexible loop between the domains is colored red for both panels. Bottom, reaction catalyzed by GH3.12 with salicylic acid as the carboxylate substrate. The AMP-salicylate conjugate is formed in the first reaction. This activated intermediate is then conjugated to an amino acid in the second reaction.

The diverse structural and biophysical techniques available through the PSB provide critical tools to explore many aspects of enzymatic catalysis at different resolutions and in crystalline and solution states. As demonstrated by the study of the GH3 enzymes, the combination of protein crystallography and bio-SAXS is a powerful method to investigate conformational changes under different biologically relevant conditions.

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[1] Okrent, R.A., Brooks, M.D., and Wildermuth, M.C. (2009). The Journal of biological chemistry 284, 9742-9754.

[2] Westfall, C.S., Zubieta, C., Herrmann, J., Kapp, U., Nanao, M.H., and Jez, J.M. (2012). Science 336, 1708-1711.

[3] Round, A., Brown, E., Marcellin, R., Kapp, U., Westfall, C.S., Jez, J.M., and Zubieta, C. (2013). Acta crystallographica. Section D, Biological crystallography 69, 2072-2080.

Production and Analysis of Perdeuterated Lipids from *P. pastoris* Cells

A collaborative effort involving the ILL, the Free University of Brussels, the CNRS Plant Molecular Biology Institute (Strasbourg), the Laboratory for Cellular and Vegetable Physiology (CNRS/CEA/Univ. Grenoble Alpes/INRA), as well as the European Spallation Source (Lund) has been put together to optimize ways for producing biologically relevant models of cell membranes. Collecting experimental data using the same lipids found in vivo is not always possible since isotope-labelled lipids are needed for a range of biophysical techniques (in neutron scattering or NMR studies, for example), given that these can be very difficult to prepare via synthetic routes and are not commercially available.

The ability of the yeast *species Pichia* pastoris to grow in fully deuterated media, widely exploited in expression systems for producing deuterium labelled proteins, has now been used to produce fully deuterated unsaturated glycerolipids. Perdeuterated phospholipids and sterols grown in deuterated media were extracted and analysed. It was found that when yeast cells are grown in a deuterated environment, the phospholipid homeostasis is maintained - but the fatty acid composition and unsaturation level is modified. The ergosterol synthesis is not affected by the deuterated culture medium. However, remarkably, an accumulation of C18:1 fatty acid is strikingly triggered in perdeuterated lipids from this micro-organism. Lowering the growth temperature of *Pichia* in deuterated medium reduces this effect, but the level of polyunsaturated fatty acids obtained in unlabelled medium is still higher. The results [1] confirm that the production of well-defined natural unsaturated perdeuterated lipids is possible and gives new insights into the process of desaturase enzymes.

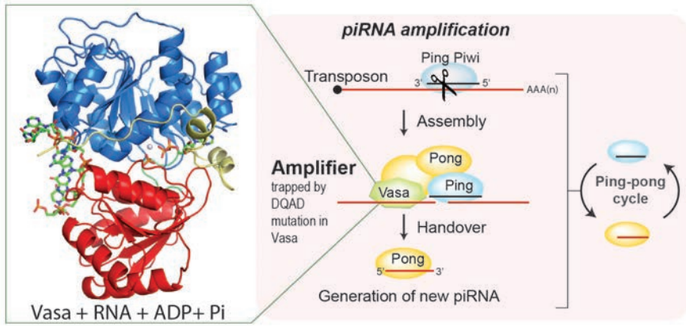
V. Laux and M. Haertlein (ILL Life Sciences Group)

[1] A. de Ghellinck, H. Schaller, V. Laux, M. Haertlein, M. Sferrazza, E. Maréchal, H. Wacklin, J. Jouhet, G. Fragneto (2014). PLoS One 9(4): e92999. (2014) PLoS ONE 9(4): e92999.

Small RNA amplification for germline genome defense

Transposons or "jumping genes" constitute a large part of eukaryotic genomes and play an important role in development and genome evolution. However, due to their potential for genomic destruction they must be tightly controlled. This regulation is normally achieved by maintaining such elements in a reversibly inactive state through the chemical modification of histones and DNA, termed "epigenetic silencing". Germlines are entrusted with the task of transmitting genetic information from one generation to the next and formation of germ cells (egg or sperm) usually involves a complete rewriting of these epigenetic markers, generally leading to a significant increase in transposon activity. To protect their genomes, animal germ cells have evolved dedicated genome defenders in the form of Piwi proteins and a set of small, 24-30 nucleotides, RNAs called Piwi-interacting RNAs (piRNAs). In all animals studied so far, piRNAs are perfectly complementary to endogenous transposons present in the host genome. Since some of the Piwi proteins are small RNA-guided endoribonucleases, target recognition by piRNAs result in their cleavage by Piwi, contributing to transposon silencing. In such a role, piRNAs can be considered an RNA-based innate immune system against internal genomic threats.

