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PSB et al

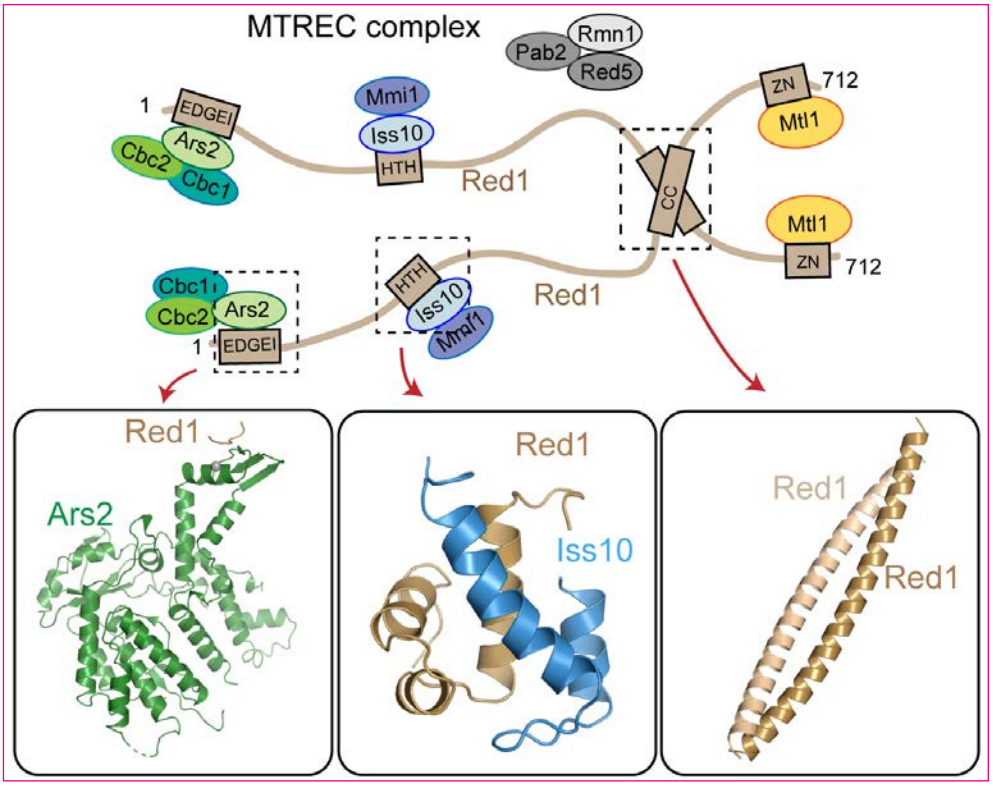
Partnership for Structural Biology Newsletter

Scientific highlights

How cells eliminate aberrant RNA transcripts

Eukaryotic cells produce large amounts of RNA transcripts but only some of them are functional and useful for cellular activities. The aberrant and unwanted transcripts need to be eliminated. To achieve this, cells rely on nuclear RNA-targeting complexes that specifically detect these RNAs and deliver them to the RNA degradation machinery. The fission yeast complex MTREC and its human counterpart PAXT are such RNA-targeting complexes and are essential for this RNA surveillance. MTREC/PAXT are large complexes consisting of a scaffold protein Red1/hZFC3H1 that recruits several protein modules required for the RNA recognition, and an RNA helicase that delivers the transcripts for degradation. The mechanisms by which these complexes perform their function remained poorly understood.

In this study, Anne-Emmanuelle Foucher and Ariadna Juarez of the Kadlec team at the IBS, structurally characterized several key aspects of the MTREC/PAXT complex (Figure 1) [1]. Together with the group of Montserrat Soler Lopez of the ESRF they used yeast two-hybrid assays to identify interactions that Red1 forms with the other subunits within the complex. One such interaction links the Red1 scaffold to the Iss10 subunit involved in specific elimination of meiotic transcripts that are produced in fission yeast in time when meiosis should not be initiated. The NMR structure of the complex determined together with Cameron Mackereth of IECB in Bordeaux, combined with follow-up *in vivo* assays performed in Verdel group at the IAB, improved our understanding of how the MTREC complex specifically targets fission yeast meiotic transcripts. In addition, the validated AlphaFold model of the Red1/hZFC3H1 dimeric coiled-coil revealed that these complexes function as dimeric assemblies. Finally, the crystal structure of Red1 bound to Ars2 of the RNA cap-binding module was determined by X-ray crystallography using the ESRF beamline ID30A-3. The structure showed how Ars2 specifically recognizes a short conserved motif of Red1. Joint analysis with Stephen Cusack of EMBL revealed that the human cap-binding module also interacts with equivalent short motifs of multiple other RNA factors, which eventually defines whether the specific RNA should be processed, exported or degraded. Overall, this work provided novel insight into the architecture of the RNA-targeting complexes and their role in RNA metabolism.



A schematic model of the MTREC complex summarising current structural and biochemical information. Lower panels show the structures of Red1 complexes obtained in this study. Red1 binds Ars2 via its "EDGEI" motif and Iss10 with its helix-turn-helix (HTH) domain. It dimerizes through the coiled-coil region (CC) and associates with the Mtl1 helicase with its Zn-finger domain. Atomic details of the interactions with the remaining domains remain unknown.

J. Kadlec (IBS)

[1] A. E. Foucher, L. Touat-Todeschini, A. B. Juarez-Martinez, A. Rakitch *et al.* (2022). Nat Commun., **13**, 4969

CONTENTS

Strikingly different roles of SARS-CoV-2 fusion peptides uncovered by neutron scattering

SARS-CoV-2 is an encapsulated virus, thus it is surrounded by a lipid membrane with embedded structural proteins, such as membrane, envelope and spike proteins. The SARS-CoV-2 most exploited infection pathway is the direct membrane fusion mechanism between viral and cell (plasma) membranes, which is promoted by the spike protein. While the S1 spike subunit bears the receptor-binding domain for the angiotensin-converting enzyme 2 (ACE-II) [1], essential for binding to the host cell, the S2 subunit contains a so-called fusion domain that is responsible for triggering the membrane fusion mechanism. Indeed, after binding, a series of proteolysis leads to S2 conformational changes that free the fusion domain [1], allowing its association with the plasma membrane and the subsequent initiation of the membrane fusion. However, molecular mechanisms driving this fusion pathway were not deeply understood. Thus, the study of the interaction of SARS-CoV-2 S2 fusion domain with model membranes that could mimic the plasma membrane has become of outmost importance, since it could shed light on the key step of the infection process.

Our work aimed at a better understanding of the membrane fusion mechanism, by simplifying the systems down to its core elements and using neutron techniques [2]. Small peptides from the fusion domain (thus called fusion peptides, FP) were employed, and their interaction with model membranes composed of both natural and synthetic phospholipids was investigated, by employing interfacial techniques such as Langmuir trough, Brewster angle microscopy (BAM) and neutron reflectometry (NR), alongside with scattering and spectroscopic techniques, such as small-angle neutron scattering (SANS), quasi-elastic neutron scattering (QENS), neutron spin echo (NSE) and circular dichroism (CD). The collaborations with the ILL deuteration facility (D-Lab) and Lipid-platform (L-Lab) were essential since they provided the natural lipids extracted from yeast cells that were exploited to mimic the eukaryotic plasma membrane.

