PSB et al.
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# Down to atomic-scale intracellular water dynamics

Below: the Venice lagoon between the Italian coast and the Adriatic Sea. Water embraces macromolecular structures and flows in live cells in nourishing channels in between, almost as free as in pure water.

**Water has extraordinary properties compared to other liquids, which make it a fascinating and important theme for study, especially as it is common biological knowledge that water is essential for all living organisms.**

In modern biology, an understanding of life processes is sought at the level of atoms and molecules and their interactions, and it isn't surprising to discover that the special properties of water play an important, if not vital, role at this level.

Understanding the structural and dynamic properties of water on the molecular scale constitutes a main scientific challenge, in particular within the intracellular matrix in which biological molecules interact with each other in metabolic processes. Interestingly, the properties of intracellular water continue to provoke controversy after more than fifty years of study, so it is important to underpin statements about these properties with strong experimental data.

In a paper published in *EMBO Reports* in May of this year (see below), Marion Jasnin and her co-workers described neutron scattering experiments using deuterium labelling to directly address the nature of water dynamics *in vivo* in the cytoplasm of *Escherichia coli* bacteria. A wide range of experimental timescales was covered by the experiments, for motions on the atomic length scale.

In contrast to the widespread, if controversial, opinion that water is 'tamed' by macromolecular confinement, the measurements established that water diffusion within the bacteria is similar to that of pure water at physiological temperatures. It was concluded that liquid water flows freely inside cells, within the crowded macro-

(see page 2)

## CONTENTS

molecular environment, like the water around the islands of the Venice lagoon (see picture, previous page).

The study represents a prime example of successful collaboration between member institutes of the PSB: the IBS, the ILL and the D-Lab, which pooled their expertise to establish an important property of water in an extremely complex environment.

The first author of the study was a PhD student at the IBS; the D-Lab was essential to prepare the isotope labelled samples, which allowed the focus on the dynamics of different components; and the state-of-the-art spectrometers and expertise at ILL were essential for successful neutron scattering experiments.

**Marion Jasnin (IBS) and Giuseppe Zaccai(ILL)**

*Jasnin M, Moulin M, Haertlein M, Zaccai G, Tehei M (2008). Down to atomic-scale intracellular water dynamics.* EMBO Rep., *9: 543-7*

# Membrane remodelling by the ESCRT-III

## Members of the Weissenhorn group and their colleagues set out to reconstitute the ESCRT-III polymer to discover its potential role in the final steps of membrane fission

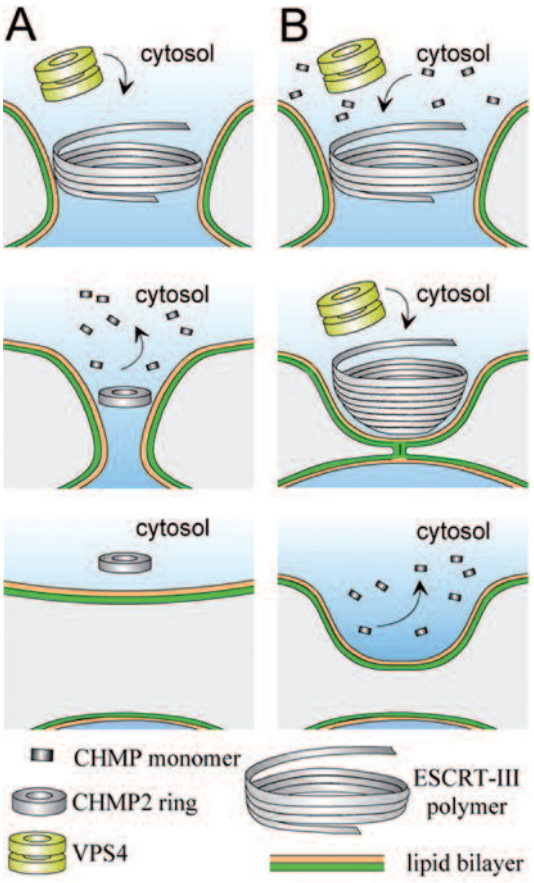
Dynamic genesis of membrane bound structures is fundamental to life. For example, during cell division, the mother cell undergoes major membrane remodeling and finally membrane scission to give rise to two independent daughter cells. Similarly, within the host cell, enveloped retroviruses such as HIV, assemble on the plasma membrane (thus becoming wrapped in an envelope derived from the host cell membrane) and by hijacking cellular protein machineries undergo membrane scission- a step essential for the release of newly formed infectious viral particles.

Interestingly, a family of proteins termed as the endosomal sorting complex required for transport (ESCRTs) have been implicated in membrane remodeling during (i) cell division, (ii) HIV-1 release and, (iii) multivesicular body biogenesis (MVB). Although seemingly unrelated, all three processes involve membranes budding away from the cyotosol. Therefore, recruitment of a common protein machinery i.e. ESCRTs is hardly surprising.

Accumulating data over the last years suggested that a sub-set of the ESCRTs called ESCRT-III form a protein lattice on cellular membranes that is subsequently disassembled by a AAA-type ATPase called VPS4. We hypothesized that such assemblies might be responsible for catalyzing the final steps of membrane fission during cell division, HIV-1 budding and MVB biogenesis.