Like a true immune system, the piRNA pathway can memorize every transposon encounter and rapidly ramp up defense against the most abundant (hence most active) transposons. This is achieved via the so-called Ping-Pong piRNA amplification mechanism [1], which links the act of transposon transcript cleavage to the birth of a new piRNA. How this is achieved is not known. Our study now describes how the highly conserved germline RNA helicase Vasa acts as a RNA clamp to assemble a piRNA Amplifier complex on transposon transcripts. This complex can collect one of the transposon cleavage fragments generated by Piwi cleavage (the Ping partner) and deliver it to a new Piwi protein (the Pong partner), where it matures as a new piRNA. Thus, the target itself becomes a substrate for new piRNA production, serving as a record of



A schematic illustration of Vasa's role in the piRNA Ping-Pong cycle and the crystal structure of mutant Vasa in complex with RNA and ADP+Pi.

this encounter. We managed to capture a snapshot of this highly dynamic event by introducing a point mutation (DQAD) in the ATPase catalytic site (DEAD) of Vasa. The crystal structure of this mutant in complex with RNA and ATP was determined in collaboration with the Cusack group using data collected at the ID14-EH4 beamline at the European Synchrotron Radiation Facility (ESRF). This structure revealed that the mutation prevents release of the ATP hydrolysis products (ADP + Pi), subsequently freezing the enzyme on the target RNA with associated protein partners and allowing their identification. This work greatly benefited from the unique interdisciplinary environment provided by the PSB and its proximity to the ESRF.

These results highlight how an RNA helicase provides an ATP-controlled protein assembly platform that is critical for small RNA amplification, thereby protecting the germline genome from transposon activity and maintaining animal fertility. A similar mutational strategy may be employed for studying other RNA helicases, most of which are engaged in dynamic association of ribonucleoprotein complexes *in vivo*.

J. Xiol, P. Spinelli and R. Pillai (EMBL / UVHCI)

Xiol *et al.* (2014) Cell **157**, 1698-1711
[1] Brennecke, J. *et al.* (2007). Cell **128**, 1089-1103.

Seeing the invisible: low-populated transient conformations of proteins in the focus of solid-state NMR

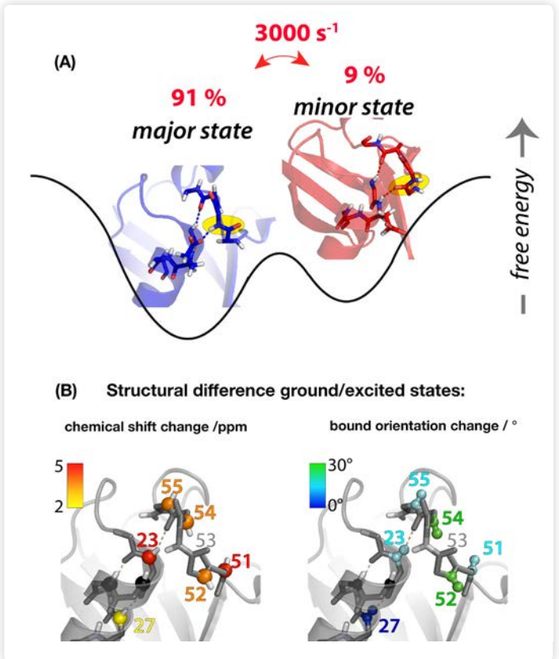
The three-dimensional structure that a protein spontaneously adopts in its environment is dictated by a subtle balance of numerous interactions, which are all individually weak. At physiologically relevant temperatures, these interactions are cont nuously rearranged, such that proteins visit a range of different conformations. The function of proteins often crucially depends on conformational states that are transient and low-populated, in exchange with a major conformer. Such transient states with life times often in the range of only a few milliseconds may, for example, correspond to binding-competent conformers in allosteric proteins [1] or to the actual functional states in enzymes [2]. Due to their transient nature, detecting and characterizing these alternative structures, that co-exist with the predominant state, represents a significant experimental challenge. Over the last years, Nuclear Magnetic Resonance (NMR) spectroscopy in solution-state has shown to be powerful to detect such states, and even determine structural models of these "excited" states [3]. Solution-state NMR, however, is faced with an inherent technical limitation: it is restricted to soluble proteins and generally only deals with proteins of rather small size. Many interesting systems in biology, such as membrane proteins embedded in liposomes, viral capsids, secretion systems, amyloids and many other supramolecular assemblies are generally out of reach for solution-state NMR.

A recently emerging variant of NMR spectroscopy, solid-state NMR (ssNMR), is not bound to these limitations, and is rapidly emerging for atomic-resolution studies of biological objects that are inaccessible to X-ray crystallography and solution-state NMR. For technical reasons it has so far been difficult to detect conformational dynamics, such as the above-mentioned rare excursions to low-populated states, by ssNMR. Researchers in the NMR group at IBS have established a novel method that provides insight into short-lived conformational states. The technique can provide information about the life times and population of these conformers, and – importantly – it can also provide atomic-resolution insight into structural propensities of these states, such as the difference in bond orientation between the readily visible "ground" state and the excited state. In a first application, the method has been applied to the crystalline protein ubiquitin, for which conformational dynamics can also be seen in solution-state, thus allowing to benchmark the method. A minor conformational state, populated to about 9 % could be revealed, in which a peptide plane is flipped, resulting in hydrogen bond and side chain rearrangements. Interestingly, the study revealed that the dynamic process in the microcrystals – which is also present in the protein in solution – is significantly impacted by the crystalline environment (Figure).

The methodology, tested on a well-characterized system in a first step, paves the way to studying challenging biomolecules that inherently rely on motion for their function: for example, it may provide important clues about the function and conformational rearrangements of membrane proteins in native-like lipid environments, or megadaltonlarge enzymatic assemblies.