Our multi-technique approach revealed that the different segments of the S2 fusion domain exploited (FP1, FP2 and FP4) bind to plasma membrane models and assume different functions in the infection process. In particular, we proved the insertion of FP1, the N-terminal segment of the fusion domain after proteolysis, in the hydrophobic core of the plasma membrane, causing an increase in the dynamics of the lipid acyl chains on a picosecond time and Ångström length scales, thus weakening the membrane and rendering it more prone to rupture. Importantly, this membrane weakening had previously been interpreted as a first step towards viral penetration into the host cell [3]. Besides, such behavior has been shown to be calcium-dependent. In fact, the increase in the concentration of calcium ions drives FP1 to fully cross the plasma membrane (Figure 1), thus creating pores that can be identified as fusion points. Moreover, FP2, and especially FP4, can bind the head-group region of the cell membrane causing dehydration, which would contribute to membrane weakening and promote fusion. Finally, we could depict a clear picture of the membrane fusion mechanism [2]: while FP2 and FP4 bridge the plasma and viral membranes together, the fusion points created by FP1 would allow lipids to diffuse between the two membranes (Figure 2).

A. Santamaria (ILL)

[1] M. Hoffmann, H. Kleine-Weber, S. Schroeder, N. Krüger *et al.* (2020). Cell. **181**, 271-280.
[2] A. Santamaria, K. C. Batchu, O. Matsarskaia, S.F. Prévost *et al.* (2022). J Am Chem Soc. **144**, 2968-2979.
[3] W. T. Heller (2021). Chem Phys Lipids. **234**, 105022.

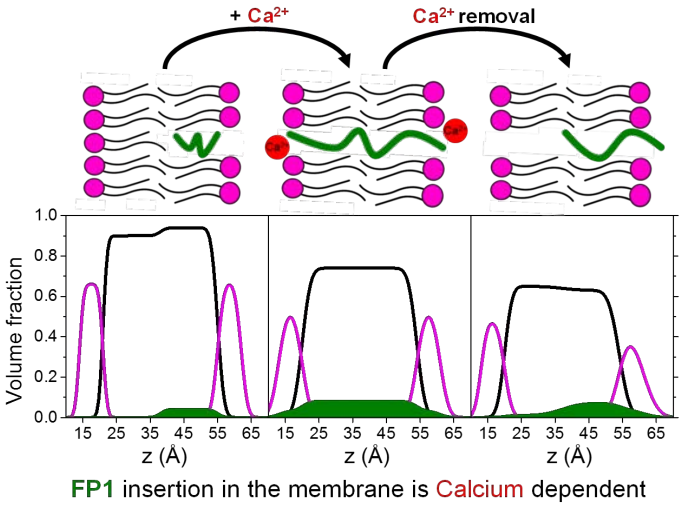


Figure 1: Sketches of FP1, depicted in green, interacting with plasma membrane model. The hydrophilic head-groups are depicted in magenta, the hydrophobic acyl chains are depicted in black. Moreover the influence of calcium ions (depicted as red spheres) is also shown. Each sketch is referred to a volume fraction profiles normal to the interface showing the insertion of FP1 in the bilayer. The volume fraction profiles are obtained from the analysis of NR data.

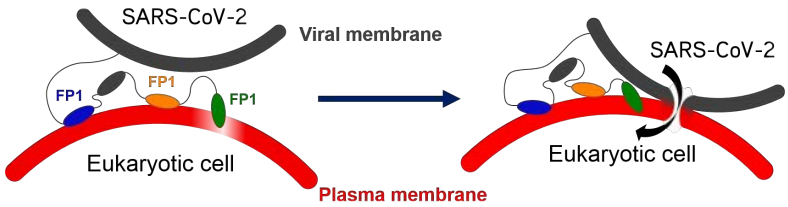


Figure 2: Model of the membrane fusion mechanism. The plasma and viral membranes are depicted in red and grey, respectively. FP1 (green) punctures the plasma membrane; FP2 (orange) and FP4 (blue) bridge the two membranes together. After the fusion, the viral genetic material can enter into the host cell.

Imaging the complex between a snake toxin and a nicotinic receptor

Snake venom is a mixture of toxins that is produced by specialized glands in the snake's head. It is composed of a staggering variety of toxins, and it can have a wide range of effects on prey or predators. Neurotoxins in the venom often target ion channels and can cause rapid paralysis, seizures, or death. Some neurotoxins have been found to have applications in human health (e.g. Ziconotide for severe and chronic pain, derived from a cone snail toxin) and agriculture (e.g. the insecticide Spear, derived from a spider toxin).

Here, a team of researchers lead by the lab of Chris Ulens at KU Leuven focused on the interaction between a short-chain α-neurotoxin and the nicotinic acetylcholine receptor [1]. The receptor was isolated from the electric organ of the Torpedo fish, where it is abundant, and stabilized in lipid nanodiscs. It is a close homologue of the human nicotinic receptor found at the neuromuscular junction. The complex between the synthetic toxin ScNtx (a recombinant toxin with a consensus sequence based upon alignment considering the eleven most toxic short-chain α-neurotoxins from elapid snakes) and the receptor was imaged at the cryoEM facility of the IBS.

The toxin binds to the neurotransmitter binding site, at the interface between two subunits (Figure 1), in a site overlapping with the binding site of long-chain α-neurotoxins. The novelty of the work lies in the detailed description of the differences between the binding modes of these two types of toxin, which in turn permits a better understanding of their specificity for different subtypes of nicotinic receptors.

More than 100'000 deaths occur from snakebite each year. The results also pave the way for the development of new antivenom therapies consisting in a decoy-receptor approach. The structural knowledge could be used to better engineer recombinant proteins that resemble the nicotinic receptor and can capture toxins [2]. Those decoy-receptors might be cheaper to produce and easier store than the antibody cocktails currently used to treat snake bites.

H. Nury (IBS)

[1] M. Nys, E. Zarkadas, M. Brams, A. Mehregan *et al.* (2022). Nat Commun. **13**, 4543.
[2] L.O. Albulescu, T. Kazandjian, J. Slagboom, B. Bruyneel *et al.* (2019). Front Pharmacol. **10**, 848.

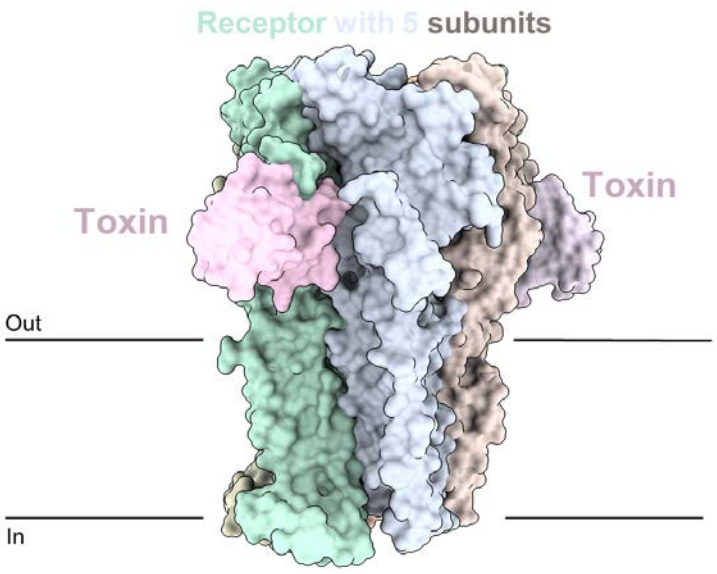


Figure 1: Surface representation of the ScNtx toxin bound to the nicotinic acetylcholine receptor.

