Beamline biology
In great shape

Reports from the user meeting
Phase II upgrade enters execution phase
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A compact, photon counting area detector
• 1200 frames per second
• 28 x 28 mm sensitive area
• 24 bit counter
• 8 energy thresholds
• No readout deadtime
• No dark noise
• High resolution imaging from 55μm pixels
• Based on Medipix 3RX
• EPICS, TANGO and Labview

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A revolutionary fluorescence and mapping detector readout system with 30X improvement on industry standard
• Significant improvements in rate and data quality for x-ray fluorescence
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• 125 eV at 30 kcps with Fe55 with Vortex ASIC
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• “An order of magnitude increase in dynamic range and peak count rate” Matt Newville, APS/Argonne
• “30X more productive. It’s huge!” Sam Webb, SSRL/Stanford

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MX170-HS
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MX300-HS
MX340-HS
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The only X-ray detectors designed with cutout for simultaneous SAXS/WAXS

LX170-HS
LX255-HS
A powerhouse for biology

The ESRF has a 20-year history in providing world-leading facilities for structural biology, in particular macromolecular crystallography (MX). Among the first ESRF beamlines were ID09, ID02, ID13, which were partially dedicated to the technique, and BM14. The success of these beamlines led directly to the construction of four dedicated MX beamlines at ID14, to the creation of the ”MAD” beamline at ID29 and, later, to the construction of ID23-1 and ID23-2 – the latter being the world’s first micro-focus beamline fully dedicated to MX. Coupled with advances in automation, sample tracking and online data analysis, these end-stations have helped drive the explosion of structural biology research since the 1990s.

Diffraction data collected at the ESRF has led to more than 10,000 deposits in the Protein Data Bank including those for crystal structures of the ribosome and G-protein coupled receptors – work that has been recognised by Nobel prize committees. Taken as a whole, these depositions provide structural information on a wide range of biologically important systems including membrane proteins and molecular machines with medical relevance such as influenza polymerase (p17).

Despite the success of the MX facilities at ESRF, which are routinely exploited by major pharmaceutical companies (p24), we have not rested on our laurels. The Phase I Upgrade Programme has seen the aging ID14 complex replaced by a combination of beamlines at ID30 and BM29, of which three are currently operational: ID30A-1 (MASSIF-1) provides high throughput, fully automatic data collection (p13); ID30A-3 (MASSIF-3) is suited to serial crystallography experiments of the type recently carried out on ID13 (p19); while BM29 is aimed at high throughput BioSAX.

Beyond MX

The ESRF also provides a wide range of non-MX techniques in biology including X-ray fluorescence, X-ray diffraction, X-ray absorption and infrared spectroscopy. These probe the chemistry and conformation of biological samples at the micrometre and enable a full understanding of biological systems. Clinically oriented research is also at the forefront of life science activities at the ESRF. Users are able to study processes such as drug metabolism and breathing in real time under different physiopathological conditions, for instance, and researchers are also working on the development of microbeam radiation therapy at the ID17 medical beamline. Identifying the different crystal forms of proteins – particularly those, such as insulin, used as therapeutic agents – is not always straightforward, but new instruments recently developed at ESRF mean that powder diffraction, for example, can now offer a powerful alternative (p18).

The availability on the EPN campus of platforms for protein production, crystallisation and biophysical characterisation – in addition to facilities for X-rays, neutrons, NMR and electron microscopy – is unique in Europe, and is strengthened by the Partnership for Structural Biology (p21). Combining these resources, we can expect that the study of the dynamics, as well as the structures, of single molecules or large complexes will soon become mainstream in Grenoble.

The additional brilliance and stability of the Phase II upgrade storage ring will bring increased resolution and sensitivity for the bioimaging community, while for MX it will allow beamlines optimised for serial crystallography and time-resolved studies. Combined with partnerships such as the PSB and the Partnership for Soft Condensed Matter this will ensure that biology remains a pivotal activity on the EPN campus to 2020 and far beyond.

“Biology will remain a pivotal activity on the EPN campus to 2020 and far beyond”

Gordon Leonard, Head of the ESRF’s structural biology group
Jean Susini, Director of research for life sciences
Lost letters rise from the ashes

Writing that has lain undiscovered for centuries inside a scroll charred in the eruption of Mount Vesuvius in 79 AD has been uncovered thanks to advanced X-ray imaging at the ESRF’s ID17 beamline. The papyrus scroll was discovered in 1754 in a library from the Roman town of Herculaneum, which was buried beneath thick volcanic debris after the famous eruption. But the risk of damaging the delicate object has prevented researchers from opening the scroll up.

Vito Mocella of the Italian CNR’s Institute for Microelectronics and Microsystems in Napoli and co-workers used a powerful technique called X-ray phase-contrast tomography (XPCT) to unravel the contents of the scroll without opening it (Nat. Commun. 6 5895). Previous non-destructive attempts to do so were unsuccessful because the ink used to write on papyrus, which was obtained from smoke residues, has a similar density to the carbonised remains of the scroll.

XPCT is extremely powerful in distinguishing between materials with such limited contrast relative to one another. It exploits the refraction of X-rays as they pass through matter to detect small variations between the refractive indices of ink and paper, which exist because the ink did not penetrate into the botanic fibres of the paper.

“After several trials to select the most readable samples from the scanned images, there is no longer any doubt,” said team member Daniel Delattre from the Institut de Recherche et d’Histoire des Textes. “We can see deformed or twisted Greek letters. Victory!” The team was able to extract words lying under several papyrus layers, and managed to reconstitute an almost complete Greek alphabet from the rolled-up papyrus. They were even able to hypothesise that the text could be by the Epicurean philosopher Philodemus.

Emmanuel Brun of the ESRF and the Ludwig-Maximilian-Universität explains that without the ESRF’s X-rays properties it would have been impossible to see inside the Herculaneum scroll without damaging it. “This work proves, once again, the extremely high efficiency of phase contrast imaging,” he said. “Now we just have to face the analysis of this huge volume of data.”

The study, which generated media coverage all over the world, offers new possibilities for deciphering hundreds of so far untouched texts. “We can celebrate that we will eventually be able to read these precious past testimonies while, at the same time, preserving them,” said Mocella. “This is the beginning of our superb adventure to read lost literature!”

Dark field X-ray microscopy is unveiled

A new imaging technique invented at the ESRF enables complete 3D maps of orientation and strain inside a material with unprecedented resolution. Traditionally, the internal 3D structure of a material is predicted from the analysis of surfaces or thin sections cut from the sample. As crystalline solids are made up of thousands or millions of intricately arranged individual grains, however, these sections are not necessarily representative of the full internal structure.

Now it is possible to map the 3D structure of an entire sample in one stage. Lead author Hugh Simons of the ESRF and co-workers used an annealing study of aluminium to demonstrate the technique at ID06. They first mapped the full specimen using 3D X-ray diffraction contrast tomography, after which the dark field technique was used to zoom in and produce a magnified high-resolution 3D map of individual grains (Nat. Commun. 6 6098).

The bespoke X-ray optics work in a similar way to dark-field electron microscopy: the Bragg condition allows a single grain to be selected based on its orientation and lattice spacing. Unlike electrons, however, X-rays can penetrate deep into the material to allow in situ studies. The result is a 3D map that revealed details of orientation and strain at the nanometre scale with a resolution of 0.001°.

“It means we can map the complex nanostructure of materials in 3D without having to cut or damage the sample,” says Simons. “Better still, we can even do this under a variety of loads and forces, recording minute structural changes during important processes such as annealing or deformation.”

Surprise transition in metallic glass

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Surprise transition in metallic glass

Novel behaviour in metallic glasses has been discovered using synchrotron X-ray diffraction at the ESRF’s ID27 beamline. Unlike conventional window glass, metallic glasses can contain several elements, which offers greater scope to tune their properties for applications.

The dense atomic packing of metallic glasses and the non-directional nature of metallic bonds would suggest that such systems cannot undergo a phase transition from one amorphous state to another. But in 2007 such a transition was observed in cerium-aluminium glass, motivating qualitative explanations based on the delocalisation of 4f electrons. In search of a more quantitative explanation, Qiang Luo of Tongji University in Shanghai and co-workers used the ESRF to investigate the structural evolution of a cerium based metallic glass as a function of pressure using in situ X-ray diffraction both at room temperature and near the glass transition temperature.