P. Ma and P. Schanda (IBS)

Ma P. *et al.*, *Angew Chem Int Ed Engl.* 2014; **53** (17), p. 4312-4317.
[1] Bruschweiler, S.; Schanda, P.; Kloiber, K.; Brutscher, B *et al. J Am Chem Soc* **2009**, *131*, 3063.
[2] Henzler-Wildman, K.; Thai, V.; Lei, M.; Ott, M. *et al. Nature* **2007**, 450, 838.
[3] Bouvignies, G.; Vallurupalli, P.; Hansen, D. F.; Correia, B. E. *et al. Nature* **2011**, 477, 111.

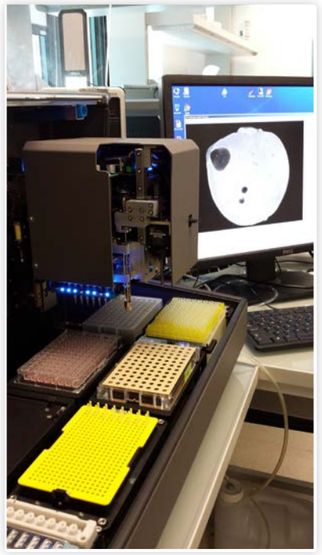


Thermodynamic, kinetic and structural insight into a transient conformational state in the microcrystalline protein ubiquitin. (A) Model of the exchange process, in which the ground state – readily observed by X-ray diffraction – is in exchange with a minor state, populated to 9%. The flipped peptide plane is highlighted in yellow. (B) Structural information of the "excited" state obtained from this technique, extracted as differences between the observable major state and the transient excited state. Left: chemical-shift differences, which are sensitive to the local environment around each nitrogen atom (spheres). Right: difference of the orientation of the H-N bonds between major and minor states. As expected, the largest bond orientation changes arise around the peptide plane which flips by ~180°. These data allow testing structural hypotheses of the excited state.

News from the platforms

High Throughput Membrane Protein Crystallisation Platform

Recently V. Gordeliy's team installed the High Throughput Membrane Protein Crystallisation (HTMPC) platform at IBS Grenoble. This crystallisation facility is equipped with state-of-the-art instrumentation dedicated to membrane protein sample characterization and nanovolume high throughput crystallisation. FTIR spectrometer and UV-Vis spectrophotometer allow a precise determination of protein, lipid and detergent content prior to crystallisation. The HTMPC platform provides a gain of two orders of magnitude in the amount of the protein needed and opens a way to structures of high impact human membrane proteins (which are normally only available in small amount). To test 96 conditions, only 5-10ul of protein are needed. The technical setup allows different techniques for crystallisation of membrane proteins: directly from detergent using standard methods used for soluble protein, and from lipidic mesophases (*in meso*, bicells and other methods, where membrane protein crystals grow inside the lipidic matrix). One of the main factors for successful *in meso* crystallisation is the ability of the protein to move laterally along the membrane bilayer. A pre-crystallisation screening assay is available at the platform to determine the diffusion rate and mobility of membrane proteins in the lipidic membranes in an automatic mode with the fluorescence recovery after photobleaching (FRAP) system. The platform utilizes an automated imaging system which uses visible, polarised and UV lights to detect crystals. The smallest crystals which can be confidently detected are about 5-10 microns. In combination the suite of devices employed provides the necessary information required for successful membrane protein crystallisation. HTMPC is in the process of becoming incorporated to the UMS. Access to user results soon will be available in real-time through the Crystallisation Information Management System (CRIMS) interface.
Situated next to the large scale facilities of the (ESRF) and the Institute Laue Langevin (ILL), the HTMPC platform is in an ideal place to take full advantage of the state-of-the-art experimental tools. This enables rapid testing, development and implementation of novel approaches to membrane protein crystallisation which can then be applied to a wide range of membrane proteins.



The HTMPC platform provides services, access to equipment and expertise to both internal and external users, from both academic and industrial communities.

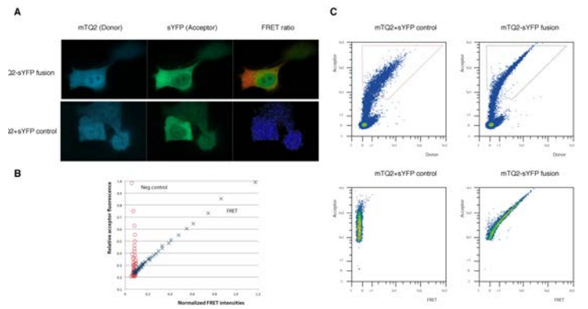
Contacts for HTMPC platform:
ibs-plateforme-htmpc.contact@ibs.fr
People responsible for the HTMPC platform: **V. Gordeliy, E. Round, F. Dupeux and V. Polovinkin**

In Cellulo Protein-Protein Interactions Revealed by FRET

To demonstrate the physiological relevance of protein-protein interactions analyzed by biochemical or structural approaches, it is often of interest to study their actual interactions in more physiological conditions. Förster Resonance Energy Transfer (FRET) utilizes the short distance interplay of well-chosen fluorophores or fluorescent protein chimera to probe for protein-protein interactions. The cell-imaging platform (IBS) provides two complementary approaches to acquire FRET signal from living cells: confocal microscopy for direct imaging of the interactions in a single cell, and flow cytometry for a statistical analysis of FRET intensities in whole cell populations.

With the help of Antoine Royant (IBS) and Joachim Goedhart (van Leeuwenhoek Centre for Advanced Microscopy, University of Amsterdam), who provided us with the enhanced Turquoise (mTQ2) and Yellow (sYFP) variants of the green fluorescent protein [1], we recently set up the optimal experimental conditions to measure FRET signal in living cells.

