JULY 2022 - NUMBER 29

PSB et al

Partnership for Structural Biology Newsletter

Scientific highlights

High-pressure crystallography unravels noble gas intervention into protein-lipid interaction and suggests a model for anaesthetic action

We have discovered at the ESRF that noble gases have great potential in exploring membrane protein (MP) functionality [1]. Using the high-pressure MX (HPMX) facility at the ESRF, we generated high-pressure atmospheres of argon and krypton to produce noble gas derivatives of MP crystals, a technique initially intended to study channels and voids in protein structures. In our structural study, in collaboration with the IBS, we have revealed that noble gas atoms have a high tendency to occupy pockets on the hydrophobic surface of MP by non-specific binding (see Figure). These noble gas atoms compete with lipids whose hydrophobic acyl chains usually occupy the grooves on the protein landscape. However, we have observed that under pressurised conditions, gas atoms alter some of the lipid positions (Figure) and displace lipid molecules bound to their surfaces. This phenomenon has a huge significance for the studies related to MP, because lipid environment is one of the main determinants for MP functionality, and some MP species require special lipids to exercise their function properly.

Furthermore, our study also sheds light on anaesthesia, since noble gases are known to cause anaesthesia (xenon at atmospheric conditions, argon and krypton at hyperbaric conditions). The symmetric shape and chemical inertia of noble gas atoms imply that the interaction mechanism observed with well-established MPs should be the same for anaesthetic MP neuronal targets or MPs in general. On this line, our study suggests that the anaesthetic state could actually be generated by decreasing the conformational dynamics of neuronal ion channel MPs. Subsequent molecular dynamics *in silico* analysis of MPs embedded in a model lipid bilayer have shown that, indeed, MP structure fluctuations decrease in presence of the noble gases argon and krypton. Taken together, our results provide an insight to the anaesthetic effects of noble gas atoms, where their non-specific binding to an anaesthetic MP at several sites on the hydrophobic surface, often in grooves between alpha-helices, should force the protein structure to solidify, i.e. making the transition to a different state more difficult.

I. Melnikov (ESRF)

[1] I. Melnikov, P. Orekhov, M. Rulev *et al.* (2022) Commun Biol, **5**, 360

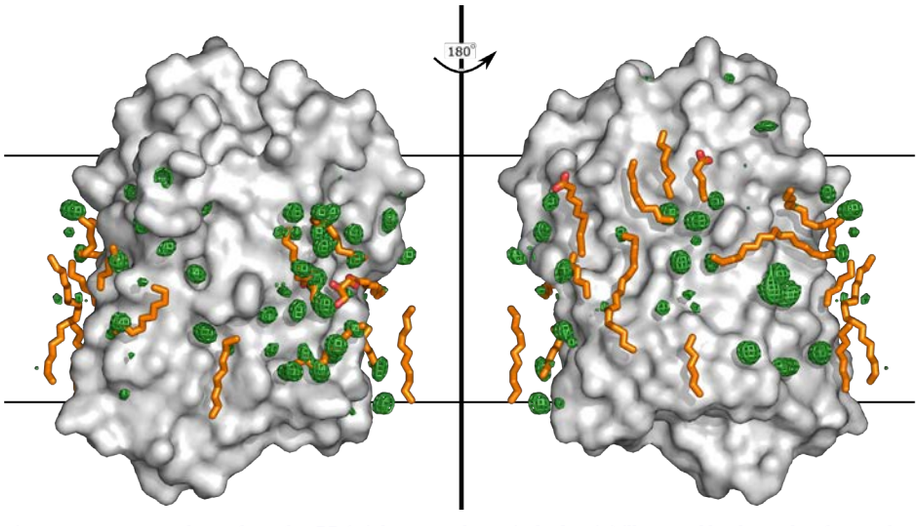

Figure: Argon binding sites arrangement on the surface of tmBR (triple mutant bacteriorhodopsin) illustrated by the peaks of anomalous difference map (contoured at the 3.5 r.m.s.d. level, green mesh). At some sites, argon atoms displace lipid molecules from their initial positions in the native structure (lipids in their native positions are superposed here as yellow sticks). Hydrophobic region in the structure is bounded between two horizontal lines.

CONTENTS

Visualizing protein breathing motions associated with aromatic ring flipping

Nuclear magnetic resonance (NMR) studies carried out in the 1970s surprisingly demonstrated that aromatic amino acids in proteins can undergo so-called ring flipping, i.e. 180 degree rotations of the aromatic side chain [1]. Paradoxically, these aromatic amino acids are in many cases located in the tightly-packed protein core, where they engage in multiple interactions to maintain the protein fold and thereby ensure function. At that time, it was proposed that large-scale protein "breathing motions" of the core would be necessary in order to accommodate these ring flipping events [2]. However, until now the structural details of these motions have remained enigmatic.

By combining NMR spectroscopy and X-ray crystallography, we have been able to map the structural changes associated with aromatic ring flipping in the core of a protein [3]. Using NMR, we could show that the SH3 domain of the JIP1 scaffold protein undergoes exchange between two conformational states in solution – a major state populated to 97%, corresponding to the crystal structure of the SH3 domain, and a minor state populated to only 3%. A mutation of tyrosine 526 (Y526) to alanine entirely abolished this exchange showing that the minor state is somehow related to the side chain of Y526.

In the major state, Y526 is located in the hydrophobic core, where the side chain of Y526 adopts an eclipsed conformation (Fig. 1A). This conformation is normally unfavorable due to steric interactions of the aromatic side chain with the protein backbone; however, it appears to be stabilized by multiple CH-π and π-π interactions with surrounding residues (Fig. 1A). By perturbing this delicate interaction network by single point mutations, we were able to capture the high-resolution structure of the minor state by X-ray crystallography. The structure reveals that the minor state corresponds to a staggered conformation of the side chain of Y526 with the aromatic ring being stabilized by CH-π interactions from L519 (Fig. 1B). The transition from the eclipsed to the staggered conformation of Y526 is associated with large-scale structural rearrangements, in particular in the surrounding β-strand (Fig. 1C).

By calculating the volume of the Y526 pocket, we could show that a substantial void volume is generated around the tyrosine ring during the structural transition from the major to the minor state. The size of this void volume corresponds to determined activation volumes measured for ring flipping events of buried aromatic residues in other proteins by pressure-dependent NMR. Our data

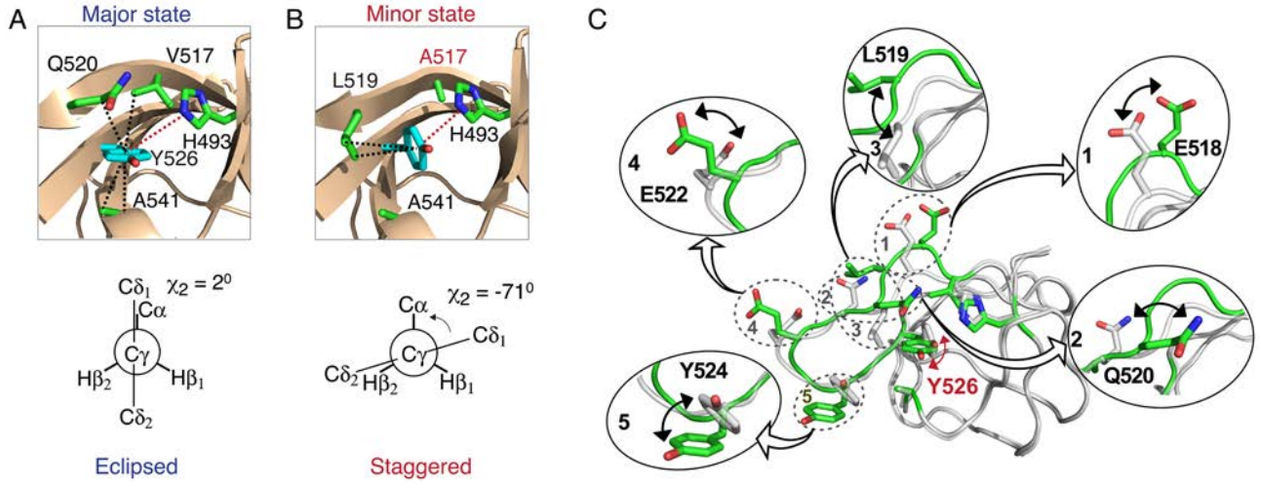

Figure 1: (A) Structure of the major state of JIP1-SH3 showing the conformation of Y526 and its stabilizing interactions with H493, V517, Q520 and A541. Dashed lines indicate CH-π (black) and π-π (red) interactions. An eclipsed conformation of the Y526 side chain is observed. (B) Structure of the minor state of JIP1-SH3 showing the staggered conformation of Y526 that is stabilized by CH-π interactions with L519. (C) Illustration of the structural changes between the major (green) and minor (gray) conformation of JIP1-SH3.