The data revealed two unexpected features. As the sample was heated at constant pressure the team found that instead of expanding it was compressed. Such a negative thermal expansion has not been observed in other glasses at high pressure, says team member Gaston Garbarino of the ESRF. The team also found a jump in volume when the sample was compressed to pressures of around 5.5 GPa and heated to 390K. This is in contrast with the continuous and smooth process for the low- to medium-density amorphous state transition at room temperature, and suggests that a first-order phase transition occurs. “It is a huge effect of around 1.8% considering that the volume difference between a bulk metallic glass and its crystalline counterpart is usually 0.3–1%,” says Garbarino.

The conclusion, he says, is that cerium metallic glass has a hierarchical structure in which different “clusters” in the system behave differently (Nat. Commun. 6 5703).
ESRF and ILL invite applications for second joint student programme

Following the success of the inaugural ESRF-ILL joint summer student programme last year, the two facilities are offering undergraduate students the opportunity to spend four weeks living and working as scientists on the EPN campus this summer.

Throughout their time in Grenoble, which this year lasts from 6 September to 3 October, the students will be based in a research group at either the ESRF or ILL and will take part in experiments and attend introductory lectures on the principles and applications of both X-ray and neutron science.

“After the success of the scheme last year we are pleased to be offering this opportunity to another set of undergraduate students,” says event organiser Patrick Bruno of the ESRF. “We like to give them a taste of what it is really like to work among scientists in a real scientific setting. We know last year’s undergraduates also appreciated the chance to make new friends in the process.”

The summer school is open to undergraduate students from any member or scientific associate country of the ESRF or the ILL, and the deadline for applications is 1 April 2015. Competition is likely to be strong, with just 20 places available. All successful applicants will have their accommodation paid for and receive financial support during their stay in Grenoble. More details can be found at www.ill.eu/summerschool2015.

Users’ corner

The next Beam Time Allocation Panel meeting to review proposals submitted for the 15 January (long term projects) and 1 March (standard proposals) deadlines will be 23 and 24 April 2015, respectively.

The next deadline for standard proposal submission is 10 September 2015. Please note the new date for the September round submission.

The 25th ESRF User Meeting & Associated Workshops took place on 9–11 February 2015. More can be read about the meeting in the dedicated article on p10–11.

News from the beamlines

- After 10 months of shutdown, ID01 began commissioning in November 2014 and went into user mode in December. The new vertical axis monochromator was found to be of excellent stability and the conditions for coherent diffraction imaging and nanofocusing are perfectly met by this new device. In total, about 40% of the instruments are considered operational and during the next six months user mode will alternate between installation and commissioning time. It is expected that the beamline will be fully open to users for the proposal deadline of 1 March 2015.

- ID15 stopped operation on 17 December 2014 as part of the upgrade. Removal of the beamline optics hutch and the experimental hutch ID15B is underway and construction of the new hutch began in March 2015. ID15 will be back in user mode in the summer of 2016.

- In order to move to ID15B, the high-pressure diffraction beamline ID09A will cease operations at the end of 2015. The new beamline ID15B will be available for user operation after the summer shutdown in 2016 for period 2016/I (proposal deadline 1 March 2016). No proposals will be accepted for period 2016/II, for which the deadline is 10 September 2015.

- From August 2015, BM20 (ROBL) will double the beamtime available to users on the XAS spectroscopy station dedicated to actinide and environmental research (ROBL-RCH). The materials science station (ROBL-MRH) will discontinue operation for external users at the same time, although some in-house activity will be possible until July 2016.

- For the high-resolution powder diffraction beamline ID22, a large 40 x 40 cm² Perkin Elmer 1611 medical imaging detector has been acquired that will be operational for users in June 2015. The detector, which has a pixel size of 100 x 100 μm², will allow complementary measurements using photons of an energy up to 80 keV, allowing much faster PDF measurements and the ability to check the texture and granularity of samples prior to high-resolution analysis.

- A new MAXIPIX 2 x 2 chips (S16 x S16) detector has been recently installed and commissioned at SpLine, the Spanish CRG beamline at BM25. The experimental setup, which is installed at the second focusing point of branch B, is devoted to hard X-ray photoelectron spectroscopy (HAXPES) and surface X-ray diffraction (SXDM). It includes a heavy 25+3D diffractometer, a UHV chamber and an electrostatic analyser able to handle electrons with kinetic energy up to 15 keV. The new detector completes and improves the performance of the SXD/HAXPES station.

- Two new mirrors were installed at the microfocus beamline ID23-2 during the winter shutdown. The primary goal of this upgrade was to make more efficient use of the Pilatus3 2M detector by improving the photon flux from 2.5 x 10¹⁰ photons/s to 10¹¹ photons/s at 200 mA. Additionally, the new mirrors produce a more Gaussian, tightly focused beam at the sample position, which is now approximately 5.5 x 9.5 μm².

- The first user experiment exploiting a new device to record Raman spectra from protein crystals directly at the MX beamline ID29 has been successfully performed. The team combined X-ray diffraction data with Raman spectra to investigate the reaction mechanism of the enzyme urate oxidase, which catalyses the degradation of uric acid (Angew. Chem. Int. Ed. 53, 13710).

- The end-station is equipped with a brand new MD2-S microdiffractometer that will soon allow in situ crystallisation plate screening and data collection with a PILATUS3 6M detector capable of operating at a frame-rate of 100 Hz. Although ID30B currently has an SC3 sample changer installed, this will soon be changed to a newly developed FLEX CD sample changer with a high-capacity Dewar capable of holding 12 SPINE pucks and 12 Unipucks.

- The soft X-ray beamline ID32 was inaugurated in November, and further details can be found on p19.
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Phase II upgrade project team appointed

Inspired by studies of “ultimate” or fourth-generation storage rings, where the equilibrium emittance reaches the X-ray diffraction limit and opens the door to completely new experiments, the ESRF’s Phase II Upgrade Programme entails a major upgrade of the source in terms of reliability, stability and brilliance.

A new, hybrid multi-bend achromat (HMBA) lattice design will substantially decrease the storage ring’s horizontal emittance to around 134 pm rad, with a corresponding increase in the brilliance and transverse coherence of the photon beam. Following approval by the ESRF Council, the project was launched in the summer of 2014.

On 1 January 2015, the project entered the execution phase. It is managed by the newly created Accelerator Project Office (APO), which is headed by the director of the accelerator and source division, Pantaleo Raimondi. He is assisted by Dieter Einfeld, a pioneer of the HMBA concept, who has designed several leading light sources and also works on the Max-IV project in Sweden.

Paul Mackrill is responsible for planning and implementation as execution manager, while Jean-Claude Biasci has been appointed technical manager in charge of the engineering design. As infrastructure coordinator, Rudolf Dimper will oversee buildings and infrastructure, vacuum and alignment issues.

At the facility level, the APO team is supervised by a project management board and the board of directors, and is also advised by the Machine Advisory Committee comprising accelerator specialists who will review the project periodically.

The APO is assisted by a support team: Philippa Gaget as project assistant; Anne Dely as administrative and finance assistant, and Anya Joly as communication and documentation assistant.

The project is organised into 14 work packages. These are responsible for: management; beam dynamics; magnets; accelerator engineering; power supplies and electrical engineering; radio frequency; control system; diagnostics and feedback; photon source; injector upgrade; vacuum system; buildings and infrastructure; reliability and operation; and radiation safety. Project planning is now under way with the aim of announcing a schedule later this year detailing the dates and duration of the facility shutdown. During this period, the present storage ring will be removed and the new storage ring assembled, tested and commissioned in the existing tunnel.

Critical prototypes are already being validated and large-scale procurement is expected to start in September. It is hoped that pre-assembly of key components can begin in September 2017, followed by installation in autumn 2018 and commissioning in 2019. User service mode operations are expected to begin in 2020.

Triple success for Phase I upgrade beamlines

Three new experimental stations – ID30A, ID32 and ID02 – were inaugurated during the meeting of ESRF’s Science Advisory Committee in November 2014, marking the final steps to completion of Phase I of the ESRF Upgrade Programme.

ID30A will eventually provide three three end-stations replacing the highly productive ID14A and ID14B beamlines, which closed at the end of 2012. The inauguration of ID30A-1 (MASSIF-1), which is the only public experimental station for macromolecular crystallography in the world that is completely automated, and of ID30A-3 (MASSIF-3) will save hours or even days of beam time and provide new research opportunities for the ESRF’s structural biology users (see p.13).