Using Hela cells transfected with fusion constructs of mTQ2 and sYFP, or with independent mTQ2 and sYFP proteins as a control, we show that FRET can be visualized in real time in living cells (Figure panel A). In this experiment, the acceptor protein (sYFP) is sequentially FRAPped in the nucleus region to slowly bleach its fluorescence, and the relative donor fluorescence (mTQ2) is imaged simultaneously.

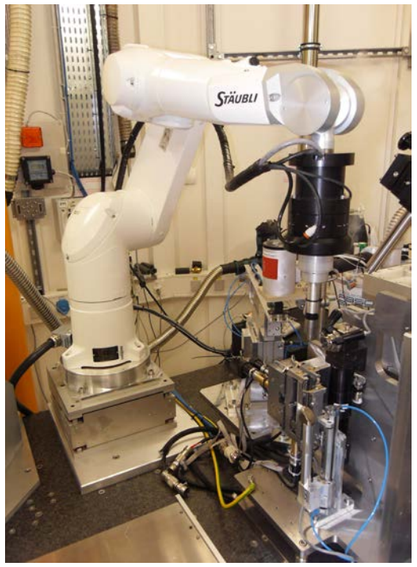


A, FRET imaging of mTQ2-sYFP fusion or control proteins expressed in Hela cells. FRET ratio is represented in pseudo-colors (rainbow LUT) where blue is the lowest value, and red the highest. B. sYFP fluorescence intensities (acceptor) plotted against FRET intensities during the sequential photobleaching of the acceptor in the cells showed in A, using a FRAP device pointed towards the cell nucleus, and set at the maximum acceptor excitation intensities (515nm). C. FRET signal measured by flow cytometry in the same transfected Hela cells expressing controls or mTQ2-sYFP fusion proteins. Top panel represent the double transfected cell population gated (dashed box) using the direct fluorescence intensities of both fluorescent proteins. Bottom panel is the relative FRET intensity channel of the gated population. The signal shift to higher values in positive cells, clearly highlight the FRET occurring in the entire cell population. Data acquisition were performed on the spinning disk confocal system and the flow cytometer of the M4D platform.

The figure shows typical cells a few seconds after the bleaching event. The plot (B) clearly shows the FRET signal correlation with the bleaching of the acceptor in the nucleus of the positive cell. In parallel, the cells can be analyzed using the multichannel flow cytometer of the cell-imaging platform (C). The dot-plots presented illustrate the high yield FRET obtained in positive cells (right) but not in control population (left). Unlike imaging that can only illustrate a few cells, and may need photobleaching strategy, flow cytometry enables a direct FRET analysis of the whole cell population in a few seconds.

F. Lacroix, RL. Revel-Goyet, and J.P. Kleman
Cell imaging platform – M4D, IBS
[1] Goedhart, J. et al. (2012). Nature Communications, 3, 751–759.
[2] http://www.ibs.fr/platforms/other-facilities-developments/time-laps-3d-4d-epifluorescence/

News from the MASSIF beam lines



The first of the three *MASSIF* (Massively Automated Sample evaluation and Screening Integrated Facility) beam lines, *MASSIF-1*, will be available for ESRF users from July 2014. This unique and highly automated end station is designed to perform sample evaluation *i.e.* sample screening and diffraction data collection using automatic pipelines based on users' demands. The beam line is equipped with RoboDiff, a robot acting both as a sample changer and goniometer in tandem with a high-capacity dewar (240 current SPINE standard vials) and a Pilatus2 3M detector. A pilot study for *MASSIF*-1 will be launched in September, and to continue for six months, to evaluate user demand and advance the level of reliability of automated diffraction data analysis and collection pipelines. During this phase, scientists from ESRF BAGs, including members of the PSB are strongly encouraged to send their samples to *MASSIF*-1 to have them automatically evaluated. Feedback on the experiments performed and results will be returned to users via ISPyB. More information on these processes can be found here: www.esrf.eu/MXPressE

MASSIF-3, which will become operational from December 2014, will provide a highly intense beam of about 10µm in diameter in size at the sample position and will be equipped with an EIGER detector capable of recording images at a frame rate of 750 Hz. This setup not only allows diffraction experiments from very small samples, but also opens perspectives to perform time-resolved experiments at both cryogenic and room temperatures.

C. Mueller-Dieckmann (ESRF)

Events

GRAL-48H

Within the framework of the Labex GRAL (Grenoble Alliance for Integrated Structural Cell Biology), the first GRAL-48H Workshop took place in Autrans on April 7th and 8th 2014.

This meeting gathering 100 participants from the GRAL community (iRTSV, IBS, UVHCI and their PSB partners), was a great opportunity to get a global overview of the multidisciplinary research performed in the GRAL partner institutes. The workshop consisted of four oral sessions including key lectures by three outstanding international speakers, Prof. Paul Jarvis (Oxford University, UK), Prof. Guy Cornelis (Namur University, Belgium) and Prof. Dorit Hanein (Sanford- Burnham Medical Research Institute, USA), as well as a poster session. Three of the oral sessions were dedicated to the presentation of the Labex's pilot projects on Host-Pathogen Interactions; the Compartments of the Chloroplast and its Substructures; and Methodological Developments. The fourth session was dedicated to other ongoing projects developed within the GRAL community.