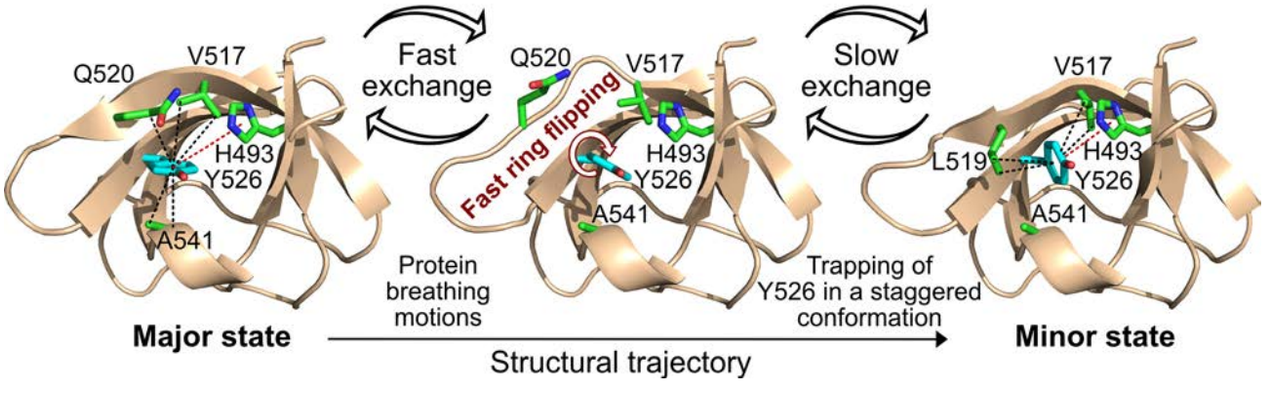

Figure 2: Illustration of the protein breathing motions along the structural trajectory from the major to the minor state. A void volume is created around Y526, which allows fast ring flipping to take place. The ring flipping is occasionally interrupted by trapping of Y526 in a staggered conformation through formation of CH-π interactions with L519.

are therefore consistent with a model by which protein breathing motions along the structural trajectory from the major to the minor state allow fast time-scale ring flipping events to take place (Fig. 2). Our results visualize for the first time the structural details of the protein breathing motions associated with ring flipping of core aromatic residues – structural details that have remained elusive for almost five decades.

M. Ringkjøbing Jensen (IBS), A. Palencia (Institute for Advanced Biosciences)

[1] K. Wüthrich, G. Wagner (1975). FEBS Lett., **50**, 265-268.
[2] I.D. Campbell, C.M. Dobson, R.J. Williams (1975). Proc. R. Soc. B, **189**, 503-509.
[3] L. Mariño Pérez, F.S. Ielasi, L.M. Bessa, D. Maurin *et al.* (2022). Nature, **602**, 695-700.

Intron removal in the spotlight

In eukaryotes, genes are often punctuated with non-coding segments known as introns. In order to produce functional messenger RNAs, which can be later translated into proteins, introns between the coding sequences (exons) need to be removed. This removal is catalysed by a large and dynamic RNA-protein complex - the spliceosome. Spliceosome assembles *de novo* on each intron from five canonical subunits - small nuclear ribonucleoprotein particles (snRNPs) and several dozen of other factors.

One of them is U2 snRNP, which has a very specific role in splicing - to find and recognise the so-called branch site. The branch site is one of the key positions within every intron as it helps to define the exon-intron boundaries and therefore influences the outcome of the splicing reaction. It is estimated that up to 50% of all genetic disorders are related to problems with splicing signals or malfunctions of the splicing machinery. This makes spliceosome a relevant drug target.

Recent work by Jonas Tholen, a PhD student in the Galej group at EMBL Grenoble, used cryo-EM to determine the structure of the human U2 snRNP in three different conformational states, providing new insights into the mechanism of branch site recognition [1].

For this Tholen et al. first developed a new method of 17S U2 snRNP purification from CRISPR/Cas9 engineered human cell lines, which allowed a high-quality 20+ subunit RNA-protein complex for further structural analysis to be obtained. The cell culture part of this work was performed at the Eukaryotic Expression Facility PSB platform. The structure of the 17S U2 snRNP was determined at near 2 Å resolution, allowing modelling of some of the previously unknown interactions within this complex. Following up on these results the authors reconstituted branch site recognition *in vitro* from purified components. This allowed the isolation and subsequent structure determination of two other previously unknown assembly intermediates on the splicing pathway (Figure 1).

One very exciting finding concerns the processing of weak branch sites. In humans most of the branch sites do not have any clear sequence signatures that would allow them to be easily identified. Most likely, a context-dependent combinatorial readout of several weak signals allows the spliceosome to recognise them properly. How exactly this is achieved remains elusive. One of the structures determined shows that a small protein, SF3B6, plays an important role in stabilising the branch site-U2 snRNA duplex by enforcing its helical geometry. This might be particularly relevant for sequences with a weak U2 base pairing potential and provides a conceptually new idea explaining how such recognition could be accomplished.

Overall, these new findings contribute to a better understanding on how introns are recognised and processed by the human spliceosome.

J. Tholen and W. Galej (EMBL)

[1] J. Tholen, M. Razew, F. Weis, W.P. Galej (2022). Science, **375**, 50-57.

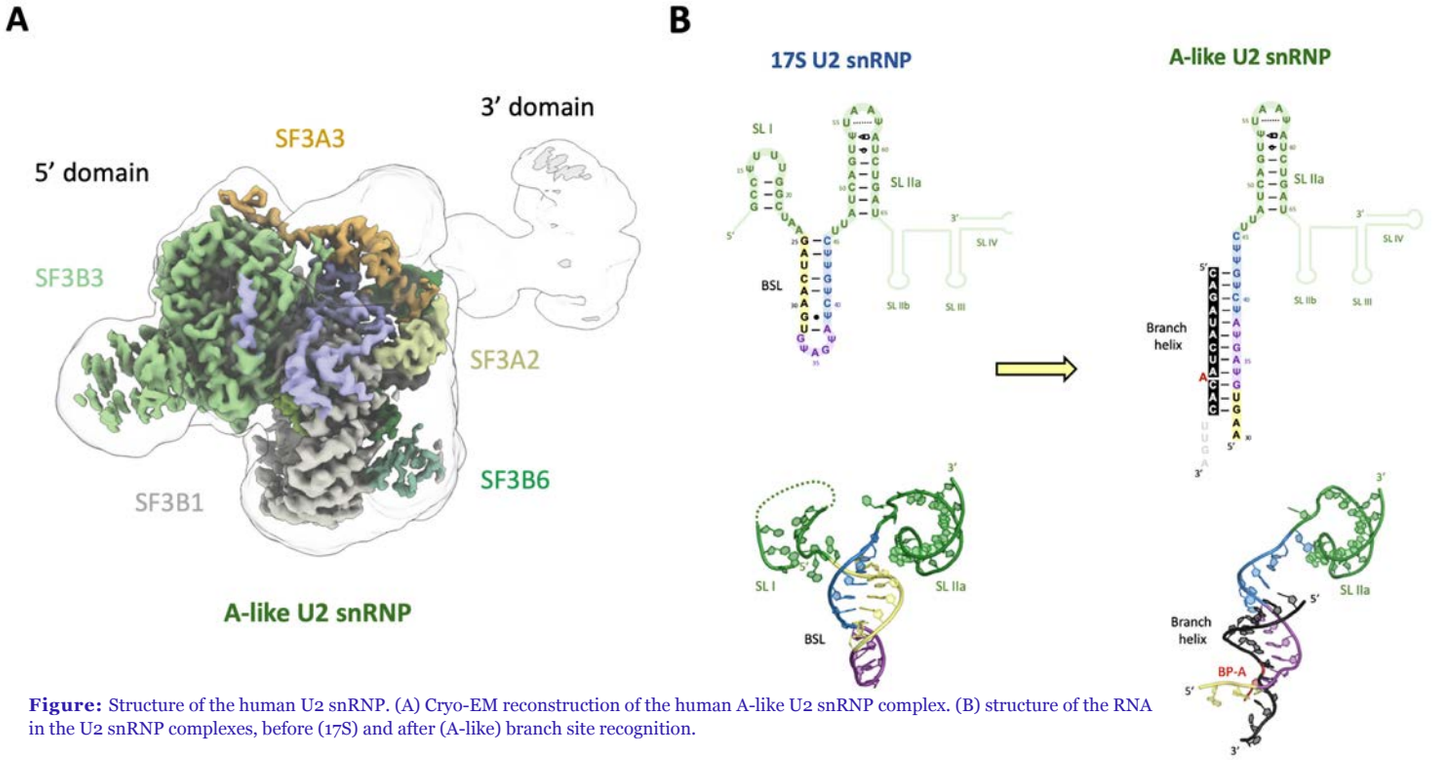

Figure: Structure of the human U2 snRNP. (A) Cryo-EM reconstruction of the human A-like U2 snRNP complex. (B) structure of the RNA in the U2 snRNP complexes, before (17S) and after (A-like) branch site recognition.

