CISB et al.

Centre for Integrated Structural Biology Newsletter

N° 1

September 2006

A European Centre for Integrated Structural Biology

On January 13, 2006 the Carl-Ivar Brändén Building was inaugurated on the International Site in Grenoble, France. The new laboratories house the Centre for Integrated Structural Biology (CISB) which operates as a collaboration between major international and national partners based in Grenoble and is an important step in the development of the region as a European centre of excellence for structural biology.

The Carl-Ivar Brändén Building.

The CISB comprises two complementary units: the Partnership for Structural Biology (PSB), whose members include the European Molecular Biology Laboratory (EMBL), the European Synchrotron Radiation Facility (ESRF), the Institut de Biologie Structurale (IBS) and the Institut Laue-Langevin (ILL), and the Institut de Virologie Moléculaire et Structurale (IVMS, associated with the Université Joseph Fourier and the CNRS). Valuable funding has come from Europe's Framework 6 Programme (Construction of New Infrastructures) to help finance the building of the laboratory and building-up the unique set of technological platforms.

Together the CISB partners offer an amazing range of expertise in the life sciences and benefit from the presence of some of the world's most important instruments for structural biology: the ESRF's X-ray source is one of the most powerful in the world and the ILL offers the world's leading source of neutrons. For many years the ESRF, ILL and EMBL have collaborated in offering scientists services and training connected to these instruments, already making the site a pivotal contact point for large European research projects and interdisciplinary collaborations. The enhanced facilities available now include laboratories for high-throughput protein purification and expression, robotic crystallisation facilities, deuteration and isotopic labelling, nuclear magnetic resonance, mass-spectrometry and cryo-electron microscopy. The power and heart of the CISB lies in the assemblage of all these components in a unique platform under one roof with scientists sharing the same coffee room – which is the breeding ground for new ideas and scientific collaborations!

Interdisciplinary and international scientific collaboration is necessary today to push forward fundamental disease research. The CISB does not have everything in-house and is not a closed club. Bridges are being built to scientific academic and industrial communities, both locally and Europe wide, to enable the experience of the Centre to be shared and to bring in expertise from outside. For the future, the International Site is also considering other Partnerships and Centres of Excellence to add to the life science portfolio of the CISB in areas such as soft condensed matter and engineering.

Ed Mitchell (ESRF)

CONTENTS

Structure of rabies virus nucleocapsid

Rabies is a disease known from the early middle ages. In Europe, terrestrial rabies is carried by foxes. The infected foxes bite dogs and these then bite humans. 100 percent of the people that get ill with rabies will die. This is because the disease only becomes apparent when major damage of the brain has already occurred. Death by rabies is a terrible death with people in the end-stages of the disease attacking and biting other people, thus transmitting the infection. More than 100 years ago, Pasteur developed the first, primitive vaccine against rabies. Today there are very efficient vaccines, both for humans and for animals, with little or no side effects. In Western

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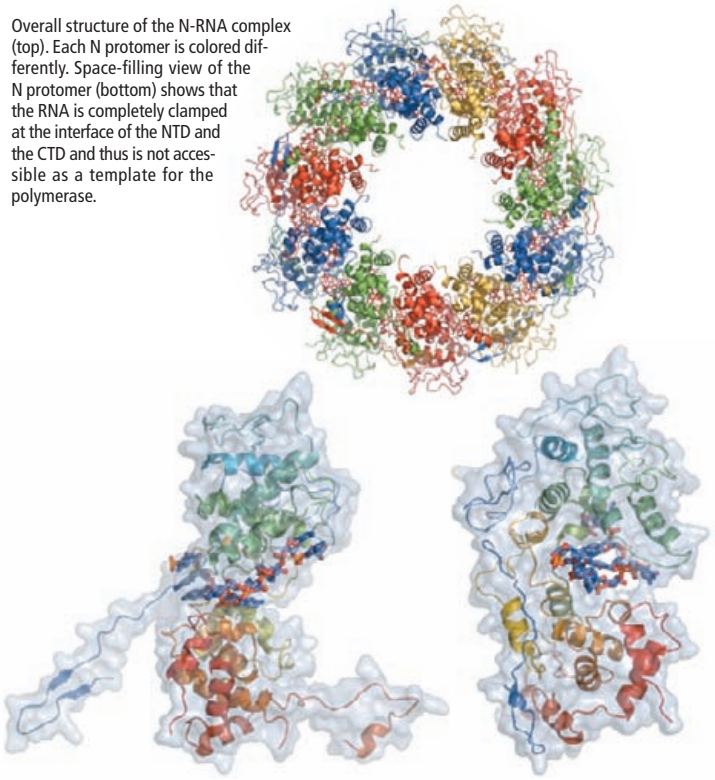

Overall structure of the N-RNA complex (top). Each N protomer is colored differently. Space-filling view of the N protomer (bottom) shows that the RNA is completely clamped at the interface of the NTD and the CTD and thus is not accessible as a template for the polymerase.

Europe the foxes are vaccinated so that our dogs do no longer get infected. However, outside of the vaccination zone, in Eastern Europe and in particular in Africa and Asia, rabies is still very common and more than 50,000 people die from rabies in this part of the world, because public health care is absent or poorly organised. Note that for keeping a vaccine you need a fridge that works all the time. For the moment there are no drugs for treating rabies infected people.