In line with the sentiment expressed by Richard Feynman, "What I cannot create, I do not understand", we set out to reconstitute ESCRT-III polymer and study its interaction with membranes and VPS4 in a collaborative effort with Guy Schoehn from UVHCI/IBS, Jacob Piehler from the Goethe University Frankfurt am Main and Heinrich Gőttlinger from University of Massachusetts (Worcester). Access to the electron microscopy platform and the fluorescence spectrometer at the PSB were critical for the feasibility of the project.

Density gradient ultra-centrifugation demonstrated that the ESCRT-III sub-units CHMP2A and CHMP3 together formed high molecular mass structures (polymers) which recruited VPS4 and disassembled upon addition of ATP. The disassembly of these polymers in real time was further probed by a fluorescence dequenching assay, thereby consolidating the notion that the energy of ATP, which is harvested during ESCRT-III disassembly, upon coupling with the membranes could potentially lead to membrane scission. Membrane binding properties of the ESCRT-III was measured in real time on solid supported lipid bilayers of defined composition using reflectometric interference spectroscopy which demonstrated that the negatively charged lipids were essential for binding. The co-polymer bound stable to the membranes and did not exchange with either soluble or membrane bound CHMP2A or CHMP3.




A model for ESCRT-III helical polymer function. Short helical tubular structures assemble on the inside of a newly formed bud with their C-termini exposed towards the 45 nm wide cavity, which allows VPS4 interaction.

Having studied the biochemical properties of the ESCRT-III polymer the structural characterisation was done by negative stain and cryo electron microscopy. ESCRT-III formed closed and open ended helical tubular structures with a diameter of ~50 nm which is comparable to the neck width of a budding virus. VPS4 was found to be recruited through the central pore of the tube which interacted with the lipid membranes via its outer surface - a property which ensures simultaneous recruitment of membranes and VPS4. These findings directly suggest two mechanisms for membrane scission:

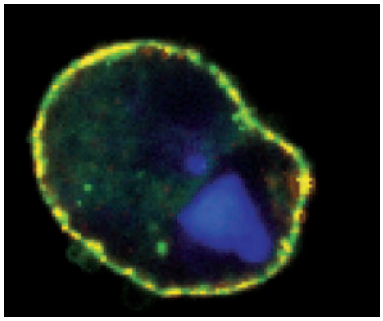
1) The ESCRT-III polymer assembles within the neck of a budding membrane and upon the action of VPS4 undergoes constriction thereby shrinking the pore and eventually fusing it to generate two separate membrane bound structures (Figure 1A).
2) The ESCRT-III polymer assembles within the neck of a budding membrane in an asymmetric manner, the membrane follows the closed end of the polymer thereby shrinking the pore. The action of VPS4 reduces the tension at the upper side of the funnel leading to abscission of two membrane enveloped structures (Figure 1B).

We now aim to test these models for further clarifying the mechanism of membrane constriction.

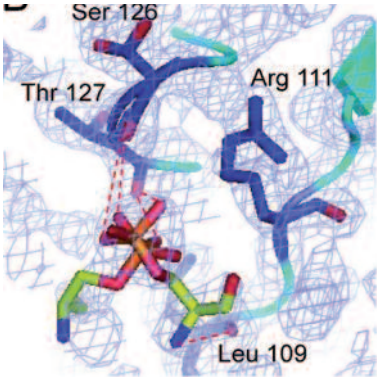
**Suman Lata and Winfried Weissenhorn (UVHCI)**

*Lata S, Schoehn G, Jain A, Pires R, Piehler J, Gottlinger HG, Weissenhorn W. (2008) Helical structures of ESCRT-III are disassembled by VPS4.* Science, *321, 1354-1357.*

# C1q tracks down early apoptotic cells



PS and C1q co-localize within membrane patches on apoptotic cells (2 h after UV irradiation).
Phosphatidylserine (annexin V labeling in green), C1q (red) and colocalization in yellow. Nuclei were labelled with Hoechst (blue).



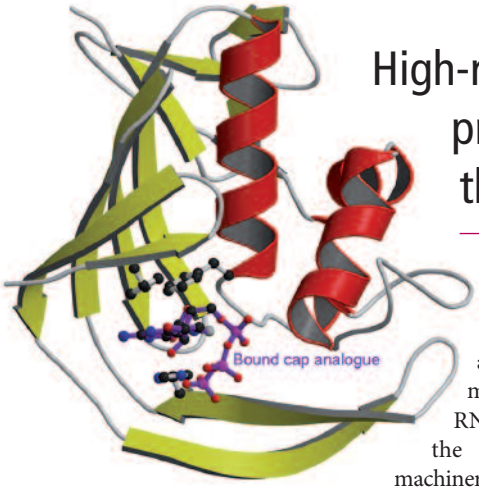

X-ray structure of a phosphoserine-C1q GR complex obtained by soaking of native C1q GR crystals into ligand-containing solutions. Detailed view of the interactions between subunit C and phosphoserine. The two alternative conformations of phosphoserine are shown. Electron densities (2mFo-Fc) are contoured at the 1 σ level.