A selection of SARS-CoV-2 scientific highlights from IBS

Two and a half years after the outbreak of the SARS-CoV-2 pandemic, IBS researchers are actively working on the development of new vaccines and the identification of novel therapeutic targets.

Adenovirus-inspired vaccine platform tackles SARS-CoV-2

Although mRNA-based vaccine solutions have emerged very quickly to manage the SARS-CoV-2 pandemic, they have logistical limitations such as freezing storage and use within 6 hours after thawing that do not allow for their widespread deployment. Moreover, a sterilizing immunity preventing a vaccinated person from transmitting the virus does not exist to date. A rapidly adaptable vaccine platform that can be deployed on a large scale is thus still desirable.

The 'Adenovirus Team' at IBS has designed a new type of self-assembling vaccine that is easy to produce and very stable at room temperature. For this, a 60-mer non-infectious adenovirus-inspired particle was modified to allow the spontaneous and irreversible display of up to 60 copies of a glycosylated SARS-CoV-2 antigen (RBD: Receptor Binding Domain of the spike protein) [1]. The clustering of these antigens on the 30nm wide vaccine platform thus mimics well the external structure of the coronavirus, while being harmless (Figure 1).
Immunizations have been performed in mice with these new-generation vaccines [1]. Characterization of the immune response in vaccinated animals showed that the clustered antigens on the vaccine platform induced antibodies against SARS-CoV-2 already after the first immunization and titers higher than those of patients infected with SARS-CoV-2 were reached after the second immunization. Remarkably, the serums of the immunized animals enabled a total neutralization of the SARS-CoV-2, showing that a functional and effective response was achieved (Figure 1).

This new vaccine technology, which can be rapidly adapted to any pathogen is a new tool for pandemic preparedness.

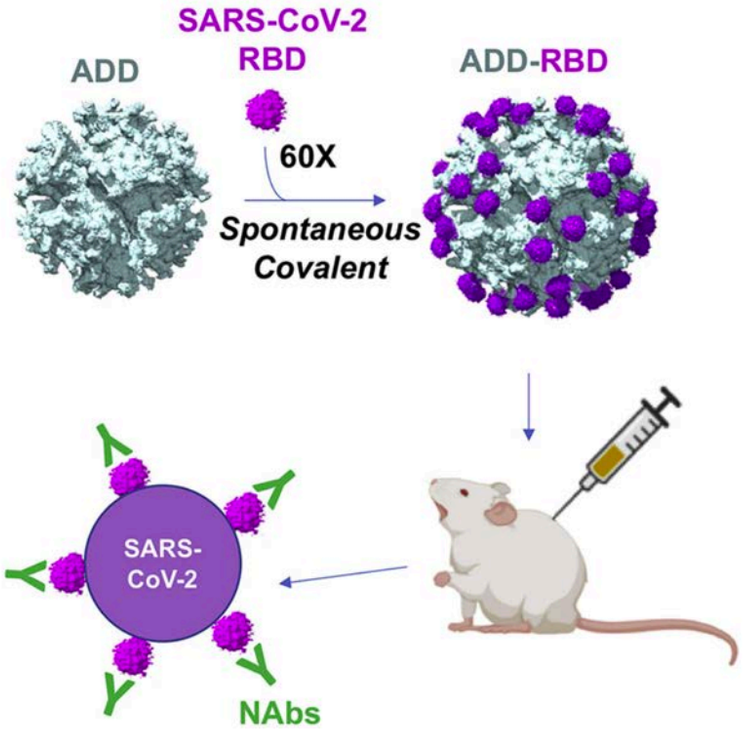

Figure 1: The 'plug and display' vaccine platform enables the spontaneous binding of 60 copies of the 'Spike-RBD' coronavirus antigens. Prime and boost immunizations of mice result in a total neutralization of the pathogenic SARS-CoV-2.

P. Fender (IBS), P. Poignard (IBS), D. Hannani (TIM-C)

[1] C. Chevillard, A. Amen, S. Besson, D. Hannani, *et. al.* (2022) Mol Ther, 30, 1913-1925.

Vaccination with synthetic SARS-CoV-2 virus like particles induce sterile immunity in macaques

The recent pandemic caused by SARS-CoV-2 virus highlighted the role of vaccines in improving public health. In an extraordinary international effort and based on technology previously developed, different SARS-CoV-2 vaccines have been developed and are currently in use. However, the emergence of variants of concern, with progressing accumulations of mutations and higher transmission rates increase the risk of vaccine escape and impose the need for a vaccine inducing sterile immunity, preventing disease and transmission.

The IBS EBEV group in collaboration with several IBS groups (CAID, MEM and M&P), IDMIT (Institut de biologie François Jacob), Amsterdam University and the Pasteur Institute developed a two-component platform adapted to present viral glycoproteins as multimeric antigens on lipid nanoparticles [1]. Formaldehyde cross-linking was used to stabilize the SARS-CoV2 spike (S) glycoprotein. Cryo-electron microscopy structure determination

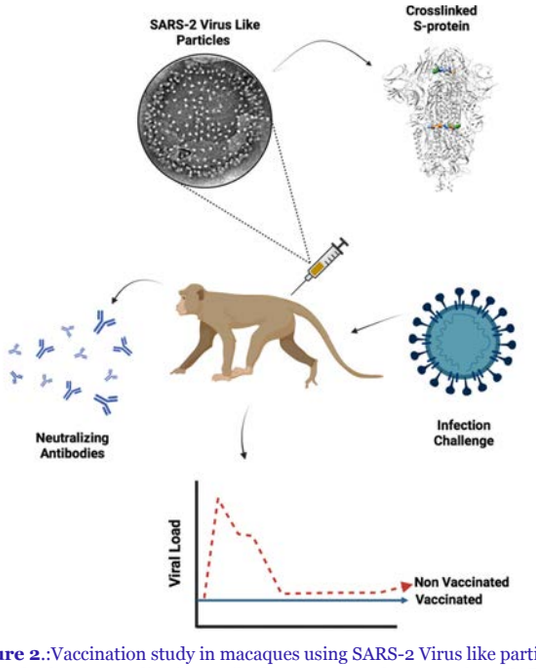

Figure 2.:Vaccination study in macaques using SARS-2 Virus like particles (S-VLPs) induced robust neutralization antibody response and protects from SARS-2 infection.

confirmed the presence of two major cross-linking sites that keep S in the closed native pre-fusion state. S is then linked to lipid vesicles thereby producing S-coated lipid nanoparticles that resemble virus-like particles (S-VLPs).

Vaccination of macaques with S-VLPs induced both strong antibody responses and *in vitro* neutralization activity against the Wuhan vaccine strain as well other variants [1]. Analysis of the immune response showed T helper CD4+-biased T cell responses as well as robust mucosal immunity. The challenge of vaccinated and control animals with the SARS-CoV-2 Wuhan strain indicated that vaccination induced sterile immunity since no virus replication could be detected in the vaccinated group as compared to the infected control group. This proof-of-concept study renders S-VLPs an efficient and safe vaccine candidate for further development.

G. Sulbaran (IBS)

[1] G. Sulbaran, P. Maisonnasse, A. Amen, G. Effantin, *et. al.* (2022) Cell Rep Med, 3, 100528.

NMR characterises the intrinsically disordered SARS-CoV-2 nucleoprotein in dynamic complex with its viral partner nsp3

The processes of replication and transcription of viral RNA represent important targets for inhibition of SARS-CoV-2, and the development of rational strategies to achieve this end requires a molecular understanding of the viral replication cycle. The nucleoprotein (N) is an essential cofactor of the replication machinery, encapsidating the viral genome, providing protection from the host cell environment, and playing an essential role in regulating gene transcription. N is also highly dynamic, with more than 40% of its primary chain being intrinsically disordered, regions that link the folded RNA-binding and dimerization domains. The disordered regions are nevertheless functionally active, comprising essential phosphorylation sites and containing important mutations associated with variants of concern.