Rabies virus has RNA as chemical molecule to store it genetic information. A large group of RNA viruses, called positive strand RNA viruses, has an RNA molecule inside the virus particle that codes directly for proteins, using the cellular protein translation machinery. However, when rabies virus infects the cytoplasm of the host cell, its RNA has first to be transcribed into messenger RNA, which then codes for the viral proteins. Because of this characteristic, the RNA is called negative strand RNA. For this first step in the infection process, the virus needs to carry its RNA in a large and complicated protein-RNA complex, called nucleocapsid, which contains the nucleoprotein plus the viral RNA dependent RNA polymerase complex. We hope that, when we know the structure of this protein-RNA complex, we are able to define specific sites against which anti-viral drugs can be developed.

We have now been able to produce the RNA-nucleoprotein complex of rabies virus in a recombinant system. Because the nucleoprotein was expressed in the absence of the other viral proteins and RNA, this recombinant protein did not bind to viral RNA but to cellular RNA. In fact, we were able to purify a complex of 11 nucleoprotein molecules plus an RNA molecule of 99 nucleotides. Because the RNA molecule is so short, the nucleoprotein molecules at the ends of the RNA molecule interact with each other and a circle is formed. This recombinant, circular complex could be crystallised and the structure was solved with a resolution of 3.5 Å. Although some regions of the protein are rather flexible and at this resolution poorly defined, the RNA molecule is clearly visible and also how the protein recognises this RNA. Each nucleoprotein protomer interacts with exactly 9 ribonucleotides and almost all phosphates and ribose units of these nucleotides are contacted. This means that the RNA is bound in a sequence independent manner. The most surprising feature of the structure is that the nucleoprotein forms two jaws that totally close over the RNA molecule. This means that the RNA is not accessible and that the nucleoprotein has to undergo a significant conformational change for transcription and replication of the viral RNA. The reason for this hiding of the RNA is probably that the virus does not want to show its RNA to the cellular innate immune system. There are a number of cellular molecules, called Toll-receptors, which can recognise RNA that does not have the clear signatures of cellular RNAs and that, once activated, start a cascade of cellular defence mechanisms that are harmful for the replication of the virus.

This structure directly suggests two ways in which one could inhibit viral replication. The first would be to stabilise this closed structure. In this way the viral polymerase complex would have difficulty to transcribe or replicate the viral RNA. The second would be to stimulate the conformational change of the nucleoprotein so that the viral RNA would be released and could then either be recognised by the Toll receptors or become a target for cellular RNA degrading enzymes. A collaboration with virologists and chemists from the Pasteur Institute in Paris has started in order to investigate these possibilities. The final aim is to define or design cheap drugs to combat this disease in poor countries.

Aurélie Albertini et al. (IVMS, FRE 2854 UJF-CNRS/ EMBL)

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News From the platforms

ID23-2: The first microfocus beamline dedicated to Macromolecular Crystallography: When size really matters!

With the development of high-throughput Macromolecular Crystallography, new challenges arise. A major one is the problem of the protein crystal size. Obtaining suitable crystals can be time consuming, especially for difficult projects (large protein complexes in particular). It has become clear in past years that focussing the beam to the size of the smallest crystals would greatly enhance the quality of the diffraction by dramatically reducing the background and therefore increasing the signal/noise ratio. The feasibility of such an approach has been demonstrated beautifully in the past on ID13 at ESRF (see Small is beautiful: protein micro-crystallography; Cusack S, Belrhali H, Bram A, et al. Nature Structural Biology 5: 634-637 Suppl. S AUG 1998).

The decision was made in July 2001 to construct the new ESRF beamline ID23, in part in view of the development of the Partnership for Structural Biology. ID23 has two independent end stations: ID23-1 as a MAD beamline (from 5 keV to 20 keV energy) and ID23-2 as a fixed energy (14.2 keV) microfocus beamline fully dedicated to Macromolecular Crystallography (MX).

The key challenge of ID23-2 is to provide the user community with a highly reliable beamline with a beam size smaller than 10 µm in diameter whilst retaining the same easy-to-use point-and-click environment as the other ESRF MX beamlines. Beamline construction and commissionning started in 2004 with the main optics hutch components installed during late 2004 and completed in autumn 2005 with the installation of the minidiffractometer and a Mar-Mosaic225 CCD detector. The beamline was opened to the user community on the 14 November 2005.

Currently, the beam size at sample position is 7.5 (horizontally) microns by 5.3 (vertically) microns (FWHM). Due to the size of the beam, some drift in the position is of course natural (maintaining a 7 micron beam on a 7 micron crystal is hard work!) but, crucially for the users, the beam position can be checked and realigned by a non-expert via the user friendly environment. Data from crystals smaller than 20 µm in the largest dimension are routinely collected on ID23-2 with exposure times in the range of 0.5 to 2s per image . The small beam is also used to probe small blocks of crystals larger than the beam (pic-

ture) to find the best section of an imperfect large crystal. The beamline is therefore also appropriate for needle-like crystals which can be very long but less than 10 µm thick. The next step is to collect data using an even smaller, around 1 micron, beam. At this level is a 1 micron protein crystal really a crystal? How many unit cells are needed to make a Bragg diffraction pattern? Stability becomes even more of an issue. A project to develop hardware and techniques to enable this is currently underway at the ESRF with ID23-2 as the test bed.