Efficient apoptotic cell clearance is critical for maintenance of tissue homeostasis, and to control the immune responses mediated by phagocytes. Little is known about the molecules that contribute 'eat me' signals on the apoptotic cell surface. C1q, the binding subunit of the C1 complex of complement, plays a crucial role in the detection and scavenging of a wide variety of potentially noxious substances ranging from pathogenic non self to altered self, such as β-amyloid fibrils, the pathological form of the prion protein, necrotic and apoptotic cells, or modified forms of the low-density lipoprotein. In this paper a variety of cellular and molecular approaches were used to investigate the nature of the target(s) recognized by C1q at the surface of apoptotic cells. This study provides the first experimental evidence that C1q recognizes phosphatidylserine (PS) exposed at the surface of apoptotic cells. This conclusion is based on the following concordant observations: (i) C1q binds to apoptotic cells at early stages of apoptosis, well before cell permeabilization, a property that is crucial in terms of maintenance of immune tolerance. (ii) C1q binding and PS exposure proceed concomitantly, and annexin V inhibits C1q binding in a dose-dependent manner. (iii) As shown by co-sedimentation and SPR analyses, C1q recognizes PS specifically and avidly, through multiple interactions between its GRs and the phosphoserine head of PS. (iv) The majority of the C1q molecules are remarkably distributed in membrane patches where they co-localize with PS. This latter observation validates the observed C1q/PS interaction at the cellular level. The ability of C1q to recognize PS is further substantiated at the atomic level by the X-ray crystallographic analysis of a phosphoserine-C1q GR complex, revealing that phosphoserine is bound to subunit C of the C1q GR, through a site located inside the C1q cone and oriented towards the target surface.

This finding, together with the known binding versatility of C1q, sheds new light on the physiological role of C1q and provides insights into the recognition mechanism of this major actor in the clearance of unwanted self-cells and control of immune tolerance.

**Philippe Frachet (IBS)**

*Païdassi, H. et al (2008). C1q binds phosphatidylserine, and likely acts as an early, multiligand bridging molecule in apoptotic cell recognition.* J Immunol. *15;180(4) 2329-2338*




# High-resolution influenza virus protein image opens the way to antiviral drugs

Viruses are masters of cunning when it comes to hijacking the host cell. Now, in the case of the influenza virus, scientists are one step ahead.

The groups of Stephen Cusack and Darren Hart at EMBL Grenoble, in collaboration with others in the joint Unit of Virus Host-Cell Interaction (UVHCI), have identified and produced a high-resolution image of a key component of the polymerase – PB2 – that copies the genetic material of the virus and multiplies it. PB2 steals an important 'cap' molecule from host cell RNA molecules to direct the protein production machinery towards the synthesis of viral proteins, and binds it by sandwiching it between its amino acids. The cells are then tricked into making viral instead of host proteins.

The atomic resolution structure of a PB2 domain bound to a cap they generated reveals these amino acids for the first time. Whilst the recognition mechanism is similar to other cap-binding proteins, its structural details are distinct. The influenza virus uses this cap – a modified RNA base which must be present at the beginning of all messenger RNAs (mRNAs) to direct the cell's protein-synthesis machinery to the starting point – "like a password to gain access to the cell's protein-making machinery for its own purposes," as Head of EMBL Grenoble Stephen Cusack puts it. The viral polymerase binds to host cell mRNA, cuts the cap off and adds it to the beginning of its own mRNA.

Collaborators at the Centro Nacional de Biotecnologia (CSIC) in Madrid then showed that disruption of the PB2 cap-binding site prevents the influenza virus from replicating, and all the findings led to a paper published in the 4 May issue of *Nature Structural and Molecular Biology*. "This suggests that the PB2 cap-binding site is a promising target for anti-influenza drugs," says Darren. "Our new insights will help us design mimics of the cap that would inhibit viral replication and hence reduce the spread and severity of the virus."

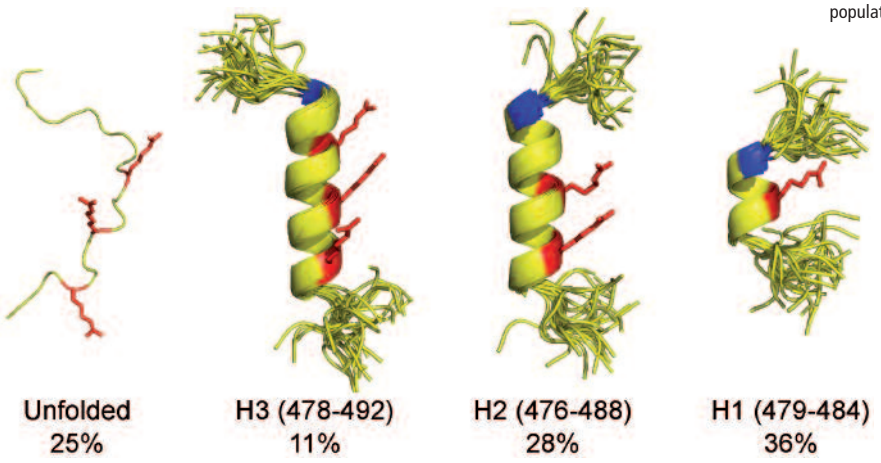
The UVHCI comprises EMBL, the University Joseph Fourier and the National Centre for Scientific Research, and was launched last year to strengthen the collaborative work going on in virology between the outstation and its neighbours. Ties with Grenoble Hospital will bring patients into the picture too.