The FDP group at IBS used NMR spectroscopy and small angle X-ray scattering to describe the dynamic properties of N, and to describe for the first time its interaction with the N-terminal domain of the viral protein nsp3 (nsp3a) (Figure 3), an interaction which appears to position the nucleoprotein at the replication site of the viral genome, prior to encapsidation [1]. The interaction implicates two distinct "linear motifs" within the central disordered domain of N, which wrap around the partner nsp3a (Figure 3). This also results in a substantial collapse of the dimensions of N, forming a highly compact, but still dynamic molecular assembly, which is also shown to regulate RNA binding. The identification of distinct linear motifs and interaction sites that mediate this essential interaction between viral proteins provides future targets for development of novel strategies against COVID-19, as well as shedding new light on the viral replication process.

M. Blackledge (IBS)

[1] L. M. Bessa, S. Guseva, A. R. Camacho-Zarco, N. Salvi, *et al.* (2022) Sci Adv, 8, eabm4034.

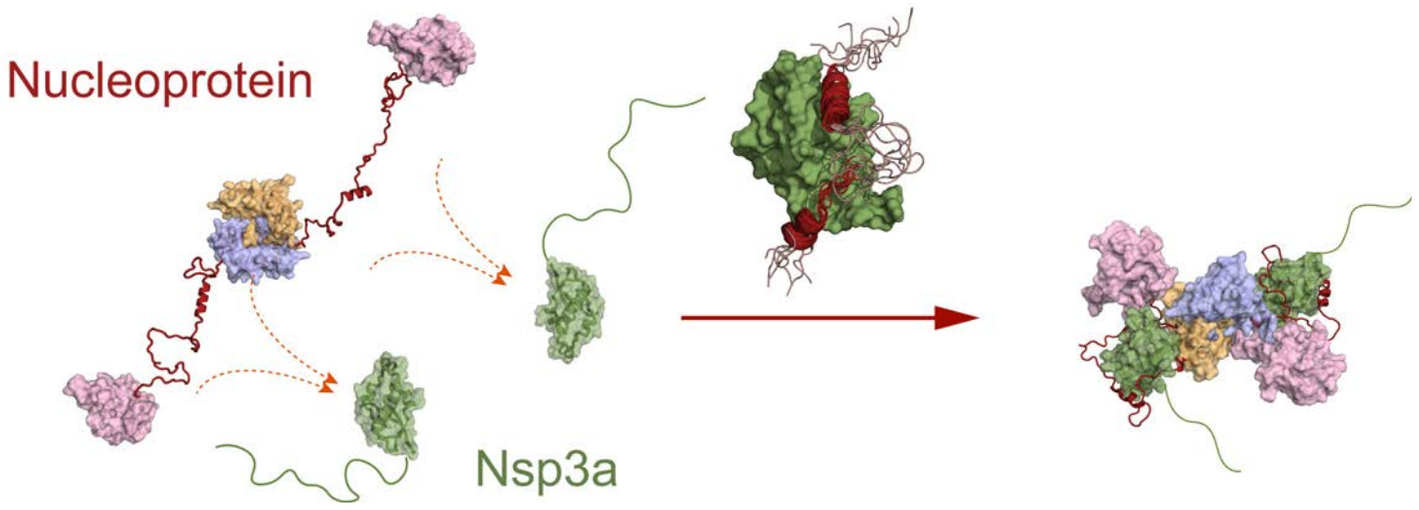

Figure 3: Interaction of the largely disordered N nucleoprotein from SARS-CoV2 with its viral partner nsp3a (green).

Neutron crystallography reveals novel mechanisms used by *Pseudomonas aeruginosa* for host-cell binding

Antibiotic resistance is one of the major global threats of the 21st century and requires the development of new effective treatments. *Pseudomonas aeruginosa* is a human opportunistic bacterial pathogen and one of the most common pathogens associated with hospital-acquired infections. Due to its increasing resistance to antibiotics, it can also cause fatal infections in immunocompromised or cystic fibrosis patients. *Pseudomonas* bacteria produce many virulence factors including sugar-binding proteins called lectins that are involved in the initial stages of host infection. One of them, called LecB, is specific to fucose, a simple monosaccharide that is present on the surface of host cells in the form of glycosylated proteins and lipids. Lectins help bacteria to read this complex 'glycocode' by targeting specific carbohydrates during attachment to host cells.

LecB lectin is currently viewed as a potential drug target for glycomimetic compounds used in anti-adhesive therapy, which is advantageous compared to antibiotic therapy, since it does not promote emergence of resistance. LecB displays an unusually high affinity towards fucose with a unique binding site that contains two calcium ions directly involved in sugar binding.

Neutron macromolecular crystallography (NMX) offers unique insights into ligand-binding as it directly locates and allows visualization of all hydrogen (or deuterium) atoms involved in the interactions. Perdeuteration, where all hydrogen atoms are replaced by deuterium atoms, enhances their visibility in the neutron maps. While perdeuteration of recombinant proteins is almost routinely carried out in dedicated deuteration facilities, the production of perdeuterated sugars is still very challenging [1].

The team at ILL, together with researchers from CERMAV institute in Grenoble, have used perdeuteration (carried out in the D-lab facility at ILL) and NMX, resulting in a neutron structure of the perdeuterated LecB/fucose complex that was collected on the LADI-III instrument at ILL. The structure gave new insights into the tight binding between the sugar and the protein in unprecedented detail [2].

The study enabled a complete description of the hydrogen-bonding network between the sugar and the protein (Figure 1). We could observe that all of the charged amino acid residues involved in the calcium coordination were non-protonated. Interestingly, we also observed a low-barrier hydrogen bond, a special type of strong hydrogen bond between one of the fucose hydroxyl groups and the protein. Neutrons played an important role as all of these details could not be revealed using X-ray crystallography alone. The new structural data may help in the design of new potent glycomimetic compounds for fighting antibiotic-resistant bacteria.

L. Gajdos (ILL)

[1] L. Gajdos, V.T. Forsyth, M.P. Blakeley, M. Haertlein *et al.* (2021). Glycobiology, **31**, 151-158.
[2] L. Gajdos, M.P. Blakeley, M. Haertlein, V.T. Forsyth *et al.* (2022). Nat Commun, **13**, 194.

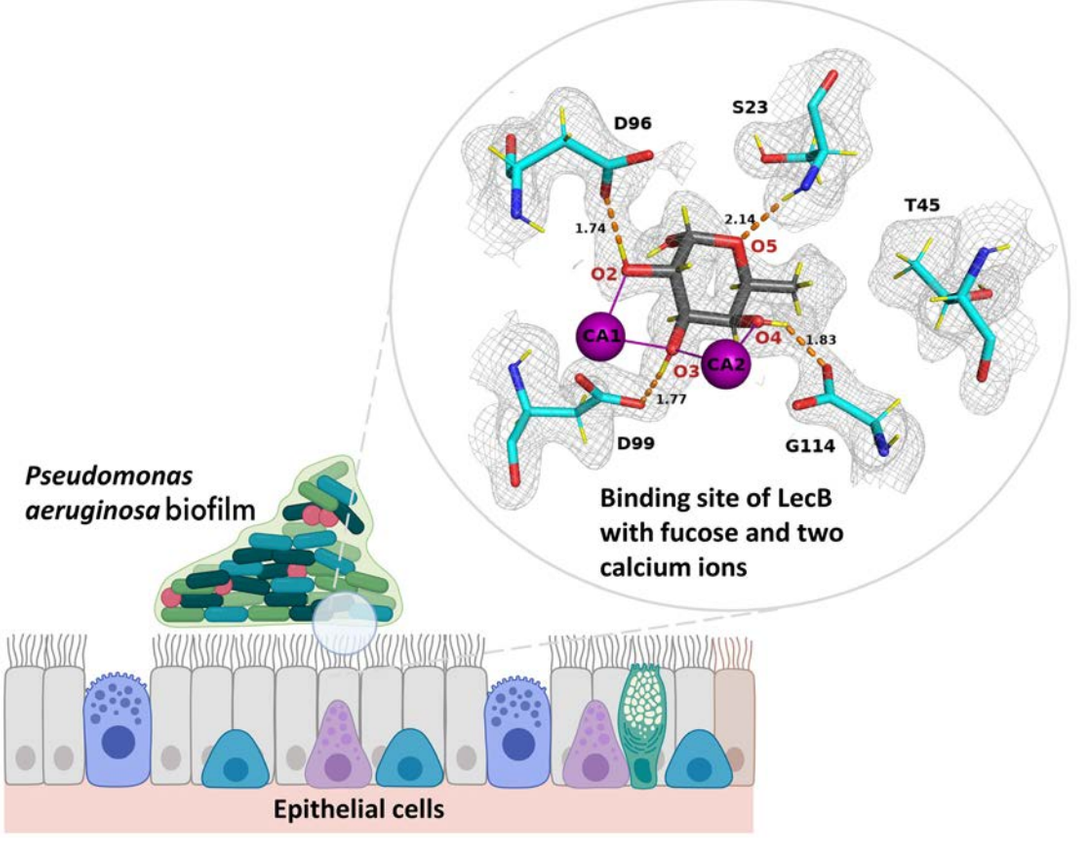

Figure: Schematic illustration of a *Pseudomonas aeruginosa* biofilm colonizing epithelial cells. Inside the circle are presented the details of the fucose-binding site of LecB lectin. The neutron density is represented by grey mesh, hydrogen bonds by dashed orange lines (Å). Fucose is shown as black sticks, protein as cyan sticks and calcium ions as purple spheres.