So keep watching this space and when size matters, think of ID23-2!

David Blot (EMBL), Edward Mitchell and Laurent Terradot (ESRF).

Overview of ID23-2 experimental hutch. On the right and from top to the bottom. X-ray beam on the fluorescent screen. Picture of a microcrystal during data collection and the corresponding diffraction pattern measured.

The ILL-EMBL Deuteration platform – Directions for use

Deuteration Laboratory group at the ILL.

You want to study a biological macromolecular complex in solution, see how subunits are arranged, but you cannot crystallize it? Are you interested in knowing the protonation states of active site residues or in identifying water molecules important for catalysis? You are interested in macromolecular dynamics ? The use of neutron scattering is the solution for you.

If you feel you need some background please have a look at:

http://www.ill/deuteration
http://www.sfn.asso.fr/PromoNeutron/Bio.pdf or
http://www.ill.fr/neutbio2005/frameset_accueil.html

where you can learn more about Neutron applications in Biology.

Sample preparation (i.e.deuteration) is crucial if you want to study biological systems with neutrons. The ILL-EMBL Deuteration platform has been set-up to help you with this task. The access to this platform is easy and free of charge if your proposal is accepted. Fill in the proposal form that you can download from http://www.ill/deuteration (please do not hesitate to contact the platform responsible Michael Haertlein) and send it anytime to the ILL Scientific Coordination Office (SCO@ILL.fr).

Your proposal will be peer-reviewed and you will know within 4 weeks if it has been accepted, in which case you will be invited to use the platform to carry out your deuteration experiment. At the Deuteration Laboratory you will be counting on the help and advice of staff with the appropriate expertise.

The deuteration experiment usually involves obtaining high cell density cultures of recombinant E.coli in fermenters using deuterated minimal medium or other specific labeling schemes depending on the purpose of your project. Once your labeled solution, fibre or crystal sample is ready you have to submit a proposal for beam time on a suitable ILL instrument.

If you have a potential neutron project please do not hesitate to contact one of the following persons:

- Michael Haertlein (haertlein@ill.fr)
- Peter Timmins (timmins@ill.fr)
- Trevor Forsyth (tforsyth@ill.fr)
- Jo Zaccai (zaccai@ill.fr)

Michael Haertlein (ILL)

Advances for neutron protein crystallography at the ILL

Proteins are the key workhorses of life and are responsible for many chemical reactions as Nature's catalysts. They are therefore major targets in drug discovery programmes of pharmaceutical companies. Neutrons are optimal for locating hydrogen atoms, often key atoms in biochemical reactions, and which, because of their low mass, are very difficult to place with other techniques such as X-ray crystallography. The neutron Laue diffractometer LADI was developed for neutron protein crystallography at medium-resolution (1.5–2.5 Å) and provides 10 to 100 fold gains in efficiency compared with conventional neutron diffractometers. LADI made feasible studies of larger, more complex systems for which the protonation state of amino-acids and details of hydration are of key interest. Nevertheless, neutron protein crystallography remains a demanding and intensity limited technique. In order to increase the accessibility to the structural biology community, further orders of magnitude improvement in instrument performance need to be provided so that the requirement for large crystal volumes can be further decreased. To this end, an upgraded neutron Laue diffractometer LADI-III, based on the design of VIVALDI (or LADI-II) has been constructed and will be available for users from September 2006. Major modifications to the neutron detector design and readout system have been incorporated, so that the image-plate area is scanned by a miniaturized reading head, located inside the drum. The transferal of the image-plates and readout