ID32 is the ESRF’s soft X-ray beamline, with one end station featuring a spectrometer that provides unprecedented energy resolution for resonant inelastic X-ray scattering studies. The instrument, which is able to rotate a full 100 degrees with the help of air pads, will be used primarily for investigating the magnetic and electronic properties of materials, including high temperature superconductors. A second UHV high magnetic field end station for magnetic dichroism studies took its first users at the end of November.

The third newly inaugurated beamline, ID02, is a direct replacement for its predecessor. It includes a 34 metre-long, two metre-diameter detector vacuum tube that houses multiple detectors for small, wide and ultra-small angle scattering techniques. In addition to the improvements in source properties and detector performance, one of the highlights of the ID02 upgrade is its new ultra-small angle scattering technique for radiation-sensitive samples such as living biological cells and functional soft matter.

The upgrade beamlines provide users with a performance at the cutting edge of what is achievable, says ESRF director of research Harald Reichert. “They open up new possibilities; they save users time and offer unique capabilities,” he says. “The inauguration of these experimental stations is testament to the hard work of the many people involved in developing them from design to completion.”
More than 300 people attended the 25th ESRF User Meeting on 9–11 February to discuss the latest science and opportunities at ESRF beamlines. Brand new results revealing the conditions in the Earth’s core were a highlight, as were other keynote lectures focusing on dynamical and structural biology.

Four and a half billion years ago, announced ESRF user Catherine McCammon of Bayreuth University in Germany to a packed and silent auditorium during this year’s user meeting, the Earth formed. This remarkable process began with the Earth’s core, she continued, so by studying the nature of this extreme environment 6370 km beneath our feet, we can learn more about the history of our planet – and, more importantly, about conditions on its surface.

“Without the magnetosphere to shield us from the solar wind, probably no adaptive life forms would survive, and that has a lot to do with the core,” said McCammon, adding that the Earth’s magnetic field randomly flips once every few hundred thousand years or so. “There are suggestions that such flips are responsible for mass extinction events, and the signs are that we are heading towards another one.”

By revealing structural and chemical changes to matter under extreme pressures and temperatures, synchrotrons are proving vital tools for investigating planetary interiors. Since the Earth’s interior is almost entirely inaccessible to direct sampling, geoscientists traditionally have relied on seismic waves to infer depth profiles. McCammon and her group are using the ESRF’s ID09 and ID18 beamlines to measure the structure and elastic properties of potential core materials to compare with seismic data, using diamond-anvil cells and lasers to squeeze and heat samples so as to replicate the very high pressures and temperatures in the Earth’s interior.

“We know that the core of the Earth is primarily made of iron, but we also know from many other measurements, including those made here at the ESRF, that the density profile of pure iron is just too heavy to explain the geophysical data,” she explains. Several arguments point to iron being mixed with light elements to match the core’s lower density, with carbon being a popular contender. But so far none have managed to explain the unusual elastic properties (more technically, the high Poisson’s ratio) of the solid inner core, which McCammon described as being similar to that of tyre rubber despite the core being under pressures of several million atmospheres.

Experiments carried out very recently by her group at ID18 have suggested a possible explanation for this density difference (Nature Geoscience, published online on 23 February). By mixing graphite and iron powders inside a large volume press and then compressing and heating them, the team found that carbon dissolves in iron to form a previously unknown phase (a new structure of Fe₃C₇) that is stable under the conditions at the core. “Carbon alloying causes profound changes to the elastic properties of iron at Earth’s core conditions that explains the high Poisson’s ratio in the inner core,” says McCammon. “We nearly fell off our chairs at ID18 when we saw the recent results.” Her group is also developing new methods to study the core, in particular via melting, so stay tuned for further exciting news about the world below our feet.

Into the biosphere

The meeting kicked off with a topic even closer to home: biology. Hyotcherl Ihee of KAIST and the Institute for Basic Science in South Korea described the latest developments in picosecond pump-probe X-ray diffraction and scattering techniques, which allow his group to study structural dynamics and spatiotemporal kinetics of varied molecular systems over timescales ranging from picoseconds to milliseconds. He emphasised how time-resolved X-ray diffraction can complement the crystallographic results to provide insights into the structural dynamics of proteins.
Winner of the 2015 Young Scientist Award: Beatrice Ruta

The 20th ESRF Young Scientist Award has been awarded to beamline scientist Beatrice Ruta, 34, for her studies of atomic dynamics in glasses using coherent X-ray techniques. The award was presented by John Evans of the University of Southampton and Diamond Light Source in the UK on 10 February during the plenary session of the 2015 User Meeting.

Understanding the behaviour of atoms in glasses is a major research goal. Glasses are highly disordered systems that are typically produced by cooling a liquid very rapidly, causing a kinetic transition that depends on the system’s thermal history. As such, glasses can be viewed as liquids that have lost their ability to flow, leaving them trapped in a metastable state from which they spontaneously evolve towards a more stable configuration. Remarkably, however, glasses all have similar properties – a fact that has fascinated Beatrice for the past 10 years. “It’s almost like a philosophical thing: you have to find order amongst disorder,” she says. “I really love searching for the mechanisms responsible for this.”

Born in Rome, Beatrice initially studied mathematics at Sapienza University of Rome but switched to physics because she felt it was more team orientated. She completed her PhD, with a thesis titled “Vibrational properties of glasses at the transition from microscopic to macroscopic regime”, in 2010 at Joseph Fourier University during which she spent three years working at the former ID16 beamline (now ID20). In 2010 she was appointed as a postdoc at ID10, where she has been a full scientist since August last year.

Glasses come in many forms such as silica, metallic and chalcogenide glasses, she explains. By building up a general picture of the microscopic dynamics researchers can understand how to manipulate the process functioning in their natural environment, and also presented recent data showing the agreement between results obtained at synchrotrons and at X-ray free electron lasers.

Moving from dynamical to structural studies, Tim Salditt of the Universität Göttingen presented an overview of 3D structural analysis from bacterial and eukaryotic cells to nerve fibers, and lens-less diffractive imaging are functioning in their natural environment, and has used the ESRF’s ID01, ID02 and ID10 beamlines to investigate membrane fusion and synaptic vesicles, among other biological systems.

New format

A progress report on Phase II of the ESRF Upgrade Programme, and a directors’ report that included clips of the 10 new upgrade beamlines from Phase I that are now back in user operation, showcased the wealth of opportunities on offer today and in the near future as the ESRF continues to transform.

Four microsymposia were also held during the three-day meeting, which adopted a new format compared to previous years: novel routes for the study of strongly correlated electron systems; new possibilities for chemical studies; hierarchical imaging of biological, biomimetic and bio-compatible materials; and structural biology at the ESRF. A total of 350 delegates attended the microsymposia, during which scientific goals were discussed and researchers encouraged to take advantage of the full ESRF beamline portfolio.

Additional “poster clip” sessions gave users the chance to present their work ahead of the poster session. Selected from 110 contributions, the best poster award went to Anastasios Pateras from the Institut Fresnel in Marseille for his poster titled “Coherent X-ray 3D Bragg shadowgraphy on a III-V compound semiconductor thin film”. The February event also saw the ESRF communication group deliver two well received “scientists and the media” workshops, and the new format of the 2015 User Meeting was hailed a success.

Matthew Chalmers
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detecting the future
Rise of the robots transforms MX

A new era of automation in structural biology has begun with the opening of the upgrade beamlines MASSIF-1 and MASSIF-3.

In September 2014 the first of the ESRF’s MASSIF upgrade beamlines opened – but users will never physically come to the beamline. MASSIF, which stands for “massively automated sample selection integrated facility”, is a suite of beamlines that operates with minimal human intervention. Highly intense X-ray beams and complex workflows offer unprecedented sample throughput rates. The first beamline to open was MASSIF-1, offering a unique automatic service that allows data to be collected from a wide variety of samples and strategies that are tailored to individual crystals.

MASSIF-1, which is operated by the ESRF and EMBL Joint Structural Biology Group, offers a fully automated service for sample evaluation and data collection from macromolecular crystals. The new service is not designed to replace all user visits to the ESRF but rather to do the hard work of screening crystals during the night, thereby freeing up researchers to spend time solving challenging data-collection problems and studying the underlying biology.