News from the platforms

News from the ILL Deuteration Facility

After 20 years as the head of the ILL Deuteration Laboratory, Dr. Michael Haertlein is retiring at the end of June 2022. With his departure, the D-Lab team – Martine Moulin, Valérie Laux and Juliette Devos - is continuing its activities with the same enthusiasm and dedication. The platform's staff remains available to assist you with your sample preparation.

The ILL D-LAB team. From left to right: Martine Moulin, Valérie Laux and Juliette Devos.

The Deuteration Laboratory (D-Lab) is run as a user platform and is part of the ILL Life Sciences Group located on the second floor of the CIBB. It allows users in the area of life sciences and structural biology to seek tailor-made deuterated biomolecules in support of neutron scattering, protein crystallography, dynamics and reflectometry.

The D-Lab is one of the many technical platforms of the PSB. Access to the platform is by a rapid electronic peer-review system. In order to optimize our communication and collaborations with the scientific community, a new email address has been set up (dlab-proposals@ill.fr) for easy access to the team of the facility. Applicants and new users are invited to contact the D-Lab team before submitting proposals to discuss their scientific projects and technical feasibility.

For further information, you can consult the webpage on the ILL website dedicated to the platform (https://www.ill.eu/users/support-labs-infrastructure/deuteration-laboratory) or you can contact the D-Lab team (dlab-proposals@ill.fr). We are looking forward to hearing from you.

M. Moulin, V. Laux and J. Devos (ILL)

Opening of the French MX beamline BM07-FIP2

The French Macromolecular Crystallography beamline BM07-FIP2 officially opened last October 1st, welcoming its first user from the Laboratoire Information Génomique et Structurale (IGS) in Marseille (UMR 7256 Université Aix-Marseille – CNRS). FIP2 is the successor of beamline BM30A-FIP [1], which operated between 1999 and 2018. All optical elements (mirrors, monochromator) as well as most of the experimental setup (robotic arm-based sample changer, minidiffractometer) have been transferred from BM30 to the BM07 port in the context of the EBS upgrade of the ESRF. The X-ray beam is now produced by a 2-pole wiggler, which results in a 10-fold increase in flux compared to FIP, approximately up to 1 x 10^{12} photons/s for the full beam at 12.7 keV. The size of the top-hat beam can be adjusted between 50 x 50 μm^2 and 250 x 250 μm^2. The beamline is now equipped with a Hybrid Pixel Coupled X-ray detector (Pilatus2 6M, Dectris), which allows for 2 to 3 min data collections, a very significant improvement compared to the typical data collection times on FIP. The sample changer uses the SPINE-SC3 standard of pucks (an upgrade to Uni-Pucks is planned for next year) and will soon be able to screen crystallization plates for room temperature *in situ* data collections. Everyone from the PSB is welcome to come and test crystals on the beamline so as to evaluate the increased ease in data collection. Regular beamtime access can be granted either through a request for MX beamtime at the SOLEIL committee possible every 6 months (national access) or through the MX BAGs of the ESRF (international access).
More details are available on the FIP2 website [2].

Top left: picture of the optical hutch. **Right**: picture of the experimental hutch featuring the X-ray detector Pilatus2 6M, the minidiffractometer MD2M, the robotic arm of the sample changer and its sample storage dewar. **Bottom left**: picture of the control cabin.

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[1] M. Roth *et al.* (2002) *Acta Crystallogr. D*71, 15-26.
[2] https://www.esrf.fr/home/UsersAndScience/Experiments/CRG/BM07.html

"L-Lab" – A lipid deuteration platform at the ILL

The Lipid deuteration platform "L-Lab" at ILL has been established to cater to the needs of the ILL user community, initially through making available a wide range of pure natural phospholipid class mixtures in their deuterated form. The idea behind creating this facility within the realms of ILL was conceived back in 2013, with the main objective of maximizing the application of neutron scattering techniques in understanding biological membranes and their behavior. The platform has resulted from years of pioneering research at ILL within the PSCM, under a team led by Giovanna Fragneto in collaboration with the D-Lab and Hanna Wacklin-Knecht. The ability to deuterate natural lipid molecules definitely adds a powerful dimension to neutron scattering studies but studies involving deuterated biomimetic membranes are currently limited by the low availability of several biologically relevant unsaturated phospholipid species. This could be overcome by the use of D2O and labeled carbon sources in the growth medium of the host organism followed by extraction and purification of the target molecules from the labeled biomass, through chromatographic separation.

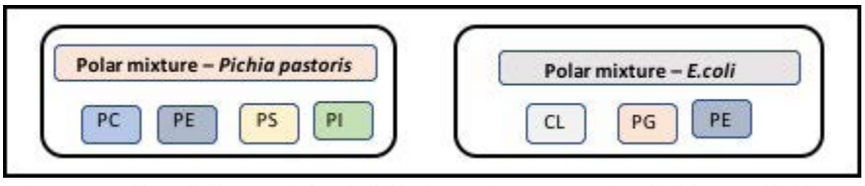


Figure 1: Readily available natural phospholipid mixtures that were HPLC-purified from total lipid extracts.

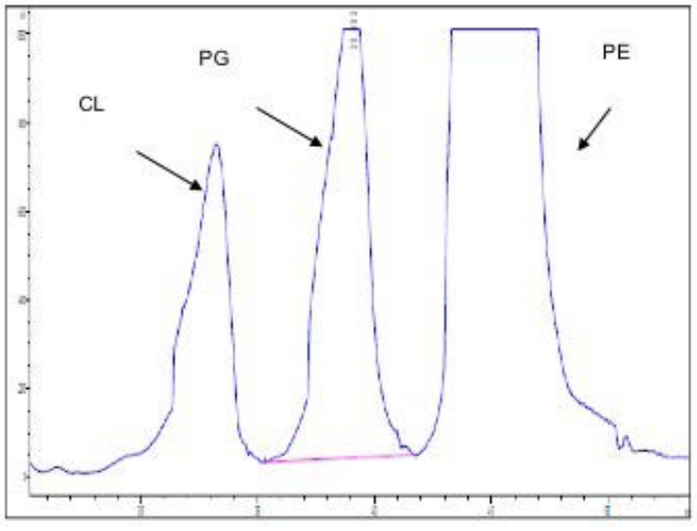


Figure 2: An HPLC chromatogram showing separation of phospholipid class mixtures from *Escherichia coli*.

To achieve these goals, a strong emphasis has been placed in our facility on development of novel extraction and isolation techniques with a focus on (a) separation of individual molecular species from purified natural polar mixtures and (b) separation of other neutral lipid molecules and various other metabolites. So far, we have been successful in extracting and separating various phospholipid mixtures from total lipid extracts after producing them biologically in *Pichia pastoris* and *Escherichia coli* (Figure 1). These deuterated polar mixtures, that consist of acyl chains of varying lengths, enable researchers in mimicking natural bilayers and thereafter pursue neutron scattering investigations. Substantial efforts at the PSCM have been deployed to well-characterize these mixtures by various analytical (Figure 2) and neutron scattering techniques in order to increase our understanding of lipid membranes resulting from them [1]. To achieve our goals, the lab has recently acquired a 1260 Infinity II Semi-prep LC purification system that has been coupled to an Agilent Mass detector allowing for mass selective detection of the purified molecules.

K. Chaithanya Batchu (ILL)

[1] Santamaria *et al.* (2022) J. Am. Chem. Soc. 144, 2968-2979.