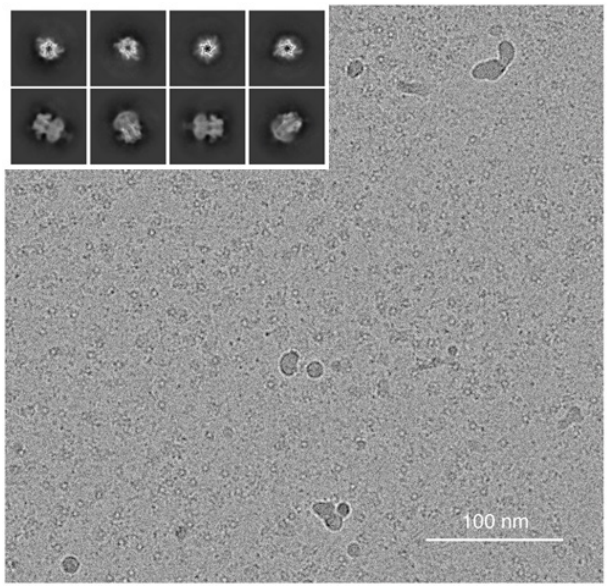


Figure 2: Representative micrograph and 2D classes of the sample obtained at the IBS Glacios microscope.

Slow protein dynamics probed by time-resolved oscillation crystallography at room temperature

In serial crystallography (SX), data sets are composed from (most often) still diffraction patterns collected on many crystals. The development of SX over the last decade has fostered the resurgence of time-resolved macromolecular crystallography (TR-MX). SX-based TR-MX has initially focused on the fast time scales of protein dynamics, from milliseconds down to hundreds of femtoseconds. There are also slower processes that can be studied by collecting partial or complete oscillation datasets at room temperature, which has been made routinely possible by the availability of a humidity-controlled sample environment and fast readout and noiseless X-ray detectors.

Phototropins are plant blue-light photoreceptors mediating the response to excessive or insufficient light levels, which use LOV (Light-Oxygen-Voltage) domains as light-sensing modules, using a Flavin MonoNucleotide (FMN) as chromophore. Upon blue light illumination, a covalent bond is formed in microseconds between the FMN and a nearby cysteine residue, constituting the 'photoadduct' intermediate state of the LOV domain. The formation of the photoadduct triggers a series of structural rearrangements of phototropin, eventually culminating in the build-up of the active, signalling state of the photoreceptor that can last for tens of seconds after the end of light illumination. The mechanism of phototropin activation is studied for both fundamental and biotechnological reasons, to explain the varied light sensitivity in plants and to be able to modulate it by engineering [1].

In a previous study, we monitored the build-up of the photoadduct population in crystals of the LOV2 domain of phototropin 2 of

Arabidopsis thaliana and could produce a 4.2 s-long electron density movie showing protein movements with a time resolution of 63 ms [2]. In the present study, we have monitored the thermal relaxation of the LOV2 photoadduct after interruption of the blue-light induced photoequilibrum in crystals. We first characterized spectroscopically the decay of its population at the *in crystallo* Optical Spectroscopy (*ic*OS) Lab at the ESRF and showed that the phenomenon is significantly slower in crystals than in solution (Figure 1A), which can likely be explained by the increased rigidity of the protein owing to crystal contacts. We then probed the structural evolution of the protein over a ~30 min period with a 1.2 s time resolution, which corresponds to the duration of a complete oscillation data set recorded with the Eiger X 4M detector on beamline ID30A-3 at the ESRF (Figure 1B). The decay time constants derived by both methods from the green curves are remarkably similar.

One unexpected result of the study was the identification of a crystal phase transition 60 to 80 s after the end of illumination, as the diffraction data cannot be reduced any more in the original tetragonal space group $P4_32_12$ with one molecule in the asymmetric unit. A loss of a 2-fold symmetry element leads to a change to the orthorhombic space group $P2_12_12_1$ with two molecules in the asymmetric unit differing in the conformation of the C-terminus. One C-ter progressively reorders into the original alpha-helical structure, while the other one eventually adopts a hook-shaped conformation (Figure 2). This can be explained by the side chain flipping of a conserved tryptophan residue occurring at one crystal contact upon relaxation of the photoadduct.

This study paves the way for routine time-resolved crystallography experiments probing slow components of protein dynamics, from seconds to minutes, to hours [3].

N. Caramello, S. Engilberge, C. Mueller-Dieckmann (ESRF), A. Royant (IBS, ESRF)

[1] J. E. Hart & K. H. Gardner (2021). J Biol Chem. **296**, 100594.
[2] S. Aumonier, G. Santoni, G. Gotthard, D. von Stetten *et al.* (2020). IUCrJ. **7**, 728-736.
[3] S. Aumonier, S. Engilberge, N. Caramello, D. von Stetten *et al.* (2022). IUCrJ. **9**, 756-767.

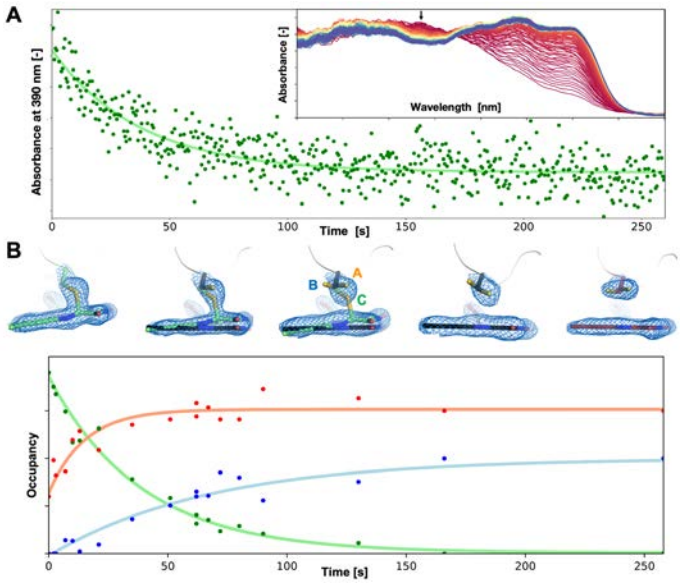


Figure 1: Joint monitoring of LOV2 photoadduct decay by (A) time-resolved *in crystallo* UV-vis absorption spectroscopy at the *ic*OS Lab and (B) time-resolved oscillation X-ray crystallography on beamline ID30A-3.

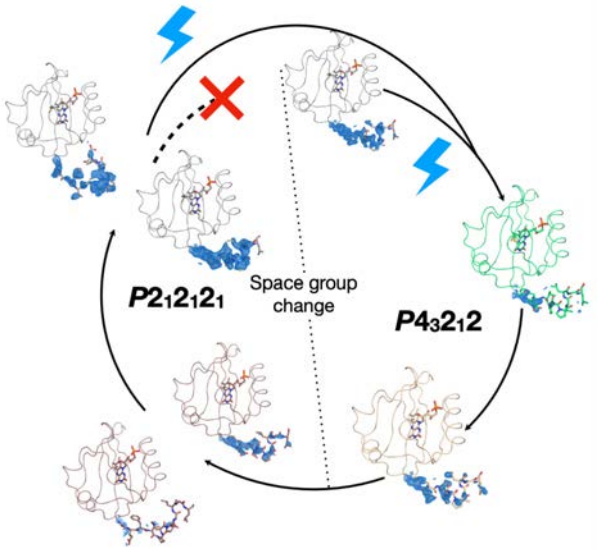


Figure 2: *In crystallo* photocycle of LOV2 featuring a reversible crystal phase transition.