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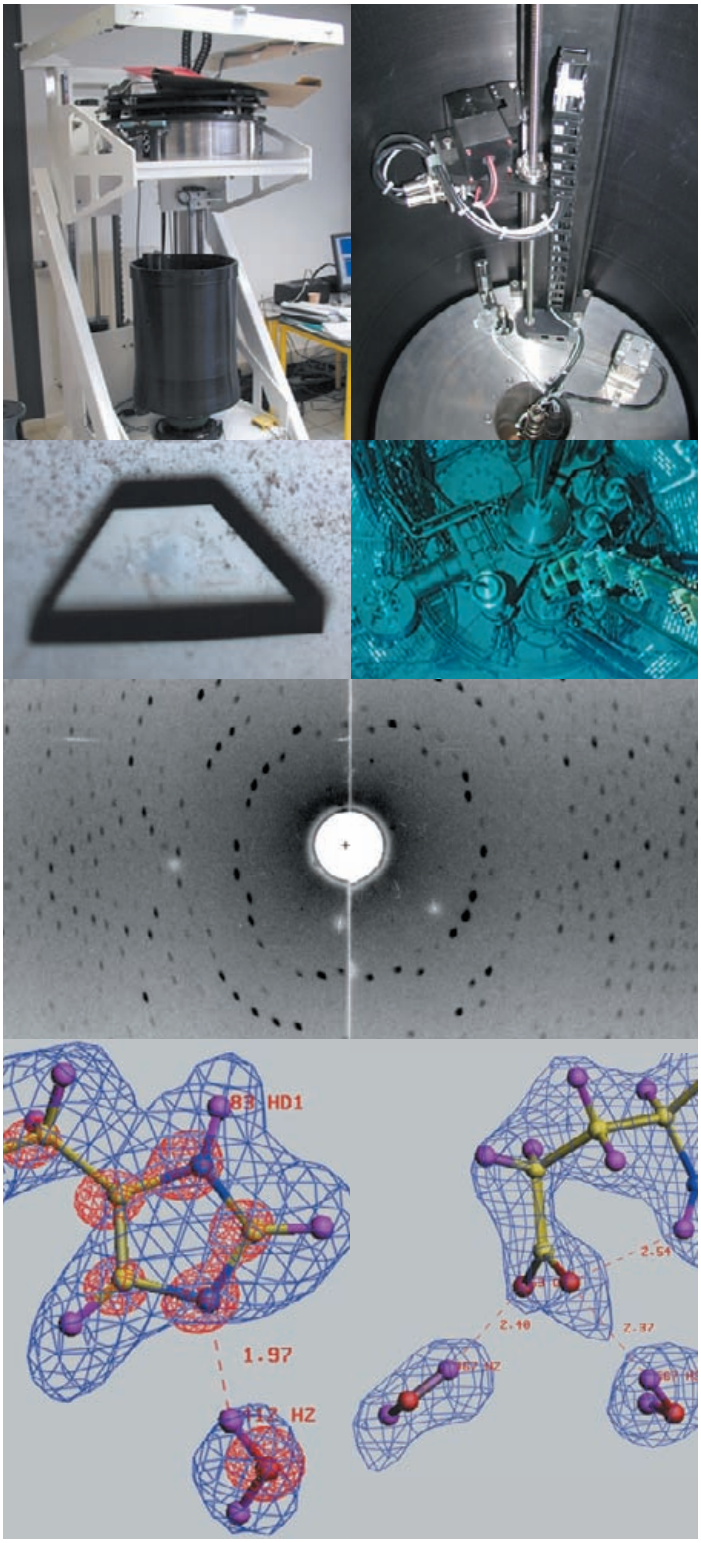

system internally, provides a 3-fold gain in neutron detection in comparison to LADI. Furthermore, in order to help reduce the number of spatially overlapped reflections, the dimensions of the drum have been increased and the entire detector height can be adjusted to allow for relocation to a higher intensity beam, providing yet further gains. Further improvements are planned, such as the use of proven supermirror focusing optics to concentrate the beam at the sample (2-4 fold) and new processing tools for weak and/or spatially overlapped reflections in Laue data software. The gains derived from the technical improvements coupled with parallel advances in preparation (expression, purification and crystallization) of fully (per)deuterated samples will clearly lead to more diverse and challenging projects in the future.

Matthew Blakeley (EMBL/ILL)

Figure 1. Neutron protein crystallography at the ILL.
Top left, the Laue diffractometer, LADI-III.
Top right, the readout system located inside the drum.
Centre left, a crystal (0.15mm^3) of the fully deuterated ternary complex of human aldose reductase-NADPH-IDD594.
Centre right, the reactor core at the ILL.
Centre, a neutron Laue diffraction pattern from concanavalin A at 293K.
Bottom left, the $2F_o - F_c$ nuclear density at 2.2 Å in blue and the $2F_o - Fc$ X-ray density at 0.66 Å in red for the side-chain of histidine 83 and a D_2O molecule in fully deuterated aldose reductase.
Bottom right, the $2F_o - F_c$ nuclear density at 2.2 Å in blue for glutamic acid 53 and two D_2O molecules in fully deuterated aldose reductase.

Events

The ILL "Millennium Symposium" and European Users Meeting, 27-29 April 2006.

As announced previously, the second Millennium Symposium was held at the Grenoble *Centre de Conferences et d'Affaires*. Results of a modernisation program that started five years ago were discussed by engineers and scientists with a strong emphasis on the user feedback. Among the delegates was the future British ILL director Andrew Harrison who has reasons to be optimistic, at this point when the reactor restarts after the 10-month shutdown. New strategies and plans were drawn for the next decade, to ensure that the ILL neutron scattering instruments maintain their world leadership. You can find more details on the discussions that took place and on the improvements that may affect your work, as a present or future user, on the websites:

http://vitraill.ill.fr/symposium/welcome.jsp or http://www.ill.fr

Susana Teixeira (ILL)

Report

Report from the CISB Science Advisory Board

On January 13, 2006 not only the Carl-Ivar Brändén Building was inaugurated, but also the CISB Science Advisory Board met for the first time. This committee meets annually and reviews, discusses and advises the CISB partners on issues related to our different technical platforms, our organisational structure and the science we are trying to do together. Members of the committee are Janet L. Smith (Chair), Miguel Coll, Jean-Marie Frère, Rob Kaptein, Werner Kühlbrandt, Felix Rey, Joel L. Sussman, Georg Büldt, David Stuart and Anthony Watts. At the first meeting in January the committee took a tour of the new building and platforms and was introduced to the CISB through a mix of scientific presentations and round table discussions.

