PSB et al
Partnership for Structural Biology Newsletter

SCIENTIFIC HIGHLIGHTS

Mutually exclusive CBC complexes determine the fate of Pol II transcripts

Within the cell nucleus RNA polymerase II (Pol II) transcribes many different classes of RNA (e.g. mRNA, histone mRNA, snRNA, snoRNA, miRNA), each of which has its own distinct maturation (e.g. splicing and 3' end processing) and transport pathways. A central question in RNA biology is how all these processes are coordinated, regulated and quality controlled and how aberrant transcripts are detected and degraded. One process common to all Pol II transcripts is the addition of an m^7G cap structure at the 5' end which is immediately bound by the heterodimeric nuclear cap binding complex (CBC), comprising subunits CBP80 and CBP20. CBC then acts as a platform to assemble co-transcriptional complexes that help determined transcript fate. Whereas some CBC associated proteins such as NELF-E (negative elongation factor subunit E) and ARS2 (arsenite-resistance protein 2) seem to associate with RNA-CBC complexes regardless of the type of RNA, others are important for distinct RNAs, such as PHAX, which is required for snRNA and snoRNA transport.

To better understand the nature of the different CBC complexes, we used various biochemical, biophysical and structural methods to analyse the individual interactions between human CBC and ARS2, PHAX as well as NELF-E at the molecular level [1]. For instance, isothermal titration calorimetry (ITC), fluorescence anisotropy and SEC-MALLS analyses, provided by the PSB biophysical platform, were used to show that a CBC-ARS2-PHAX ternary complex can be formed, whereas NELF-E binding to CBC excluded ARS2 and PHAX. To determine the structural basis for this observation, extensive crystallisation screens of many different CBC complexes were performed using the EMBL HTX crystallisation platform. Eventually crystals of CBC in complex with m^7GTP and C-terminal peptides of

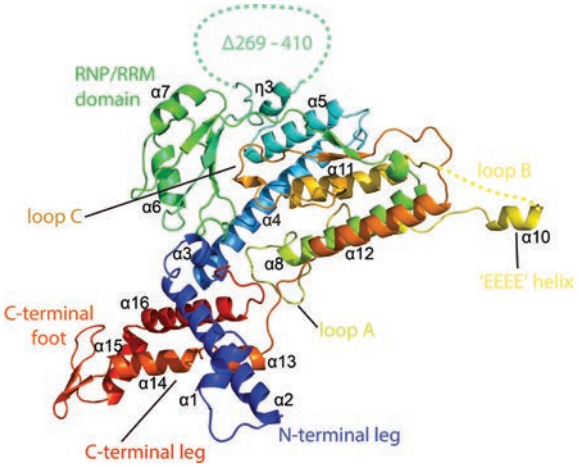


Figure 1. Crystal structure of human ARS2 core in rainbow colours (blue N-terminus to red, C-terminus). The RNP/RRM domain is a metazoan-specific addition.

ARS2 (845-871) or NELF-E (360-380) were obtained. The automated ESRF beamline MASSIF-1 allowed us to test many crystals searching for those exhibiting the best peptide occupancy. Interestingly, it was found that the C-terminal peptides of ARS2 and NELF-E bind to the same pocket on CBC, at the CBP20/CBP80 interface, this by virtue of the fact that they actually share a previously unrecognized sequence homology [1]. This explains the incompatibility of ARS2 and NELF-E binding to CBC.

More recently we set out to better characterize the structure of human ARS2, a highly conserved metazoan protein involved in numerous aspects

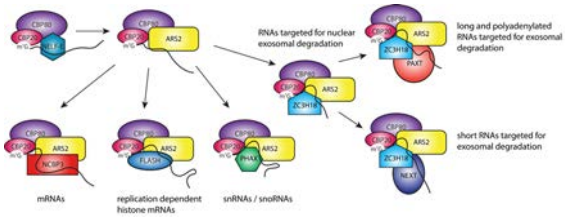


Figure 2: Mutually exclusive CBC-ARS2 complexes. Early in transcription CBC is proposed to bind to NELF-E which is subsequently replaced by ARS2. Then, depending on the type of RNA and the corresponding 3'' end processing and export pathways, alternative and mutually exclusive CBC-ARS2 complexes assemble. Other complexes target the RNA for exosomal degradation.

of nuclear RNA metabolism [2]. It is an adaptor protein between CBC and multiple RNA processing machineries such as Drosha (miRNA processing) and FLASH (histone mRNA processing). Using limited proteolysis we identified the stable core of ARS2 comprising residues 147-270 and 408-763. After multiple rounds of crystallisation and construct optimization, the structure of the human ARS2 core could eventually be solved by selenomethionine-SAD phasing at 3.2 Å resolution, using a genetic algorithm to optimally merge different datasets (Figure 1). This allowed us to identify and map regions involved in RNA and protein-protein interactions. Additionally, proteomics analysis of ARS2 binding partners in cells led to the identification of a higher order CBC complex containing ARS2 and a novel cap-binding protein called NCBP3 (CBC-ARS2-NCBP3), which seems to be mutually exclusive with the binding of PHAX or FLASH to CBC-ARS2.

Taken together, these results enable us to propose a model of co-transcriptional processing involving mutually exclusive CBC-RNA or CBC-ARS2-RNA complexes that dynamically evolve depending on the RNA class and maturation state (Figure 2).

W. Schulze and S. Cusack (EMBL)

[1] W. M. Schulze & S. Cusack (2017) *Nat. Commun.* **8**:1302.
[2] W. M. Schulze, F. Stein, M. Rettel, M. Nanao, S. Cusack (2018) *Nat. Commun.* **9**:1701.