One enzyme to fit them all

Transfer RNAs (tRNAs) are essential molecules for decoding the genetic information from messenger RNA (mRNA) during protein translation. Here, tRNAs recognize a codon, a three-letter code present in the mRNA, via a complementary three-letter code anticodon present in the tRNA, like pieces of a puzzle fit together. Surprisingly, for some of the codons in mRNA, no tRNA with the exact anticodon sequence exists. But how can these be correctly translated? In humans, for example, 19 of the 64 possible codons don't have a tRNA with a complementary anticodon. Three act as stop signs for protein production. For the other 16, Francis Crick proposed in 1966 that their codon-anticodon recognition had room for fuzziness. He suggested a set of rules for tolerable mismatches to allow non-canonical base pairs, so-called "wobble base pairs" [1].

One of the rules proposed by Crick explains half of the 16 codons with missing anticodons: that a tRNA with the modified nucleotide inosine in the first position of the anticodon would be able to recognize three different codons. In eukaryotes, this inosine is present in 8 different tRNAs. Inosine is generated by the post-transcriptional enzymatic deamination of an adenosine base. This essential reaction is catalyzed by a heterodimeric enzyme ADAT2/3 – short for adenosine deaminase acting on tRNA [2].

The Kowalinski group at EMBL Grenoble has become intrigued by how this enzyme, ADAT2/3, is able to recognize 8 chemically different tRNA substrates and not off-targets on similar RNA structures. How can the enzyme's active site be promiscuous enough to accept the diversity of several tRNAs with different sequences, but at the same time specific enough to avoid mutagenesis of any mRNAs that would result in aberrant protein production?

In our most recent work [3], the group was able to resolve this dilemma by delivering the Cryo-EM structure of ADAT2/3 bond to tRNA (Figure 1). The structure, along with biochemical experiments, demonstrated that ADAT2/3 has a gate closing the active site, which in principle, is an obstacle to RNA binding. The interaction with the tRNA needs to be initiated by two additional domains that are distant from the active site. These two domains are independently able to recognize features in the 3D structure of the tRNA; in concerted action with the active site, all three sites together "measure" the geometry of the whole tRNA, which serves as a verification mechanism. Only once recognized as a

bona fide tRNA, the extra domains work together to force the tRNA anticodon through the gate and position it in the active site to trigger the deamination reaction. Altogether, based on structure, biochemical experiments and previous data, a multi-step mechanism of tRNA recognition by ADAT2/3 was proposed that excludes recognition of off-target RNAs (Figure 2).

L. Dolce and E. Kowalinski (EMBL)

[1] F. H. Crick (1966) J Mol Biol. **19**, 548-55.
[2] A. P. Gerber, W. Keller (1999) Science **286**, 1146-1149.
[3] L. G. Dolce, A. A. Zimmer, L. Tengo, F. Weis *et al.* (2022) Nat Commun. **13**, 6737.

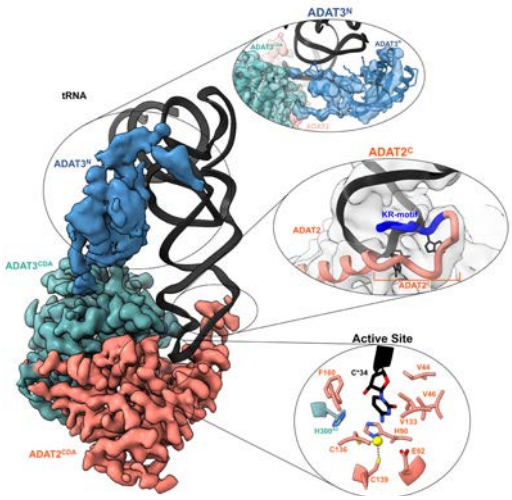


Figure 1: Cryo-EM structure of ADAT2/3 bound to tRNA, with highlights for the flexibly attached domain (ADAT3^{N}), the intrinsically disordered motif (ADAT2^{C}), and the active site.

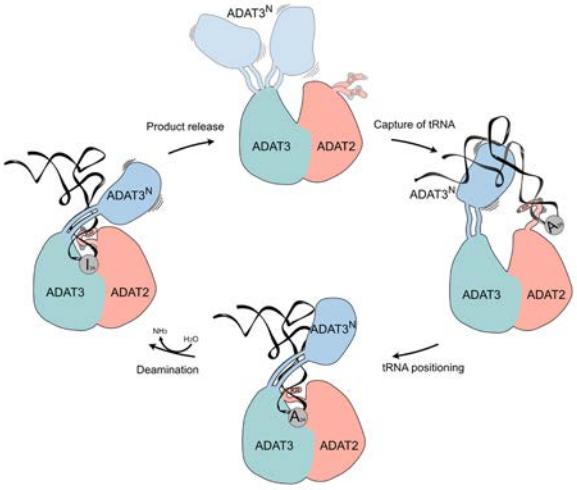


Figure 2: Proposed multi-step mechanism for tRNA recognition and deamination by ADAT2/3.

News from the platforms

ID29 first users

Last September, the brand-new beamline ID29, dedicated to time-resolved serial crystallography, opened to its first users. Thanks to the EBS upgrade, the beamline generates a 10 to 100 µs pulsed beam at a repetition of 925 Hz, with a flux of about 10^{15} ph/sec. The new ID29 is designed to determine the structure of macromolecules at room-temperature, revealing the conformational changes that take place during reactions. With these characteristics, this beamline is unique in the world and provides a new tool for structural biologists to study enzymatic processes that occur in the micro-to-millisecond time range. During the first experiments, diffraction data from protein microcrystals were collected using various different methods including tape-drive, acoustic levitator, high viscous injector and fixed-target (Figure a and b). All these sample delivery methods were used to study different biological systems, ranging from membrane protein receptors to antibiotic resistance and COVID-19 multi-domain non-structural protein (nsp) targets in physiological conditions.

J. Orlans (ESRF), S. Basu (EMBL), D. De Sanctis (ESRF)

Left: Example of a tape-drive experimental set-up. **Right**: A high-viscosity injector experiment.

Cryo-cooling of protein crystals under anoxic conditions at the HPMX Laboratory

Since 2015, the High Pressure Freezing Laboratory for Protein Crystals (HPMX) allows ESRF MX users to study interactions between proteins and light gases (e.g. He, Ar, Kr, Xe, O_2, CO_2) [1]. Among the latter, molecular oxygen (O_2) is an essential molecule, kinetically stable but which can be thermodynamically activated to power cellular metabolism. O_2 interacts with many proteins and is a substrate for various enzymes involved in different biochemical reactions. On the other hand, a number of enzymes are O_2-sensitive and their active sites are damaged when exposed to atmospheric oxygen (e.g. enzymes containing iron-sulfur clusters). In the atmosphere, the reactions of O_2 with these proteins (O_2-dependent and -sensitive enzymes) often result in undefined mixed states. Crystallographic studies of such structures require these proteins to be in proper states and thus need to be crystallized under anoxic atmosphere in gloveboxes. However, extracting crystals from gloveboxes to perform diffraction experiments is a tricky operation, during which samples might be ruined if they are accidentally exposed to atmosphere. To improve this step, we have designed a new type of miniature airlock that facilitates the harvesting, recovery and flash-cooling of protein crystals grown in gloveboxes [2]. This airlock system is based on a narrow pipe passing through the glovebox-wall, in which the crystals mounted on SPINE-supports are shuttled rapidly from the glovebox anoxic atmosphere directly into a liquid nitrogen bath outside, while always remaining protected from O_2 (Figure 1A). This anoxic cryo-cooling system simplifies the handling of crystals and thus improves sample preparation rates compared to former methods, which required the cryo-coolant to enter inside the glovebox. This improved flexibility opens new experimental perspectives such as the cryo-trapping of intermediates in anoxic crystals and the use of *in crystallo* spectroscopy to monitor protein states. Figure 1B shows the protein structure and Figure 1C the UV-vis spectrum of a reduced crystal of the tRNA modifying enzyme MiaE [3]. The crystal was prepared and flash-cooled in the HPMX laboratory (O_2 level < 30 ppm), the diffraction data collected on the beamline ID30B and the spectrum measured on the same crystal in the icOS laboratory. This development is the result of a collaboration between the ESRF PSCM, the ESRF SB-Group and the LCBM.

