JANUARY 2021 - NUMBER 26

PSB et al

Partnership for Structural Biology Newsletter

Scientific highlights

Using an integrated structural biology approach to study the delicate balance between the two life cycles of a temperate bacteriophage

Bacteriophages are bacterial viruses and the class of temperate bacteriophages that can enter into two different life cycles following infection of a host. In the lysogenic lifecycle the viral genome is integrated into the host genome, where it stays dormant as a prophage. In the lytic life cycle the cellular machinery is exploited to produce new phages leading to cell lysis and release of new phage progeny. One intriguing question is how the delicate balance between the two life cycles is controlled at the molecular level?

To answer this question, we have studied the temperature bacteriophage TP901-1 that infects the gram-positive bacterium *Lactococcus lactis,* which is important in the dairy industry where it is used to produce cheese. It is estimated that around 60-70% of technological problems associated with cheese production are caused by bacteriophage infections of *Lactococcus* bacteria. The choice between the lysogenic and the lytic lifecycle in TP901-1 is controlled by two proteins, the repressor CI and the anti-repressor MOR and their interactions with the phage DNA genome. It has been shown that CI is a trimer of dimers that in the lysogenic state binds to three DNA sites on the phage genome via its N-terminal helix-turn-helix domain. This particular conformation efficiently suppresses transcription from the lytic promotor, and we previously determined the structure of a dimer of CI in complex with one of the DNA sites using X-ray crystallography combined with small angle X-ray scattering (Fig. 1) [1].

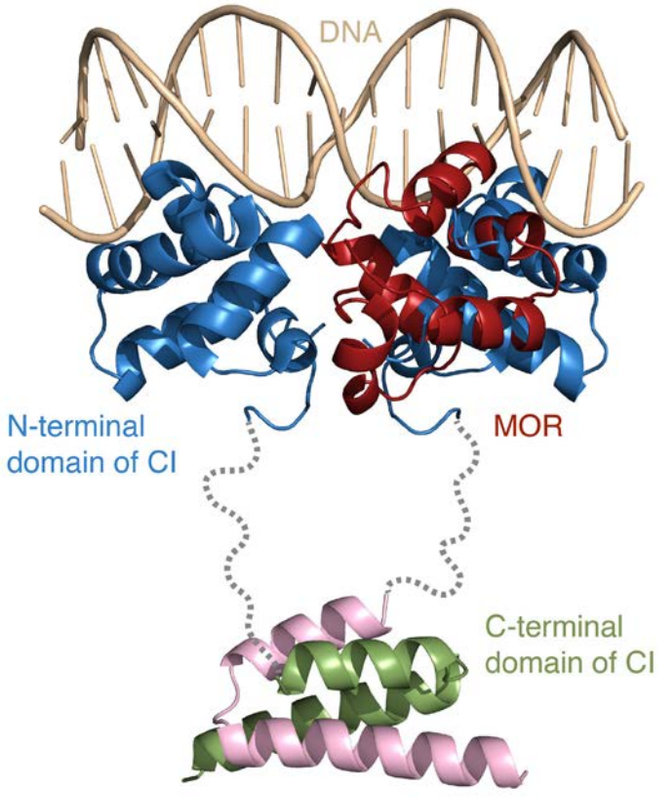

Figure 1. Model of the dimer of CI of the temperate bacteriophage TP901-1 showing the structure of its C-terminal dimerization domain and its two N-terminal domains that bind to the phage DNA. The crystal structure of the CI:MOR complex is superimposed showing how MOR inhibits binding of the N-terminal domains of CI to the two adjacent major grooves on the DNA.

In our most recent work, we revealed the structural details of the switch from the lysogenic to the lytic life cycle in collaboration with researchers at the University of Copenhagen, the Technical University of Denmark and the Institute for Advanced Biosciences in Grenoble [2]. Thus, we obtained the NMR solution structure of MOR revealing a five-helical bundle domain. To our surprise, we discovered that MOR does not enter in competition with CI for binding to the DNA sites, as observed for example in the λ phage infecting *E. coli*, but rather binds directly to the N-terminal domain of CI as observed by NMR and ITC experiments. In collaboration with the Viral Infection and Cancer group (VIC) of the IBS, we could show using native mass spectrometry that the CI:MOR and CI:DNA complexes are mutually exclusive. To understand this in detail, we determined the crystal structure of MOR in complex with the N-terminal domain of CI at 2.3 Å resolution. Superposition of the CI:DNA and the CI:MOR structures reveals how MOR inhibits binding of CI to DNA by occupying the position of the N-terminal domain of CI in one of the major grooves of the DNA (Fig. 1). We also designed single point mutations of CI and MOR leading to the disruption of the interaction between the two proteins. When tested *in vivo*, the bacteriophage was unable to enter the lytic life cycle demonstrating the importance of the CI:MOR complex for maintaining the bistability of the genetic switch. Our results have far-reaching implications as the structural features of the CI:MOR complex are likely conserved in bacteriophages from a number of human pathogens implicated in transfer of antibiotic resistance.

M. Ringkjøbing Jensen (IBS)

[1] K.K. Rasmussen *et al.* (2018). FEBS Lett., **592**, 1738-1750.
[2] K.K. Rasmussen *et al.* (2020). Proc. Natl. Acad. Sci. U.S.A., **117**, 20576-20585.

CONTENTS

Neutron studies of perdeuterated urate oxidase further the understanding into the hydration step of catalysis

Urate oxidase (UOX) is an enzyme that catalyses the O2-dependent degradation of uric acid (UA) to 5-hydroxyisourate (5-HIU). As humans no longer possess UOX due to a pseudogenization event that resulted in the inactivation of the uricolytic pathway, recombinant UOX can play an important therapeutic role as a protein drug. This is applicable in cases where levels of UA are high as seen in severe hyperuricemia, and tumour lysis syndrome, a possible side effect of chemotherapy. In addition, UOX is of interest mechanistically as it contains no metal or co-factor, commonly used to facilitate the reaction of organic molecules with O_2: instead UOX relies on its substrate, UA. The reaction proceeds via two stages: oxidation, followed by a hydration. However, the method by which UA is able to react with O_2, and the role that UOX plays, are still under investigation.