CONTENTS

Tools for macromolecular crystallography: mesh scan analysis routine and crystal phasing via radiation damage

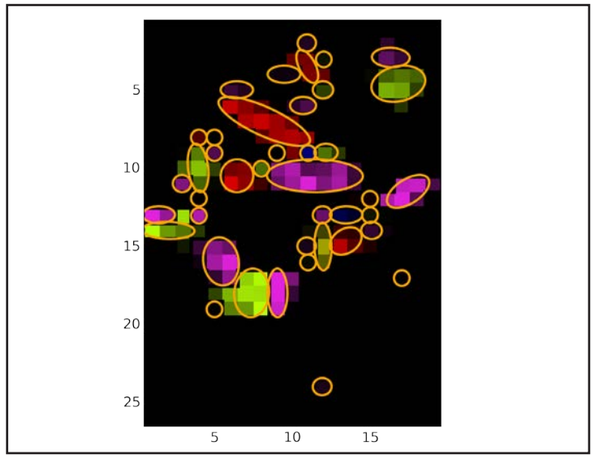


Figure 1. A crystal map generated by *MeshBest* based on the mesh scan of NarQ crystals. Adjacent crystals are illustrated with different colours to be visually separable. Crystal shapes are approximated with ellipses, which are drawn above the map.

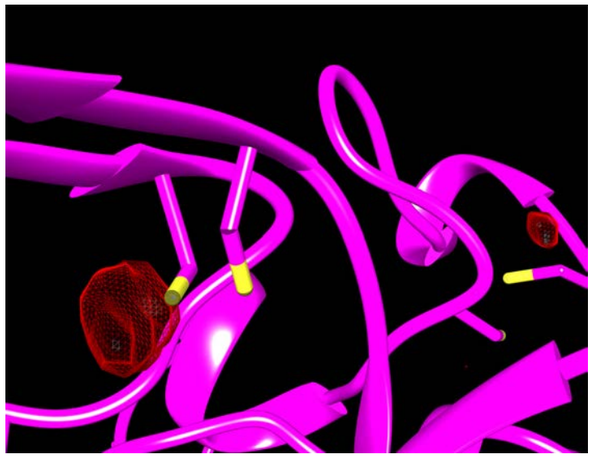


Figure 2. Negative electron density peak in vicinity of disulfide bridges in Thaumatin.

In macromolecular crystallography the structural information is extracted from diffraction intensity data. To obtain the data of a best possible quality, the diffraction experiment should be properly organized. A tool to characterise the sample area is often used to determine the positions, sizes and strengths of diffraction of protein crystals mounted on the sample holder. For this purpose, at the ESRF we have developed *MeshBest* [1] – a software that performs the sample characterisation algorithms based on the 2D area raster diffraction scan (commonly known as mesh scan) routine. Based on the estimations of diffraction quality from another software *Dozor*, *MeshBest* produces a 2D map of the crystals contained in the sample area (Figure 1). Multiple strategies of analysis are available here dependent on the sample type: either crystal positioning for large crystal samples or preparations for multi-crystal data collection with possible aperture size variation to adapt to different crystal size.

The success of an X-ray diffraction experiment depends not only on the data quality – how the diffraction intensities have been measured – but also on the possibility to extract their phase information, which is a crucial step in building 3D models of macromolecules. Several strategies are available to overcome this problem. We have developed a method to obtain phase information using a phenomenon that is usually avoided, radiation damage. In our method, the so called RIP (radiation induced phasing), we use specific radiation damage induced by X-ray beam or by UV light exposure of the samples [2]. This specific radiation damage occurs on disulfide bonds and carboxylic groups from acidic amino acid residues (Figure 2). We determine the substructure of damage and then calculate and extend the phases to the whole protein. To expand the field of application of this method, we have also used partial datasets from extremely small crystals and compensating for by merging multiple partial datasets [3]. Merging of a large number of partial subdatasets (hundreds or more) is however not straightforward because of non-isomorphism between crystals. Thus, to optimize the data merging, we use a global optimization strategy based on a genetic algorithm. This method is the key for a successful determination of the data groups that produce good datasets for phase determination (and building a 3D model of the macromolecule). Our approach demonstrates the possibility of performing RIP experiments with partial datasets collected in a serial approach. Moreover, we show that the genetic algorithm is a powerful tool for data selection in such a challenging phasing method.

I. Melnikov, O. Svensson, G. Leonard, A. Popov, N. Foos and M. H. Nanao (ESRF)

[1] I. Melnikov *et al.* (2018). The complex analysis of X-ray mesh scans for macromolecular crystallography. *Acta Cryst.* **D74**, 355-365.

[2] D. de Sanctis *et al.* (2016). Radiation-damage-induced phasing: a case study using UV irradiation with light-emitting diodes. *Acta Cryst.* **D72**, 395-402

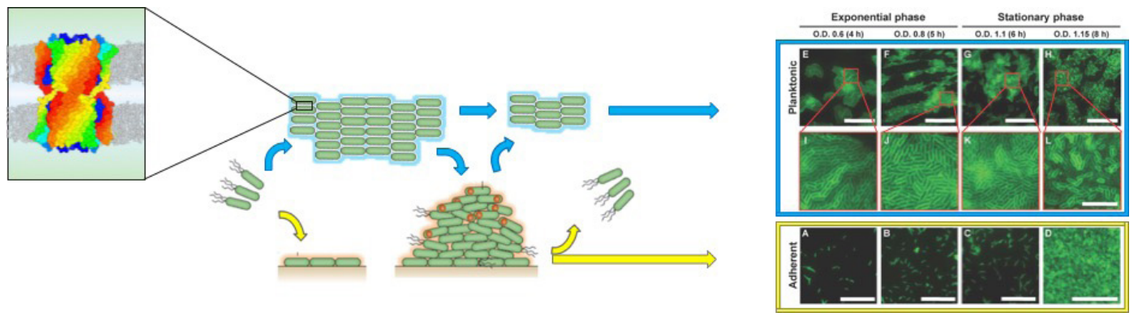
[3] N. Foos, *et al.* (2018). X-ray and UV radiation-damage-induced phasing using synchrotron serial crystallography. *Acta Cryst.* **D74**, 366–378

Porin self-association enables cell-to-cell contact in *P. stuartii* floating communities of cells