A strong emphasis was dedicated to young researchers that were able to present their work through the poster session and a 2-minutes "flash presentation". The participants were very pleased with the content, the location and the organization of these two days and wished for the meeting to take place again every 18-24 months. The organizers would like to thank all participants and confirm that this will be done!

C. Guillouet (GRAL Executive Manager)

Inauguration of the new IBS Buildings

Friday 21st February saw the inauguration of the new IBS building, which was funded by local and regional authorities within the framework of a CPER contract (Contrat de Projets Etat-Région) and the Plan Campus. The building, which is beautifully finished, fits in well within its environment as it reflects surrounding mountains and the changing colors of the sky.

It offers a unique environment for researchers to decipher the living world at a molecular level. The fifteen IBS groups occupy four floors of laboratories, platforms and offices. Administration, support units and two platforms (NMR and electron microscopy) share the ground floor, which offers also a seminar room for up to 120 persons and a large hall, very convenient for conferences and meetings. That's where the inauguration and common reception took place in the presence of Geneviève Fioraso, French Minister for Higher Education and Research, high officials from Rhône-Alpes and Grenoble area, many dignitaries as well as representatives of the PSB partners.

As 2014 has been declared by Unesco the "International Year of Crystallography", the IBS groups now have at their disposal a unique environment for furthering knowledge of the molecular and cellular architecture and dynamics of living organisms. Exciting times ahead!

O. Kaikati (IBS)

PSB student day 2014

On the 27th of January 2014, the sixth edition of the PSB student day was opened by a scientific seminar given by Hugues Nury (IBS).

Throughout the day, the Chadwick Amphitheater has seen second and third year PhD students: Nick Aschman (UVHCI), Filippo Romoli (ESRF), Samuel Lenton (ILL), Jorge Dias (EMBL) and Louise Lassalle (IBS) giving talks about their research projects. During two clip sessions, 27 first year PhD students also introduced themselves. A large audience attended to the event and took the opportunity to discuss around poster sessions, during lunch and coffee breaks, in a friendly atmosphere.

Philippe Chinkirch opened the afternoon by a presentation of the "ValoriDoc" association (http://www.valoridoc.net/) which is designed to help PhD students jumpstart their future carreers. As usual, the closing ceremony rewarded the Best Poster and the Best Clip. Alice Tissot (UVHCI) was awarded the Best Clip prize and Widade Ziani (IBS) was rewarded for the Best Poster.

On behalf of the PSB student committee, I would like to sincerely thank our PSB scientific coordinator, Florent Bernaudat, for his precious help. Congratulations to all the participants and we look forward to seeing you next year!

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

Fluorescence Day

On March 17, the first "Fluorescence Day" gathered about 70 people in the IBS2 seminar room. The goal of the meeting was to get to know who does what in the field of biological fluorescence in the Grenoble area and to encourage new collaborations. The different instruments/platforms and advanced methods available in several Grenoble institutes (IBS, EMBL, IAB, iRTSV, LiPhy, IAB) as well as many exciting biological projects that significantly rely on the use of these techniques were presented by 13 speakers. It appeared that biological fluorescence-based research in Grenoble is very active, from fluorescen protein's developments and super-resolution microscopy to spectacular live cell imaging and optogenetics applications.

The audience appreciated the mixing of both sides of the Grenoble fluorescence community (developments and applications, but also East and West) and it was suggested that the meeting should take place every year. In 2015 it will be organized by the "East" laboratories.

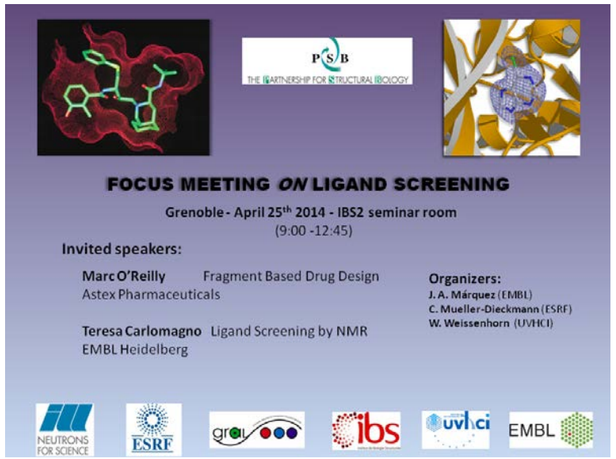
D. Bourgeois (IBS)

FOCUS MEETING *ON* LIGAND SCREENING

The PSB "FOCUS MEETING *ON* LIGAND SCREENING" took place on the EPN campus on the morning of the 25th of April 2014 and gath red more than 50 participants in the IBS seminar room. The purpose of this meeting was to help identify unique opportunities to establish pipelines for efficient ligand screening within the Grenoble area. The meeting opened with a lecture by Marc O'Reilly (Astex Pharmaceuticals, U.K) on the challenges of fragment based drug discovery, then followed by the presentations of several technical platforms located at the PSB and iRTSV (CEA Grenoble), on their latest developments and possible applications in ligand screening. After coffee, the meeting continued with 5-minutes clips from PSB and iRTSV groups on potential high-value targets that could be studied in pilot projects for the development of new ligand-screening pipelines. A closing lecture by Teresa Carlomagno (EMBL, Heidelberg) informed the audience on the structural mechanisms of drugs studied by NMR.

In the afternoon, an open round table was organized with the external speakers, group leaders and platform managers (approximately 20 participants). The external speakers contributed greatly to the discussion which proved to be very lively and fruitful, leading to potential future new collaborations and interactions.