**Vienna Leigh (EMBL)**

*Guilligay, D. et al. (2008). The structural basis for cap-binding by influenza virus polymerase subunit PB2.* Nature Structural and Molecular Biology, *15(5):500-6*

*(This article first appeared in issue 45 of* EMBL&cetera, *June 2008)*

## Quantifying nascent structure in intrinsically unfolded proteins from NMR residual dipolar couplings




Rather than randomly fraying in free solution, the interaction site of NTAIL populates a totally unfolded form (25%of the population) plus three helical conformers, each stabilized by a different N-capping interaction (blue). This projects the flexible chains in the direction of the partner protein, allowing temporary stabilization of the complex, and promoting efficient interaction of the positively charged amino acids (red) of NTAIL with the negative surface of P.

A broad category of proteins (around 35% of the human proteome) do not fold into stable three-dimensional structures but are either fully unfolded, or contain unfolded regions of significant length. Classical approaches to structure determination necessarily fail to adequately describe these Intrinsically Unfolded Proteins (IUPs) due to their inherent flexibility, and they can only be described by an ensemble of rapidly interconverting conformers. NMR spectroscopy is the only structural technique that can study the conformation and dynamics of IUPs at atomic resolution and is actively being used in the Blackledge group at the IBS to develop an understanding of these proteins.

A particularly intriguing feature of IUPs is their proposed capacity to undergo a disorder-to-order transition upon interaction with a physiological partner. In collaboration with Rob Ruigrok at the UVHCI the conformational behavior of partially unfolded NTAIL, the C-terminal domain of the nucleoprotein (N) of the Sendai virus (SeV) a paramyxovirus that causes bronchiolitis in mice and primates, has been investigated. This partially folded chain folds upon binding to another IUP, the Phosphoprotein (P), a co-factor of the viral polymerase. For the polymerase to move forward during transcription or replication P-NTAIL interactions need to be made and broken very rapidly. The mechanism of this highly dynamic interaction between two inherently flexible proteins remains poorly understood and is the subject of an active collaboration between the Ruigrok and Blackledge groups.

Here NMR, in particular Residual Dipolar Couplings that are highly sensitive to levels of structural order in IUPs, has been used to quantitatively analyze the level and nature of helical conformation in the interaction site of the pre-recognition form of the partially folded NTAIL. Rather than fraying randomly, the molecular recognition element of NTAIL preferentially populates three specific helical conformers, each stabilized by a different N-capping interaction. In addition to providing experimental evidence for the molecular basis of helix formation in partially folded chains, carrying clear implications for understanding early steps of protein folding, these results also provide novel insight into the mechanism of interaction between these flexible proteins: The specific capping of the helix results in a controlled projection of the unfolded peptide chains adjacent to the helix in the direction of the partner protein, identifying a mechanism by which the unfolded protein can achieve non-specific encounter interactions prior to binding. These transient interactions may temporarily stabilize the partners, thereby promoting fast interaction of the projected positively charged amino acids of NTAIL with the negative surface of P. This experimental evidence adds to our understanding of the much neglected, but surely ubiquitous Flexible-Interactome.

**Martin Blackledge (IBS), Rob Ruigrok (UVHCI)**

*Ringkjøbing Jensen et al. (2008).* JACS, *in press.*

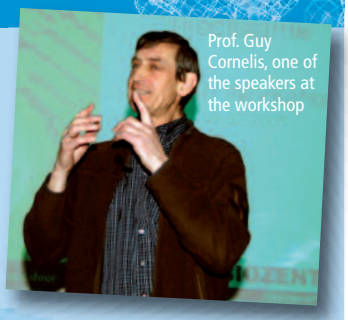
# Training at the PSB

## International Workshop on "Structural and Molecular Biology of Host Pathogen Interactions"

This workshop was a satellite of the ESRF Users' Meeting on 5-7 February 2008 at the ESRF. Organised by the PSB and generously sponsored by ESRF, SPINE-2 complexes, GE Healthcare, Dutsher Instrumentation and VWR, it attracted more than 150 participants and highlighted the strengths and interest of the scientific community in Grenoble, with significant participation by several PSB students and scientists.

The three half-day sessions were divided into three central topics: Bacterial adherence and invasion; Virus and Host cell factors; and Immune response. By combining molecular, cellular and structural biology, the aim was to show how structural biology studies have complemented those using cellular and molecular biology during the last decade.

The first session on bacterial pathogens showed how different techniques, combined with macromolecular crystallography, have provided insight as to how bacteria assemble different adhesion and secretion machines to infect eukaryotic cells. The session on virus entry and assembly presented a variety of mechanisms' viral pathogens such as HIV, influenza and rabies used in virus assembly and replication. The contribution of structural biology, particularly synchrotron-based crystallography, has helped define some of the mechanisms viruses use to take advantage of the host cells. During the last session, several presentations described how host cells respond to these pathogens.


Prof. Guy Cornelis, one of the speakers at the workshop

The program also allowed for two lively poster sessions.