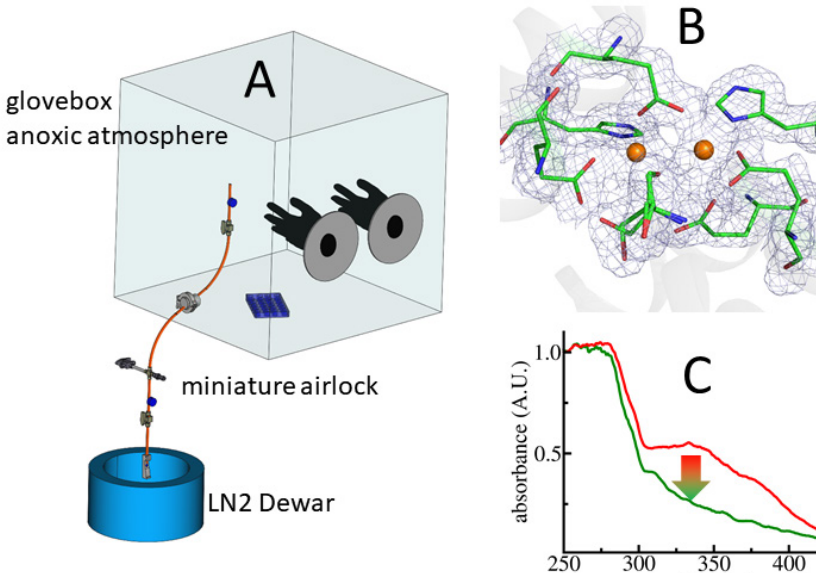


Figure 1: **A**. Technical drawing of the anoxic cryo-cooling system. **B**. Structure of the enzyme MiaE at 1.6 Å. The reduced crystal was prepared in a glovebox cryo-cooled with miniature airlock. **C**. Absorption spectrum recorded on the same reduced crystal in green (oxidized reference in red).

P. Carpentier (ESRF/LCBM-CNRS)

[1] B. Lafumat, C. Mueller-Dieckmann, G. Leonard, N. Colloc'h, *et al.* (2016). J Appl Crystallogr. **49**, 1478.
[2] P. van der Linden, S. Engilberge, M. Atta, P. Carpentier (2022). J Appl Crystallogr. **55**, 1212.
[3] P. Carpentier, C. Leprêtre, C. Basset, T. Douki, *et al.* (2020). Nucleic Acids Res. **48**, 9918

EVENTS

Grenoble Host-Pathogen Interactions Club

Close to fifty researchers gathered at the Institut de Biologie Structurale on 30 November 2022 for the 6th meeting of the Grenoble Host-Pathogen Interactions Club (HPIC). The programme featured talks by Olivier Terrier (Centre International de Recherche en Infectiologie, Lyon) who described the interactions between SARS-CoV-2 and other respiratory viruses in human epithelium, and by Cécile Breyton (IBS) on the Structural basis of bacteriophage T5 host recognition, infection trigger and *E. coli* cell wall perforation. Three junior scientists were also invited to present their work: Elena Buelow (TIMC) described her studies of the microbiome, resistome, and mobilome components in hospital and urban wastewater biofilms, Luciano Dolce (EMBL) detailed the structure of *Trypanosome brucei* ADAT2/3 bound to tRNA (see Highlight on page 4-5), and finally Luis Vigetti (IAB) presented his PhD work on how to decipher the high-speed motility of the eukaryote *Toxoplasma gondii*. The meeting was well received by all participants who were also pleased to have the opportunity to meet again in person after a series of virtual events. Do not miss the next HPIC meeting that will be held on 4 May 2023 (for more information on the club and future events, please check: https://hostpathogen.fr.

F. Bernaudat (PSB)

Grenoble Drug Discovery Club

On 25 November 2022 the Grenoble Drug Discovery Club (GDDC) (https://grenobledrugdiscovery.fr) organised its fourth (but first in-person) scientific meeting, which took place at the IAB. The meeting was very well attended with 105 registered participants from the various Grenoble research institutes: CERMAV, CHUGA, DCM-UGA, DPM-UGA, EMBL, ESRF, IAB, IBS, ILL, and IRIG. The programme included an exciting collection of invited speakers of international stature: Valérie Lamour (IGBMC, Strasbourg, FR), Jordi Mestres (IMIM, Barcelona, ES), Florence Mahuteau (Institut Curie, Orsay, FR), and Didier Leroy (Medecines for Malaria Venture, Geneva, CH), together with a selection of local speakers Gilles Degotte (DCM/UGA), Guillaume Hoffmann (IAB), Rahila Rahimova (EMBL), and Flora Clément (IRIG). The oral presentations, posters and lunch/coffee breaks were the occasion for many lively scientific exchanges. The organisers wish to thank the speakers for sharing their work and all those who contributed to the success of this day: the participants, the funders (UGA, Labex GRAL, Labex Arcane and PSB), and the sponsor CDD Vault, and look forward to see everyone again during the future events of the GDDC in 2023.

F. Bernaudat (PSB)

Cryo-EM SYMPOSIUM

On 22 November, staff from ESRF, IBS, EMBL, and ILL gathered in the ESRF Auditorium to celebrate 5 years of CM01 operation with a dedicated CM01 Cryo-EM Symposium. As a hallmark of the birthday celebration, this hybrid event was intended to mark the success of a unique and true PSB collaboration, wherein all the above four institutes of the EPN campus joined forces to support the user program of CM01. Scientists from across the campus and CM01 staff gave an overview of the research carried out and the continuing advances being made at this key part of EPN infrastructure.

Directors from all four institutes (ESRF, EMBL, ILL and IBS) gave presentations on their institute's participation to the CM01 project, as well as providing some perspectives on the cryo-EM field going forward. Researchers from each institute were invited to showcase their work that has benefitted from CM01. Crucial areas of research such as Alzheimer's disease, the pharmacology of neurotransmission receptors, mechanisms of transcription regulators and the conformational dynamics of bacterial membrane proteins were all discussed. CM01 beamline staff, essential to the continuing operation of the beamline, also gave presentations on the ongoing developments. In addition, due to the resolution revolution and the growing number of cryo-EM focused projects both by staff present on the EPN campus, but also from external users, it was made clear that the need for cryo-EM facilities will continue to increase in the coming years, and hence the need to reinforce the existing collaboration of the campus institutes and perhaps foster further future collaborations towards cryo-EM developments.

Eaazhisai Kandiah (ESRF), beamline responsible of CM01 and organiser of the event, extends a thank you to those that participated in the event by giving talks and to those that joined the symposium both in person and remotely by zoom.