The committee summarized the impressions of its first visit in a report to the PSB/IVMS Steering committee. They thought that the CISB was an "impressive effort to bring together expertise and unique facilities in the field of structural biology" and commended the partners "to synergize their strengths while respecting their individual scientific and administrative identities". On the more concrete side they advised us to develop a long-term plan to retain and further develop expertise in running the platforms and to consider

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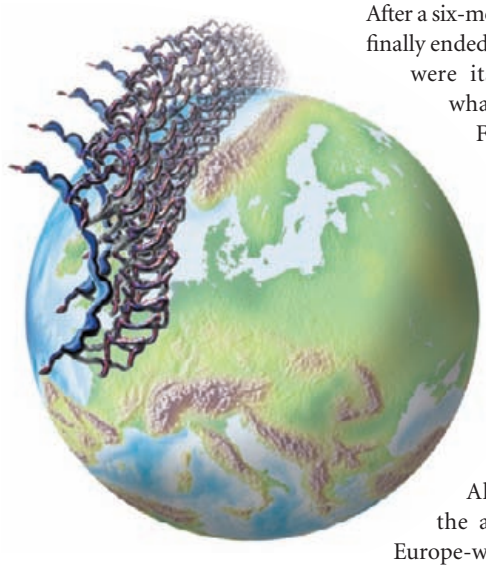
opening the platforms to a broader academic community.

With respect to specific platforms the Science Advisory Board thought that the establishment of a mammalian and/or baculovirus protein expression facility should be motivated by ambitious scientific projects of the platform responsible; they advised on concentration and upgrade of different equipment for biophysical characterization of proteins under one platform umbrella and recommended the purchase of a cryo-electron microscope for the CISB. The deuteration facility was seen as an especially important platform for neutron diffraction studies and NMR. Following this report the CISB steering committee is now considering an additional small-angle scattering platform, with both SAXS and SANS components, which would facilitate study of large complexes in solution, and complement both crystallographic and electron microscopic techniques.

The CISB Science Advisory Board also contributed to the discussion about a common biological theme and collaborative projects, which they thought "would be an excellent way to facilitate integration of the technical components of the CISB" and would "leverage local strengths to make a strong international biological research unit". – Obviously, something to demonstrate at the next advisory board meeting, which will take place beginning of 2007.

Christoph Müller (EMBL)

Adieu SPINE, bonjour SPINE2-COMPLEXES!



SPINE (Structural Proteomics in Europe) began in October 2002 as one of the three EU FP5 pioneer integrated projects. At that time there was little coherent, large scale funding of "structural genomics" in Europe (apart from some uncoordinated national efforts), contrary to what was happening in the USA and Japan and the risk was that Europe would fall behind particularly in the development and implementation of high throughput techniques applicable to structural biology. SPINE, coordinated by Dave Stuart in Oxford, received 13.2M €, spread between 15 to 20 labs, for an ambitious three year project, which aimed to cover everything: from methods development in high-throughput prokaryotic and eukaryotic expression systems, crystallisation, NMR, synchrotron crystallography technology, bioinformatics and LIMS, to solving lots of structures in the target areas of bacterial and viral pathogens, cancer, neurobiology and immunology.

After a six-month extension, SPINE finally ended in March 2006. What were its achievements and what was its impact? Firstly, over the lifetime of SPINE, it has become commonplace rather than exceptional for European structural biology laboratories to run high-throughput cloning, expression and nano-volume crystallisation robots. Although this "was in the air", SPINE, with its Europe-wide network of motivated laboratories and excellent training workshops was an important catalyst in stimulating the development and democratisation of novel high throughput techniques, beta-testing new methods and disseminating the necessary technical know-how. Secondly, SPINE has encouraged standardisation of high throughput methods to facilitate inter-change between labs, for instance in the formulation of a European standard for handling frozen crystals (linked to the development of sample changers), generalised use of Gateway cloning and accepted data models for LIMS. Thirdly, by its policy of an open, de-centralised network and focus on high value, human-health related targets, it has gone beyond the potentially divisive dichotomy between the "traditional" way of doing structural biology ("one post-doc/one project" with in depth complementary functional investigations) and "factory-style" structural genomics (multiple parallel projects, abandoning of failures, targets often of unknown function, only E. coli expression). The SPINE model of structural proteomics whereby high throughput techniques are exploited for high value targets is likely to become the norm for structural biology. Fourthly, SPINE laboratories have actually produced a lot of structures (217 crystal structures, 56 NMR, according to http://www.spineurope.org)!

What is SPINE's legacy in Grenoble? There is no question that SPINE, in particular its stimulus to implementing high throughput platforms, was an important integrative factor underpinning the establishment of the PSB/CISB, and was embraced by the initial PSB science programme. Firstly, although EMBL and ESRF were the officially funded partners, SPINE also included the IBS (through Frank Kozielski's kinesin project) and the IVMS (through Wim Burmeister's EBV project). Secondly, the stimulus and resources from SPINE were critical to EMBL in establishing what are now the PSB platforms in high throughput expression (ESPRIT platform of Darren Hart) and crystallisation (Josan Marquez team, with initial important input of the IBS). Thirdly, SPINE was one of the driving forces behind the automation programme on the ESRF beamlines, a joint venture involving ESRF, EMBL and MRC-France. SPINE funds contributed to the development and mass construction of the sample changer as well as great progress on other aspects of automation (automatic crystal alignment, ISPyB database etc), often in collaboration with other projects (e.g. eHTPX, DNA and BIOXHIT).