Providencia stuartii is a Gram-negative pathogen involved in increasingly frequent urinary-tract infections in residents of nursing homes and intensive care units [1]. Treatment of *P. stuartii* infections are complicated by its stringent antibiotic-resistant phenotype and by its ability to form biofilms attached on indwelling catheters and uroepithelial cells, explaining their long (up to 6 months), chronic and sometimes fatal (up to 33%) nature [1]. Recently, we showed that *P. stuartii* stays social through its whole growth cycle, hardly ever occurring as isolated cells, but rather forming floating communities of tightly-packed cells that only later sediment-onto, attach-on and colonize surfaces (Fig. 1, middle and right insets) [2]. It had yet remained elusive how intercellular contact is maintained within the floating communities of cells. We found that *P. stuartii* porins, which form water-filled trimeric channels in its outer-membrane, are adhesive proteins that can self-associate, supporting the formation of floating communities of cells [3]. Indeed, reconstitution of *P. stuartii* porins in artificial bilayers results in aggregation of the latter, while recombinant expression in model planktonic cells results in these forming floating communities of cells. Crystal structures of the porins reveal that they can form dimers of trimers (DOTs), assembled through self-matching interactions between their extracellular loops (Fig. 1, left inset). Within the DOTs, facing channels remain open, suggesting that they could allow a direct circulation of small hydrophilic solutes between adjacent periplasms. Hence, DOTs could not only support intercellular contact, but also enable basal communication within floating communities of cells. By shedding light on a novel function of porins, i.e. to form adhesive junctions between cells, our results put them forward as potential new targets in the quest for novel therapeutic agents aimed at disrupting biofilms leading to more antibiotic-sensitive isolated cells [3].

M. El Khatib, J. Lopes , G. Tetreau and J.-P. Colletier (IBS) and C. Nasrallah (University of Oxford).

[1] J. W. Warren. *Rev. Infect. Dis.* **8**, 61–67.
[2] M. El Khatib, Q.-T. Tran, C. Nasrallah, J. Lopes, *et al.* (2017) PLoS ONE, **12**, e0174213.
[3] M. El Khatib, C. Nasrallah, J. Lopes, Q.-T. Tran, *et al.* (2018) PNAS, **115**, E2220-E2228.



Porin self-association enables cell-to-cell contact in the *P. stuartii* floating communities of cells which precede (and later sediment into) surface-attached biofilms.

The Localisation of Cholesterol in Deuterated Lipid Bilayers

Cholesterol is a vital constituent of mammalian membranes and is known to induce changes within the lipid bilayer. These changes include creating liquid crystalline (ordered) phases within the bilayer; increasing the thickness and compositional order with more rigid packing and the equivalent level of lateral diffusion across the membrane, as compared to disordered phases. Depending on the saturation and thickness of the membrane in question the presence of cholesterol can localise itself either closer to the head-tail interface (saturated lipid membranes) or towards the inner interface between the two bilayer leaflets (polyunsaturated lipids).

In this study we used neutron reflectometry (all data obtained on FIGARO, ILL) to look at the localisation of cholesterol in a supported lipid bilayer comprised of *in vivo* produced phosphatidylcholine (PC) and both unlabelled and per-deuterated cholesterol samples. The PC lipids were produced from a genetically modified strain of *E. coli* engineered to synthesise PC and adapted to grow in deuterated medium [1]. Specific deuterated conditions allow the overall neutron scattering length density (which depends on the level of deuteration) of the lipids to match that of D_2O. The deuterated cholesterol was produced from a genetically engineered strain of *P. pastoris* in specific deuterated conditions [2]. All of the deuteration of samples was carried out in collaboration with the ILL Deuteration-lab.

The specific deuteration of the components alongside contrast variation techniques showed the cholesterol localised closer to the head-tail interface rather than in the centre of the core of the bilayer [3]. When the cholesterol content was increased from 0 to 10 and subsequently to 20 mole %, the thickness of the bilayer increased. The increase in cholesterol from 10 to 20% also altered the distribution from symmetric localisation at 10% to an asymmetric distribution at 20% (Fig. 1). This work will facilitate the modelling of bilayers containing cholesterol and other cellular membrane interactions with biomolecules.

S. Waldies (ILL)

[1] S. Maric *et al.* (2015) Appl.Microbiol. Biotechnol. 99, 241–254
[2] M. Moulin *et al.* (2018) Chemistry and Physics of Lipids, 212, 80-87
[3] S. Waldie *et al.* (2018) Langmuir, 34, 472–479

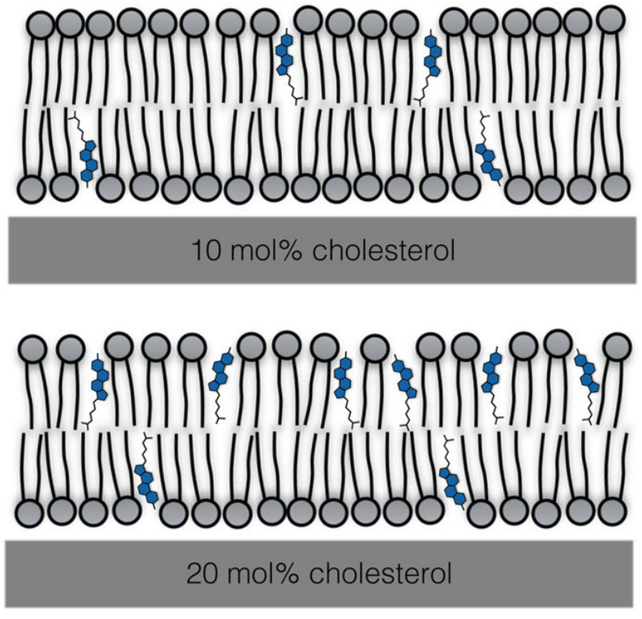


Figure 1. Schematic of cholesterol distribution in match-out deuterated PC containing 10 or 20 mol % cholesterol. The thickness of the bilayer increases from 43 to 46 and 48 Å in the absence and presence of 10 and 20 mol % cholesterol, respectively.

Tracing molecular oxygen routes in O_2-dependent enzymes

Since 2015, a high pressure laboratory dedicated to the use of gases in structural biology is open to users of the MX beamlines at the ESRF. This service is located in the environment of the MASSIF beamlines and allows for the flash-cooling of crystals without the addition of a cryoprotectant, the preparation of noble gas derivatives for structure phasing and the tracking of small molecule routes within proteins.