L. McGregor and E. Kandiah (ESRF)

Grenoble Epigenetics Club

For the last 11 years, the Grenoble scientific community has been demonstrating a growing interest in the field of chromatin, transcription, epigenetics and RNA biology. To facilitate interactions and spark off new collaborative projects within this community, the Grenoble Epigenetics Club – initiated by Jérôme Govin and André Verdel (now both at IAB) – has led to the successful organization of 26 meetings and 3 symposia, also with the contribution of PSB scientists from EMBL and IBS.
This Club organizes 3-4 half-day meetings each year, usually in autumn, winter and spring, whose location rotates between IAB and the PSB Campus. In line with this calendar, the last two events were organized at EMBL on November 17 2022 and at IAB on January 26 2023. These meetings, which are open to anybody and for which no registration is required, gather about 50 local scientists at all career stages and speakers from Grenoble, surroundings and beyond.

On November 17, Patrick Lomonte (University of Lyon) presented the latest data from his group on mechanisms of viral defense through chromatin modifications, Christopher Swale (IAB) his exciting data on spliceosome targeting in parasites, and Kiran Padmanabhan (IGFL Lyon) recent results on the epigenetic regulation of the mammalian circadian clock.
On January 26, Jan Kadlec (IBS) presented structural insights into nuclear RNA fate determination, Monica Dolega (IAB) principles of folding of nuclear proteins, and Julie Carnesecchi (IGFL Lyon) how cell fate is decided by transcription and RNA processing networks.
If you are interested in following the activities of this Club, please sign up for our mailing list to make sure you don't miss any event or communication!

J. Govin (IAB), M. Marcia (EMBL), J. Kadlec (IBS), A. Verdel (IAB), C. Zubieta (PCV).

ESS-ILL USER MEETING

In the first week of October 2022, the Institut Laue-Langevin (ILL) together with the European Spallation Source (ESS) brought together 320 neutron researchers in Lund (Sweden) to share and discuss neutron research with a broad range of areas including life science, energy solutions, materials and the environment. The ILL, as a globally leading neutron source, has enabled crucial scientific discoveries related to materials, energy and health for 50 years. The future flagship facility ESS will be in full operations by 2027, providing new opportunities for cutting-edge science through its unprecedented brightness and state-of-the-art instruments. The two neutron facilities jointly hosted the 3rd ESS-ILL User meeting, a big conference for European neutron research community from both academia and industry. Over the course of three days, participants could watch exciting scientific presentations on neutron research as well as the current status of ILL and ESS.

The structural biology talks focused on recent challenges in health including COVID-19 pandemic. For instance, Marianna Yanez Arteta from AstraZeneca shared results on investigation of lipid nanoparticles in mRNA delivery using small angle-neutron scattering at the ILL. Andrey Kovalevsky from the Oak Ridge National Laboratory showed how neutron protein crystallography could uncover SARS-CoV-2 main protease function and help in the design of potent inhibitors.

The ESS-ILL User meeting is held every two years and provides an important platform for collaboration and exchange of scientific ideas to further shape the future of European neutron research.

L. Gajdos (ILL)

Fête de la Science 2022

Photo credit: UtopikPhoto_PDS2022 and O. Cavoret (bottom right)

For the 31st edition of the national science festival "Fête de la Science", which took place from 4 to 8 October 2022, the partners of the EPN Campus have reiterated their teaching and outreach actions. During the week, the ESRF welcomed pupils from 12 different primary schools from the Grenoble area in the MINATEC building, and offered them the opportunity to make their own kaleidoscope in order to explain how mirrors can reflect and direct light, as a first approach to synchrotrons and X-rays. For its part, the IBS visited three local primary schools and initiated over 150 pupils to the study of the molecules of life. Three high school classes were able to visit IBS laboratories and participated to several workshops to explore proteins at the atomic level. A series of videoconferences were also organised with 4 high schools from the Rhône-Alpes region, and enabled the students to discuss directly with scientists in order to discover the world of research and career opportunities.

On Saturday 8 October, the EMBL, ESRF and ILL shared a common stand at the Parvis des Sciences (https://parvis-des-sciences.com/), and proposed several activities including protein crystals fishing, and fluorescence microscopy. With over 1000 visitors, the Parvis des Sciences was once again a success and the volunteers animating the booth greatly appreciated the interaction with the public, especially after the last two years during which the exchanges were mainly virtual.

F. Bernaudat (PSB)

Advances Isotopic Labeling Methods for Integrated Structural Biology International Workshop (AILM 2022)

From September 13th to 16th, the fourth edition of the AILM workshop was held at IBS. This meeting gathered 100 researchers: 60 national participants, including 30 researchers from the EPN campus, 40 international visitors from Europe, USA, Canada, India, Australia and Japan. A total of 40 oral presentations and 38 posters covered the latest advances in isotope labeling and their applications to the study of complex biological systems by NMR, EPR and neutron sciences. The AILM2022 workshop, co-organized by IBS, IBPC (Paris), IGBMC (Illkirch) and the University of York (UK), was supported by GRAL, Infranalytics, the International Society of Magnetic Resonance, Instruct, Frisbi, CNRS and the University of Grenoble Alpes. Thanks to the support of these sponsors, the organizers were able to offer free accommodation to all young researchers who requested it. 16 young trainees were selected to pursue advanced practical training in isotope labeling from September 16 to 23 and had the opportunity to be trained in advanced techniques using their own constructions. The next edition of the AILM workshop is scheduled for spring 2024.

J. Boisbouvier (IBS)

ANNOUNCEMENTS

Montse Soler Lopez and **Max Nanao** were appointed Head and Deputy Head, respectively, of the ESRF structural biology group in July 2022. They succeed Gordon Leonard, who will retire in September 2023 (see profile), and Christoph Mueller-Dieckmann, respectively. Montse Soler Lopez jointed the ESRF in 2014 from the IRB in Barcelona to become the ESRF molecular biology laboratory scientific manager in the CIBB. Max Nanao joined the ESRF in 2016 from EMBL Grenoble to become beamline scientist responsible for ID23-2.

Gergely Papp has been appointed head of the Instrumentation team at EMBL Grenoble. Gergely is originally from Debrecen in Hungary and studied at INSA engineering school in Lyon. He started work in the instrumentation group as an Automation Software Engineer in 2010, becoming interim head in July 2020 after the retirement of Florent Cipriani. Gergely has expertise in the development of innovative scientific instruments for structural biology experiments. Examples include the FlexHCD and bioSAXS sample changers used at the EMBL-ESRF Joint Structural Biology Group Beamlines. The team is currently working on the development of EasyGrid, an instrument for the preparation of Cryo-EM single particle and Cryo-ET grids, which is also being tested for the preparation of samples for X-ray imaging techniques.

Malene Jensen, team leader in the Protein Dynamics and Flexibility (FDP) group at the IBS, is one of the 7 researchers in life sciences distinguished by the first Impulscience grant program of the Bettencourt Schueller Fondation. The foundation supports each of these researchers, for a period of 5 years, with 2.3 MEuros. Malene is specialized in the study of intrinsically disordered proteins, a class of proteins that remains functional despite the absence of a stable, three-dimensional structure. The work of Malene will allow to observe more closely the capacity of cells to respond continuously to internal and external signals, thanks to so-called scaffold proteins. Malene will use nuclear magnetic resonance (NMR) spectroscopy to study intrinsically disordered scaffold proteins in the MAPK cell signalling pathways, and she will visualise their assembly with kinases and GTPases. The work will reveal how scaffold proteins contribute to enzymatic activity and signalling specificity. The study of the specific mechanisms of cell signalling is particularly relevant because deregulation of many signalling pathways is associated with diseases, such as metabolic disorders and cancer. The work will therefore open new avenues for drug discovery by targeting the recruitment of enzymes to scaffold proteins.