So what about the future? SPINE2-COMPLEXES, which is due to start in July 2006, received 12M € for 3.5 years and basically builds on and continues the philosophy of SPINE. However since in the meantime several EU FP6 structural biology integrated projects with related interests have begun, notably BIOXHIT (high throughput X-ray technologies), VIZIER (RNA virus replication machinery), 3-D REPERTOIRE (X-ray and EM studies of multi-subunit yeast complexes), E-MeP (membrane proteins), SPINE2-COMPLEXES will focus on protein expression technologies (e.g. directed evolution expression screening strategies and eukaryotic expression systems) on the one hand and the target area of "From receptor to gene: structures of complexes from signalling pathways linking immunology, neurobiology and cancer". There is much less emphasis on structure determination methods (X-ray and NMR) and LIMS/PIMS developments, which are largely funded by other projects. The targets are exclusively eukaryotic (indeed mainly human proteins and viral proteins that interfere with signalling pathways). A novelty in SPINE2-COMPLEXES is the inclusion of two labs from new EU member states in Eastern Europe, from Prague and Budapest (where the recent planning meeting was hosted in May 2006). There is also 0.5M € additional funding for a project called TEACH-SG that will pay for training workshops on SPINE2-COMPLEXES related technologies.

In SPINE2-COMPLEXES, EMBL and ESRF are again the official partners. ESRF will work on new technologies for measuring very small crystals and EMBL will develop an improved on-line humidity device for screening improvements in diffraction that should be set up on BM14. In addition EMBL will pursue target areas in innate immunity, and, with the IVMS, on viral proteins that interfere with host cell processes.

Stephen Cusack (EMBL)

A two-day journey into the world of crystallography

A practical session of the ELLS course for high school teachers.

As Marie Curie funded predoctoral students Elena Seiradake and I were asked to contribute to the science for teachers activities of the EMBL.

Together with Alexandra Manaia from the European Learning Laboratory in Heidelberg and Mary Jane Villot we organized a two-day course about structural biology for high school teachers from the Grenoble area. We organized a program covering topics ranging from protein purification to structure analysis. The 3-4th March we hosted 12 biology and physics teachers. Many researchers from the EMBL and the IVMS participated either by lecturing or by helping out during the practicals.

The day began with Christoph Müller giving a general introduction about structural biology. The teachers then divided into four groups and headed to the wet lab to experience protein purification first hand. Thanks to Elena's fine tuned protocol, they all managed to purify the fibre head protein of adenovirus, resulting in a nice gel summarizing the different purification steps. Nicolas Tabouriech had perhaps the most inquisitive group, who already within fifteen minutes were clamouring to see the *E. Coli* rare codon table… Thus began the steady stream of questions that would challenge us for the next 48 hours. That afternoon Josan gave a much appreciated talk about crystallisation, after which one group of teachers manually set up crystallization experiments using the lysozyme kit provided by Alexandra while another group visited the crystallization robot facility. During the coffee break, Hassan set up two stations for flash cooling crystals: microscopes, loops, magnets, cryosolutions, crystal trays,… After a brief discussion of what should be done to flash cool crystals we followed Hassan's advice and let the teachers alone to decide how to proceed. The strategy prooved successful: with a bit of help all the teachers managed to fish and freeze at least one crystal.

The second day started with a presentation of the ESRF by Dominique Cornuejols. We all learned a lot but a half an hour was really not enough. Dominique then brought us to beamline BM-14 for a demo of data collection. Once again Hassan decided to leave them on their own in the optics hutch to try to figure out how the X-ray beam proceeded along the tubes and devices facing them. It was the day of the physicists. The automatic sample changer impressed them a lot. However, when they tried to collect diffraction images of their beautifully mounted crystals, just like in real life…. no beam! But that didn't matter, the weather was nice and so we could enjoy a long coffee break in the sun after lunch. The teachers showed each other fossils and various other geological objects. It was not too surprising since they had already exchanged petri boxes full of mating fungi… That afternoon Carlo told us about the Epstein-Barr virus and the structure of a DNA-bound transcription factor called Zebra. Then we headed to the computer graphics room to have a closer look at Zebra as well as at other structures and electron density maps using the programs Coot and Pymol. The teachers were greatly interested because they only knew the (less sophisticated) program Rasmol for looking at atomic structures with their students. The presentation of the Protein Data Bank also interested them a great deal as a source of example structures. Some teachers asked Thibault Geoui to see the electron microscope and Celine Fabry happily obliged with a short presentation. It was about 6 pm and they decided to return to BM-14 since Hassan told them the beam was back. He collected images and showed them how to process them. Later they still wanted to see the crystals they had made on the first day. A few more questions, lots of thanks, and then they left, bringing with them crystal trays and protein gels to show to their fellow teachers and pupils.

The teachers were very pleased with what they learned, and went off thinking about how they could use their new knowledge later in their classroom. It was undoubtedly a great experience for all those involved.