In our recent study, we employed room temperature X-ray (MX) and neutron (NMX) macromolecular crystallography, in conjunction with simulations, to investigate the hydration step of the UOX reaction mechanism [1]. Our data were collected using the LADI-III beamline at the ILL and BM30A at the ESRF. NMX can be used to determine the hydrogen positions in proteins, providing information on water positions, hydrogen bonding partners, and the protonation states of residues. The isotopic substitution of hydrogen (^{1}H) with deuterium (^{2}H or D), known as deuteration, can also be conducted as deuterium possesses more advantageous properties for neutron scattering experiments. The replacement of hydrogen with deuterium was achieved during protein expression (perdeuteration – 100% D) in collaboration with the Deuteration Lab (D-LAB) at the ILL (Fig. 1 a, d, e). The incorporation of deuterium at non-exchangeable positions in ^{D}UOX was then validated using the Mass Spectrometry platform at the IBS, where the level of deuteration was found to be >99% (Fig. 1 b, c). By studying ^{D}UOX in complex with the inhibitor 8-azaxanthine (AZA), we were able to trap a water molecule (W1) situated in the H_2O/O_2 binding site above the inhibitor molecule (Fig. 2 a). The identification of deuterium atom locations in the ^{D}UOX-8AZA-W1 complex confirmed that W1 bonds to Thr57* and Asn254 as an H_2O molecule (Fig. 2 b, c). The NMX and QM/MM calculations were able to determine the protonation states of residues Thr57* and Lys10*, suggested to form a catalytic dyad [1, 2]. We found that the Thr57*-OH forms a hydrogen bond with the oxygen atom of W1 and the orientation of this hydroxyl group is stabilized by the -NH_3+ of Lys10*. These protonation states agree with a recent proposal for the role of this catalytic dyad in the hydration step of the reaction [3]. It has been suggested that the Thr57*-OH is central to a Lys10*-NH_3+-assisted proton relay system (Fig. 2 d). We also found evidence of structural heterogeneity in the tripeptide Pro253-Lys255, involving W1-bonded Asn254. Our studies show that the major conformation of these residues favours W1 binding and therefore may suggest that this dynamic behaviour can help to promote W1/O2 exchange in the oxidation reaction step, increasing the activity of this co-factor independent enzyme. This work allowed us to visualise for the first time the exact nature of a key active site water molecule and understand its interactions with catalytically important residues, with relevance to a key hydration step.

L. McGregor (ILL)

[1] L. McGregor, T. Földes, S. Bui, *et al* (2021). IUCrJ, **8**, 1.
[2] R. Imhoff, N. Power, M. Borrok, *et al.* (2003). Biochemistry, **42**, 4094-4100.
[3] D. Wei, X. Huang, Y. Qiao, *et al.* (2017). ACS Catalysis, **7**, 4623-4636

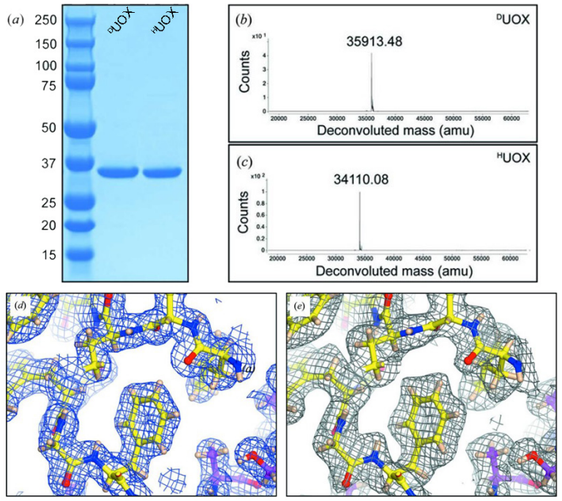

Figure 1. ^{D}UOX and examples of electron and neutron density maps for the ^{D}UOX–8AZA–W1 complex. (a) SDS-PAGE analysis of purified ^{D}UOX and ^{H}UOX samples. (b), (c) ESI-MS analysis of purified (b) ^{D}UOX and (c) ^{H}UOX, showing a mass difference of 1803 a.m.u. As the calculated mass difference (assuming complete deuteration) is 1820 a.m.u., this corresponds to >99% deuterium incorporation in the ^{D}UOX sample. (d), (e) Representative examples of $2mF_o$-DF_c (d) electron density and (e) neutron maps contoured at the 1.0σ level. X-ray and neutron data extend to 1.33 and 2.10 Å respectively.

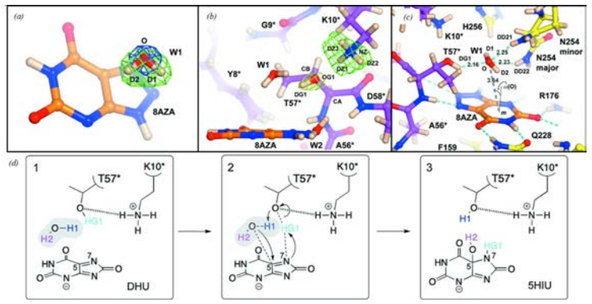

Figure 2. (a) W1 is present as a neutral H_2O molecule. $2mF_o$-DF_c electron density map shown in blue at the 3.0σ level clearly define the W1O position. An omit mF_o-DF_c neutron map calculated only with W1O contribution indicates the presence of two deuterons (D1, D2) as suggested by the elongated positive density (in green at the +3.0σ level) next to the oxygen atom. (b) The Lys10*–Thr57* dyad in the active site. Difference neutron density calculated in the absence of deuterons bound to Lys10*NZ and Thr57*OG1 is shown at the +3.0σ (green) and +2.0σ (lemon) levels, respectively. (c) W1 is sandwiched between Thr57* and Asn254. The latter is modelled in two conformations that refine at 0.8 (major) and 0.2 (minor) occupancy, respectively. W1 is oriented such that its D2 atom forms an O—H···π hydrogen bond with 8AZA. (d) reaction mechanism of the hydration step of UOX catalysis.

Cryo-EM structures of La Crosse polymerase give insights into genome replication of a human pathogenic virus

Bunyavirales is a very large and diverse order of segmented negative-strand RNA viruses (sNSV) comprising more than 500 species classified into twelve families [1]. It contains serious human pathogens such as La Crosse virus, Hantaan virus, Crimean-Congo haemorrhagic fever virus, Rift Valley Fever virus and Lassa fever virus, against which no effective treatment is currently available. Viruses from the *Bunyavirales* order are related to other sNSV and in particular to influenza virus, a major human pathogen belonging to the Orthomyxoviridae family.

Replication and transcription of the RNA genome constitute essential processes performed by the virally encoded RNA-dependent RNA polymerase. Replication generates full-length genome or antigenome copies (vRNA and cRNA respectively), whereas transcription produces capped viral mRNA that is recognized by the cellular translation machinery to produce viral proteins. Transcription is initiated by a "cap-snatching" mechanism, whereby host 5′ capped RNAs are bound by the polymerase cap-binding domain, cleaved by the polymerase endonuclease domain several nucleotides downstream, and then used to prime synthesis of mRNA.

The Methods and Electron Microscopy group (MEM) at IBS, in collaboration with Stephen Cusack's group at EMBL, have determined the structure of the complete La Crosse virus RNA-polymerase obtained at 3 Å resolution by cryo-electron microscopy [2], using data collected on the Glacios (IBS) and Krios (ESRF) cryo-microscopes (Fig. 1). This structure reveals for the first time the presence of key protruding C-terminal domains, notably the cap-binding domain, which undergoes large movements related to its role in transcription initiation, and a zinc-binding domain that displays a fold not previously observed (Fig. 1).

In order to be active, the polymerase needs to bind the 5' extremity of the genomic RNA in a specific binding site located at the surface of the polymerase [3]. This organizes the active site that becomes ready to bind the 3' end extremity to be copied during replication. Replication initiation generates a double-stranded RNA in the active site cavity that will then be split into single-stranded template and product RNA by the polymerase [3].