F. Bernaudat (PSB Coordinator)



Neutrons in Biology and Biotechnology 2014 meeting

The Neutrons in Biology and Biotechnology (NIBB) meeting took place at the ILL (19th-21st February). This biannual meeting has evolved over the years from technical aspects relating to neutron techniques to become a more science driven event with strong emphasis on interdisciplinary methods with neutrons occupying a central role alongside the other major techniques including X-rays, NMR, EM, ... This is very well illustrated within the PSB.

The meeting focused on highlighting recent results in fundamental biology and biotechnology, as well as considering the future of the field and the implications for the type of science that can be considered as well as for the instruments required. The 2 day long meeting offered cutting edge scientific talks on recent results with great examples of the unique information obtained by using interdisciplinary techniques. A colloquium by Tim Hunt (UCL, Nobel Prize 2001) entitled "Switches and latches: The control of entry into mitosis" followed the meeting. With 105 participants on a wide variety of subjects, NIBB 2014 was a very successful meeting. Younger scientists were very well represented and contributed greatly to the dynamism of the event.

E. Mossou (ILL/Keele University)

PSB Scientific Advisory Board Review

The biennial PSB review by the Scientific Advisory Board (SAB) took place on 30-31 October 2013. The SAB is an international committee composed of eminent scientists in the field of structural biology and the members present were: Anthony Watts (Chair, Oxford U., UK), Félix Rey (Institut Pasteur, France), Helen Saibil (Birkbeck College, UK), Michael Sattler (Helmholtz Zentrum München, Germany), Gunter Schneider (Karolinska Institute, Sweden), Titia Sixma (Netherlands Cancer Institute, The Netherlands), Vladimír Sklenář (Mazarik U., Czech Republic) and Joel Sussman (Weizmann Institute, Israel) - David Stuart (Oxford U., UK) and Elena Conti (Max-Planck Institute, Germany) were also invited but unable to attend.

On the 1st day of the review the SAB visited various PSB platforms as well as the newly opened IBS building. The SAB then attended a clip session during which 20 PhD students and postdocs, from all five institutes of the PSB, presented their work through 90 seconds presentation. The session was then followed by a cheerful PSB Get Together in the IBS foyer that enabled everyone to meet with the SAB members in an informal setting.

On the 2nd day, the SAB attended a series of presentation describing some of the latest developments on PSB platforms as well as two scientific talks by young researchers, Bárbara Calisto (ESRF) and Guillaumme Communie (IBS, UVHCI). The SAB members also shared a coffee break with a dozen of students (in the absence of supervisors) and interviewed them in order to get a feeling of their working experience in the PSB.

The review ended by a closed working lunch for the SAB committee which then fed back the PSB Steering Committee and Science Board with comments and advices for the future of the PSB. The SAB was especially impressed by the level and quality of the work presented, as well as by the enthusiasm of the students, and we thus would like to thank all the participants for their contribution.

F. Bernaudat (PSB Coordinator)

ESRF UM2014 and fond farewell to ID14

A special symposium on the 3rd of February 2014 to commemorate the closure of the ID14 complex was organized in conjunction with this year's ESRF users' meeting. Over 100 people attended the "Celebrating 15 years of scientific achievement on ID14" symposium at the IBS2 and heard how the vision of ID14 was realized (S. Wakatsuki) and some of the amazing structural discoveries it contributed to.

The talks ranged from molecular details on the fundamental biology of photosynthesis (I. Andersson and N. Nelson) and protein synthesis (Nobel laureate Prof. Venki Ramakrishnan) to the intricacies of how the innate immune complement system works. The success of the symposium is probably best summarized by Prof. Piet Gros, who remarked that "ID14 probably made my scientific career in the complement field".

A. McCarthy (EMBL) - on behalf of the organising committee

Training the next generation of structural biologists

The month of June saw twenty young scientists from seventeen different countries spend a week at the PSB learning how to solve challenging problems in structural biology. The group was attending the EMBO Practical Course on the Structural Characterization of Macromolecular Complexes, an event organised biennially on the EPN campus since 2002.

Hailing from laboratories from across Europe, the US and Mexico, the twelve PhD students and eight postdoctoral fellows all shared a keen interest in the 3D architecture of macromolecular complexes. During their stay, course participants learned how to identify, produce and purify complexes, analyse macromolecular interactions through biochemical and biophysical techniques, and structurally characterise

complexes using X-ray crystallography, small-angle scattering, electron microscopy, NMR and mass spectrometry. Course lectures were given by 23 local and external speakers, including many leading experts in their field. Many speakers recounted their own personal experiences as they struggled to characterise the structures of multi-subunit assemblies such as the spliceosome, RNA polymerase III, and nucleosome- or ribosome-bound complexes.
The talks were complemented by practical sessions run by local tutors, allowing students to gain hands-on experience with techniques and instruments presented during the lectures. For example, students prepared sample grids for electron microscopy, analyzed a protein-protein interaction by NMR, and carried out measurements by CD spectroscopy and microscale thermophoresis – in many cases using their own samples.
Past editions of this course have consistently received highly positive feedback and this year was no different. In the final course evaluation, one workshop participant wrote "Overall this course was just like I hoped it would be. The lectures were given by outstanding experts in their fields, the tutorials and practicals were interesting and helpful and the social events were very entertaining." Another wrote: "I personally benefited immensely towards my project from the experts as well as from fellow students' experience. I liked the most that the tutors were very friendly and helpful. It was great that we did social activities together so we could ask any question any time. Thank you for organizing such a great course!" The continuing success of this and other related courses held on the EPN campus bears witness to the outstanding research environment of the PSB and its privileged position as a training center for budding scientists aspiring to pursue ambitious projects in structural biology.