Jeanne Moriniere (EMBL)

Training structural biologists from around the world

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

The 15 pre-docs and 5 post-docs represented 17 nationalities – mostly European, but there were also students from Israel, the U.S., Brazil, Malaysia and Azerbaijan. With backgrounds as diverse as crystallography, electron microscopy, mass spectrometry and bioinformatics, they all shared a keen interest in the 3D structures of macromolecular complexes. During their stay, the students learned about the different strategies and techniques required to produce, purify, and characterize multi-protein and protein-nucleic acid complexes. They also learned that tough problems in structural biology are often best solved through innovative thinking and the combined use of complementary techniques that span diverse disciplines.

Teaching the course were over twenty highly motivated speakers from across Europe and the U.S. They lectured on topics that covered the gamut from basic laboratory protocols to the description of complex, macromolecular assemblies. Some speakers focused on practical matters – how to express recombinant protein in baculovirus, for example, or to determine binding constants by isothermal calorimetry. 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 chronology of events and logic by which the structure of a difficult macromolecular complex was eventually cracked. Several speakers who had participated in previous editions of the course returned again this year, contributing greatly to the course's success.

The talks were followed by practical sessions that let students gain hands-on experience with techniques and instruments presented during the lectures. Among other things, students got the chance to collect and process synchrotron data at the ESRF

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beamlines, prepare sample grids for electron microscopy, carry out measurements by CD spectroscopy and dynamic light scattering, and screen for protein expression in a high-throughput format.

After a hard week's work, the participants could finally relax and enjoy some fine cuisine at a mountain-side restaurant in the nearby village of le Sappey. Feedback on the course has been very positive. "I have plenty of new ideas about my project," said one student, "and can't wait to try them out back in the lab." One speaker described the course as "highly informative, friendly and fun for students and instructors alike. This meeting seemed to fly by so quickly, probably because of all the great conversations I had throughout the week."

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

Carlo Petosa (EMBL)

Participants of the EMBO course on protein complexes.

Spotlight

Efficient clearance of dying cells, also called apoptotic cells, is crucial to the organism and plays a central role in immune tolerance. Removal of apoptotic corpses relies on the recognition of "eat me" signals, displayed at the dying cell surface, by phagocyte receptors, followed by intracellular signalling, eventually leading to engulfment and inflammatory responses. The various events involved in these processes and the numerous molecules implicated are largely unknown and still highly controversial in higher eukaryotes. Therefore, the ambition of the project initiated by the IBS team at CIBB is to shed light at the cellular, functional and structural levels on the molecular actors of the cascades of events that take place from the recognition of "eat-me" signals to the engulfment.

The project combines different complementary approaches.

A cellular model is currently being developed with LEM/IBS team. This includes HL-60, a pro-myelocytic cell-line that can be easily differentiated into macrophages, and apoptotic corpses generated after UV irradiation from the epithelial Hela cells in culture. In a first approach, transcriptional expression of proteins will be studied by quantitative real-time PCR in order to identify novel protein targets. We recently defined differentiation conditions for HL-60 cells in culture. Preliminary studies by PCR suggest that specific DOCK proteins could be present only after macrophage differentiation.

Cloning, expression and purification of already known targets is also further developed for biochemical and structural studies of either full-length proteins or protein domains, either alone or in complex. Known targets involved both in "eat-me" signal recognition and in signalling (C1q, MBL, ficolins, DOCKs, ELMO, GULP) are our primary objectives for X-ray crystallography and NMR structure determination of the different domains and their interaction with their protein partners. Other techniques such as small angle X-ray scattering will also be used to assess structural changes upon activation of protein assemblies that are too large

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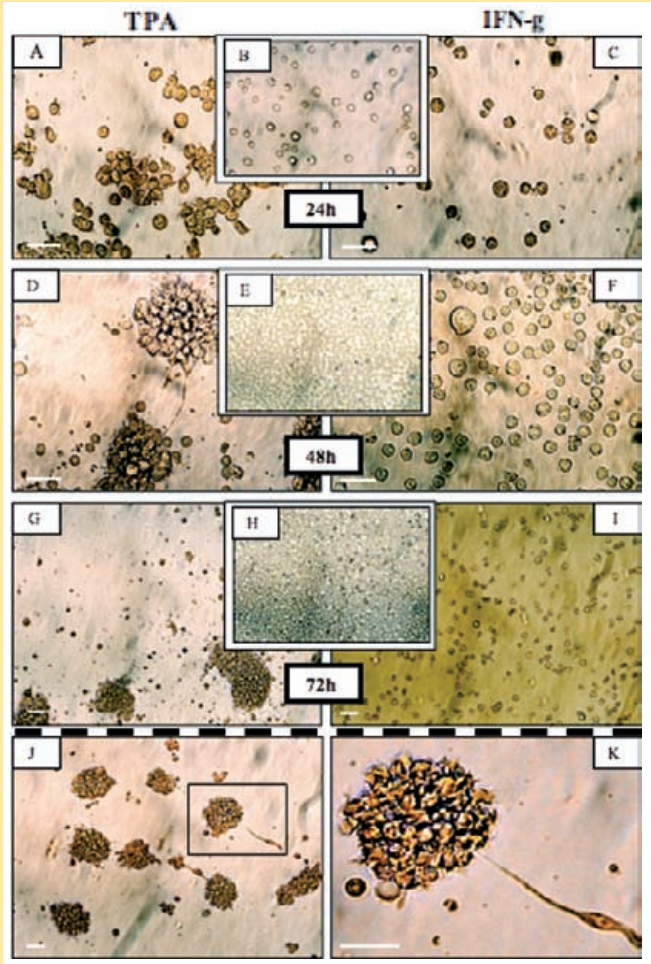