NEWCOMERS

Nicolò Paracini joined the ILL in January 2023 as an instrument scientist on the FIGARO reflectometer. Nico received a PhD from Newcastle University in 2019, then worked as a postdoc at Malmö University for 3.5 years. His research focuses on model biological membranes and protein-lipid interactions, including the Gram-negative bacterial outer membrane and its role in antibiotic resistance, curvature effects on lipid & protein sorting and novel sample environments for biomembrane research with neutrons. Interested in collaborating? Get in touch!

Lukáš Gajdoš joined Matthew Blakeley as a co-responsible for the LADI/DALI neutron macromolecular crystallography beamline at the ILL, in February 2023. Lukáš completed his PhD at the University Grenoble Alpes under supervision of Anne Imberty from CERMAV, CNRS. The thesis was focused on studying protein-carbohydrate interactions in bacterial infection using neutron crystallography. After that, he joined the Large Scale Structures group at the ILL as a postdoctoral researcher to study radiation damage in protein crystals using neutron and X-ray crystallography.

Rosicler Barbosa Lazaro joined EMBL Grenoble in September 2022 as a research technician in Marquez team and will contribute to the operation of the High Throughput Crystallisation (HTX) facility. Rosicler obtained her PhD at the Brazilian Synchrotron Light Laboratory and spent three years as a postdoc at Synchrotron Soleil working on functional and structural characterization of proteins related to expression regulation. "I'm really happy to be part of Marquez team and contribute to the development of new technologies and the operation of the HTX platform".

Romain Linares joined EMBL Grenoble in January 2023 as a staff scientist and will contribute to the operation of the PSB operated Cryo-EM (CM01). Romain obtained a PhD in Biochemistry in 2016 in CBMN (Laboratory of Chemistry and Biology of Membranes and Nano-objects, Bordeaux) under the supervision of Pr. Alain Brisson, working on the characterisation of extracellular vesicles from blood plasma, mainly by electron microscopy. In 2017, he joined IBS for a postdoctoral fellowship. Over the past 5 years, together with Drs Cécile Breyton and Guy Schoehn, he studied the structure and the infection mechanisms of bacteriophage T5, a virus infecting *E. coli*, using cryo-EM.

Felix Weiss joined the IBS in October 2022 as a CEA scientist specialized in cryo-EM. Félix did his PhD work in Reynald Gillet's team at the University of Rennes 1 on the structural elucidation by cryo-EM of the trans-translation mechanism of ribosomal complexes. He then moved to the MRC Laboratory of Molecular Biology in Cambridge as a research associate to work on eukaryotic ribosome biogenesis failures in cancer biology. Between 2017 and 2022, Félix was a staff scientist on the cryo-EM platform at EMBL-Heidelberg, providing support in sample preparation, grid freezing, microscope operation, images acquisition and data processing. As a scientist within the MEM group at IBS, Félix will provide guidance to groups with no prior experience in cryo-EM. He will also be involved in the installation and operation of the new Titan Krios CM02, while developing new methods for sample preparation.

Sylvain Engilberge joined the IBS in October 2022 as a CEA scientist on beamline BM07-FIP2. Sylvain completed a PhD in physics for life sciences in 2017 at the University Grenoble Alpes under the guidance of Dr. Eric Girard, working on the development of molecular glues for protein crystallization and *de novo* phasing. He then moved to the University of Galway in Ireland to continue his investigations on crystal engineering. After another postdoc at the Swiss Light Source, he joined the Structural Biology group of the ESRF to shift his research focus to the study of protein dynamics by time-resolved spectroscopic and crystallographic methods. In addition to support users' experiments on BM07-FIP2, he will contribute to the refurbishment of the beamline and to the implementation of new methodologies in room temperature protein crystallography.

PROFILE

Photo: Chantal Argoud

Gordon Leonard has joined the ESRF in 1996, and has been involved in the PSB since its creation in 2002. Gordon became Deputy Head of the ESRF Structural Biology group in 2000, and was then Head between 2013 and 2022. Gordon will officially retire next September, and the PSB Newsletter met with him to know more about his career and his experience on the EPN campus.

Could you tell us a bit about yourself, your history and your scientific interests?

I started off by doing an undergraduate BSc in Chemical Physics at the University of Surrey in Guilford, where in my third year we merged with the Chemistry department and I had my first crystallography course - so I guess this is where it started! Once I'd finished that degree, I was asked by Dr David C. Povey if I wanted to do a PhD in inorganic chemistry and crystallography, co-supervised by M.F.C. Ladd and L. F. Larkworthy and working on the synthesis and crystal structure determination of chromium (II) complex halides and thiocyanates with a view to explaining their magnetic properties. After completing my PhD in September 1984, I moved to Switzerland to join the group of Jean-Claude Bünzli at the University of Lausanne, working on the synthesis of complexes of rare earth elements

with compartmental ligands to see what had interesting magnetic properties. During this time it became clear, to me at least, that inorganic chemistry was not really my forte and that I would rather move into what was at that time (around 1986!) the relatively new field of macromolecular crystallography. So, I left Switzerland after about 18 months and went looking for somebody who would take a chance on me. That person was Tom Brown at the University of Edinburgh who was at that time setting up a lab specialising in the synthesis and X-ray structural analysis of mismatch containing DNA oligomers. I stayed in Edinburgh for about 5 years, gaining experience in MX and trying to improve my golf game (the former worked, the latter much less so!) and then took up a position in Bill Hunter's group at the University of Manchester. Initially, my time was spent mostly on the structure determination of short fragments of DNA with mismatched base pairs or mutation-inducing modified bases. I also started to help a PhD student, Serena Cooper, who was working on the structure determination of class II Zn-aldolase which turned out to be a bit of a challenge. We were eventually successful when we combined phases from isomorphous replacement and MAD experiments. At that time, in the early 1990s, MAD was just becoming a technique that people were aware of and our MAD experiment (SeMet derivative, more than 10 crystals to get a complete data set) was one of the first carried out on the new ESRF BM14 and we were helped by Sean McSweeney and Andy Thompson, who had both been at SRS, Daresbury – very close to Manchester - so I already knew them quite well. Quite soon after this, I saw an opening of a beamline scientist for BM14 and so I came to the ESRF on a 5-year scientist position in 1996, working with the beamline responsible Andy Thompson. When Andy moved on to start the design and construction of ID29, I was made BM14 beamline responsible then appointed as a permanent scientist at the ESRF. After a relatively long stint as a BLOM I was made the beamline responsible for ID29 and, from the very early 2000s, was Deputy Head of the ESRF Structural Biology group. When Sean McSweeney left as group Head, I applied for the vacant position and was successful. From then on, I moved a bit further away from day-to-day beamline activities, focused on organisation and strategy – mostly management responsibilities. Finally, after nearly 27 years at ESRF I decided to retire, voilà!

Why did you decide to come and work at the ESRF?

A position became available on the MX anomalous scattering beamline (BM14). Thanks to my experience in Manchester with Bill Hunter (and John Helliwell), anomalous scattering and how to use it in MX was something I understood really well. So I successfully applied. Moreover, I liked being on the beamline - a lot of experiments carried out at Daresbury - and preferred this to being in the lab and so was becoming to see myself more as a beamline person. At the beginning on BM14, MAD was a hard thing to do, the instrumentation and software wasn't like what it is now, and users also needed a lot of help, both for data collection and subsequent structure solution. Even scanning an absorption edge scan was a bit of a fine art, but it was this aspect I enjoyed. Moreover, I had a few nice collaborations at the time, including with Bill Hunter and Jim Naismith. I also liked having the opportunity to develop the beamline to make it increasingly more user friendly with higher throughput. For example, the move from image plates to CCD changed the length of data collection from days to hours! Lastly my wife Kate was very happy to move to France, even though we had two small children at the time, and, initially, the position was not permanent.