**Laurent Terradot (ESRF)**

# Peter Timmins

*Peter Timmins is co-responsible for the low resolution crystallography instrument DB21 at the ILL, where he has worked for more than 33 years. With retirement close and plans to travel around the world, he told us what made him come to Grenoble and how he sees the future of the PSB.*

Hasn't changed a bit: The 'three ages' of Peter

**Peter Timmins is the head of the Large Scale Structures group at the ILL.** Interview by Susana Teixeira (ILL/Keele University) and Dominique Housset (IBS)

**Why did you decide to develop your career in Grenoble?**

I made no really deliberate decisions…except the decision to come to the ILL. I did want to come here for two or three years, believing I would then go back to an academic post in the UK, as was usual at the time. But it didn't turn out thay way. I guess much of it was luck: in 1978 I was just about to apply for a position at Daresbury Laboratory when I was offered a permanent post here at the ILL.

**Did you always work with neutrons?**

No – I was a chemist, originally. My PhD was in small molecule and protein crystallography. I did protein crystallography with X-rays at low resolution and a bit of electron microscopy. I was at Birkbeck and we had a rather interdisciplinary group of crystallographers, biochemists, chemists and a theoretical physicist. We were wondering about the role of water in life. John Finney, a young lecturer there at the time, got a grant to study water in biological systems. I thought this sounded great and I took a postdoc to work with him. I often went to Oxford to look at Dorothy Hodgkin's electron density maps to measure, by hand, distances between water peaks!

In 1974 after attending a meeting at Harwell on Neutrons in Biology I saw an advertisement saying the ILL was looking for scientists. I applied and was amazed I actually got an interview!

I was interviewed by Prof. Mössbauer, who was a physicist and asked me questions about protein crystallography that didn't seem to make any biological sense to me. Despite my scepticism at 'physicists' questions I got the job.

Soon after I arrived at ILL, Bernard Jacrot, who was Senior Scientist for biology at that time, suggested that I work on the structure of tomato bushy stunt virus (TBSV). The structure of TBSV was at the time being solved by X-ray crystallographic techniques but it was clear that the disordered RNA would not be visible. Jacrot's idea was that I should extend the contrast variation technique to single crystals and hence visualize the missing RNA – which we eventually did. Along with Michel Roth, Anita Bentley and Graham Bentley in collaboration with John Finch from the MRC in Cambridge we went on to study the nucleosome core particle and establish that the DNA was bound arund the outside of the histone core – something which was not known at the time and could only be done with neutrons contrast variation.

**What kind of breakthrough do you expect from neutrons in biology in the coming years?**

Techniques that don't require too much sample will develop faster, such as protein crystallography and small angle scattering, particularly when combined to look into very large complexes. The other very promising area is reflectometry: it has the potential to look at samples that are very biologically relevant. Studies of proteins in lipid bilayers can reveal quite a lot: the way they pack, the profile through the membrane, and so on. Structural biology is moving more and more towards large complexes, and neutrons have a part to play here. I also believe that inelastic scattering for understanding dynamics will have a bigger role to play if sample quantities can be reduced to 10 mg or less.

**What are your hopes for the SAXS/SANS PSB platform?**

My dream is that one day users will be able to write a joint proposal to the ESRF and the ILL for SAXS and SANS, so they can come and use both techniques, get data in the same format, and use the same software to get the structure! In the coming months we hope there will be time available at the ESRF at the same time as the scheduled experiments at D22, so some users will be encouraged to use the same sample on both facilities. It's a new way of working for both institutes, but so were systems like the Block Allocation Groups (BAGs) once and these are working very well.

**How will the Soft Matter laboratories interact with the PSB?**

At the moment the Partnership for Soft Matter is mainly driven by two institutes, ILL and the ESRF. Many scientific projects and complementary techniques will overlap with the work of the PSB. For example, there'll be a lot of work involving model and real membranes, which is of interest to physicists, chemists and biologists. Techniques such as light scattering and DSC will be available.

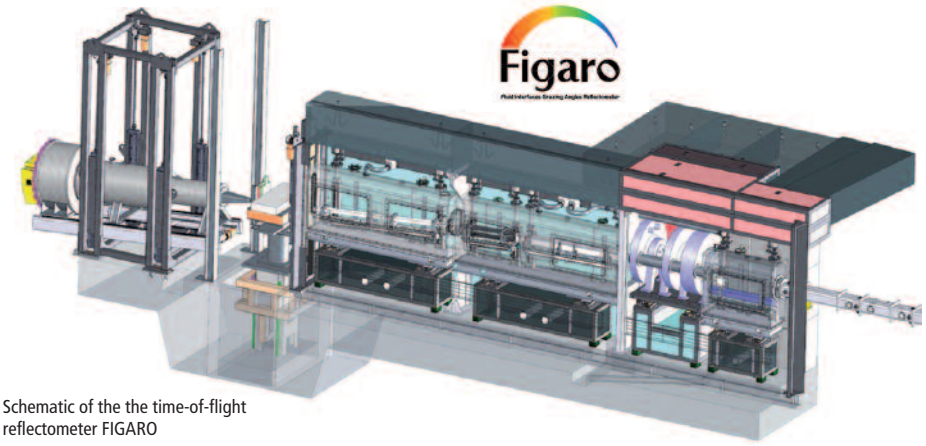
# Newcomers

**Christiane Schaffitzel** from ETH Zurich (Switzerland) has joined **EMBL** as a Team Leader. She studies ribosomal complexes involved in co-translational targeting and translocation by cryo-electron microscopy and single particle analysis. Using this methodology, her team will continue analyzing large macromolecular machines involved in gene expression.