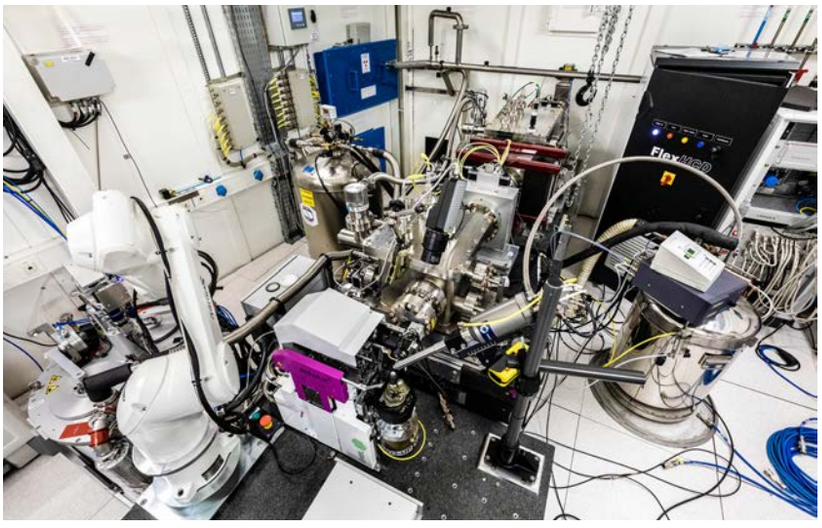
ID23-2: an automated and high-performance microfocus beamline for macromolecular crystallography

The ID23-2 microfocus beamline was recently almost entirely rebuilt [1]. After re-commissioning the beamline during the COVID-19 lockdown in spring and summer of 2020, ID23-2 now has dramatically improved beam properties. We have recently described this upgrade in the Journal of Synchrotron Radiation [2] in which we discuss the optical upgrade and design, as well as the measured beam properties from the Extremely Brilliant Source (EBS) and the new sample environment including a state of the art MD3-Up high-precision multi-axis diffractometer. The upgraded beamline offers beam sizes down to 1.5 x 3.0 microns (horizontal x vertical) that are well suited to single microcrystals, larger crystals with non-uniform diffractive properties and multi-crystal sample holders. Additionally, the vertical beamsize can be changed in seconds to fixed sizes up to 25 microns. The MD3-Up microdiffractometer provides extremely fast and reproducible translations, which allows for rapid diffractive sample characterization via mesh scans in MXCuBE3 [3]. Additionally, an overview of the software suite, including automatic phasing and

automatic serial crystallography processing is discussed. Finally, by mining automatic processing results, we show some initial data showing the improvement of data quality post EBS.

M. Nanao (ESRF)

[1] www.esrf.fr/UsersAndScience/Experiments/MX/About_our_beamlines/ID23-2
[2] Nanao M, Basu S, Zander U, Giraud T, *et al.* (2022) J. Sync. Rad. 29, 581-599.
[3] http://www.mxcube3.esrf.fr/



ID23-2 Experimental hutch. Photo credits: S. Cande

Ultimate automation in MX – CrystalDirect at MASSIF-1

The ESRF-EMBL JSBG operated beamline MASSIF-1 started service in 2014 as the world's first beamline to collect data automatically using a system that makes smart decisions in order to collect the best possible data [1, 2]. At the same time, the EMBL HTX Lab and Instrumentation teams started to develop fully automated crystal harvesting, using laser photo ablation, with the CrystalDirect harvester (CDh) and the CRIMS software, establishing automated protein-to-structure and fragment screening pipelines operated over the internet [3, 4]. With the new beam properties promised by the EBS accelerator, it was decided to upgrade MASSIF-1 to a MD2-S diffractometer and Flex robotic sample changer and to develop operational integration between the HTX Lab and MASSIF-1 with funding from both EMBL and ESRF. The EBS upgrade provides an improved beam with tunable diameter between 100 and 10 µm, while the integration of these two world-leading platforms brings unique opportunities for end-to-end automation of the protein to structure pipelines. A CDh device has now been installed and incorporated into the beamline software environment. Users can set up the experiment by defining a harvesting plan in CRIMS, that is then used to queue samples in MXCuBE3. An automatic workflow, selected according to the experiment requirements, is then used to collect data. This development not only brings a higher level of automation, but also allows new complex experiments to be automated – in particular, room temperature data collections can now be performed, at scale, in a consistent manner, using a humidity control device. The first experiments performing fragment screening at both cryo- and room-temperatures are now underway. These new experiments are currently available on request; please contact us for more information if you are interested.

The installation of the EMBL CrystalDirect harvester on MASSIF-1. Samples can be mounted from the dewar in the classical manner or directly from crystallization trays mounted in the CDH2. The gripper of the Flex robot is either maintained in liquid nitrogen for cryogenic experiments or, as pictured, at room temperature, where the crystal is transferred to a humid air-stream provided by the HC-lab humidity control device.

M.W. Bowler (EMBL), D. Nurizzo (ESRF), S. Rocchio (EMBL) and J. Marquez (EMBL)

[1] Svensson, *et al.* (2015). *Acta Cryst.* **D71** 1757-1767
[2] Bowler, *et al.* (2015). *J. Sync. Rad* **22**, 1540-1547
[3] Zander, *et al.* (2016). *Acta Cryst.* **D72**, 454–466
[4] Cornaciu *et al.* (2021) *JoVE*, **e62491**, doi:10.3791/62491

EVENTS

50 years of neutrons: happy anniversary to ILL

On the 31st of August 1971 the Institut Laue-Langevin (ILL) research reactor delivered neutrons for the very first time and have continued successfully to do so for 50 years! Founded in 1967, the ILL is Grenoble's first international institute and has shown an exceptional agility in its capacity for renewal. To celebrate this important anniversary a ceremony was held on the 16th of June at the World Trade Center (Grenoble). The event started with historical documents and interviews about the first neutron delivery, followed by a series of round tables explaining all the different professions enabling the reactor to safely and efficiently run since 50 years. The audience met the watch team, mechanics, technicians, electricians, safety and radioprotection engineers as well as researchers who gave a complete overview of the complexity and impressive infrastructure of the ILL. The program also covered a section dedicated to the most important upgrades done at the ILL to meet the highest safety standards, without forgetting its important impact for Grenoble's development over the years. A common message from all the contributors was clear: teamwork is what makes the ILL function since 50 years. We wish the ILL many more years of science and innovation, driven by its enthusiasm, talent and ambition.

L. Gajdos and M. Oliva (ILL)

30th Anniversary of IBS

The IBS celebrated its 30th anniversary on June 16th 2022. For this occasion, IBS organized a special "IBS Day" at the Maison Minatec. The day included flash poster presentations, a series of short talks by IBS and ESRF staff on X-ray crystallography and its applications, a presentation of two IBS/PSB platforms and several exciting scientific talks, including one by the laureate of the 2022 IBS Young Scientist Award, Benoit Arragain from the MEM group (now post-doc at EMBL, Grenoble). After the afternoon coffee break, Juan Fontecilla-Camps and Eva Pebay-Peyroula retraced the history of IBS since its creation in 1992 and Winfried Weissenhorn, current IBS director, discussed the perspectives and challenges of structural biology research for the years to come. The day ended with an insightful talk by two astrophysicists from the Observatoire des Sciences de l'Univers in Grenoble, who presented the outcome of ten years of research at the surface of Mars. The celebration of the 30th anniversary then continued in the evening on the EPN campus with drinks, food, outdoor games, live music and dancing! With more than 210 participants during the day and 170 for the evening event, the 2022 IBS Day was a real success!

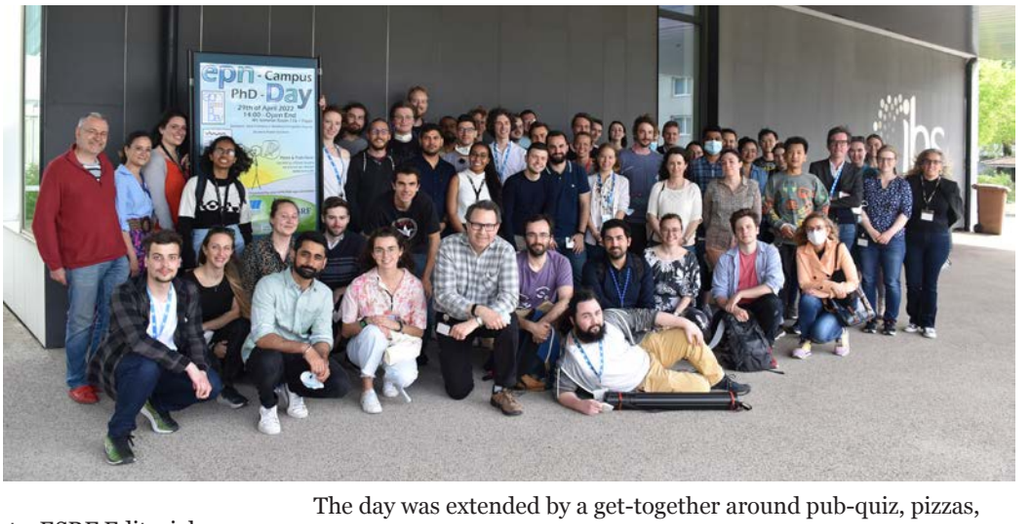
J. Timmins (IBS)