Course details and lecture notes are available at
http://www.embl.fr/embo2014/
C. Petosa (IBS)

Native mass spectrometry

Native mass spectrometry (MS) represents a powerful tool to study the architecture of protein complexes when high-resolution structural data are not available. On January 23rd 2014, the IBS hosted the first symposium "Native MS and its Applications in Structural Biology", organised by the MS team of the IBS and sponsored by the company MS Vision. The 60 participants listened to the presentations given by three international speakers and five local ones. In the first session Dr Barran (Manchester University) illustrated the use of MS to characterise biosimilars; Dr Snijder (Utrecht University) described his work, probing the limits of MS to analyse megadalton assemblies (mainly viruses); Dr Uetrecht (European XFEL, Hamburg) discussed the combination of native MS with X-ray free-electron lasers to resolve the structure and dynamics of biomolecules. Then, Dr Masselon and Hentz (both at CEA, Grenoble) illustrated their efforts to develop the next generation of instruments whose sensitivity will be boosted by nano-electromechanical detectors. Dr Signor and Dr Forest (both from the IBS) provided an overview of the IBS MS facility activity and hydrogen/deuterium exchange MS, respectively. Finally, Dr Boeri Erba discussed her research activity at the IBS using native MS and gave also a practical demonstration of the IBS mass spectrometer specially modified to analyse intact protein complexes (see photo). Encouraged by the excellent feedback from attendees, the IBS MS team will continue to communicate the latest developments in the field of native MS and to promote its use within the structural biology community.

Photo: © CEA/www.denis-morel.com

E. Boeri Erba (IBS)

IYCr 2014 International Year of Crystallography

Crystallography encompasses a range of areas and techniques where developments are often symptomatic of general technological and conceptual trends. The IYCr sets the scenario for timely discussions on the role of Science in society. Crystallography has a pivotal role within Structural Biology and is also a powerful dissemination tool, where the naive beauty of crystals is an undeniable asset.

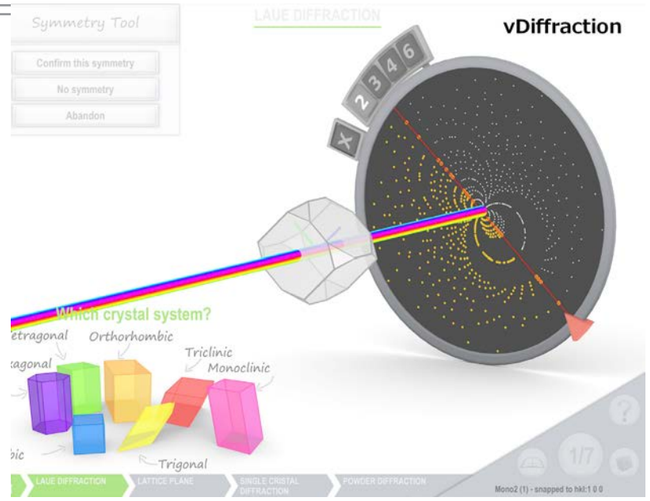
Early this year an official inauguration ceremony (where the PSB partners were present) took place in Paris to set the pace for a busy year. Worldwide events and crystallographic tools are listed on the IYCr[1] and other websites, such as "100 ans de Cristallographie"[2], delivering information to scientists and the general public. Local events included seminars last March by Ada Yonath and Jean-Marie Lehn, 2009 and 1987 Chemistry Nobel prizes, respectively. Companies such as STOE, DECTRIS and Xenocs also adhered by setting up an Open Factory, to take place in September 2014. This is an intensive training and will be split between Grenoble (ESRF and Xenocs) and Darmstadt.

Pedagogical projects and audio-visual tools for all levels of Crystallography expertise have been set up apropos the IYCr, such as an international crystal growing competition. An interactive application to teach the principles of crystal diffraction – vDiffraction (see image for a sneak peek) - was also designed by Alain Filhol (ILL) and introduced at the inaugural ceremony of the IYCr. A test version will soon be released, where a collection of photos of biological crystals will provide examples of research samples.

The PSB community is here called to contribute! Send us images of unique crystals for consideration either to vDiffraction or to decorate future issues of the PSB Newsletter. Not yet graced with a beautiful crystal? Then you can visit the IYCr website to find out more about the many reasons that make us Crystallographers proud.

S. Teixeira (ILL/Keele University)

[1]http://www.iycr2014.org/events/lectures/lectures-la-cristallographie,-une-clef-dela-connaissance
[2]http://www.echosciences-grenoble.fr/sites/100-ans-de-cristallographie



The 1st PSB Blue Symposium

The 1st PSB Blue Symposium took place at the EPN Campus on June 27th, 2014. During the morning session, John Mattick (Garvan Institute, Sydney) and Martin Jinek (University of Zurich) explained the role of RNA in genome programming and editing and Yvonne Jones (University of Oxford) described surface assemblies in signaling systems. The afternoon session featured Christopher Dobson (University of Cambridge), illustrating mechanisms of amyloid formation and their role in disease, and Chris Tate (MRC, Cambridge), describing structure and function of G protein-coupled receptors. Young local scientists from EMBL, UVHCI, IBS, ILL, ESRF and IAB presented their work on structure, dynamics and RNA biology throughout the day. The Symposium provided a platform for scientific exchange and was followed by a barbecue at the ESRF chalet.