**Carlo Petosa** has moved from the EMBL Grenoble to set up a new research group at the **IBS**. The group will use X-ray crystallography and complementary techniques to study HIV proteins involved in viral transcription and their interactions with the nuclear transport machinery of the host cell.

**Adam Round** has joined **EMBL** Grenoble as Staff Scientist, following two years at the EMBL Hamburg outstation working on automation of data collection for solution scattering experiments. He will work within the EMBL/ESRF Joint Structural Biology Group as a beamline scientist for the new Bio-SAXS beamline and will continue previous efforts on automation in collaboration with EMBL (Grenoble and Hamburg) and the ESRF to implement an automated data collection system and automated data processing pipeline in order to offer user friendly high throughput access for the users of the Bio-SAXS beamline (ID14-3).

## FIGARO: The ILL's new reflectometer




Schematic of the the time-of-flight reflectometer FIGARO

FIGARO (Fluid Interfaces Grazing Angles ReflectOmeter; www.ill.eu/figaro/home/) will be a high flux, flexible resolution, time-of-flight reflectometer with a vertical scattering plane, to be commissioned at the ILL by the end of 2008. It will be used for studies of thin films at air/liquid and liquid/liquid interfaces mainly in the realms of Soft Matter and Biology. Applications involve the study of the interaction of proteins with lipid monolayers, surface behaviour of surfactants, polymers and other amphiphiles at liquid/air and liquid/liquid interfaces. No polarized neutron option will be available on day one, although this is an option not excluded for the future.

Unique features of the instrument include the simultaneous use of a Brewster Angle Microscope during the reflectivity measurements, and the possibility to strike the interface with neutrons from above and below in a wide q-range. With an incoming beam of wavelengths comprised between 2 and 30Å and possibility to have incoming angles from -2.5 to +3.9°, it will be possible to attain a q-range from ~0.002 to ~ 0.39Å-1 when deflecting the beam downward and from ~0.002 to ~ 0.27Å-1 when deflecting the beam upward. The beam will be pulsed by four choppers (see image). The distance between the discs is such that 6 different wavelength resolutions can be obtained, ranging from 1.2 to 10%, by keeping the projected chopper openings equal to zero.

For liquid/liquid interfaces it is important that the incoming beam can approach the interface from above or below the horizon as one liquid phase may be far more easily penetrable than the other.

The sample stage is equipped with active and passive anti-vibration systems, x-y-z translation axis, goniometer for solid samples. Sample environment will include adsorption troughs, Langmuir trough, humidity chamber, solid/liquid cells.

A two dimensional Aluminium monoblock multitube detector will be positioned at 2850 mm from the sample. This detector will allow measurements of specular and off-specular reflectivity as well as GISANS.

At the last ILL proposal round FIGARO had 14 requests of beam-time, mainly in in the areas of Structure and Dynamics of Biological systems (College 8) and Condensed matter (College 9). 25 days of beam-time have been awarded for the first time and will be scheduled partly in December 2008 and partly next year. Richard Campbell (campbell@ill.fr) has been appointed as responsible of the instrument and started his « adventure » on FIGARO last February.

**Giovanna Fragneto (ILL)**

# Training at the PSB

## Training the next generation of structural biologists

Twenty young scientists from thirteen different countries spent a week at the CISB last June to learn how to tackle challenging problems in structural biology. The group was attending the fourth biennial EMBO Practical Course on the Structural Characterization of Macromolecular Complexes, jointly organized by EMBL/UVHCI, ESRF and IBS.

The 12 pre-docs and 8 post-docs came primarily from laboratories in Europe, but there were also students from Israel and Brazil. With backgrounds as diverse as crystallography, electron microscopy and mass spectrometry, they all shared a keen interest in the 3D architecture of macromolecular complexes.

During their stay, the students learned about the different strategies and techniques required to produce, purify and characterise multi-protein and protein-nucleic acid complexes. They also learned that tough problems in structural biology are often best solved through the combined use of complementary techniques spanning diverse disciplines.

Teaching the course were twenty-four highly motivated speakers from across Europe and the US, including several local researchers. Topics covered basic laboratory protocols to the description of complex macromolecular assemblies. Some speakers focused on practical matters – how to express recombinant protein in mammalian cells or to determine binding constants by isothermal calorimetry – while others outlined an entire discipline; small-angle scattering, nuclear magnetic resonance or single particle analysis by electron microscopy. Still others presented case studies, outlining the strategy used to crack a tough structure.

The talks were complemented by practical sessions run by local tutors, allowing students to gain hands-on experience with the techniques and instruments presented in the lectures. Among other things, students got the chance to prepare sample grids for EM, carry out measurements by CD spectroscopy and dynamic light scattering and optimize protein buffer conditions in a thermofluor stability assay.