In this study, we describe the polymerase structure in two states: in a pre-initiation state in which the 5' RNA and the 3' extremities are visible (Fig. 1) and an elongation-mimicking state that reveals the position of a 10-base-pair template-product RNA duplex in the active site cavity (Fig. 2). The two structures reveal the coordinated movement of several domains and loops, namely the priming loop, the mid-thumb-ring linker and lid domain, between pre-initiation and elongation. They reveal the essential amino acid of the lid domain that triggers separation of the template-product RNA duplex and their channelling towards their respective exit tunnels. These structures pave the way towards a detailed understanding of the essential conformational changes that are required for the polymerase to perform replication and transcription. These structural details and the observed dynamics of key functional elements will be instrumental for structure-based development of polymerase inhibitors that are currently lacking to counteract these life-threatening diseases.

B. Arragain, G. Schoehn and H. Malet (IBS) and S. Cusack (EMBL)

[1] Y. Sun, J. Li, G. F. Gao, P. Tien *et al.* (2018) Crit. Rev. Microbiol., **44**, 522–540.
[2] B. Arragain, G. Effantin, P. Gerlach, J. Reguera *et al.* (2020) Nat. Commun. **11**, 3590.
[3] P.Gerlach, H. Malet, S. Cusack, & J. Reguera. (2015) Cell **161**, 1267–1279.

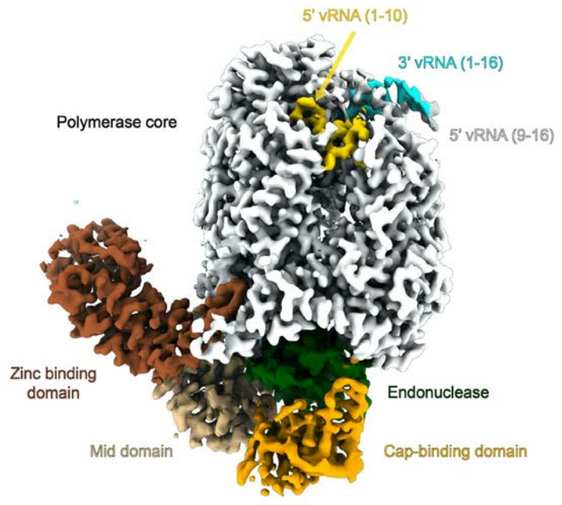

Figure 1. La Crosse virus polymerase structure

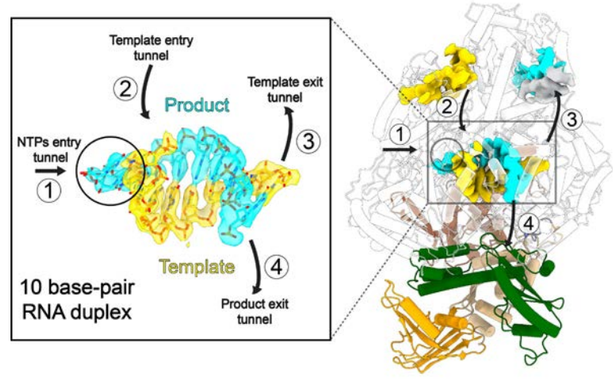

Figure 2. RNA present in the elongation-mimicking state of La Crosse virus polymerase structure

tRNA-adenosine-37 decorated by the monooxygenase MiaE

Transfer RNAs (tRNAs) are essential adaptors that mediate the genetic code translation for protein synthesis. To be fully functional, tRNAs undergo post-transcriptional modifications catalyzed by different tRNA-modifying-enzymes. Up to date, over 100 different tRNA modifications have been identified in various organisms, which modulate tRNAs structure, function and stability (Fig. 1A). Importantly, modifications at position 37 on the anticodon loop are shown to improve translational fidelity, and their benefits are crucial for organism survival. In humans, defects in these modifications seem to be associated with cancer or diabetes. The chemistry of tRNA modifications is usually basic (isomerization or methylation) but strikingly, some of the modifications result from multistep enzymatic reactions involving several enzymes, substrates and intermediates. The enzyme MiaA catalyzes the conversion of adenosine A37 into N6-isopentenyl-A37 (i6A37), which can be further transformed into methyl-thio-i6A37 (ms2i6A37) by MiaB. The latter modification might finally be transformed into 6-hydroxy-ms2i6A (ms2io6A37) by the di-iron monooxygenase MiaE (Fig. 1B), which has only been identified in some bacteria.

Our work addresses the biochemical pathway of MiaE from *Pseudomonas putida* (Pp-MiaE). The crystallographic structure of Pp-MiaE displays a compact single domain that consists essentially of a four-α-helix-bundle housing a di-iron catalytic center. Molecular oxygen (O2) in the solvent is required to transit via a dedicated functional diffusion route to reach the buried catalytic di-iron cluster for the oxidation to proceed. This oxygen diffusion channel, of hydrophobic nature, was mapped out (Fig. 1C, in red) with krypton atoms (O2 mimics) from pressurized Pp-MiaE crystals using the "soak-and-freeze" methodology [1]. In contrast, a wide hydrophilic cavity (Fig. 1C, in cyan) was detected, and it is proposed to accommodate stereo-selectively the tRNA A37 within the active site in order to facilitate the hydroxylation of its isopentenyl moiety. A computed docking model of MiaE-tRNA complex, based on the crystallographic structures and supported by *in vivo* activity of site-directed mutants, showed that a non-canonical flexible loop of the enzyme (Fig. 1C, in blue) positively charged is likely involved in tRNA recognition and grasping processes. The Pp-MiaE active site consists of 2 iron atoms coordinated by histidine and glutamate residues and bridged by an oxygen atom, assigned as μ-oxo by *in cristallo* spectroscopy. This observation suggests that the crystal structure corresponds to the oxidized resting state [FeIII–O–FeIII] of Pp-MiaE (Fig. 1D). Conclusively, these studies highlight the structural elements of Pp-MiaE specifically involved in the interaction and processing of the tRNA substrate and O2 co-substrate [2].

The structural studies of Pp-MiaE result from a collaboration between M. Atta of the laboratory CBM-BioCat (CEA-Grenoble) and the ESRF Structural Biology group, and benefit from access to the PSB platforms: ESRF beamlines ID29 and ID30B, laboratory for *in crystallo* spectroscopy (icOS) and ESRF MX high-pressure laboratory (HPMX).

P. Carpentier (ESRF/CEA)

[1] B. Lafumat, C. Mueller-Dieckmann, G. Leonard, N. Colloc'h *et al.* (2016). J. Appl. Crystallogr., **49**, 1478-1487.
[2] P. Carpentier, C. Leprêtre, C Basset, T Douki, S Torelli, V Duarte *et al.* (2020). Nucleic A. Res., **48**, 9918-9930.