Were you here during the creation of the PSB?

Yes, I was actually. Ed Mitchell took charge of the PSB during its creation but I suppose I was tangentially involved as the Deputy Head of the Structural Biology. In the beginning of the PSB, the MX beamlines were seen by the community as more of a technical platform, but ESRF presence in the PSB has grown over the years. Indeed, the involvement of all PSB partners in the operation of ESRF beamlines and other facilities has clearly proven its benefits to the ESRF and the wider structural biology community. All in all, I think that the PSB has been an excellent resource for providing a series of technical platforms for SB research on the EPN campus. In addition, it has helped to cross fertilise collaborations between the different institutes by proving a common ground for people working on projects with similar goals.

How have you used the PSB?

It has been an invaluable resource for students and especially those working at the ESRF. Having access to the biophysical platform to do ITC experiments, screening crystallisation conditions using the HTX platform has contributed a lot to many projects. In particular, in recent years the membrane protein crystallography platform has been critical to the success of several PhD projects. And now with the access to CM01, the benefits to some of the inhouse research projects have been clear through some interesting results.

How do you see the evolution of SB and the use of integrative approaches over the coming years?

Interaction. I think there will be an increase in complementarity and an increase in super resolution microscopy techniques like confocal microscopy, soft X-ray imaging and cryo-electron tomography. MX, SAXS and SANS will also remain 'go-to' techniques for the foreseeable future. An obvious, and very useful, complementary strategy would be to make more use of the combination of MX and NMX. Currently this is challenging due to the sample requirements but with advances in neutron techniques and instrumentation, this might be more possible in the future.

I think the next step for the PSB is that it could be to be more external user orientated. This would allow such users access to more than just one platform, for example, not just the MX beamlines but incorporating the HTX platform, CM01, the ILL and the D-LAB when necessary too. This is definitely the way that research is going: you will need to have multidisciplinary research in order to publish good papers. Many labs already have access to the type of facilities offered, but many don't. A peer-reviewed but externally accessed service in a centralised facility could be really interesting for the EPN campus in general.

What advice would you give structural biologists at the beginning of their career?

Definitely don't get disheartened too quickly! Research can be hard and there are many disappointments along the way but it's worth it for the successes. I'd say this is one of the benefits of working in the PSB environment, there are people working there with expertise in almost everything who can help you with the challenging aspects of your projects, so help is at hand. Don't be put off by apparent failure and sometimes thinking outside the box will help you a lot. Keep going!

What are your plans for your retirement?

I think I'm going to start off by having a bit of a rest, taking it easy, reading, listening to very loud music, training for the étape Loch Ness and generally enjoying my free time. Kate and I have got plans to do a bit of travelling and I'll hopefully get to see a lot more of friends and family. I might even take up the bagpipes! It was something I had the chance to do when I was at school but didn't want to at the time – but now might be the perfect opportunity. If the neighbours complain too much about the noise, there's always the more socially acceptable bass guitar to learn instead. It turns out I might actually keep myself rather busy...

L. McGregor (ESRF)

DATES FOR YOUR DIARY

27th February to 31st March 2023 – Hercules European School
This 1-month school, coordinated by the Université Grenoble Alpes, is designed to provide training for students, postdoctoral and senior scientists from European and non-European universities and laboratories, in the field of Neutron and Synchrotron Radiation for condensed matter studies (Biology, Chemistry, Physics, Materials Science, Geosciences, Industrial applications). It will include online lectures and practicals in small groups. More information: https://hercules-school.eu.

6th March to 10th March 2023 – Tutorial in Macromolecular Crystallography 2023 edition
Fundamental aspects of crystallography will be treated in 22 hours of theoretical sessions (lectures and exercises), 3 practical sessions of 2 hours each on graphical workstations and a practical session of 2 hours in data collection on a synchrotron beamline. The tutorial is limited to 20 participants and it is aimed in the first place at graduate students at Université Grenoble Alpes and on EPN campus who have a priority in registration. The tutorial counts for 3 ECTS credits needed for the "Ecole doctorale" of the UGA. he tutorial is furthermore open to post-docs and staff of the EPN / PSB partners. It will take place in the CIBB seminar room on the EPN campus on the “Presqu'île scientifique”. For more information, please contact the course organiser Wim Burmeister (wim.burmeister@ibs.fr).

27th to 28th March – 48h of GRAL
GRAL, the Grenoble Alliance for Integrated Structural and Cellular Biology, organizes a 2-day seminar open to all staff from the GRAL labs (= BGE / BioSanté / IBS / LCBM / LPCV), as well as close collaborators in Grenoble, including PSB members. It will be an excellent opportunity to strengthen interactions and build collaborations. The programme includes 3 keynote talks by Andreas Diepold (Max Planck Institute, DE), Julia Cuellar Santiago (Université de Lausanne, CH), and Panagiotis Kastritis (Martin-Luther University Halle-Wittenberg, DE), as well as 9 presentations by local scientists, and 2 poster sessions: one for PhD students to present their work and the other for platform staff to present their expertise + for scientists to present their work done using platforms. More information: https://www.labex-gral.fr/events/

27th April - The 26th Epigenetic club of Grenoble at the IBS
Confirmed speakers: Jamie Hackett (EMBL Rome), Sabrina Fritah (Luxembourg Institute of Health), and Auriane Rakitch (IAB)

24th to 26th May 2023 - Inserm Workshop 271 'Using machine learning for protein structure prediction in your research'
This workshop about AI applied to protein structure prediction, is co-organised by Juan Reguera (AFMB), Eva Kowalinski (EMBL Grenoble) and Cameron Mackereth (Inserm). It targets scientists with interest in this new technology, not exclusively structural biologists but also those who would be willing to use these powerful AI tools in their research and don't know exactly how. Top developers, editors, and academic and industrial users will give an idea of the state of the art and a glance on the future perspectives of AI on structural biology and beyond. The workshop consists of 3 days talks in Bordeaux 24-26 of May plus the option of practical training to choose between Grenoble (7-9 June), Bordeaux and Marseille. More information and registration: https://ateliersinserm.dakini-pco.com/en/

31st May 2023 – PSB 20th Anniversary
To mark the 20th Anniversary of the signature of the Memorandum of Understanding (on 15 November 2002) that established the PSB, a celebration opened to all PSB members will be organised on Wednedsay 31 May at the IBS and the Chalet. This event will also be organised in parallel to the PSB Science Advisory Board review that will take place on 30-31 May. Further information will be distributed soon.

6th to 7th July 2023 – PSB Symposium “Dynamics in Structural Biology”
The fourth edition of the PSB biennial Symposium will take place in person on the EPN campus and will focus on 'Dynamics in Structural Biology'. It aims to highlight progress in protein dynamics and to illustrate how big biological questions can be resolved in structural biology through the application of (interdisciplinary) methodological approaches, enhancing our understanding of the dynamic behaviour of macromolecules. More information and registration: https://www.esrf.fr/psbsymposium2023

2nd to 4th October - The 4th Symposium of the Grenoble Epigenetics club (co-organized by EMBL, IBS, IAB and EMBL French Partners from Ecole Normale Superieur and College de France Paris).
Preliminary programme available online at: https://epigenetics.fr/sympo-iv-signaling-through-chromatin-oct-2-4-2023-grenoble-fr/
Registration platform to open soon.

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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.