Numerous enzymes have buried active sites which require substrates and products to diffuse via a tunnel network. The tunnel architecture impacts enzymatic processes, since it acts as a filter to select the proper substrate, reject inhibitors and regulate their diffusion rates. Hence, a proper understanding of the mechanism of these enzymes requires these diffusion routes to be deciphered in detail. Many oxydoreductase enzymes, such as oxidases, oxygenases, hydroxylases or catalases, contain such functional tunnels, which regulate the transport of O_2 to, or away from the buried active site. However, determining the existence of diffusion pathways for a gas molecule as light and labile as O_2 is a challenge. To address this question, we have developed a novel gas pressure cell on the basis of the 'soak-and-freeze' methodology [1]. The method uses high pressures of oxygen or krypton, which can be considered as a mimic of O_2, to populate the binding sites in crystals of O_2-dependent-proteins. Once produced, these gas-derivatives, still under pressure, are immediately flash-cooled at cryogenic temperature (Figure 1a). These frozen gas-protein complexes are stable in time, and can then be used for diffraction data collection.

[NiFe] hydrogenases reversibly catalyze the oxidation of protons into molecular hydrogen. Hydrogen molecules diffuse to, and away from the active site through dedicated hydrophobic tunnels. Most hydrogenases require anaerobic conditions to operate, since molecular oxygen can also take these routes and inactivates the enzyme. The [NiFe] Hydrogenase from *Ralstonia eutropha* (*Re*MBH) is O_2-tolerant, and as such, is a target of high interest in the field of renewable energies. The architecture of the gas tunnels was initially believed to play a role in its O_2-tolerance properties, possibly by filtering out molecular oxygen. The gas tunnels in *Re*MBH were first indirectly revealed by krypton labelling using the 'soak-and-freeze' method [2]. The Kr-derivative *Re*MBH structure displays 19 Kr sites, which maps out the gas network and reveal two tunnels connecting the active site to the enzyme surface (Figure 1b). To confirm that these tunnels are indeed relevant to O_2 diffusion, binding sites in *Re*MBH were also directly probed by oxygen molecules using the 'soak-and-freeze' method [3]. Seven O_2 molecules were unambiguously identified within the hydrophobic gas tunnels and the four molecules with highest occupancy are represented on Figure 1c. Tunnel analysis showed that O_2'tolerant hydrogenases contain less, and narrower channels than their sensitive counterparts. This feature presumably allows for a tighter control of the access to active sites and contributes to the O_2-tolerance. Molecular dynamics simulations confirmed that O_2 and H_2 molecules both diffuse specifically within the same gas tunnels, and corroborated the high occupancy of O_2 positions determined by crystallography. Simulations also demonstrated that O_2 diffuses preferably away from the active site, and confirmed that the gas tunnel network of *Re*MBH holds a mechanism to separate H_2 from O_2 and protects the enzyme from inhibition.

The studies of tunnels in *Re*MBH is a collaboration between the group of P. Scheerer's group at Charité - Universitätsmedizin Berlin and members of the Structural Biology group at the ESRF. The project was led by P. Scheerer, the fragile crystals were grown in the PSB wet lab (G. G. and D. v. S.) and the soak-and-freeze method was developed at the ESRF with support from the PSB and the Partnership for Soft Condensed Matter (P. vd. L., A. R. and P. C.).

P. van der Linden (ESRF-PSCM), G. Gotthard (ESRF-SB), D. von Stetten (ESRF-SB), A. Royant (IBS/ESRF-SB), P. Carpentier (CEA-LCBM/ESRF-SB)

[1] B. Lafumat, C. Mueller-Dieckmann, G. Leonard, N. Colloc'h *et al.* (2016). Journal of Applied Crystallography, 49, 1478-1487.
[2] J. Kalms, A. Schmidt, S. Frielingsdorf, P. van der Linden *et al.* (2016). Angewandte Chemie international Edition, 55, 5586-5590.
[3] J. Kalms, A. Schmidt, S. Frielingsdorf, T. Utesch *et al.* (2018). Proceedings of the National Academy of Sciences USA, 115, E 2229-E2237.

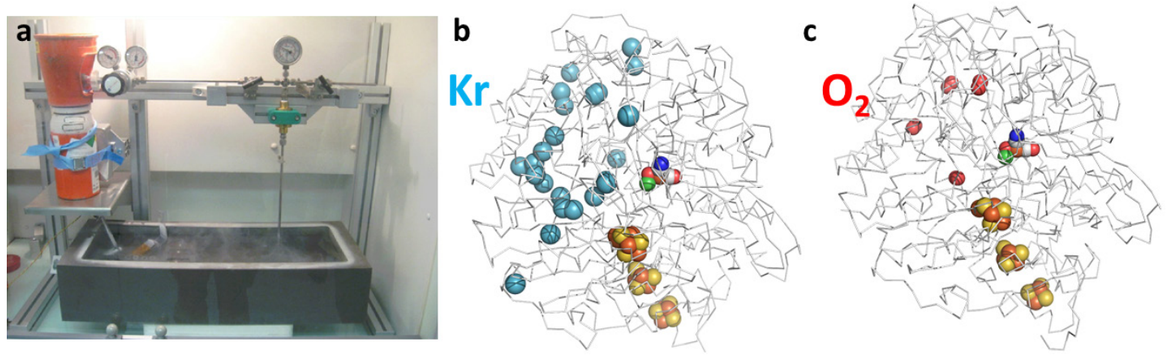
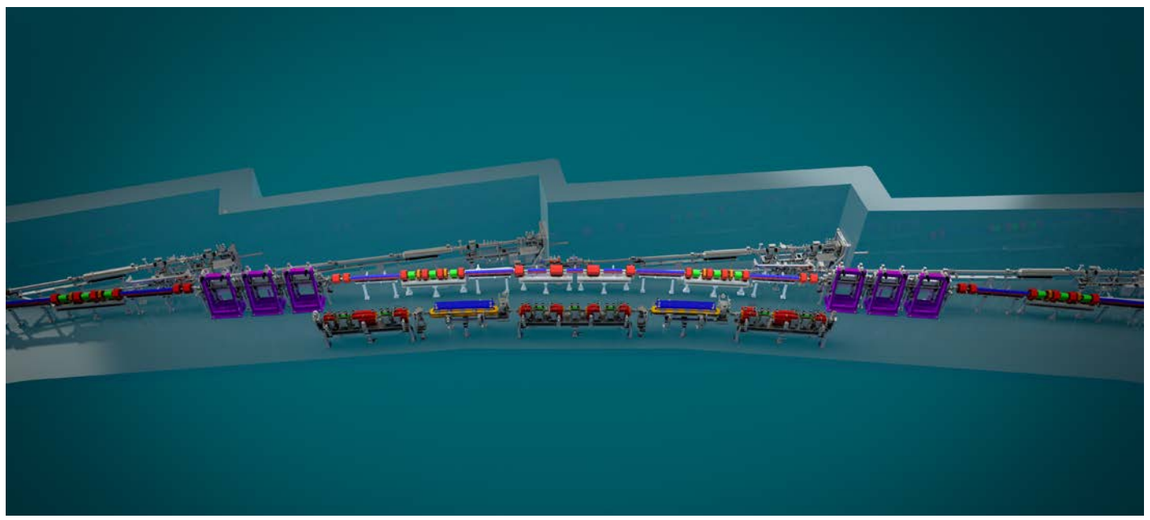