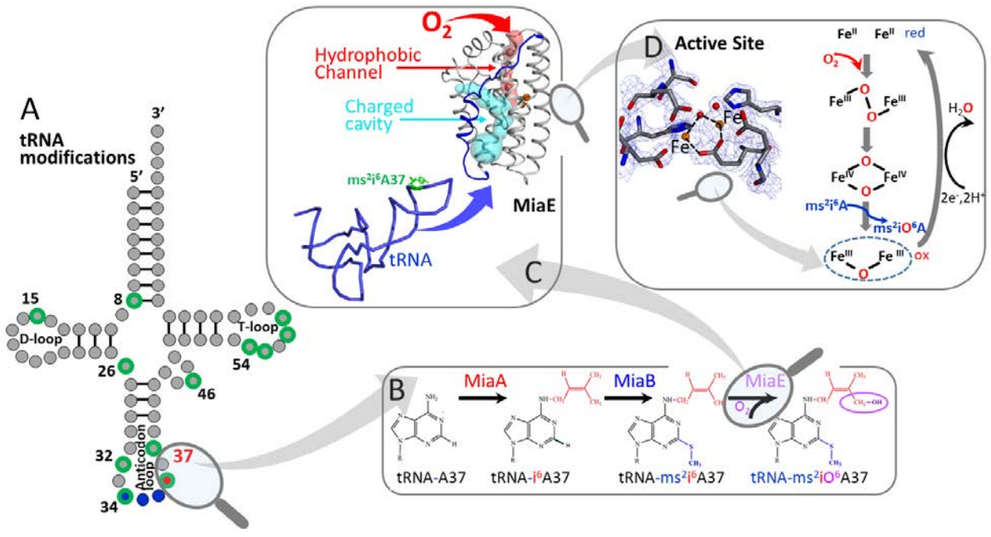

Figure 1. A. Typical modifications of tRNAs (in green) and modification at position 37 on the anticodon loop (in red). B. Details of hypermodifications of tRNA-A37 catalyzed successively by the enzymes MiaA, MiaB and MiaE. C. Structural details of MiaE showing tRNA docking and O2-channeling. D. Active site and catalytic cycle of MiaE.

News from the platforms

Accurate and rapid 3D printing of microfluidic devices using wavelength selection on a DLP printer

The use of microfluidic devices and techniques as a basis for sample preparation and experimentation is, nowadays, reaching state-of-the-art status at synchrotron X-ray beamlines [1,2]. Microfluidics, in comparison with traditional sample handling approaches, provides access to shorter length and timescales while using smaller sample quantities. Some examples in the areas of soft matter and life sciences include nanoparticle and crystal growth, protein folding and fiber alignment. The construction of microfluidic devices in PDMS or Kapton for use on X-ray beamlines is often a time consuming and complex multi-step process. Recent developments of 3D printing technologies have opened possibilities for rapid fabrication of complex microfluidic devices. Additionally, 3D printing allows easy implementation of channels in the third dimension. The presently preferred approach for 3D printing of microfluidic devices is the solidification of a resin under UV light irradiation using Digital Light Processing. One of the major challenges in 3D printing of microfluidic devices using this process is that the static liquid resin trapped in the channels during printing easily polymerises, which obstructs the channel after completion of the printing process. We have shown that it is possible to gain better control over the resin polymerization and improve the quality of the devices, which has led us to a single day turnaround from design to use of a device [3] (Fig. 1).

Different microfluidic components were printed and tested (Fig. 1a). These components, including particle filters, droplet generators and droplet traps, were integrated into the microfluidic devices, which were tested by performing biomineral and protein crystallization experiments. After filling the devices with the relevant reagents, the flow was stopped (Fig. 1b), the devices were sealed and stored, and crystals grew in all of the droplets (Fig. 1c). Two proteins tested on an X-ray beam showed that the as-printed devices are suitable to obtain in-situ diffraction data from microcrystals trapped inside the channels (Fig. 1d). The developed method is thus particularly suitable for X-ray studies and opens the path for the use of 3D printed microfluidic devices on X-ray beamlines [3].

P. van der Linden, A. Popov and D. Pontoni (ESRF)

[1] S. Köster and T. Pfohl (2012). Mod. Phys. Lett. B 26(26), 1230018
[2] A. Ghazal *et.al.* (2016) Lab Chip 16(22), 4263
[3] P. J.E.M. van der Linden, A. Popov and D. Pontoni (2020) Lab Chip 20(22), 4128

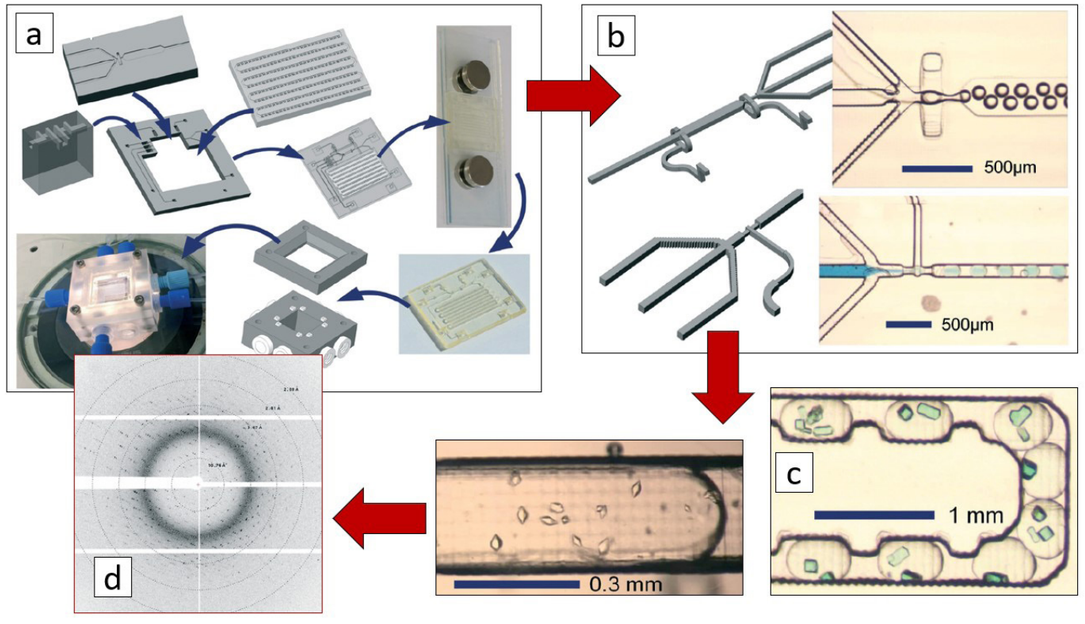

Figure 1.(a) Different elements such as filters, a droplet generator and droplet traps are digitally assembled into the frame. The completed design is printed, post cured between glass slides, made transparent and clamped in the support for the experiment. (b) Flow focusing droplet generator design and operation. T-junction droplet generator design and operation. (c) Channel constrictions with trapped droplets. Lysozyme crystals and thaumatin crystals. (d) X-ray diffraction of a thaumatin crystal deposited on a resin slab and measured at the ESRF beamline ID30-A3.

A new detector for electron diffraction of protein nano-crystals

Last June, a new hybrid pixel detector from Amsterdam Scientific Instruments was installed on the 200 kV cryo-electron microscope F20 of the IBS electron microscopy platform (Fig. 1), with the financial support of IBS and ILL. This detector is dedicated to the development of electron diffraction for protein nanocrystals at cryogenic temperature. With this technique, complete high-resolution diffraction data can be obtained from a few crystals with sizes not exceeding 100 or 200 nm in each dimension. Moreover, charge information can be obtained on metal ions or chemical group [1].