M. Stelter (IBS)

PSB Dynamics

The PSB now and then: a perspective from W. Stirling

I consider the PSB to be one of the most important initiatives during my period as Director-General of the ESRF (2001-2008). The idea of uniting the different actors in structural biology on our site with external colleagues, including strong linkage to international user communities and the pharmaceutical industry, was already being discussed when I arrived. Over the next few years the project evolved to be more centred on EMBL, ESRF, ILL, and the IBS (involving the CEA and the CNRS), working alongside scientists within the UVHCI unit. Today more than 300 scientists are involved in biology within the PSB. I always believed that the Partnership for Structural Biology – the unimaginative but accurate title that we agreed on – was a very important step for the ESRF and the ILL. Pooling our resources, both intellectual and practical, in the crucially important fields of life sciences seemed totally evident to me, even with a physicist's background. This was not, however, the opinion of everyone and I had to battle some rather negative opinions from certain of the ESRF's governing organisations. In the end we convinced them of the interest for our institutes and their users. Working out how to finance the Carl-Ivar Brändén Building required patience and application, but working together with EMBL and IBS we managed what seemed impossible at the outset and eventually this wonderful laboratory building was constructed. It was gratifying to see the EPSRC commitment to the PSB in their funding for the Deuteration Laboratory. The CIBB was officially opened on January 13th 2006. Having the indefatigable Sine Larsen pushing me – and everyone else involved – was a great help. The development of the PSB has continued apace leading to the vibrant and productive partnership of today. The technical platforms have grown in number and complexity from the 10 that were installed at the outset to the 23 that we now have. Visiting the CIBB is an uplifting experience. Seeing all of these young (and some not so young) people working together on problems of very real importance makes all the interminable meetings and discussions a decade ago seem very worthwhile. I am proud to have been involved with many colleagues in the foundation of this remarkable joint laboratory. For the future I think it is essential that we continue to maximise the exploitation and development of the capabilities we have within the PSB, with a particular emphasis on outreach and the delivery of high impact scientific results to our international user communities. It is by responding to the needs of our users that our methodologies and scientific scope will be enhanced in this rapidly changing field.

W.G. Stirling (ILL Director)

PROFILE: Monserrat Soler-López

I received my PhD from the Universitat Politècnica de Catalunya in 2000, based on the structural biology of DNA and DNA-drug complexes. I gained further experience in nucleic acid crystallography at the Hauptman-Woodward Medical Research Institute in Buffalo and at Northwestern University Medical School in Chicago. I was a postdoctoral fellow from 2000 to 2005 at the EMBL, where I worked on the structure determination of proteins involved in transcriptional regulation and nuclear transport. I was subsequently involved in the set up of a semi-automated cloning and protein expression pipeline for structural studies on the bromodomain protein family. In March 2005, I joined a structure-based drug discovery biotechnology company, Crystax Pharmace ticals, and was appointed Unit Head to manage and expedite the structural analysis of diverse pharma-ceutical targets. In January 2008, I joined the Experimental Bioinformatics Lab (EBL) at the Institute of Research in Biomedicine (IRB Barcelona) as a Lab Director, where we evaluated the structural organization of chromatin at the molecular and genome-wide levels. Furthermore, we implemented diverse large-scale methods and 'omic' technologies to identify and characterize protein interaction networks associated with complex diseases, in particular Alzheimer's disease. Since April 2014, I am the Manager of the Molecular Biology Laboratory within the Structural Biology Group at the ESRF. In parallel to the support of in-house research, my aim is to combine large-scale biology approaches with more focused structural biology efforts in order to achieve a detailed, mechanistic understanding of neurodegenerative pathologies.

Newcomers

Florine Dupeux joined Valentin Gordeliy's team at the IBS in December 2013 as a CNRS research engineer to further develop the membrane protein crystallisation facility. (see "News from the Platforms" on page 5).
For more information you can contact Florine at: florine.dupeux@ibs.fr

Previously at EMBL, **Philippe Mas** has joined the CNRS-ISBG to run ESPRIT and the Thermal Shift Assay platform at the PSB. For more information about platform applications you can contact him at: mas@embl.fr

Following the closure last year of the French Armed Biomedical Research Institute (IRBA) site in La Tronche, several scientists arrived on the EPN science campus as visitors. Dr Florian Nachon, Dr Marie Trovaslet and Dr Xavier Brazzolotto joined the DYNAMOP group and Dr Anne-Laure Favier the IRPAS group at the IBS.
Dr Céline Contesto-Richefeu, Dr Frédéric Iseni and Corinne Ducournau joined the UVHCI.

ANNOUNCEMENTS

GTBio2014 Conference

The GTBio2014, the meeting of the Biology thematic group of the French Crystallographic Association (AFC) will be held in Grenoble on the EPN Campus (IBS and ESRF auditoriums), from Tuesday October 7TH until Friday October 10th, 2014. Registrations are open and abstracts for either oral or poster presentations can be submitted. Languages of the meeting will be both French and English.
Important dates to remember:
June 30th, 2014: deadline for submitting an abstract for a talk
June 30th, 2014: deadline for requesting student fellowships (see web site).
September 7th, 2014: deadline for registration and for submitting an abstract for a poster.
For more information please visit the website: http://gtbio2014.ibs.fr
Contact: gtbio2014@ibs.fr

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

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