Users describe their experimental requirements in the beamline database ISPyB, and this information is then used by the beamline software to set data collection parameters. Rather than the rigid scheduling that exists on other beamlines, slots on MASSIF-1 are booked flexibly to allow fast turnaround.

World leading

In less than three months of operation, more than 5 million diffraction images have been collected from over 4500 samples at MASSIF-1. These range from initial hits from crystallisation experiments to large-scale data collection for drug discovery programmes. This world-leading service is made possible by RoboDiff, a new ESRF-developed sample changer that also acts as a goniometer, and by workflows that fully evaluate samples, centre the best volumes and then collect diffraction data optimised for maximum resolution with minimal radiation damage.

The automatic routines developed are often able to locate crystals more effectively than the human eye and in many cases produce higher resolution datasets, as all positions within a sample can be evaluated for diffraction quality. Initial experiments have been very successful, with users complimenting the accuracy, efficiency and simplicity of the facility (see box, above). The beamline is also proving highly popular with the pharmaceutical industry, as rational drug design requires large numbers of datasets in order to search for fragments bound to proteins.

Using the fast X-ray centering routine, samples are located and centred systematically at the position of highest diffraction signal. Important parameters for sample characterisation such as flux, beam size and crystal volume are automatically taken into account – a process that is often too time-consuming when users are present. The beamline should therefore allow the accumulation and comparison of a large amount of information that was previously unknown, including the exact dimensions of crystals and deeper information about their quality. Over time, MASSIF-1 will provide a treasure trove of additional information to feed back into crystallisation experiments.

Hybrid operation

MASSIF-3, which provides a micro-focused beam 15 μm in diameter and a flux exceeding $10^{13}$ photons/s, entered user operation in December 2014 with a provisional experimental setup. Since February, it has pioneered the use of a new generation of pixel detectors in structural biology: the Eiger 4M, which is capable of recording images at a frame rate of 750 Hz.

The detector allows full datasets to be collected in a matter of seconds using the full beam intensity, which may help avoid radiation damage. It will also open new avenues in synchrotron serial crystallography and in time-resolved and room-temperature experiments. MASSIF-3 will likely be run in a hybrid mode that allows users either to send their samples for automatic data collection or to perform experiments while they are physically at the beamline.

Together, the operation of MASSIF-1 and MASSIF-3 represents a major step forward in macromolecular crystallography experiments at the ESRF. Matthew Bowler is a scientist at the EMBL Grenoble Outstation; Philippe Carpentier and Didier Nurizzo are in the ESRF Structural Biology Group.
Imaging all features

The ESRF beamlines enable bioimaging at many different lateral resolutions and contrasts, and increasingly offer in vivo and in situ capabilities.

Ever since its development in the visible light regime more than 300 years ago, microscopy has played a pivotal role in advancing our understanding of biological systems. In recent times, synchrotron imaging and analysis techniques using light ranging from hard X-rays to the infrared have become increasingly powerful in addressing biological problems. Bioimaging has always been a core driver of the ESRF’s life sciences programme, and its imaging capabilities have gone from strength to strength during the past two decades.

At the outset in 1987, coronary angiography was identified as one of the four targets for the ESRF’s imaging beamlines. The technique is much less invasive than conventional, hospital-based coronary angiography and it success motivated the construction of the ESRF’s biomedical beamline ID17. During the 1990s, when phase contrast imaging techniques started to outperform absorption-based imaging, the ESRF pioneered the development of holotomography at ID19, which is particularly important for non-destructive 3D imaging of soft materials. The potential of X-ray microscopy for cell biology motivated the construction of the ID21 beamline in the tender X-ray domain, and today hard X-ray nanobiology is one of the two main goals of the upgrade nano-imaging beamline ID16A.

The inherent nature of soft materials calls for hierarchical investigations because macroscopic function can only be understood by knowing the microscopic structure and dynamics of samples over all length scales – right down to the atomic.

Biomedical insights
At the largest scales, full human organs and even small animals can be studied by 3D imaging using the wide energy range (20–150 keV) and large beam size (150 × 5 mm²) of ID17 (figure 1). It is one of the few beamlines in the world dedicated to biomedical applications, notably clinically oriented research in biomedical imaging, radiobiology, radiotherapy and radiosurgery. Images can also be acquired in vivo to allow users to study processes such as drug metabolisation and breathing in real time under different physiopathological conditions. Such capabilities are highly relevant for preclinical studies of lung pathologies, including asthma and chronic obstructive pulmonary disease. In 2013, for instance, an international team led by the University of Picardie used ID17 to investigate the effects of allergen challenges to the local lung ventilation, for instance.

The long beamline ID19 also offers microtomography for macroscopic objects, thanks to a large beam size of up to 50 × 15 mm². ID19 can reach spatial resolution in the submicrometre and sub-second temporal resolutions, while offering exceptional contrasts. This has allowed users to reveal the dentin substructure in fossils teeth, for example, and to follow in real-time the decay of protein-based foams or visualise tumours in soft tissue with much higher levels of detail than MRI instruments. In 2007, users from Charité Berlin came to ID19 to study a human jawbone six months after bioceramic particles had been implanted, allowing them to investigate how the degradation speed, microporosity and other properties of bioceramic particles affect bone regeneration (figure 2).

A variety of analytical techniques at the ESRF allow researchers to probe the chemical composition of biological samples at the micrometre – in particular at ID13 and ID21. Micro-X-ray fluorescence (XRF), X-ray diffraction (XRD), X-ray absorption spectroscopy (XAS) and infrared (FTIR) spectroscopy can be used to map elements, phase, species and molecular groups. In 2013 a team from the Istituto Italiano di Tecnologia in Genova combined Raman spectroscopy with micro-XRD at ID13 and micro-FTIR at ID21 to measure conformational changes in amyloid (Aβ) peptides, which are relevant for the study of Alzheimer’s disease.

Indeed, micro and now nano-probe XRF has become a key tool in the new field of “metallomics”, which allows the distribution, concentration and chemical state of metals inside a cell to be mapped at the organelle level. This is an essential step towards the full...
great and small

understanding of certain cellular physiological or toxicological processes. Experiments usually aim at localising and quantifying metals, either naturally present in cells or tissues or following the uptake of exogenous metals originating from drugs, cosmetics or environmental pollutants (figure 3).

Towards the nanoscale
Since most biological processes occur at the sub-cellular and organelle level there has been a continuous effort to push the spatial resolution to the nanoscale. The ESRF’s upgrade nanofocus beamlines “NINA” opened to users last year. The nano-analysis beamline ID16B offers a multi-analysis nanoprobe for spectroscopic studies that allows in situ experiments at selective submicrometre scales. The nano-imaging beamline ID16A offers quantitative 3D characterisation of the morphology and elemental composition of specimens in their native state by combining coherent imaging techniques and X-ray fluorescence analysis. The beamline has a beam size as small as 35 x 20 nm² at 17 keV and a flux as high as 10¹² photons/s. The first experiments demonstrated unprecedented capabilities in the localisation of metals inside organelles and improved sensitivity in the 3D imaging of the ultrastructure of bone tissue for different pathologies.

Coherent scattering is an alternative way to access smaller cellular structures. It exploits a lens-less approach where the image is reconstructed numerically from high resolution scattering data while the sample is coherently illuminated. The ID10 beamline exploits high-resolution coherent diffraction microscopy to image biological specimens such as bacteria in different growing phases (figure 4). So far, isolated objects smaller than 7 µm can be imaged at 40–20 nm resolution but this technique is expected to improve towards the atomic resolution offered by macromolecular crystallography.

Since intense X-ray nanobeams can provoke radiation damage and modify the chemistry of a sample, the ESRF has developed a range of off-line and in-line equipment to help users. By the end of this year, ID16A will offer a cryogenic sample environment at temperatures below 100 K that will allow scientists to better preserve samples from radiation damage. Laser-based optical tweezers have been developed at ID13 for manipulating single cell model organisms and allowing live cell X-ray elemental imaging, while at ID21 the cryo-microtome and the cryo-stage are regularly used for preparing cryo-sections of roots or leaves from plants, preserving the tissue structure and reducing the risk of radiation damage.

In addition to the ESRF’s world-class X-ray and infrared micro/nano-spectroscopy, atomic force microscopy (AFM) instruments are also available for nanoscale characterisation of samples. In 2013, for example, a Grenoble-based team developed spectroscopic methods using in situ AFM equipment to investigate the mechanical impedance of PC12 living cells (figure 5). Work is now being undertaken to follow the modifications of these mechanical properties in real time and under physiological conditions.