The new 512x512 pixel detector uses the Medipix 3RX chip. It has no readout noise, a high frame-rate (2kHz with zero dead time between frames), a high dynamic range (24 bits) and is radiation hard. Thus, diffraction data can be collected at low dose in cryo mode, using continuous rotation of the crystal with no risk of damaging the detector with the direct beam, in a few tens of seconds.

Preliminary data have been collected on the F20 microscope using the new detector. Continuous rotation data on lysozyme crystals at liquid nitrogen temperature have been collected up to 2.9 Å resolution. The lysozyme crystals were broken up from an urchin type crystal made up of very thin needles and frozen on a grid after manual blotting. The data from two crystals were processed and merged with XDS and the structure refined with Refmac in CCP4. The Coulomb potential maps were of good quality and contained information on changes to be made on the model, as illustrated in Fig. 2.

This new equipment is now operational. Please do not hesitate to contact us if you have any kind of nanosized protein/peptide crystals you would like to investigate!

The next challenge now is to automate data collection through SerialEM, in order to speed up data acquisition of all crystals present on a grid.

T. Blum, D. Housset, M.T.B. Clabbers and E. van Genderen (IBS)

[1]] Blum *et al.* 2021 Acta Cryst.D77, 75-85.

Figure 1. View of the bottom-mounted direct detector from Amsterdam Scientific Instruments on the IBS F20 microscope.

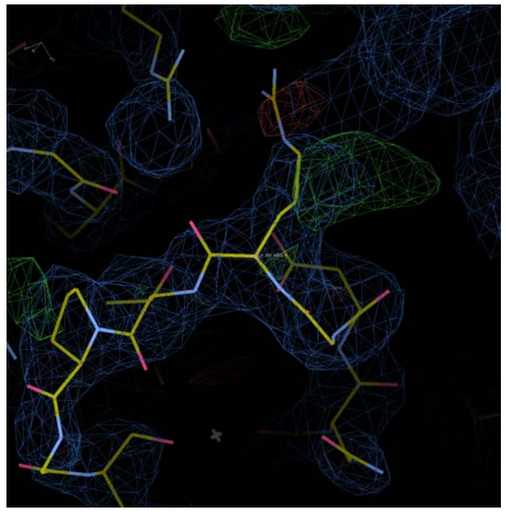

Figure 2. $\{2F_o - F_c\}$ (blue, +1σ) and $\{F_o - F_c\}$ (green, +3σ and red, -3σ) Coulomb potential maps around hen egg white lysozyme arginine 68.

Typhoon Fluorescence and Phosphorimager in the CIBB

After 15 years of dedicated service, the Typhoon Trio fluorescence- and phospor-imager, located at the CIBB, reached the end of its lifetime. Over the years, this instrument replaced all darkroom film processing activity by the PSB partners (and the darkrooms themselves), notably western blots and gel-based radioactive/fluorescent ligand-binding e.g. protein-RNA/DNA interactions. Given to the general utility of this instrument, the PSB Partners agreed to contribute jointly to its renewal, and a new generation Typhoon RGB scanner was purchased in September and installed in CIBB room 001. The instrument is fully accessible by all PSB Partners, with no charge, and new users should contact Philippe Mas (ISBG Engineer, philippe.mas@ibs.fr), for training and supervision.

F. Bernaudat (PSB)

The Typhoon RGB scanner

A mass photometer at the PSB Joint Biophysics Platform

What is the molecular weight of my protein? Is my protein complex intact, how many subunits are present? Are other species present in the sample? Are there any degradation products? Is my complex stable when I dilute it? The PSB Partners EMBL, ESRF, IBS and ILL recently joined forces to acquire a new instrument for the PSB Joint Biophysics Platform: the Refeyn One^{MP} mass photometer, which will enable scientists to answer these questions now by one more technique.

The new device uses mass photometry to estimate molecular masses of native particles in solution, ranging from around 40 kDa to several MDa in size, without the need for labels [1]. In relatively quick measurements users can determine the mass distribution of a sample to assess sample stability, complex formation, multimerisation states, and the presence of degradation or aggregates. Only low sample amounts are required, usually several microliters at a concentration of 1 µM are sufficient. This is particularly useful for rapid quality control before cryo-EM experiments [2], but also interesting for assessing the homogeneity of crystallisation samples or verification of samples prior to biochemical experiments.

In fact, scientists at the IBS have already successfully included MP One mass photometry data into their publication [3]. “The refeyn measurement was of real interest and showed that our new chimeric protein (composed of 18 polypeptide chains) was well-assembled” says Guillaume Fouët, lead author of the study. “We then performed MS and negative stain EM to confirm it, but at the time of the demo event, the refeyn measurement was our first result validating the correct assembly of our complex.”

The Refeyn One^{MP} mass photometer.

The mass photometer has been installed at the EMBL in room 152 and is now fully accessible to all PSB Partners with only a small charge to cover the required consumables. New users should contact Caroline Mas (ISBG engineer, caroline.mas@ibs.fr), for training and supervision. To check availability and booking please visit: www.isbg.fr.

E. Kowalinski (EMBL)

[1] Young *et al.* (2018) Science **360**, 423–427
[2] Sonn-Segev *et al.* (2020) Nat Commun **11**, 1772
[3] Fouët *et al.* (2020) FEBS J doi: 10.1111/febs.15543

The Structural Biology beamlines in post ESRF-EBS times

August 25th 2020 marked an important date for the ESRF, the official restart of the user programme using the new and upgraded ESRF-Extremely Brilliant Source (EBS). The preceding 18 months were used to replace and upgrade the whole storage ring to deliver the first high-energy 4th generation synchrotron: ESRF-EBS. During this shutdown, minor and major upgrades on both hardware and software have been performed on all ESRF-EMBL Joint Structural Biology Group (JSBG) Beamlines. In particular, BM29 has undergone significant instrument and control software upgrades. These include the installation of a new sample exposure unit developed by the EMBL-instrumentation team as part of a collaboration with the ESRF and EMBL-Hamburg, a new sample changer, and a new PILATUS3 2M X-ray detector. A Shimatzu HPLC system with auto-sampler for optimal experimental SEC-SAXS measurements from difficult biological systems is also available. In addition, we took this opportunity to develop a new web based graphical user interface, BsxCuBE[3] (Figure). We tasked ourselves to design and develop an intuitive user interface with a logical flow installed built with HTML 5, Javascipt 6 and React. It allows to input samples and control the beamline. Furthermore, it is linked to Exi2/ISPyB for bioSAXS where the data are processed and the results can be viewed and downloaded using a new bespoke open-source pipeline. We thank Jean-Baptiste Florial (EMBL), Raphael Cohen-Aberdam (EMBL), Marcus Oscarsson (ESRF) and Jerome Kieffer (ESRF) amongst many others for all their hard work.