Figure 1. a. Photo of the O_2-pressurization setup. **b.** Krypton atoms in the structure of Kr-pressurized *Re*MBH O_2-toleant hydrogenase highlighting the O_2-accessibility tunnels to the active site (NiFe cluster in green and dark). FeS clusters implicated in electron transfer are represented in orange and yellow. **c.** Oxygen molecules in the structure of O_2-pressurized *Re*MBH confirming the location of the tunnels.

News from the platforms

A Brilliant Future for Structural Biology at the ESRF



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2018 marks the 30th anniversary of the ESRF and a series of events will be held to celebrate this. However, perhaps the most important date in 2018 for the ESRF is 10th December. This marks the start of the 20-month shutdown ('dark period') during which the current ESRF storage ring will be disassembled and replaced - inside the current infrastructure - by a new generation of synchrotron, the ESRF – Extremely Brilliant Source (ESRF – EBS). The most important aspect of ESRF-EBS will be its new storage ring. Here, the magnetic structure (the 'double bend achromat') that makes up each of the 32 cells in the current storage ring lattice current lattice will be superseded by a new structure (Figure 1) allowing the electrons in the storage ring to remain more tightly focused, thus producing X-ray sources that are more than 100 times more brilliant (i.e. much smaller in size and much less divergent) and coherent than those currently produced.

While the 'dark period' during the EBS-shutdown may, by some, be seen as inconvenient, for structural biologists, the advantages of the world-leading properties of the ESRF-EBS will be worth waiting for. These will allow faster measurements with improved signal-to-noise ratios and to a higher (spatial) resolution from much smaller samples, whether these be crystals, solutions, or cells. One of the fields that will perhaps benefit most from the ESRF-EBS is macromolecular crystallography (MX). Indeed, the use of suitable x-ray optical configurations will result state-of-the-art beamlines at which, in addition to the current generation of MX investigations, experiments based on serial crystallography (SX) techniques will also be possible. This, in turn, will lead to a resurgence in room-temperature MX which will allow the visualisation, *in crystallo*, of functionally important conformations of biological macromolecules that are sometimes obscured as a result of the flash cooling required when data collection is carried out at cryogenic temperatures. When combined with the breath-taking increase - a factor of 100,000 or so - in flux density that will be available on the planned replacement for the current ID29 (EBSL8), SX at the ESRF-EBS will also allow time-resolved, monochromatic beam MX experiments to be performed. Although, exploration of the femto- and nano-second timescales will clearly remain the domain of X-ray free electron lasers (XFELs), EBSL8 will allow crystallographic studies at sub-millisecond time resolution, the timescale on which many conformational changes in biological macromolecules take place.

G. Leonard (ESRF)

FIP2-BM07: a new beamline at the ESRF for macromolecular crystallography

In the context of the ESRF-EBS upgrade, a new French CRG beamline, named FIP2, is being built on port BM07, in replacement of the present FIP-BM30A beamline dedicated to macromolecular crystallography [1]. FIP, located on port BM30A, will stop operations in November this year, having served the protein crystallography community for almost 20 years. Indeed, FIP hosted its first experiments in May 1999, and has contributed since its inception to almost 600 publications and as much structures deposited in the "Protein Data Bank".

In the meantime the construction of the infrastructure of FIP2 on port BM07 started in April this year. It will be completed in November, and the optics and experimental setup presently in operation on FIP-BM30A will be transferred starting in December. FIP2 will start commissioning mid-2020, and is planned to enter into full operation mode by December 2020.

FIP2 will be flexible and highly automated, and complementary to the existing beamlines at the ESRF. It is designed to host a large variety of MX experiments, including anomalous phasing, spectroscopy coupled diffraction, *in situ* diffraction, high pressure experiments, etc. FIP2 will also continue the development efforts in instrumentation and methodology already carried out on FIP, and in particular in the field of automation. But taking advantage of the new X-ray source upon ESRF-EBS upgrade, a 2-pole wiggler, FIP2 will have improved performances with respect to the present FIP beamline. One to two orders of magnitude increase in brightness (higher flux and smaller beam) will allow a whole series of new experiments, and will improve the quality of crystallography data collected on FIP2.

As for FIP, FIP2 will be managed by a team of the Synchrotron Group of the IBS and integrated into the French beamlines of the ESRF (F-CRG beamlines). It will be accessible to the community of crystallographers through a joint program committee with synchrotron SOLEIL ("national"/CRG beamtime) as well as through the ESRF program committee ("international" beamtime). FIP2 is funded by the CEA and the CNRS.

J. -L. Ferrer (IBS)

[1] M. Roth *et al.* (2002). Acta. Cryst., D58, 805-814.