Phase II of the ESRF Upgrade Programme, which will see the construction of a new ultra-low emittance storage ring, will immediately reap rewards for the bioimaging community. By increasing both the brightness and degree of coherence of the X-ray beams, it will bring significant improvements in resolution and sensitivity. These new beam properties, together with ever-faster 2D detectors, will open new opportunities in biology in particular for in vivo time-resolved imaging applications.

Marine Cotte is head of the X-ray imaging group; Sylvain Bohic is senior scientist at Inserm and scientific collaborator at ID16A.
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Flu structure opens path to new drugs

By using the ESRF, researchers at the European Molecular Biology Laboratory have obtained the first complete picture of the molecular machine that replicates the influenza virus.

Influenza virus causes a highly contagious respiratory illness, better known as the flu. Everybody on the planet is susceptible to the flu and consequently it has a high public health and economic cost (see panel). While vaccines can provide good protection against influenza, there is a need for effective anti-influenza drugs to treat severe cases and to avert pandemics where no vaccine is available.

The molecular structures of influenza virus proteins are key to developing new treatments, in particular the viral replication machinery: a large complex of three proteins called flumur polymerase that copies the viral RNA genome. After 20 years of trying, my team at the EMBL in Grenoble has determined the high-resolution crystal structure of the influenza polymerase. The results will aid the development of drugs that target this essential viral enzyme.

It was in 2007 and 2008 that experiments at the ESRF by our group and collaborators at Grenoble University and CNRS revealed the structures of two fragments of flumur polymerase. These structures were found to “cleave off” a small chemical structure called a cap from the host cell’s protein-coding RNA (mRNA), causing the machinery to instead promote viral mRNA production (Nature 458 914; Nature Structural and Molecular Biology 15 500). Now, a series of further measurements at the ESRF’s ID23-1 beamline have produced the complete structure of the polymerase and revealed how the previously isolated fragments function (Nature 516 355 and Nature 516 361).

Virus promotion
The influenza virus was first isolated from humans in 1933. Its genome comprises eight segments of negative-sense, single-stranded viral RNA (vRNA) each of which is packaged in an individual ribonucleoprotein particle (RNP). RNPs contain one RNA polymerase, which is bound to the conserved extremities of the “promoter” vRNA. The viral polymerase both transcribes and replicates each genome segment. Transcription, which produces viral mRNA, occurs by a unique mechanism among segmented negative strand RNA viruses where the 5' cap-structure is cleaved from host cell mRNAs by an endonuclease and “stitched” onto the beginning of the viral transcript. Replication produces full-length copies of the genomic vRNA and proceeds via a complementary cRNA intermediate.

One of the main experimental challenges was to obtain sufficient amounts of pure and active recombinant polymerase. To overcome this we used a novel baculovirus vector developed by Imre Berger at the EMBL in which the three polymerase subunits (PA, PB1 and PB2) are expressed as a fusion protein and cleaved into the three separate pieces. Bacteria-specific influenza A (FluA) and human influenza B (FluB) polymerases were then co-crystallised with the vRNA promoter and the structures were solved at a resolution of 2.7Å.

These are the first structures of a complete polymerase from any negative strand RNA virus and show that the polymerase subunits mutually stabilise each other through extensive and intricate interfaces. By comparing the FluA and FluB polymerase structures we can gain mechanistic insights into the transcription and replication processes. By inhibiting either of these processes, the virus can no longer multiply, and since the polymerase is very similar between different influenza strains, such inhibitors could potentially tackle a wide range of flu viruses including novel pandemic strains.

Influenza variants
- **Seasonal flu** causes annual epidemics where typically the global infection rate is 5–10% in adults and 20–30% in children, with 250,000 to 500,000 deaths occurring each year – mainly among the very young, the elderly or the chronically ill.
- **“Bird flu”** is caused by highly pathogenic avian influenza strains such as H5N1 and H7N9, which through point mutations are able to infect humans with up to 60% mortality but as yet do not transmit between humans.
- **Pandemic flu** occurs when novel human strains are generated from the recombination of avian and mammalian strains, often via an intermediate host such as pig. Pandemics are unpredictable and potentially devastating, and have occurred in 1918, 1957, 1968 and 2009.

Cap-snatching drugs
Interestingly, the FluA and FluB structures differ in the orientation of the PB2 cap-binding domain, suggesting a mechanism for cap-dependent transcription. In the FluA structure the cap-binding site faces the PA endonuclease active site, which is compatible with cap-snatching, but in the FluB structure the cap-binding domain has rotated in situ by 70° and points towards the PB1 active site. This swivelling explains how the fragment of host mRNA is first positioned to be cleaved and then directed into the polymerase active site to start the process of copying the viral genome into viral mRNA. These structures not only lay the basis for an atomic-level mechanistic understanding of the multiple functions of influenza polymerase but will also shed light on the difference between human and avian polymerase variants.

Based on the original work on the cap-snatching domains, EMBL set up a company called Savira Pharmaceuticals to exploit the structures for drug design, which has since been taken over by Roche. Despite this breakthrough we are only at the beginning, though, and our group is now focusing on a series of snapshot structures of the flu polymerase in action.

*Stephen Cusack is head of the European Molecular Biology Laboratory, Grenoble Outstation.*
Focus on: biology

The power of powder

Powder diffraction is providing novel insights into the structural characteristics of formulations used in the treatment of diabetes and other chronic illnesses.

To understand the biochemistry of life, and the onset and progression of many diseases, structural information is required about the proteins and macromolecules that control biological processes. The standard way to determine protein structure, is to grow a single crystal for crystallographic analysis. But although some 100,000 biological structures have been solved in this way, growing a single crystal is often challenging, time-consuming and success is never guaranteed. An alternative approach uses powder diffraction, for which the preparation of certain crystalline samples is often much faster and easier.

Powder diffraction is a standard technique in materials science, with samples comprising an assembly of many micrometre-sized crystals rather than a larger single crystal. A single powder diffraction pattern captures all possible crystal orientations simultaneously, rapidly delivering information about all the crystallographic phases present and improving the signal from weakly diffracting materials. Traditionally exploited for identifying different crystalline substances, powder diffraction is now being applied to numerous biological systems with impressive results.

Biological application

It was pioneering work in 1999 by Robert Von Dreele of the APS at Argonne National Laboratory, initially using the lysozyme from egg white, that demonstrated the power of powder diffraction for revealing protein structures. The method was further established by former ESRF scientist Irene Margiolaki, who is now head of our group at the University of Patras, Greece. Some of the crystalline polymorphs identified from powders are of pharmaceutical interest for the treatment of diabetes (Acta Cryst. D 68 1632; Acta Cryst. D 69 978).

Diabetes is caused by inadequate control of insulin levels in the body. Patients have to be given insulin externally via regular hypodermic injections of microcrystals or intermixtures of microcrystals and amorphous proteins, which gradually dissolve so as to augment insulin levels in the blood. With approximately 60 million people suffering from diabetes in Europe and cases expected to rise, our research is aimed at improving treatments by gaining a better understanding of the structural and other characteristics of therapeutic microcrystals used in clinical formulations. The size and morphology of insulin crystals are crucial because they affect the insulin release rate and duration of action. Synchrotron sources were previously the only facilities able to provide data with sufficient quality and angular resolution for such a detailed analysis. However, results obtained recently employing modern laboratory X-ray powder diffractometers display enhanced data resolution sufficient for performing polymorph identification studies (Acta Cryst. D 71 in press).

Following the first successful measurements at ID31, the next major achievement came in 2011 with the discovery of two new biologically active insulin complexes, which required several further experiments to confirm. Indeed, our analysis is still taking place in conjunction with ESRF beamline scientists Andy Fitch and Jon Wright. The new polymorphs appear to have dense crystal packing, thus potentially increasing the stability and lifetime of insulin formulations. This could be a key point in the development of a next-generation product comprising crystals of high protein concentration that would minimise the number of insulin injections needed by diabetes patients. If a single dose per day is enough to provide the necessary amount of insulin – stored in crystals and slowly released into the blood stream – it would be a life-quality improvement of great importance since most people need up to four injections per day.