Since the official restart of the ESRF users’ programme on August 25th, all JSBG beamlines are back to ‘normal’ user mode, all with a significantly increased photon flux. Since then, around 250 users’ sessions (comprising almost 15’000 samples) were performed on the group’s macromolecular crystallography beamlines (ID23-1, ID23-2, ID30A-1. ID30A-3 and ID30B), and almost 50 users’ sessions on the small angle X-Ray scattering beamline BM29. Given the current restrictions due to the Covid-19 pandemic, all experiments were done as either mail-in or remote experiments. The same applies for the 25 experiments scheduled on the cryo-EM. However, this is not all, the reconstruction of ID29 has evolved very well, given the current sanitary situation, with radiation tests started end of November 2020 and the commissioning phase planned to start in 2021.

C. Mueller-Dieckmann and M. Tully (ESRF)

EVENTS

Grenoble Host-Pathogen Interactions Club, Kick-off meeting on COVID-19

Grenoble boasts a large community of researchers interested in host-pathogen interactions and related fields - including basic, translational and clinical research. To help bring together this community the PSB Partners, in collaboration with the Institute for Advanced Biosciences (IAB), initiated a new "Host-Pathogen Interactions Club" (www.hostpathogen.fr), inspired by the successful Grenoble Epigenetics Club (www.epigenetics.fr) which exists since 2012. Like the latter club, the goal is to promote networking among local researchers through half-day scientific meetings (2-3 events per year), covering and reflecting the broad range of pathogens (viral, bacterial, parasitic, fungal) and host defence mechanisms of interest for the local community.

Invited speakers of Host-Pathogen Interactions Club kick-off meeting on COVID-19: Roger Le Grand (top left), Olivier Epaulard (top right), and Patrice Marche (bottom right).

The club was launched on 10th December 2020 with a kick-off (virtual) meeting on COVID-19 and three main speakers were invited: Roger Le Grand (IDMIT, Fontenay-aux-Roses) presented the work of his research unit on the prevention of SARS-CoV-2 infection in preclinical models; Olivier Epaulard (CHU Grenoble) gave a thorough overview of the evolution of the COVID-19 pandemics and the health situation in Grenoble; and Patrice Marche (IAB) explained how engineered lipid carriers can be used in vaccination approaches. Then followed a series of short (5 minutes) and flash (2 minutes) talks by young scientists which illustrated the exciting research and the broad diversity of the studies performed in the Grenoble area. The meeting gathered 131 participants connecting mainly from France, but also from the United States, Lebanon and Sweden!

The organisers, Sagar Bhogaraju (EMBL), Pascal Fender (IBS), Carlo Petosa (IBS) and Patricia Renesto (IAB), wish to thank all the speakers and attendees for making this first event a success, and look forward to meeting them again during future activities of the club. For further information on the club and to subscribe to their mailing list, please consult the website mentioned above.

F. Bernaudat (PSB)

PSB Spotlight meetings

Due to the COVID-19 pandemic, as most other events, the PSB Spotlight meetings went virtual this year and were organised as webinars.

The 7th PSB Spotlight on Mass Spectrometry (MS) took place on 13th October 2020. The meeting was opened to non-specialists and aimed to illustrate the great variety of applications of mass spectrometry, with talks from Elisabetta Boeri Erba (IBS) on the role of MS in Structural Biology; Markus Hartl (Max Perutz Labs Vienna) on the use of cross-linking MS to elucidate structural changes in proteins and their complexes; Christophe Masselon (CEA Grenoble) on the concept of ultrahigh molecular mass measurements to study intact viruses; and Yohann Couté (CEA Grenoble) who described proteomics studies and their applications to biomedical research.

Invited speakers of the PSB Spotlight on Mass Spectrometry: Markus Hartl (top left), Christophe Masselon (top right), and Yohann Couté (bottom left). Invited speaker of the PSB Spotlight on Biophysics: Patrick England (bottom right).

The 8th PSB Spotlight meeting focused on "Biophysics: Sample Characterisation and Macromolecular Interactions" and took place on 17th November 2020. The meeting started with detailed recommendations to perform biophysical characterisation of macromolecules by Patrick England (Institut Pasteur Paris) who stressed the importance of upstream quality control to ensure the success of downstream applications. Then followed talks by Dimitrios Skoufias (IBS) on bimolecular fluorescence complementation; Sigrid Milles (IBS) on single molecule fluorescence for structural biology and protein dynamics; and by Sylvain Prevost (ILL) on Small Angle Neutrons Scattering (SANS). The presentation of the Partnership for Soft and Condensed Matter (PSCM) platforms by Diego Pontoni (ESRF) and the presentation of the PSB Joint Biophysics platform by Jean-Baptiste Reiser (IBS) then concluded the meeting.

We are grateful to all the speakers for their contributions. Both webinars gathered around 60 participants each. This is similar to previous physical events, but the virtual mode enabled us to broaden the geographical scope of these meetings, as attendees not only connected from Grenoble, but also from other cities in France, as well as from Italy and Germany!

F. Bernaudat (PSB)

Fête de la Science 2020

Every year in October, during the national science festival 'Fête de la Science', the institutes of the EPN campus have a common stand at the Parvis des Sciences (PDS), which is organised in Minatec by the GIANT innovation campus and local partners, in order to enable school pupils and the general public, to meet and interact with scientists. In 2019, the PDS welcomed more than 3600 visitors over three days, but unfortunately the 2020 edition had to be cancelled due to the COVID-19 crisis. As an alternative, the partners prepared an online version of the PDS (https://parvis-des-sciences.com/pds-en-ligne) to present their work and institutes, and also initiated other actions to reach out to the public.
On 14th October 2020, the local newspaper “Le Dauphiné Libéré” presented the Grenoble synchrotron in a playful manner on a double-page poster, and the day’s edition also came with a supplement presenting the research actions of the GIANT partners (https://fr.calameo.com/read/005484845580bdf47902b2), including an interview of Stephen Cusack (EMBL) on his work on influenza viruses and the SARS-CoV-2 Coronavirus responsible for the COVID-19, as well as articles presenting the ESRF’s education programme Synchrotron@School and on nanoparticle studies at ESRF.

Top left: Poster of the ESRF Synchrotron presented in the Dauphiné Libéré (photo credit ESRF). Bottom left: Snapshot of Stephen Cusack’s interview in the Dauphiné Libéré supplement (© Dauphiné Libéré). Top right: IBS practical project in a primary school. Bottom right: videoconference between IBS scientists and high school students (photo credit IBS).

For its part, the IBS organised several teaching actions: scientists visited two primary schools to coordinate practical projects on protein discovery for a hundred pupils. IBS scientists also visited two high schools and organised a series of videoconference that enabled more than 300 high school students from the Rhône-Alpes region to participate in conference-debates with scientists in order to discover the world of research and career opportunities (a video is available on Youtube: https://youtu.be/9dganARgnKU). These events were highly appreciated by the students, and the videoconference format will likely be maintained in the future for high schools that are too far away for onsite visits.