2022 EMBO Practical course on characterization of macromolecular complexes by integrative structural biology

The 10th edition of the biennial EMBO Practical Course organised by the PSB took place from 28th May to 4th June on the EPN Campus, with a focus on integrative structural biology. Out of 150 applicants, 20 participants were selected, including 5 postdocs and 15 PhD students of 12 different nationalities and working in 20 different research centres worldwide. In the morning, course participants attended a series of seminars delivered by world-class experts on various aspects of molecular biology, biochemistry, biophysics, and structural biology. All seminars were livestreamed so that PSB and Grenoble-area researchers could follow online. In the afternoon practicals, under the expert guidance of local PSB staff, participants crystallised an RNA enzyme at the EMBL; performed ITC, MALS and a TSA assay at the PSB Biophysical Platform; collected and analysed mass spectrometry, NMR and negative stain EM data at the IBS; collected X-ray serial crystallography, SAXS and cryoEM data at ESRF beamlines; and visited the ILL facilities for SANS. Two poster sessions and a PSB Get Together evening offered further opportunities to mingle with PSB scientists. To highlight the 20th anniversary of the course, four distinguished scientists from the USA, UK and Germany gave keynote lectures on the application of integrated structural biology methods to the study of a variety of macromolecular complexes. The event was sponsored by EMBO and supported financially and administratively by all four PSB institutes, in a truly collaborative effort involving over 40 tutors and speakers.

M. Marcia (EMBL), C. Petosa (IBS), D. De Sanctis (ESRF), M. Soler Lopez (ESRF), T. Forsyth (ILL), D. Hart (IBS) and A. Thomasson (EMBL).

EPN Campus PhD Student Day



The EPN Campus and its institutions (IBS, ESRF, EMBL, and ILL) host a large number of PhD students working across a vast scientific spectrum. The EPN Campus PhD Student Day took place on the 29th of April and gathered over 50 PhD students as well as several directors, heads of graduate school and communication officers from all four institutions. This networking opportunity allowed students to come together, share their work through poster sessions, and learn about other ongoing research on campus.

To further enrich the event, two speakers were invited to share their expertise with the aspiring young scientists. ESRF Editorial Content Manager Montserrat Capellas Espuny gave a talk about "Communicating Science" and ALPX CEO and co-founder Irina Cornaciu detailed her "Experiences in changing from Academia to Industry". The event was rounded off with a "Survival Guide for your PhD" talk given by third-year PhD student and ESRF student representative, Laura Wollesen.

The scientific event was concluded by the awarding of three poster prizes in the following categories: "VIP's choice" to Rutuja Yelmar (EMBL), "Most colorful poster" to Angela Mantovanelli (IBS), and "Most creative poster" to Jennifer Graham (ILL).

The day was extended by a get-together around pub-quiz, pizzas, and drinks, organized at the ESCAPE (CROUS).

The event was organized by the PhD-student representatives (Fernanda Alvarado Galindo ILL, Mohit Agarwal ILL, Harald Bernhard EMBL, Mattia Colalongo ESRF, Simonne Griffith-Jones EMBL, Michela La Bella ESRF, Marie Lorvellec IBS, Wenke Mueller ILL, Andrea Pinto IBS, Michal Ronovský ESRF, Laura Wollesen ESRF) and financially supported by the ESRF and approved by the EPN steering committee.

EPN PhD-student representatives

Capacity Building in Structural Biology in Africa

Several scientists from the Partnership for Structural Biology (PSB) have participated in a four-day workshop dedicated to "Hands-on training in Structural Biology, a tool for sustainable development in Africa." Organised by BioStruct-Africa (co-founded by Daouda Traore, scientist at ILL and lecturer at Keele University, UK. https://www.biostructafrica.org), a non-profit organisation whose mission is to build capacity in Structural Biology for Africa-based biologists, the workshop was held at the Malaria Research and Training Centre (MRTC) in Bamako, Mali from 25th to 28th April 2022.

Top left: Daouda Traore during a lecture; **Top right and bottom**: Workshop participants observing protein crystals, and controlling and working on an ESRF beamline from Bamako. © D. Traore

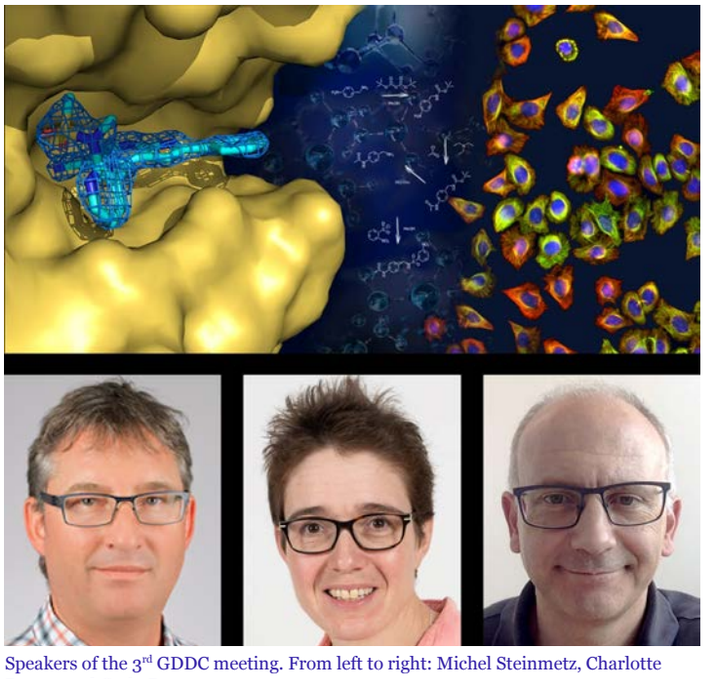
Over the four days, participants listened to lectures and presentations on Structural Biology techniques, as well as taking part in practical sessions on protein crystal growth in the laboratory. Daouda Traore presented the PSB and Structural Biology at the ESRF, Adeline Robin- Traore (EMBL) gave a talk on high-throughput crystallisation and structure-guided and fragment-based drug design, and Alessandro Grinzato gave a talk on structural determination using cryo-EM. The pinnacle of the workshop was on day three, when the participants connected remotely to one the beamline at the ESRF to collect their own X-ray diffraction data. "Access to the best facilities is key for successful science, and supporting African scientific development is a great reward," said Daniele de Sanctis, one of the ESRF staff scientists who supervised the remote data collection together with Didier Nurizzo (ESRF) and Nicolas Foos (EMBL).

"The participants really appreciated the generosity of all the instructors in sharing their knowledge and skills. We hope to have more of such workshops and for a longer period of time" said Prof. Abdoulaye Djimde, the director of the MRTC.

D. Traore (ILL)

Grenoble Drug Discovery Club

Launched in the spring of 2021, the Grenoble Drug Discovery Club (GDDC) confirmed its vitality, with once again, more than 100 participants connecting during its third virtual event, which took place on 13th April 2022, with. We were pleased to listen to Prof. Michel Steinmetz from the Paul Scherrer Institute, Villigen, Switzerland, who demonstrated, through an integrated approach combining modelling, fragment screening, crystallization and synthetic chemistry, the existence of new sites on tubulin and that it is possible to target them pharmacologically. Next, Charlotte Deane, Professor of Structural Bioinformatics at the University of Oxford presented a novel method of machine learning for fragment elaboration and early-stage drug discovery. The stage was then opened to three young local researchers, Dr. Zuzzana Macekjilkov (CHU-IAB), Dr. Paul Rivolier (CERMAV), and Dr. Cristol Fabre (CHU-IAB). Finally, Dr Carlo Petosa from the IBS captivated the audience by depicting BET inhibitors as a potential new class of antifungal drug.