Present stage of the construction of the infrastructure of FIP2 on port BM07.

Events

PSB Spotlight meeting on membrane proteins

The fourth PSB Spotlight meeting on membrane proteins took place on the EPN campus on 13th June 2018.
Over 60 participants attended the morning session in the Chadwick amphitheatre which included exciting talks by Vadim Cherezov (University of Southern California) on serial crystallography of G protein-coupled receptors, Paul Schanda (IBS) on the use of detergents for the study of membrane proteins by NMR, Hugues Nury (IBS) on structural studies of the serotonin 5-HT3 receptor by cryo-EM, Florine Dupeux (IBS/EMBL) on the latest developments at the PSB HTMP crystallization platform and Selma Maric (Malmö University) on the use of deuterated nanodiscs for structural and dynamic studies of membrane proteins and lipoproteins assemblies using neutrons.

External invited speakers to the PSB Spotlight. Left: Vadim Cherezov (USC) and right: Selma Maric (U. Malmö)

The afternoon was dedicated to practicals and a group of 15 participants, including students, postdocs and scientists, went to ID30B at the ESRF to learn more from Sasha Popov (ESRF) on X-ray diffraction data collection and data processing from membrane protein crystals, and to the PSB HTMP crystallization platform to be trained by Florine Dupeux on crystallization in lipidic cubic phase (LCP) which is the major crystallization technique for membrane proteins.
The organizers wish to thank all the speakers and participants for making this day a success.

F. Bernaudat (PSB coordinator)

Les Houches/TSRC Workshop on Protein Dynamics

The Les Houches/TSRC Protein Dynamics Workshop was held from May 27th to June 1st in the Ecole de Physique des Houches in the Chamonix valley. The workshop, with ca. 32 international speakers and a total of 60 participants was an interdisciplinary meeting bringing together researchers working on various aspects of protein dynamics using a range of techniques including X-ray crystallography, NMR, EM, AFM, MD simulations and spectroscopies. This biannual workshop aimed at bridging these various fields and fostering international collaborations. The biological questions discussed during the workshop ranged from protein folding, ligand (un)binding and drug discovery, membrane protein mechanisms as well as nucleic acid dynamics.
The meeting was financially supported by the UGA, Cecam, EBSA and the Société Française de Biophysique.

P. Schanda and M. Weik (IBS), J. Fraser (UC San Francisco), M. Heyden (Arizona State University)

EMBO Practical Course on "Characterization of macromolecular complexes by integrative structural biology"

In May, the 9th edition of the EMBO Practical Course organised by the PSB took place on the EPN Campus, with a focus on integrative structural biology. Out of 200 applicants, 20 participants were selected, including 4 postdocs and 16 PhD students of 15 different nationalities and working in 20 different research centers worldwide. Students attended morning lectures held by local and external scientists on production, purification, reconstitution and biochemical/biophysical characterisation of large macromolecular complexes. In the afternoon practicals, under expert guidance of local PSB staff, students performed ITC, DSC, native MS, and a thermofluor stability assay in the PSB Biophysical Platform, crystallised an RNA enzyme and visited the HTX facility at EMBL, collected X-ray and cryoEM data at ESRF beamlines, collected NMR and negative stain EM data at the IBS, and visited the ILL facilities for neutron protein crystallography and small-angle neutron scattering (SANS). Two poster sessions and an evening PSB Get Together offered further opportunity to mingle with PSB scientists. A visit to Vizille and a Telepherique ride to the Bastille and Per'Gras restaurant were also included as social events to encourage exchange of ideas in more informal settings. The event was generously sponsored by EMBO and FRISBI, and supported financially and administratively by all four PSB institutes, in a truly collaborative effort from all tutors and speakers.

M. Marcia (EMBL), on behalf of all organizers: D. De Sanctis & M. Soler Lopez (ESRF), E. Mossou & T. Forsyth (ILL), C. Petosa & D. Hart (IBS), and R. Tounkara (EMBL).

Group photo with students, tutors, speakers, and organizers of the EMBO course.

GiGrIll 2018 workshop: Determination of protein structure by NMR

The biomolecular NMR group of the IBS (Grenoble), the laboratory of Structural Chemistry and Biology of the ICSN (Gif-sur-Yvette) and the Structure and Dynamics team of the biological polymers by Nuclear Magnetic Resonance of the IGBMC (Strasbourg) organized from 23rd to 27th April 2018 an advanced workshop on protein structure determination by NMR. 15 participants either PhD or engineers learned about the different approaches used for protein structure determination by NMR with training on the NMR platform at IBS and in the graphics room of the CIBB for NMR data analysis. This workshop was kindly supported by IR-RMN, GRAL, FRISBI, NMR-BIO and UGA.

C. Laguri and D. Marion (IBS)

GRAL - 48H

The third edition of the GRAL-48H meeting, organised by the Grenoble Alliance for Integrated Structural and Cell Biology (Labex GRAL), took place in Autrans on 26th - 27th March 2018, and gathered 116 participants from the Biosciences and Biotechnology Institute of Grenoble (BIG) and the four Partners of the PSB.
The meeting covered the major research themes of the Labex GRAL through many exciting talks, and included three plenary lectures by keynote invited speakers: Anne Milet (Université Grenoble Alpes), Gabriel Waksman (University College London, UK) and Ove Nilsson (Umeå Plant Science Center, Sweden). Young scientists were also strongly encouraged to present their work during the meeting and two competitions were organized for "Best poster" (winner: Maha Chieb, BIG) and "Best short presentation" (winner: Benoit Arragain, IBS), and each of the winners received 500 € to fund a future participation in an international conference or a working trip.
The two-day retreat then ended by a friendly competition of biathlon, where over 40 participants could compare their (air riffle) shooting skills and their ability to remain standing when running in the snow of the Vercors.
The organizers wish to thank all the participants and look forward to seeing them in two years for the 4th edition of the meeting.