F. Bernaudat (PSB)

NEWCOMERS

José Barranco

joined the CIBB Core Team in January 2021 to become the new service technician of the CIBB building. José is an electrician by trade and he worked in different construction and industrial companies. But José is already a well-known face for many as he previously occupied the position of the EMBL building maintenance technician between 2003 and 2012 and was then employed at the ILL as an electrician until 2020.
Contact: jbarranco@embl.fr

Martin Pelosse

joined the EMBL Grenoble in September 2020 as a scientific expert in charge of the Eukaryotic Expression Facility. After a PhD student, shared between the University Grenoble Alpes and EMBL Grenoble, Martin then joined Prof. Imre Berger at the University of Bristol to continue working on baculovirus-mediated systems for expression or gene delivery involving MultiBac and ACEMBL. He most recently worked at the CEA. https://www.embl.fr/services/ht_expression/eef/
Martin is happy to discuss any facility user needs and is busily developing new methodologies to be implemented on the platform. Contact: mpelosse@embl.fr

ANNOUNCEMENTS

Joanna Wandzik (EMBL/Cusack group) is the recipient of a L'Oréal-UNESCO for Women in Science fellowship. This distinction rewards the high quality work Joanna carried out in the Cusack group at the EMBL on the mechanisms involved in Influenza virus transcription (see July 2020 edition of the PSB newsletter) and supports her next career steps. *Photo courtesy of the L'Oréal foundation.*

The European Research Council (ERC) has awarded a 'Starting Grant' to **Wojciech Galej**, a group leader at EMBL Grenoble, for his project 'MinorSplice'. This ERC project aims to provide functional and structural insights into intron excision from pre-mRNA by the non-canonical, minor spliceosome, which are largely missing for now, and may eventually have an impact on human health.

Solène Besson, PhD student at the IBS in the Adenovirology team, was awarded the 2020 SILAB - Jean Paufique Corporate Foundation prize. This foundation provides active and constant support for fundamental and applied research dedicated to the diagnosis, prognosis and treatment of skin, particularly skin cancer. Solène will receive 3 years of financial support to work on the development of vectors of immunotherapies for the treatment of melanomas.

Andrea Carfi, who's currently VP & Head of Research for Infectious Disease at Moderna, Cambridge, MA, USA, is an IBS alumnus. Andrea has been integral to the development of Moderna's COVID-19 mRNA vaccine that was recently approved for use by the European Medicines Agency (https://www.ema.europa.eu/en/news/ema-recommends-covid-19-vaccine-moderna-authorisation-eu) is an IBS alumnus. After receiving a PhD under the direction of Otto Dideberg (retired) at the IBS in 1997 he did a postdoc with Prof. Don Wiley's group at Children's Hospital (Harvard University) in Boston, MA, crossing career paths with the IBS director Prof. W. Weissenhorn. Andrea then moved to industry, joining Merck (Rome) in 2002. He returned to Cambridge, MA in the US in 2010 as a senior manager first at Novartis Vaccines and then GSK vaccines, before eventually joining Moderna in 2017. *Photo credits: https://masscpr.hms.harvard.edu/vaccines*

PROFILE

Merci et bonne retraite Florent ! Et bon courage à Gergely!

Florent Cipriani has retired from EMBL after over 28 years of inventing and constructing an amazing variety of ingenious, often revolutionary, and most importantly, highly used, scientific tools and instruments to aid macromolecular structure determination using neutrons, X-rays and electrons. His dedication to the robust automation of macromolecular crystallography (MX) and biological Small Angle X-ray Scattering (bioSAXS) experiments, in collaboration with numerous PSB and EMBL Hamburg scientists over the years, has ensured that Grenoble is at the forefront of structural biology instrumentation worldwide. His achievements are legendary, amongst the most well-known being the first precision microdiffractometer with on-axis crystal viewing (now in its third generation), the first automated neutron image-plate detector, the Spine standard for frozen crystal mounts, a crystal humidity controller, two generations of automated frozen crystal sample changers, a bioSAXS sample changer and the automated crystal harvester (CrystalDirect™). His early entrepreneurial experience allowed many of the instruments and tools developed to be commercialized through companies such as the local ARINAX as well as MiTeGen and Molecular Dimensions. This has resulted in the installation of >40 microdiffractometers at synchrotron facilities worldwide and thousands of Spine pins sold. Florent's incredible success depended on a rich combination of factors, carefully listening to scientists dreams and sheer genius in coming up with ingenious practical solutions to make them reality, mastering and integrating all relevant technologies including electronics, micromechanics, cryogenics, vacuum, robotics and computer control, seeking out and making use of the latest technical innovations, and rigorous project and team management. "Chapeau!" Florent for all your spectacular achievements! We hope you enjoy your retirement and we'll miss you! To continue the success of scientific instrument development at EMBL Grenoble, Gergely Papp has recently taken over as interim head of the Instrumentation team. Not only is Gergely a talented robotic engineer, he's also an accomplished musician! Best of luck Gergely and we're all looking forward to working with you and excited to use your team's new instruments in the future.

Florent (right) 'passe le relais' to Gergely (left). Photo courtesy of J. Sinoir (EMBL)

Interview of Florent Cipriani

Can you tell us a little bit about yourself and how you came to join EMBL?

Grenoble, the city where I was born, grew up, and studied engineering and physics is where I finally spent my entire career. No point looking elsewhere when everything is here! The 70s, it was the time of "No Nukes" concerts, and my first serious job at the CEA Grenoble, a real dilemma for me! After ten years spent in the nuclear industry, and a startup company I co-founded to build medical lasers and neutron detectors for the LETI and the ILL, I met André Gabriel from EMBL Grenoble, a leading figure in the field of X-ray position sensitive gas detectors. In 1992, André convinced me to leave industry to join him at the EMBL, to develop detector readout electronics and acquisition software - thank you André!

How much has the EPN campus evolved and what motivated you to work with PSB scientists since you joined EMBL in 1992?

Next door, the ESRF was rising out of the ground, opening up many new opportunities. For me it was an ideal time for a new challenge to help Stephen Cusack position micro-crystals in the tiny X-ray beam of ID13. Thanks to Stephen, Tassos Perrakis and Christian Riekel at the ESRF, the first member of the Micro-Diffractometer family was realised. Still growing, this family remains an incredible success. It was also the infancy of MX beamline automation at the ESRF. Essential to the efficiency of MX, automation is always in a race to catch up with the advance of synchrotron beam quality and the shorter data collection times possible with new detectors. Hours in the 1990s to milliseconds today! I was very lucky to be in the right place at the right time, at an institute and a campus where what is possible mainly depends on yourself, a big thank you to the EMBL and PSB partners.

Do you have any particular favorite instrument(s)?

I am very proud to see that most of the instruments we have developed are used on many beamlines around the world thanks to the local scientific environment, EMBL-EM (EMBL's technology transfer branch) and ARINAX. More recently with CrystalDirect™, we have contributed with Josan Marquez's HTX team to find a solution to the most challenging automation step in crystallography, crystal harvesting. I am convinced that MASSIF-1 will demonstrate in the coming years that beyond harvesting, this technology can open up a new way to operate MX beamlines - thank you in advance Matthew, Gergely and the ESRF structural biology group! Obvious, but important to recognise, is that all this is the result of teamwork - so thank you to all the hardworking members of the instrumentation team, past and present.