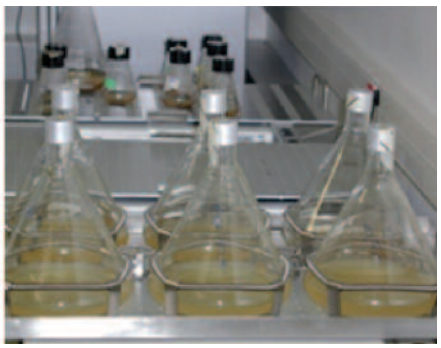
Feedback on the course was tremendously positive. "It was a real delight for me to be here," said one student. "I've met so many other scientists and had a lot of scientific interactions. I was particularly impressed with how speakers and tutors treated participants as equals." Another described it as a "highly rewarding and worthwhile course. All talks and practicals were excellent… Basically, please keep running this course!"

Thanks to everyone who helped make this year's course such a success.

**Carlo Petosa (EMBL)**

## The Eukaryotic Expression facility EEF at CIBB

Below: The EEF of EMBL Grenoble in the CIBB is fully operational since April 2008 and can now be accessed by users from the PSB (contact iberger@embl.fr)

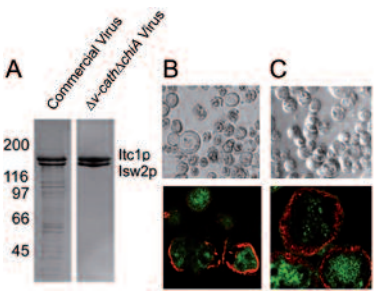


**Imre Berger joined EMBL Grenoble as Group leader in November 2007 and set up the Eukaryotic Expression Facility (EEF) in the CIBB. His group studies the structural molecular biology of eukaryotic multiprotein complexes, with focus of human transcription factors.**

At the EEF, which is overseen by Fred Grazoni, a research technician partly funded by the 3D Repertoire consortium (EU FP6), the Berger Group has installed the infrastructure for protein production using the baculovirus expression vector system (BEVS). Insect cell cultures are infected with recombinant baculoviruses to produce heterologous proteins. The group uses, and further develops, their proprietary baculovirus multiprotein expression technology, MultiBac, which relies on recombination cascades to generate transfer vectors with multigene expression cassettes. These are then incorporated into a baculovirus engineered for improved protein production properties, also developed by the group. Genes encoding for a protease and an apoptotic factor have been removed in the process with beneficial effects. In 2007, the MultiBac technology received the Swiss Technology and the W.A. DeVigier Awards.

The EEF can be accessed by all groups within the PSB (contact iberger@embl.fr). After registration, scientists interested in expressing their proteins or protein complexes are provided with reagents, instruction and know-how relating to multi-protein expression if desired, including techniques to monitor virus performance and protein production levels by means of a fluorescent marker that has been incorporated into the baculovirus backbone. Expression experiments can be performed in on plates or liquid shakers, from 5 ml expression volumes for small-scale tests up to several litres in large shaker flasks. A WAVE reactor for volumes up to 10 L will be installed in the EEF shortly.

Automation is a *sine qua non* in contemporary protein complex research. The group is also very active in developing a fully automated approach for protein complex production with BEVS, having custom-designed and installed a TECAN Freedom EvoII 200 robot at the CIBB. Recently, all experimental procedures for multigene expression vector assembly and multiprotein complex production and small-scale purification were successfully scripted into robotics routines. This method (ACEMBL) is currently limited to *E. coli* as an expression host. The group is now working on adapting the routines for baculovirus generation and small-scale production in insect cell cultures. **Imre Berger (EMBL)**




Protein production using MultiBac technology. (A) Expression with commercially available virus results in degradation of a 300 kDa yeast enzyme (left). Expression with the MultiBac deletion virus, in contrast, resulted in superior sample quality (right). (B, C) Insect cell cultures infected with commercial virus (B) and MultiBac virus (C) expressing a membrane protein, human MDR1, are shown in a comparison. In late phase, cells infected with commercial virus appear fragmented (B, top) and release heterologous protein into the medium (bottom). In contrast, cells appear intact (C, top) when infected with deletion virus, and hMDR1 protein accumulates in the vicinity of the plasma membrane (bottom). Whole cells are stained with an actin stain (green), MDR1 is stained red with a specific antibody (red).

## Remote control of MX experiments at the ESRF

The tendency of experiments on synchrotron-based MX beamlines to become shorter means that travel to and from the synchrotron often takes more time than the experiment. Remote access to such facilities would thus be highly desirable.

For several years, a form of remote access to the ESRF Macromolecular Crystallography (MX) beamlines has been available to industrial users via the highly successful MXpress data collection service developed by Elspeth Gordon and Stéphanie Monaco. However, manpower requirements mean that a similar service cannot be offered to academic users. We have therefore developed a system, accessible to industrial and academic users alike, whereby experimenters can control the ESRF's MX beamlines from the comfort of their home laboratories (pictured below).

While it is increasingly reliable automation that has made remote access feasible, the hub of the system developed is a master/slave version of the beam-line control GUI mxCuBE with the (remote) slave instances being run using desktop virtualisation software. The master version of the GUI runs on the beam-line control computers and allows local contacts to intervene at any time during an experiment.