F. Bernaudat (PSB coordinator)

28th ESRF User meeting

The annual ESRF User Meeting 2018 was held on the EPN Campus from 5th to 7th February 2018 and was dedicated to the ESRF user science and its future horizons. UM2018 attracted more than 300 participants for a program that included a plenary session held in the ESRF Auditorium, a series of beamline tutorials which included a well-attended MX BAG Meeting and, on Wednesday 7th February, three User-Dedicated Microsymposia (UDMs).


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The UDM3 entitled "Understanding neurological diseases: Synchrotron science in a multidisciplinary approach" was organised by Gabriele Giachin (ESRF), Montserrat Soler López (ESRF), Marina Mapelli (ESRF User Organisation), Paula Coan (ESRF User Organisation) and Claudine Roméro (ESRF). This symposium brought together 120 registered participants from interdisciplinary fields in the IBS seminar room. The symposium comprised 4 keynote lectures and 11 selected contributions from submitted abstracts on diverse subjects: (a) Structural Biology on proteins involved in neurodegeneration and brain development, (b) Nano X-ray Fluorescence for the imaging of bio-metals in cell, (c) and X-ray tomography as novel experimental tool for the investigation of the central nervous system and the treatment of brain disorders.

Taken together, UM2018 UDM3 delivered an excellent summary of the recent advances in neuroscience that have become available at the ESRF using synchrotron-based methods, including X-ray imaging, novel radiation therapies, X-ray crystallography, X-ray scattering and cryo-electron microscopy. We would like to thank IBS for their hospitality, all the speakers who made UDM3 such an interesting event, and all those who attended.

M. Soler López and G. Giachin (ESRF)

PROFILE

Professor Anthony Watts

Anthony Watts is the Director of the Oxford University Biomembrane Structure Unit (OUBSU) and is a Fellow of the Royal Chemical Society, the Institute of Physics, the Royal Society of Biology and the American Biophysical Society.

Since the first review of the PSB in 2006, Anthony Watts has been part of the PSB Science Advisory Board (SAB) for which he also became the Chair in 2013. He will now retire from his duties on the SAB and the PSB Newsletter editorial board asked him a few questions on his views about the PSB.

What did you feel was your mission as a member of the PSB Scientific Advisory Board?

Biophysics, as an area of science, encompasses many tools to answer specific questions in biology at the molecular level. Embracing these tools, now established as "platforms" in the PSB, so that a wider community can benefit from them, has been a mission of the Partnership. As a member and subsequently chair of the PSB SAB, I have tried to take an outsider's view of how the Partnership operates, and provide helpful suggestions of how more collaborations and interactions can be established.

For example, at poster sessions with students, I always tried to encourage students to look to different approaches for answering specific questions, and then have reported back that a new interaction was indeed helpful at a later date; this occurred more than once. PIs too, can get entrenched in their own "pet" approach and become territorial, so fertilizing new ideas by widening interactions on the Grenoble site, has been gratifying.

As Chair for the last 2 reviews, I have made it my mission to include and inform directors of the major national and international facilities of the importance and significance of the PSB. Indeed, recent developments with cryo-EM and theory groups were both strongly supported and recommended by the PSB SAB. Listening to beam line scientists, PIs, and researchers alike on a one-to-one basis, has been very informative indeed. I initiated the "student sessions" specifically for the purpose of gathering local views – this has been very useful in assessing platform success and effectiveness, or otherwise.

How does the PSB compare to other integrated structural biology research centres? What are the PSB strengths and weaknesses?

Over the last three decades, I have been fortunate to have reviewed a number of major institutions and research facilities – CERN, Diamond, chairing XFELs panels, ETH, MPI and Helmholtz reviews, most are similar in size to the PSB. But the PSB is unique. It crosses different kinds of establishments, from IBS (CNRS/CEA/University), to international subscription supported ILL and ESRF and the EMBL Outstation. Many nationalities are represented and different cultures co-exist. This diversity and cultural integration at various levels, has been fascinating to observe – indeed, it is a major strength of the PSB. Collaborations and projects come from all over (primarily) Europe, and the access to the super infrastructure, drives productivity to highly competitive levels, as can be seen in the biennial PSB reports.
Over the years, the SAB have also raised a number of concerns about the PSB and in particular regarding some of the constraints placed on local working practices by employment law. For example, the working hours of IBS are legally restricted and "out of hours" working difficult to implement. The SAB has strongly encouraged the respective managements to make all efforts to try to rationalize the situation to minimize loss of expensive resources and avoid losing the competitive edge that many of these facilities have as part of the PSB.

In my opinion, another weakness is the "subscription" argument; the stakeholders of many of the facilities expect access roughly in line with the investments they make. While there are mechanisms that seek to ensure that the best science gets done regardless of the origin of the PIs, this should be carefully monitored to ensure that this remains the case in the future.

Finally, one of the difficulties that I have observed throughout my involvement in the PSB relates to how the rather different remits of the PSB partners have been accommodated to the benefit of the partnership. The ILL and the ESRF have always been well adapted and responsive to their international service missions and the EMBL owes its origin to supporting these services missions on the EPN site. The move of the IBS onto the campus was a major step and brought with it challenges that required a shift towards the service-orientation of the platforms they brought with them. While this has taken a little time to implement, it is gratifying to see how most of these capabilities now mesh almost seamlessly as part of an immensely powerful array of abilities that is unmatched worldwide, strongly complementing and supporting the international facility operators.

Has the PSB contributed to your own research projects?

Yes, tremendously, and several postdocs and graduate students have spent time in the PSB for various activities over its lifetime. Indeed, we have brought our solid state NMR to the PSB, coupled with neutron and EM work, and the publications that resulted have been much better than the data from the individual approaches would have been. Of special mention is the D-Lab (in ILL's Life Sciences Group), where we have exploited the unique properties of deuterium in contrast matching in neutron experiments, as well as the quadrupolar interactions in solid state NMR – all on the same systems. This would have not been possible without the D-lab input. Again, from the D-lab, we have learnt and benefitted much from the resolution of labelling with deuterium (and ^{13}C and ^{15}N) in expression of challenging membrane proteins, providing expertise and a resource that is unique.

As a peripheral, but still important benefit, we have been involved with more than one EU initiative, led by or as a result of the PSB involvement. These activities have resulted in new ways of thinking about the questions we were addressing, as well as input we were able to make into other projects.