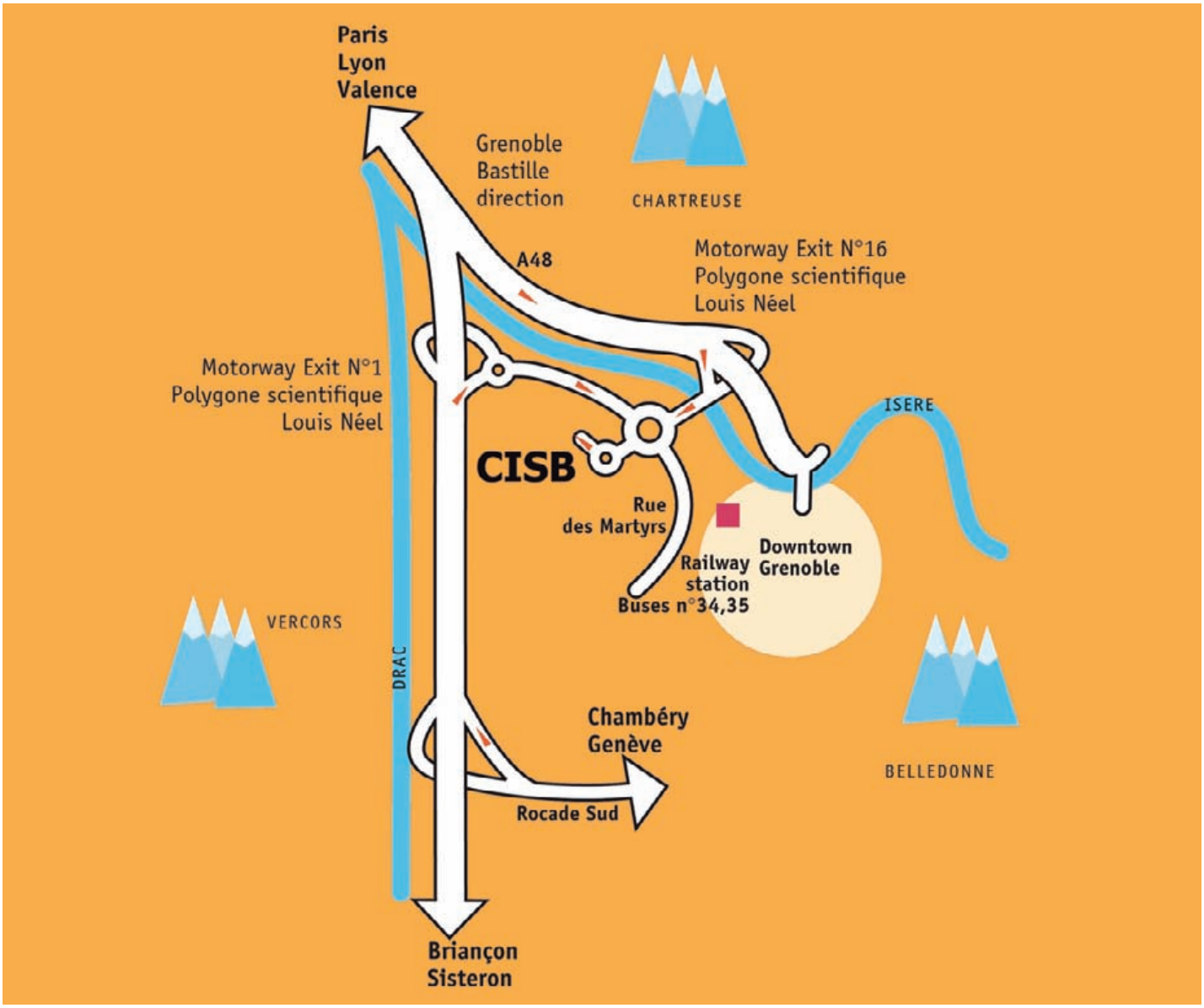
Efficient TPA and INFγ-driven cell morphology changes during macrophage differenciation.

to be studied by conventional structural studies.

The project greatly benefits from the complementarities of its participant teams, which together provide expertises in a wide variety of cellular, biochemical and structural approaches. Technically, the project takes advantages of the different high-throughput platforms (Robiomol, ESPRIT, HTX Lab, protein sample quality control, protein labelling, automated ESRF beamlines) developed within the Partnership for Structural Biology (PSB).

Thanks to the dynamism of our team partners, the first results appear less than 6 months after this new project was initiated: cell line culture and macrophage differentiation are under control, as well as apoptotic corpses production. To date, 7 protein domains have been cloned in 30 constructions, most of them are over-expressed and 3 of them are soluble and being purified.

Jean-Baptiste Reiser, Julien Pérard, Virginie Garlatti, Pierre Gans, Christine Gaboriaud, Dominique Housset, Jean-Philippe Kleman (IBS).



Announcements - Jobs

Postdoctoral positions will be available at the joint EMBL/ILL Deuteration Laboratory (see page 3).

Contact: Trevor Forsyth (tforsyth@ill.fr) or check our webpage for more information (http://www.ill.fr/deuteration).

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