More details about remote access, which is currently being rigorously tested by several of the ESRF MX Block Allocation Groups, can be found at www.esrf.fr/UsersAndScience/Experiments/MX/How_to_use_our_beamlines/remote-access

**Gordon Leonard (ESRF)**

Academic users controlling their experiment at an ESRF MX beamline from their offices at the University of York, UK

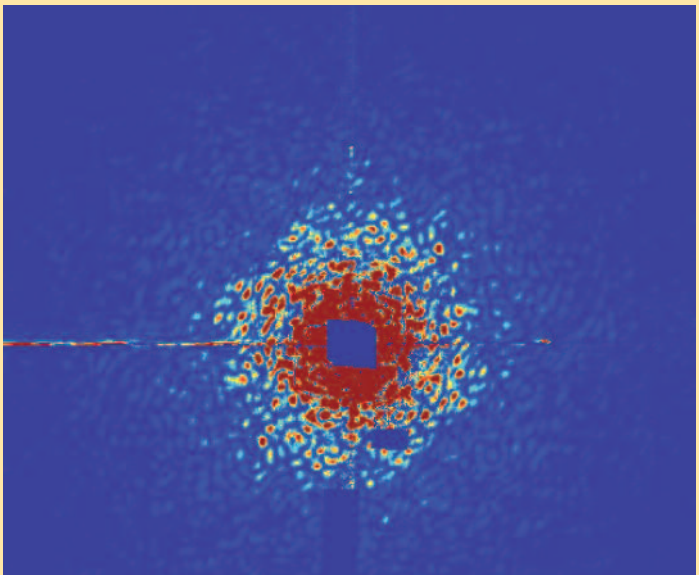
# Spotlight

## Coherent diffraction imaging applied to the bacterium *Deinococcus radiodurans*

When a coherent X-ray beam illuminates a small sample (crystalline or not), the diffraction pattern is weak but continuous. Such a continuous pattern (speckle) contains detailed information about the exact structure of the sample, limited only by the angular range over which the diffraction patterns can be collected and the degree of coherence of the beam. The phase information can be retrieved from the speckle pattern by adequate over-sampling and the use of iterative algorithms. This allows the determination of the exact spatial configuration of the sample by a simple inverse Fourier transformation.

A partially coherent X-ray beam can be produced at modern synchrotron sources by applying tight collimation and by selecting a narrow wavelength band ($\Delta\lambda$) around the central wavelength ($\lambda$) with a concomitant reduction in the intensity of the coherent beam by a factor of 103-104. In the field of biological imaging, the specimen's ability to withstand a high X-ray dose sets the resolution limit of the technique to approximately 10 nm. The potential of Coherent Diffraction Imaging (CDI) in biological X-ray imaging has been demonstrated with unstained, dried yeast cells illuminated by coherent soft X-rays (750 eV) [1].

We have recently performed very encouraging CDI experiments on unstained, frozen-hydrated Deinococcus radiodurans (DR) bacteria at the ID10C (Troika) beamline at the ESRF. A characteristic far-field diffraction pattern of DR bacteria obtained with 8 keV X-rays is shown (Figure 1). In order to illuminate the sample coherently, a pinhole is used after the monochromator to select the coherent part of the beam. The central cone of the pinhole scattering is used to illuminate the sample and unwanted fringes from pinhole scattering are blocked by secondary guard slit. The intensity of the scattered X-rays from the sample is measured by a CCD camera which is placed 2 to 3 meters downstream from the sample. This distance gives sufficient oversampling ratio to allow the convergence of the phasing algorithm.



Far-field diffraction pattern of DR bacteria. The sample-to-detector distance was 2.2 m. The square feature corresponds to the shadow of the beamstop after assembling the low and high resolution images (recorded with different beamstop positions and exposure times) limiting the achievable low frequency information.

Bacterial samples were rapidly plunge-frozen in the hydrated state and kept in a cold environment provided by a cryogenic gas stream. Sample positioning with respect to the beam is controlled by using an on-axis visible light microscope. The spatial frequencies at the edge of the diffraction pattern in the figure correspond to 25 nm resolution in real space. The visibility of the speckles in the high frequency domain is very good; however, the low frequency data suffer from parasitic scattering hindering the convergence of reconstruction algorithm (Difference map algorithm [2]). Three-dimensional imaging at nanometer resolution of the interior of non-crystalline particles in the micron size range is of vital importance in the life sciences. This technique will allow the determination of the internal structure of macromolecular assemblies, protein complexes and virus particles at a resolution sufficient to recognize known proteins and to determine the relationships between them.

While CDI potentially provides the necessary high resolution images, various experimental difficulties need to be overcome and the existing phase retrieval algorithms will be improved.

**Enju Lima, Petra Pernot, Lutz Wiegart, Anders Madsen, Joanna Timmins and Federico Zontone, MX+ID10A/C, ESRF**

*[1] Shapiro, D. et al (2005),* Proc. Natl. Acad. Sci., *102, 15343*

*[2] V. Elser, J. (2003),* Opt. Soc. Am. A *20, 40*

## *Announcements*

Information about stability and solubility is essential for structural and functional studies of protein in solution. Based on existing automated platforms available at IBS we have developed a fully automated procedure to screen protein solubility in 288 conditions. The robotized procedure includes the preparation of proteins drops (2ul of protein solution + 0.5 ul of well solution+ 0.5 ul of additives) in 288 conditions, drops imaging, automated detection of precipitate using an new in-house software. This new service will be available to all PSB members from 2009. Contact L. Imbert (IBS) at lionel.imbert@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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