What do you expect the future challenges of the PSB to be?

If one asks if structural biology has "had its day", and is now being overtaken by other areas of biology, such as chromosomal biology, quantum biology, systems biology and so on, then I suspect some re-badging of the various activities within the PSB remit might be needed to keep in step with developments. There are a few gaps in technologies on site, including single-molecule biophysics or theory and simulations, but, admittedly, not everything can be encompassed within such a Partnership. Solutions are found through collaborations, and so this is not a limitation, just a good excuse for establishing collaborations.

One challenge for the X-ray crystallography community that needs to be addressed, is the emergence of cryo-EM for structural determinations. Issues with throughput, data analysis and sample preparation, may be overcome in the medium term, and crystallographers will need to work out how current crystallographic capabilities complement the cryo-EM developments. Like other sites, ESRF, strongly supported by the PSB SAB, has very wisely embraced cryo-EM as part of its core provision (Titan Krios) and this will maximize complementarity and exploitation. It is also gratifying to see IBS, EMBL and ILL engaging in this process – as a site-wide effort on this is in the interests of all PSB Partners. Getting expertise in cryo-EM is a major issue – I see this in recruitment exercises in which I have been involved. That aside, the burgeoning development of cryo-EM represents a huge opportunity that if genuinely embraced will seriously enhance the facility core missions on the campus.

Clearly, very practical challenges including ILL instrument/science-based development, upgrading of synchrotron beam lines, upgrading of equipment, managing massive data, are all real issues. It is increasingly obvious that in the USA and in the UK running and replacement costs now operate through accounting models that occupy administrative bodies to the potential detriment of the science. This mentality has yet to arrive in the PSB and I see it as hugely important that all efforts are made to avoid it distorting the remarkable progress that has been made in Grenoble.

The PSB has to face these challenges, and resist being described as just a technical Partnership to serve the rest of the community. Senior staff should be further encouraged to be PIs and co-PIs on independent grants to answer their own questions – other peoples' science can be engaging, but your own is even more so, and more satisfying, not least when the level of support, expertise and infrastructure on the doorstep, is some of the best in the world.

J. Timmins (IBS) and F. Bernaudat (PSB coordinator)


Group picture following the students/postdocs clip session during the 2016 PSB-SAB review.

DATES FOR YOUR DIARY

25th August-15th September 2018 - ESONN 2018

The European School On Nanosciences & Nanotechnologies (ESONN) is a three-week course aimed at providing training for graduate students, postdoctoral and junior scientists from universities and laboratories all around the world, in the field of Nanosciences and Nanotechnologies. The academic and practical courses cover areas such as the elaboration, characterization and functionalities of nano-objects. IBS and ESRF scientists are involved in several practical courses for the biological session: (a) Proteins and nanoparticle assemblies and interactions by AUC and SEC/MALS (C. Ebel & A. Le Roy), (b) Cell imaging analysis of protein interactions and dynamics in living cells (F. Lacroix, J. Timmins & J.-P. Kleman), (c) Study of biomolecular interactions by surface plasmon resonance biosensor analysis (J.-B. Reiser), and (d) Cryo-Electron Microscopy: sample preparation and visualization using a Polara and a Krios electron microscope both equipped with a direct electron detector (G. Schoehn and I. Kandiah). Information & registration. https://www.esonn.fr/.

16th-19th September 2018 - Neutrons & Biology School

The "Neutrons and Biology" School organized by the Neutron French Society (SFN) will take place in Carqueiranne this year and will present an overview of the use of neutrons to probe the structure and dynamics of biological systems. Lectures will be presented by researchers from neutron international facilities and from academia and will include basic tutorials on the principles of neutron diffraction and scattering, a description of the neutron sources and instrumentation, as well as seminars on the application of neutron diffraction and scattering to different biological subjects. Students will also be introduced to the reduction, analysis and interpretation of experimental data and to the writing of an experimental proposal. The target audience includes graduate students, PhD students and postdocs or experienced scientists not familiar with the application of neutrons to biology.
More information: https://sites.google.com/view/bioneutrons2018.

10th-12th October 2018 - ILL-ESS European Neutron User Meeting

The neutron landscape in Europe is going through a period of dramatic change. Two major, national facilities will close by 2020. A substantial investment in a new European facility, ESS, will deliver transformative capabilities and extend the technique to new domains by the middle of the next decade, based on the highest peak flux in the world. ILL is currently executing phase one of the Endurance upgrade programme and is preparing a second, more extensive phase of Endurance that will be the basis of future operations well beyond 2023, exploiting its world-leading continuous flux. This user meeting (and associated satellite meetings) will be a unique occasion for European scientists to support and drive the opportunities for science with neutrons through the next decade.
Please visit www.neutrons4europe.com for more detail.

20th November – PSB Spotlight Meeting on Light Scattering and Hyphenated Techniques

The PSB Spotlight Meeting on Light Scattering and Hyphenated Techniques will take place on the EPN campus on 20th November 2018 and the aim of the meeting is to present different experimental techniques using light scattering, including dynamic and static light scattering combined with size exclusion chromatography (SEC), field flow fractionation (FFF) - multi-angle light scattering, composition-gradient (CG) - multi-angle light scattering and SEC-SAXS (small angle X-ray scattering), and their applications in biochemistry. Another PSB Spotlight meeting on serial crystallography will also be organized in October 2018 (date to be confirmed).
For more information, please visit: www.psb-grenoble.eu

ANNOUNCEMENTS

Bill Stirling, former ESRF and ILL director, has been awarded the prestigious title of Companion of the Order of St Michael and St George (CMG) for services to British science and international science collaboration.

Contacts
Editors: Andrew McCarthy (EMBL), Estelle Mossou (ILL) and Montserrat Soler Lopez (ESRF)
Student representative: Lena Anastasakou (EMBL)
Editor-in-chief: Joanna Timmins (IBS) and Florent Bernaudat (PSB)
Design: Virginie Guerard
Photos: Serge Claisse
Email: cisbnewsletter@embl.fr
www.psb-grenoble.eu

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