We have also taken advantage of the ESRF’s instrumentation and expertise to investigate another macromolecular protein: urate oxidase, which is a key component of drugs for reducing uric acid levels. Gouty arthritis is an inflammation associated with high levels of uric acid that usually affects elderly people, causing moderate to intense pain. Part of our research is dedicated to the structural characterisation of a wide range of urate oxidase polymorphs prepared under different conditions. Qualitative structural studies carried out at the ESRF have given us encouraging results concerning the improvement of purity, stability and efficiency of such formulations (Acta Cryst. D 66 539).

Better treatments

We are one of just two or three groups worldwide studying macromolecules with powder diffraction techniques, and industry participation has been an important factor in our success. The project started in collaboration with Sanofi-Aventis and today our insulin samples are based on real clinical formations provided by Danish pharmaceutical firm Novo Nordisk.

In spite of the encouraging results, many further steps are required to reach the final design of improved formulations. Our long-term plan is the full structural characterisation of the new polymorphs and to continue the exploration of the fertile phase diagrams – new crystalline forms with the potential to improve the lives of many millions suffering from chronic illnesses. The exceptional facilities offered by the ESRF will be a major contributor to the pursuit of these goals. Karavassili Fotini and Alexandros Valmas are in Irene Margiolaki’s research group at the University of Patras, Greece.
Serial crystallography takes off

Experiments at ID13 have used a novel jet injector to solve the structure of a membrane protein, marking a new phase in synchrotron X-ray crystallography.

Membrane proteins control numerous vital functions of the cell and are the targets for more than half of all drugs, including those used to treat asthma, Alzheimer’s disease and schizophrenia. Synchrotron X-rays play a major role in elucidating their structure, but membrane proteins are notoriously difficult to crystallise because they are embedded in the cell walls. Even when crystals can be obtained they are often very small, diffract weakly and can be damaged by radiation and freezing.

An alternative approach is to fire a steady stream of crystals into the path of the X-ray beam. Known as serial crystallography, this technique increases the probability of obtaining a “hit”, or producing a diffraction pattern, as the pulsed X-ray beam coincides with the sample crossing its path. A very slow version of serial crystallography has been common practice among biologists for at least a decade, but thanks to developments such as liquid cubic phase (LCP) injection, it is now possible to obtain millions or even tens of millions of exposures in a matter of hours.

XFEL territory

Modern serial crystallography began in 2010 when the Linac Coherent Light Source – an X-ray free electron laser (XFEL) – entered operation at SLAC, California. The high photon flux of XFELs allows for much shorter exposure times, which enables researchers to map small crystals before they are damaged by radiation. Researchers used femtosecond pulses to collect more than 300,000 diffraction snapshots of photosystem-I nanocrystals, which are one of the largest membrane proteins (Nature 470 73). But advances in synchrotron technology in recent years have opened new opportunities for serial crystallography at storage rings – as first demonstrated in 2013 by researchers at DESY’s PETRA III facility (IUCrJ 1 87).

The ESRF is one of the few storage rings in the world that is able to tackle such studies, on account of its high brilliance and stability. Last year, an international team came to the ESRF’s ID13 beamline with a novel LCP injector to trial the technique (see image, above). The researchers fed a stream of bacteriorhodopsin – a membrane protein that acts as a light-driven proton pump – into the path of the X-ray beam at room temperature and under ambient conditions. Tens of thousands of crystals were used and several crews worked in shifts over a four-day period (IUCr; DOI:10.1107/S2052252514026487).

The injector must be capable of producing a stable flow with a diameter of just 20–50 micrometres over long time periods without clogging or degrading the quality of the samples. It was developed by team member Uwe Weierstall at the Arizona State University and co-workers and was originally designed for use at XFELs. “We came to the ESRF expecting to test the crystals to see if they would diffract, but the results have been so amazing that we have been able to collect a complete data set and managed to solve a structure,” explains Weierstall. “The staff at ID13 have worked extremely hard to prepare for our experiment and make sure that all runs smoothly. All aspects of our experience here have surpassed expectations.”

Faster frame rate

Since the first trials, explains ID13 scientist-in-charge Manfred Burghammer, the beamline has been equipped with an Eiger 4M detector that has increased the frame rate from 17 Hz to 700 Hz, therefore providing clearer diffraction patterns.

“There is a lot of interest in establishing this technology at the ESRF,” says Burghammer. “Along with the expected upgrade of the accelerator complex in 2018–2019, the higher photon flux will maintain the ESRF as a powerful complementary facility for serial crystallography alongside XFELs.”

Synchrotrons will play a particularly important role for serial crystallography in the millisecond to microsecond time regime, he adds. “Extrapolating from the few publications since last year, my guess is that this will soon become a thriving branch of macromolecular crystallography, and probably even completely different scientific fields where ‘statistical’ diffraction methods could be applied to large ensembles of micro objects.”

Matthew Chalmers
Focus on: biology

Structure flows deep

Users from Sweden have solved the structure of human aquaporin 2, a vital water-regulating protein that could shed light on the links between protein sorting and disease.

The human kidneys produce 180 l of primary urine per day, which must be concentrated by a factor of 100 in order to achieve our normal urine output of 1–2 l. This remarkable feat is possible thanks to water channels called aquaporins located in the membranes of kidney cells, which allow water molecules to be reabsorbed by the cell while preventing the passage of ions and other solutes. By the time urine reaches the kidney collecting duct, 90% of the water has been reabsorbed by aquaporins in the nephrons (the functional units of the kidney). The remaining 10% is processed in a more regulated manner by “aquaporin 2” (AQP2), depending on whether you are dehydrated or have had too much water.

Recently our group solved the structure of human AQP2 by X-ray crystallography. Not only does it offer new insights into this vital regulatory process, it could help in the development of drugs to treat nephrogenic diabetes insipidus (NDI) – a water-balance disorder whereby patients suffer from large urine volumes (up to 20 l). Although the condition is rare, it causes severe dehydration.

Ubiquitous proteins

Aquaporins can be found in almost all species. They were first identified in human red blood cells in the early 1990s, a discovery recognised by the 2003 Nobel Prize for Chemistry. Since then, 13 aquaporin variants have been found throughout the human body. They are involved in numerous physiological processes, including bile secretion, salivation and urine concentration, and have been linked to several human diseases including cancer, cataract and brain edema.

Over the last two decades, structural studies on a number of aquaporins have shown that their core structure is the same and that they share the same passive mechanism for water transport along concentration gradients. However, small differences in amino acid sequence (and therefore structure) allow their ability to transport water across membranes to be regulated in response to different physiological triggers. In the case of human AQP2, dehydration causes the protein to be moved from intracellular storage vesicles to the cell membrane, increasing the membrane’s permeability to water and leading to a smaller urine volume. This process, known as trafficking, is tightly controlled by the pituitary hormone vasopressin. Failure to recruit AQP2 to the cell surface causes NDI.

As with all other aquaporins, AQP2 is a “tetramer” in which each of the four protein molecules function as an individual water channel. The structure revealed that, unlike other mammalian AQP structures, one end of the protein (the C-terminus) is highly flexible. Modifications of this part of the protein are crucial for AQP2 trafficking, and the AQP2 C-terminus also binds other proteins en route to and from the cell membrane. The latter is seen in the structure where a small α-helix in the C-terminus binds to the intracellular face of another AQP2 molecule, mimicking a physiologically relevant interaction between AQP2 and a regulatory protein. The interaction is further helped, our studies show, by the binding of two positively charged ions per tetramer. These most likely correspond to Ca^{2+} in vivo, indicating for the first time a direct role for Ca^{2+} in AQP2 trafficking.

Protein sorting

The structure of AQP2 is also an ideal framework for understanding the many single amino acid mutations that are known to cause NDI, S1 of which have been reported in the literature. Most of these mutants never reach the cell membrane because they are judged as misfolded by the cell’s protein quality-control system, although some are still able to transport water. By studying the effect that these mutations have on the structure, we may therefore be able to design drugs that allow the AQP2 mutants to circumvent the quality control, thereby restoring the kidney’s ability to concentrate urine.