What were the major challenges in the concept and development of new scientific instruments you had to overcome?

To those who want to know what are the main challenges in the concept and development of new scientific instruments, I would say that the first step is to ensure the problem you address is of real importance. In instrumentation, time is counted in years and the road to success is paved with many difficulties. One must never give up, so the goal has to be worthwhile! The second challenge is to be convinced on the solution you propose to obtain funding. Finally, it is essential to collaborate with visionary scientists while keeping independence on the engineering solutions. On this aspect, the EPN campus has proved to be an ideal place. Anyone who is passionate about technology and science that wants to support passionate scientists in their experimental work should really enjoy working here.

Any regrets on your retirement from EMBL?

My only regret is to leave at a very exciting time, where Cryo-EM is becoming a major tool for structural biology. As for crystallography in the nineties, the Cryo-EM sample environment needs to be re-invented. With Gergely, the project we have started a few years ago should help microscopists prepare their samples and extend the access of Cryo-EM platforms to non-experts. This development should fit perfectly with the needs of the EPN campus, which has moved from a mosaic of independent institutes to a coherent suite of services providing structural biologists with a panel of state-of-the-art instruments.

Lastly, as an engineer we imagine you already have many retirement hobbies planned, would you like to share any with us?

About my future plans, I would have to say I have no plan! Be more present for my family, shorten the home renovation work list built-up during the past 28 years at EMBL, and of course enjoy my free time! But I will not forget to keep an eye on Gergely's work, which I'm sure he will be brilliant at. Wishing him great success.

Interview of Gergely Papp

Tell us a bit about yourself and what motivates you to work as an engineer with PSB scientists?

I'm originally from Debrecen in Hungary and following my studies at INSA engineering school in Lyon I started to work in the instrumentation group as an Automation Software Engineer in 2010. I was always attracted by robotics, and science in general, since a young age. After seeing the complexity of the scientific methods used here, and the challenges required to facilitate these experiments through the development of our machines, I understood that this work was going to be a passion for the rest of my life. Our collaboration with PSB scientists is a tremendous example of how the cooperation of different scientific areas can catalyze advancement in both fields, and enable accomplishments that would be beyond the reach of each individually. Pushing each-other further, as we do within our group and with PSB scientists, is in my opinion the only way of achieving the best results from our projects.

How was it to work with Florent and what new challenging projects are the team working on?

It was a great pleasure and a privilege to work with Florent. The imagination to conceive the instruments we constructed together with all members of the instrumentation group were always striving to achieve state of the art. Florent made MX and bioSAXS accessible to a whole generation of European scientists, and largely improved the efficiency of many more beamlines the world over. The list of instruments he envisaged and developed throughout his career is testament to his tireless personality and great passion for scientific instrumentation. It's a daunting task for me to take over as interim head of the group after such a distinguished engineer and scientist. We will continue to work on MX techniques by developing instruments that can efficiently make use of the new ESRF-EBS beam. In particularly, we're developing a fast serial crystallography scanning head that is synchronized with a 1 kHz chopper for the new ID29 beamline for chip based experiments. We're also busy preparing for the installation of a CrystalDirect crystal harvester on the MASSIF-1 beamline, contributing to the world's first fully automated "harvest and collect" pipeline facility. Furthermore, motivated by the widespread development of the Cryo-EM in the last number of years, we started to develop a fully automated Cryo-EM grid preparation machine called EasyGrid in 2017. With this concept we're aiming to offer the community a reliable, reproducible and easy way of preparing Cryo-EM sample grids that will also enable time resolved experiments and sparse-matrix screening.

A. McCarthy (EMBL)

In view of the success of the PSB over the last 18 years, through fruitful collaborations and the implementation of a unique palette of technical platforms for integrated structural biology, on 1st January 2021 the Partners have renewed their engagement in the Partnership by extending their Collaboration Agreement for a further five-year period.

DATES FOR YOUR DIARY

8th to 10th February 2021 - ESRF User meeting

This year´s annual ESRF User Meeting 2021 will take place online due to COVID-19 after six months of user operation with the world's first and brightest 4th generation high energy source. Monday 8th will be dedicated to tutorials covering a wide range of practical topics providing insights into ESRF X-ray based techniques and will end with a virtual poster session. Of particular interest will be tutorials on data collection with MxCuBE3 and EXI as well as getting the best out of MASSIF-1. The Plenary Session will be held on Tuesday 9th and three dedicated microsymposia will be organized on Wednesday 10th with keynote speakers and talks from submitted abstracts: UDM1. "Emerging viral diseases: how can structural studies help to seek remedies"; UDM2. "Understanding biomineralisation: the inputs of micro- and nano-X-ray analysis"; UDM3. "Physics and Chemistry of Actinides seen by X-rays". The ESRF user community is warmly invited to participate through the submission of poster abstracts and by meeting together online for discussion, interaction and networking. More information at http://www.esrf.eu/UM2021

22nd February to 26th March 2021 – Hercules European School

This 1-month school, coordinated by the Université Grenoble Alpes, will take place online this year due to COVID-19 and 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: http://hercules-school.eu/

26th February 2021 virtual seminar at 14:00 – Tracy Brown OBE – "Challenging the Misrepresentation of Science and Evidence in Public Life"

Tracy Brown OBE from Sense about Science is the next EMBL's Science & Society Forum speaker and she will talk about how presenting and defending sound scientific evidence is important to encourage scientific dialog with the public and the people who answer to them. This is an open virtual seminar, but registration is required. More information on this seminar and a registration link can be found at https://www.embl.fr/aboutus/science_society/forum/forums_2021/26-02/index.html

8th March to 12th March 2021 – Tutorial in Macromolecular Crystallography 2021 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 from Université Grenoble-Alpes and the EPN campus who have a priority in registration. The tutorial counts for 3 ECTS credits needed for the "Ecole doctorale" of the UGA. The 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". Depending on the Covid-19 context, adaptations of the program may be required. For more information, please contact the course organiser Wim Burmeister (wim.burmeister@ibs.fr).

29th June to 2nd July 2021 - AFC2020 French Crystallography Association Congress

The AFC congress initially planned from 30th June to 3rd July 2020 in Grenoble was postponed to 2021 due to the COVID-19 pandemic. This meeting organized at Minatec will bring together ~250 scientists from the physics, chemistry and biology communities with a common interest in crystallography. Scientists from the EPN Campus are involved in both the organizing and scientific committees of this meeting. More information: https://www.afc.asso.fr/agenda-afc/1449-colloque-2020-de-l-afc.

1st to 2nd July 2021 – PSB Symposium "Frontiers in Bioimaging"

This meeting will take place online. It aims to highlight progress in 3D imaging research that bridges the gap between the atomic and cellular scales, targeting resolutions in the subnanometer to submicrometer range. Areas covered will include: cryo-electron tomography, X-ray tomography, super-resolution microscopy, and correlative light and electron microscopy. Of particular interest are applications of the above methods to the 3D determination of supra-macromolecular assemblies and subcellular structures that allow for a detailed interpretation at the atomic/near-atomic level. More information: http://www.esrf.eu/psbsymposium2021

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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.