AQP2 is one of the best-characterised membrane proteins in terms of how it is transported or “sorted” within the cell. The structure of AQP2 therefore serves as an excellent platform not only for learning about its trafficking and role in NDI, but also for studying the general mechanisms behind membrane protein sorting. Many diseases, for example cystic fibrosis, are linked to proteins not being sorted correctly yet surprisingly little is known about the underlying mechanisms. A major challenge is to move from individual membrane protein structures to capturing and determining the structures of protein complexes. These studies will help biologists answer one of the fundamental questions in human cell physiology: that of how proteins are sorted between different cellular compartments and how this is linked to disease. Anna Frick is at the University of Gothenburg and Susanna Törnroth-Horsefield is at Lund University, Sweden.
‘A wonderful tool for biologists’

The Partnership for Structural Biology (PSB) helps to maximise the scientific potential of the EPN campus, says Eva Pebay-Peyroula.

The EPNScience campus in Grenoble is unique in terms of the tools that it offers scientists – biologists in particular. It hosts four major European institutes: the ESRF, the European Molecular Biology Laboratory (EMBL), the Institut Laue-Langevin (ILL) and, since 2013, the Institut de Biologie Structurale (IBS). The EPN campus is also home to several scientific partnerships that include institutes in the wider region and is part of the GIANT campus, which comprises all scientific activities on the west side of Grenoble.

Established in 2002, the Partnership for Structural Biology (PSB) provides an integrated environment in which to carry out state-of-the-art structural biology research. Today, the PSB comprises about 300 staff scientists, students, postdocs and technicians, all of whom are now located on the same site. Sharing the canteen and other common areas is an ideal way to hold informal discussions about new scientific ideas and collaborations, and more formally it helps the PSB steering committee to draw up long-term strategies.

Common interest

The PSB is a wonderful tool. Although we all operate under very different governing bodies, we are able to address our common interest in structural biology. The provision of synchrotron radiation at the ESRF and neutrons at the ILL, combined with NMR and electron microscopy (EM), offers a rare combination of instruments and methods with which to elucidate complex biological pathways at the molecular level within a cellular environment. Indeed, the partnership has delivered an ensemble of complementary platforms including protein production, crystallisation, biophysical characterisation and structure determination. In addition to instrumentation and techniques, the PSB enables strong scientific collaborations in host-pathogen interactions, gene regulation, cell division, stress response in prokaryotes and membrane proteins.

Recently, IBS and the Unit of Virus Host Cell Interactions (a PSB member) obtained a 10-year long grant that has allowed us to upgrade our NMR and EM tools. Although an established technique, EM has progressed tremendously in the past two years and in 2014 we equipped the PSB’s Polara microscope with a new direct-detection camera that opens new ways to solve structures at relatively high resolution. Having EM and synchrotron radiation on the same site is a quite unique situation. Indeed, it would be fantastic if biologists were able to access EM platforms in a similar automated fashion as we do synchrotrons. There is a strong interest in such capabilities from several fields, in particular for membrane transporters or channels that undergo conformational changes as they function.

Nano-crystallography is another astonishing development that the PSB aims to strengthen. The emergence of X-ray free electron lasers (XFELs) has shown that we can solve a structure from numerous nano-sized crystals, each illuminated at room temperature with a single, very intense X-ray pulse. PSB scientists have already carried out several experiments at the SACLA and LCLS facilities in Japan and the US, respectively. Initially, this new capability was perceived as being in competition with synchrotrons, but in fact it has opened a new way of thinking about “serial” crystallography at synchrotrons based on the use of numerous small crystals – an important challenge in which the ESRF is at the forefront (see p19).

The dynamics, as well as the structures, of single molecules or larger assemblies also hold important secrets about their function. NMR methods developed within the PSB, for instance, have highlighted how non-structured proteins fold upon binding with a partner and thus propagate signals across the cell. Performing similar investigations inside cells is a major challenge that PSB scientists are beginning to approach. It is expected that synchrotron X-rays – in particular the increased level of coherence provided by the ongoing ESRF Upgrade Programme – will allow us to explore this vital cellular machinery in more detail than ever before.

Close contact

One of the key strengths of the EPN campus is the close contact between scientists who work on new instrumentation and methods and scientists who are striving to investigate biological questions. Several PSB teams are achieving outstanding results in targeting the mechanisms of bacterial virulence or viral infection, for instance, and these themes involve other, more medically orientated institutes in the Grenoble area. Yet our knowledge of these mechanisms at a molecular level is still very partial, and new discoveries will be needed as new pathogens emerge.

Integrating multiscale processes from the molecule to the cell and beyond still remains a major challenge for which local collaborations such as GRAL – a 10-year long project that bridges structural and cellular studies including proteomics with the neighbouring Institute of Sciences Research and Technologies – are vital. The race to achieve such integration has just started, and the PSB has an important role to play in the next 10 years to help bring it to fruition.

Eva Pebay-Peyroula is a professor at the University Grenoble-Alpes, and recently stepped down as director of the IBS.
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Life sciences under new leadership

Jean Susini describes his hybrid career path and sets out his priorities for the next five years as the ESRF’s new director of research for life sciences.

Jean Susini defended his PhD thesis in Paris on 28 March 1989. A few days later, on 1 April, he started work at the ESRF. The facility itself didn’t yet exist and he had never even been to Grenoble. It was almost like a joke, he recalls, but it determined the rest of his professional and private life.

On 1 January this year, Susini began a five-year-long term as director of research for life sciences, chemistry and soft matter science at the ESRF, replacing Bauke Dijkstra who had held the position since September 2012. Susini didn’t expect to stay so long in Grenoble, he explains. “I’ve had a funny, hybrid trajectory because I did so many things without taking time to become a real expert in a given area.”

During his career he has appeared on more than 130 publications in peer-reviewed journals and has delivered some 70 invited lectures. He has also served on the science advisory committees of other light sources, including ALBA (Spain), Diamond (UK), NSRRC (Taiwan), PSI (Switzerland), SIRIUS (Brazil), ALS (US) and also on many review committees.

From biology to beamlines

Although science was not a particular childhood fascination, Susini always liked experiments – especially if they involved fire. When he was 12, he almost burned his parents’ house down by mixing chemicals together. After a degree in biology he had planned to do a PhD in biophysics in Paris, but thanks to the influence of pioneering synchrotron scientists Yvette Cauchois and Robert Barchewitz, he started to work on advanced X-ray optics at LURE in Orsay. Although a very small field at the time, it was precisely the expertise that the ESRF needed.

Susini’s initial work in the ESRF optics group focused on X-ray mirrors and optical metrology. Five years later, in 1994, his unusual combination of expertise in X-ray optics and biology led to his appointment as a permanent beamline scientist at ID21. The then research director Carl-Ivar Brändén had charged him with building a new X-ray microscope for life sciences. “I failed!”, recalls Susini. “It was a complex instrument that was not ready yet for biologists and we didn’t compete with light or electron microscopy, but I had the right intuition to pursue X-ray fluorescence and micro-spectroscopy at the sulphur K-edge and ID21 was the first beamline in Europe to do this.”

Striving to provide ESRF users with a complete suite of complementary instruments, he co-ordinated the development of several end stations that have attracted new user communities. The hard X-ray microscope ID22-Ni was the culmination of this collective effort, serving as a prototype for the upgrade beamline ID16 (“NINA”) today. In the process, Susini helped the ESRF pursue nano-probe instrumentation and, in 2009, he became head of the newly created Instrumentation Services and Development Division (ISDD). Susini says he is driven more by developing a vision for the ESRF than by advancing his own scientific career. The exciting part about working at ID21, for instance, was that he had the freedom to guide what was studied. “I pushed a lot of cultural heritage and environmental sciences because people needed to see that synchrotrons are directly useful for society, not just for pure fundamental research,” he says. “A synchrotron is a unique infrastructure but you need collaborations across the beamlines to address a question in a holistic way.”

Three key aims

It is this sense of purpose that attracted Susini to the research director position. “I like to build things, and I felt it will allow me to continue to build something new for the ESRF,” he explains – setting out the three main challenges ahead. First, he wants to help the experiment division make the best use of Phase I of the ESRF Upgrade Programme, including greater communication with users about what the new beamlines are capable of. His second goal is to establish what will be the flagship topics in life sciences for the Phase II upgrade. The third strand is to better exploit the unique potential of the EPN campus by strengthening activities such as the Partnership for Structural Biology and Partnership for Soft Condensed Matter. “My asset is my ability to consolidate things in the long term,” he adds.

He also faces a personal challenge: to be perceived as an “insider” by the different life sciences communities. Susini is the first life sciences director who is not a structural biologist, he points out, and he says it is important to establish bridges between structural biologists and other communities. “Europe is so well equipped with beamlines that it’s very important for the ESRF to determine which areas to focus on in the next five years so that we can be ready for a new era in the field,” he says, clearly looking forward to the task ahead. “Five years flies by, so you have to establish your leadership and share your vision within the first few months of your tenure.”

Matthew Chalmers

Jean Susini in brief

**Born:** 1961, Nogent-sur-Marne.

**Education:** PhD chemical physics, University Pierre et Marie Curie (1989).


**Family:** Married, two children.

**Interests:** Hiking, cinema, draughts.

“You need to address questions in a holistic way.”
Firm finds structural solution

SAXS experiments have helped scientists from Boehringer answer a key research question in their search for new drugs.

Antibodies are a vital part of our immune system. These proteins, which circulate in the bloodstream, bind to specific antigen proteins on the surface of foreign bodies such as bacteria and viruses in order to neutralise or disarm them. Since each antigen has a different shape, it requires a different antibody to attach to it: antibodies targeting the measles virus, for example, will not be able to defend the body against chickenpox. By tailoring antibodies to attach to proteins responsible for specific diseases, pharmaceutical companies seek to develop drugs that minimise side-effects caused by antibodies binding to the wrong targets.

Boehringer, which is headquartered in Ingelheim in Germany and has more than 47,000 employees worldwide, carries out research into the structure of antibodies and other medically relevant systems to develop drugs against respiratory and cardiovascular diseases, Parkinson’s disease, HIV and many other conditions. The firm has long relied on protein crystallography and other techniques for such investigations, but recently its researchers have turned to small-angle X-ray scattering (SAXS) at the ESRF to study molecules under physiological conditions.

As is common for industrial users at the ESRF, full details of the antibody under study are protected under commercial agreement. “The structure is an antibody against a therapeutically relevant molecule in the body, but the key point is that the molecule we were interested in is used as a useful reference when we are testing new methods in our process department,” explains Stefan Hoerer, who is a principal scientist at Boehringer.

Interesting behaviour

Biochemical and other characterisation techniques in Boehringer’s laboratories had previously revealed interesting behaviour of the molecule that suggested it was unusually compact compared to other antibodies. If the antibody had such a compact conformation in solution, explains Hoerer, it would have implications for the mechanism of how the antibody works. “It would lead us to develop hypotheses about the effects of the molecule and why it is special, but we did not dare to draw such conclusions without first establishing whether the compact conformation is real or if it is an artifact of crystallisation.”

In 2012 the team used the Swiss Light Source to obtain the crystal structure of the molecule. This confirmed its compact nature, but the next step was to obtain structural information about the molecule in solution, rather than in its single-crystal form. “We wanted to use SAXS and the ESRF provides a more suitable SAXS platform for industrial customers,” says Hoerer. “We just sent our sample to the ESRF and all the rest was done by beamline staff.”

By comparing the measured scattering curve with that calculated from the X-ray structure, Emile Poudouville of the ESRF Business Development Office and Adam Round of the EMBL Grenoble Outstation confirmed that the compact conformation does not exist in solution. Instead, the molecule adopts a Y-shaped conformation commonly known for antibodies.

“This is a routine service that allows for fast results using the dedicated, highly automated facilities of BM29,” says Round. “Not only could we confirm that the structure did not match the compact form, but we could also show the shape of the extended functional conformation in solution.”

The SAXS data prevented Boehringer from carrying out “unnecessary or unintelligent” work, adds Hoerer, and demonstrates the importance of SAXS for studying antibodies in solution. “The ESRF has proven to be a very valuable partner for these sorts of question in the future,” he says.

Matthew Chalmers

Movers and shakers

Guillaume Gotthard, a post-doc in the ESRF’s structural biology group, has won the Léon Baratz & Dr Darolles prize from the French Academy of Medicine for his thesis work on the biotechnological development of enzyme-degrading organophosphorous compounds. Gotthard, who did his PhD at the University of Aix-Marseille II, collected all the crystallographic data used in his thesis work at the ESRF. He now works on the development of in crystallo optical spectroscopies at the cryobench (ID295) beamline and on the structural characterisation of coloured proteins useful for live cell imaging.

Dieter Einfeld, an expert accelerator physicist and pioneer of the multi-bend achromat lattice concept, has been appointed to the project team for the ESRF’s Phase II Upgrade Programme (see p9). Einfeld, who has worked on the Max-IV project in Sweden and been chair of the ESRF’s Accelerator Programme Advisory Committee, will be based partly at the ESRF for the next year.

Alim-Louis Benabid of Université Joseph Fourier, who was a member of the ESRF Science Advisory Committee from 2006–2008 and an observer from 2009–2011, has won a 2015 Breakthrough Prize in Life Sciences for the discovery and pioneering work on the development of high-frequency deep brain stimulation, which has revolutionised the treatment of Parkinson’s disease. The six annual Breakthrough prizes in life sciences are each worth $3 m and recognise transformative advances toward understanding living systems and extending human life.
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Beans in their element: This image shows a thin section of root nodule from the Vicia faba plant, more commonly known as the broad or fava bean. Data was acquired at the ESRF’s ID21 beamline using X-ray fluorescence imaging under cryo-conditions, and shows: chlorine (red), sulphur (green) and phosphorous (blue). Since root nodules host bacteria responsible for the fixation of atmospheric nitrogen, such studies allow comparisons between the phosphorus distribution in nodules grown in phosphorus-normal concentration conditions and those grown in phosphorus-deficient concentration conditions in order to understand phosphorus utilization in crops. The experiment was performed by Camille Rivard and Hiram Castillo Michel of the ESRF, in collaboration with Laurie Amenc, Bouchra Macoudi and Jean-Jacques Drevon from INRA laboratory Eco&Sols Montpellier, France.

In the corridors

ESRF tops Europe

The ESRF (large red band) was the number one synchrotron in Europe for the number of structures deposited in the Protein Data Bank in 2014, and ranked second in the world after the Advanced Photon Source at Argonne National Laboratory in the US (blue). Diamond Light Source in the UK (grey) was third, with the next biggest contributors shown clockwise: Swiss Light Source (yellow), Advanced Light Source (black), Stanford Synchrotron Radiation Light Source (green), National Synchrotron Light Source (red), Photon Factory (brown), Shanghai Synchrotron Radiation Facility (dark grey), SPRING-8 (dark yellow), BESSY (dark blue), Australian Light Source (purple) and the National Synchrotron Radiation Research Center (light blue). Deposits from other facilities are indicated in orange.

‘Orange Book’ now available

The technical design study for the ESRF Upgrade Programme Phase II, also known as the “Orange Book”, was published in December. In addition to full details of the upgraded source, it provides a description of the Phase II science drivers and beamlines. Electronic downloads and hard copies are available at: www.esrf.eu/home/about/upgrade/documentation/orange-book.html

New member for European XFEL

Six years after it pulled out of the project, the UK is to become the 12th member state of the European XFEL in Hamburg, Germany, with an investment of up to £30 m. Due to switch on in 2016, the European XFEL will produce extremely bright and short X-ray flashes for the investigation of ultrafast processes. A further £5.64 m will go towards a user consortium in the life sciences aimed at a dedicated serial femtosecond crystallography end station.

X-ray bootlegs

During the late 1950s, when the Soviet music industry was under tight regulation, a vibrant trade grew up in bootleg records containing forbidden music. With access to conventional recording techniques restricted, music lovers came up with an ingenious alternative: used medical X-ray fluorography sheets that were unofficially obtained from hospitals at low cost, cut into discs and then embossed with the grooves of bootlegged gramophone records. Still carrying partial images of skeletons, the disks were called “bones” and “ribs”. Now, thanks to the “X Ray Audio Project” by musician Stephen Coates and photographer Paul Heartfield, which launched in January, the scratchy sounds of this short-lived underground movement can be enjoyed by everyone: https://x-rayaudio.squarespace.com.
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- Fast response, wide dynamic range.

![Diamond XBPM](image)

Figure: Measurements at Diamond Light Source Ltd., UK, show the measured position resolutions at 1 kHz bandwidth for various beam intensities of 12.6 keV photons. A position resolution of better than 0.1% of beamsize is obtained even for an incident flux as low as $10^9$ photons/s.

![Position response](image)

Figure: Measurements on the B16 Test Beamline at Diamond Light Source Ltd, UK, show the position response for a 50 micron beam size. Note the very sharp response and the excellent scale factor at the centre of the device.

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