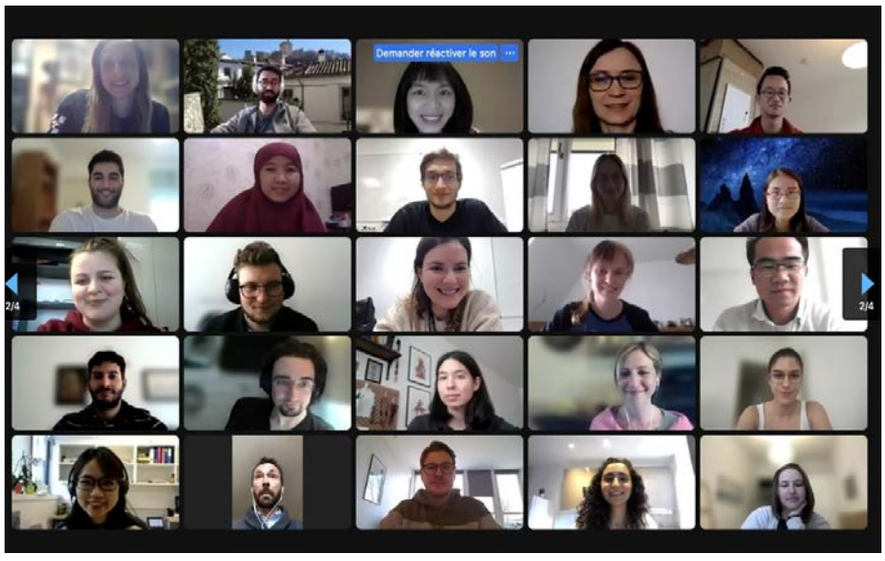


Speakers of the 3rd GDDC meeting. From left to right: Michel Steinmetz, Charlotte Deane, and Carlo Petosa

Do not miss the next GDDC meeting that will be held in person on 25th November 2022 (for more information: https://grenobledrugdiscovery.fr).

L. Lafanechère (IAB)

Hercules European School 2022



The Hercules European School 2022 edition, organized by the Université Grenoble Alpes, in collaboration with the Institut Laue Langevin, the European Synchrotron Radiation Facility and the Institut de Biologie Structurale, took place from the 28th of February to 1st of April. With almost 200 applications received, 68 participants were selected to attend as full-time (hands-on trainings and a week in one of our partner facilities - this year KIT in Karlsruhe; DESY and European XFEL in Hamburg; SOLEIL in Paris-Saclay; PSI in Villigen) and 46 as part-time (attending only the lectures in Grenoble). The majority of the participants were coming from Europe and the rest from India, Indonesia, Russia, Japan, and Taiwan, for a total of 41 different nationalities. More than 180 lecturers and tutors provided a complete training on the use of neutrons and synchrotron radiation (integrated by many other experimental techniques) for condensed matter studies, including physics, chemistry, biology, geology and materials science. Due to the Covid19 pandemic, the school was organized almost fully online, with only the weeks in Paris-Saclay and in SOLEIL held on site.

The organizing committee hopes that Hercules 2023 will be fully held in Grenoble, you will know more details for the next year organization in early 2023. Stay tuned!

G. Schirò (IBS) deputy director of Hercules European School

32nd ESRF User meeting UM2022

The annual ESRF User Meeting 2022 was held online from 7th to 9th February 2022. It tackled broad topics such as climate change, gender equality and COVID-19 vaccines, as well as highlighting more specific user research activities and updating the community on the status of ESRF beamlines. UM2022 attracted more than 900 participants from 40 different countries. On day 1, the participants could choose among a series of beamline tutorials, which included a MX BAG meeting and tutorials on remote data collection, the usage of the new MXCuBE3 interface and workflows as well as the fully automated beamline MASSIF-1. A plenary session was held on day 2 with two keynote lectures, one on plant biology and thermosensing in flowering by Chloe Zubieta (CNRS-CEA-INRAE), and a second one on novel technologies for new vaccines by Mariagrazia Pizza (GSK Vaccines, Siena). As part of the EBS Facility Report, Daniele de Sanctis presented the recent news on the new ID29 beamline (EBSL8).

Three User-Dedicated Microsymposia (UDMs) completed the UM2022 activities on day 3. The UDM2 entitled “Ligand and fragment screening: enabling technologies for pressing threats” was organised by Max Nanao, Didier Nurizzo (ESRF) and Adriana Miele (ESRF User Organisation). This symposium brought together 60 participants from interdisciplinary fields to discuss and stimulate exchange

among ESRF-EMBL Structural Biology beamline users on structure-based drug design. The symposium comprised three plenary lectures and four selected contributions from submitted abstracts. Taken together, UDM2 provided an excellent overview on both experimental and computational analysis methodology as well as case studies of protein-ligand interactions, particularly in their application to drug development.

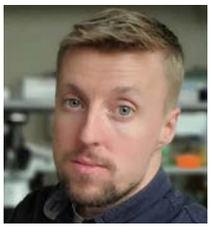
The winner of the ESRF Young Scientist Award was Claire Walsh, researcher at the University College London, for her outstanding contribution to the study of human organs and microvascular architecture of COVID-19 affected lungs by hierarchical phase-contrast tomography.

M. Soler López (ESRF)

ANNOUNCEMENTS

A new head of EMBL Grenoble.

Professor Kristina Djinović-Carugo has been appointed as the next head of EMBL Grenoble, taking over this role from Stephen Cusack from the 1st of July 2022. Awarded a PhD in Chemistry at the University of Ljubljana, Professor Djinović-Carugo has a long career in structural biology, and will join EMBL in July from the Max Perutz Laboratories at the University of Vienna, where she is currently Head of the Department of Structural and Computational Biology and full Professor of Structural Biology.
She was previously Head of Structural Biology and Crystallography Unit of the Sincrotrone Trieste from 1999 to 2004, and Director of the Laura Bassi Center for Optimized Structural Studies, Austria, from 2010 to 2016.
Kristina Djinović-Carugo's research focuses on the molecular mechanisms underlying the architecture and assembly of muscle sarcomeres, in particular Z-discs, and on understanding the molecular basis of diseases affecting muscles.



Lukáš Gajdos from ILL was awarded the Eleventh Erwin Felix Lewy Bertaut Prize of the European Crystallographic Association (ECA) and the European Neutron Scattering Association (ENSA), in recognition of his exceptional research on the characterisation of the interaction of lectins with sugars by neutron diffraction and all the experimental difficulties that he has overcome to obtain these results.
Please see scientific highlight of this work on page 6.

Sigrid Milles, ERC team leader, left IBS beginning of May 2022 to start up her own research group at the Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP) in Berlin, where she will study the intrinsically disordered proteins involved in clathrin mediated endocytosis by integrated NMR and single molecule fluorescence spectroscopy.

The Equipex+ France Cryo-EM project, which brings together the Illkirch (IGBMC), Saint-Aubin (Synchrotron Soleil) and Grenoble IBS centres, aims to equip France with three latest-generation 300kV cryo-electron microscopes. This project was successfully financed and a call for tenders for the allocation of 3 microscopes is currently underway. For Grenoble, the microscope will be installed at the ESRF and will operate on the same basis as a CRG line. Once installed, the 3 microscopes will be open to the national and international academic community as well as to industry.

PROFILE

Bonne retraite Juan!

Juan Fontecilla-Camps has been a key member of l'Institut de Biologie Structurale (IBS) since its creation in 1992, where he was head of the Laboratoire de Cristallographie et Cristallogénèse des Protéines (LCCP) and then the Metalloproteins unit until 2015, and a CEA research director until 2021. After working for more than 30 years in and around the EPN campus, and after experiencing the creation of the IBS and PSB, Juan is now retiring. His remarkable career will be highlighted by a special symposium at the IBS on July 22nd, 2022. The PSB Newsletter thought it was poignant to meet with Juan to learn more about his successful career.

Could you tell us about yourself and what brought you to IBS?

I have been interested in science since my childhood – as far as I remember I have always been fascinated by atoms, stars… After obtaining my Master degree in Biochemistry, I chose to leave Chile for political reasons to go to Barcelona and then to Birmingham, Alabama, USA, where I got my PhD degree in protein crystallography in 1980. During my thesis research, I solved the first protein structure in the lab – the one of a North American scorpion toxin! After spending one additional year in Birmingham, I moved to Denmark for a one-year post-doctoral position, which allowed me to discover Scandinavia. Next, I moved to France and more specifically to Marseille, where there was a research group interested in scorpion toxins from North Africa. In 1982, I was recruited as a CNRS researcher and 4 years later I became PI of the local crystallography lab. In 1988, I became director of a "Unité de Recherche Associée" at the Faculté Nord of the Aix-Marseille university. This is when I also started to hear about the Protein 2000 project associated with the building of the synchrotron facility in Grenoble in the early 1990's. I resigned from my CNRS DR2 position and was recruited by the CEA in 1991 to set-up a protein crystallography research group – the LCCP; this job came with generous funding for 9 staff positions, equipment and consumables. A year later, we moved into IBS created to bring together the CEA and CNRS initiatives in protein structure determination. At the start, the 'independent' LCCP wing of the IBS was completely funded by the CEA.

How did you come to study metalloproteins?

When I first came to Grenoble, most of my research was carried out in collaboration with groups from Marseille with which I had very fruitful interactions. Researchers from Marseille provided us with very high quality metalloproteins notably from sulfate-reducing bacteria. At this stage, I was fully dedicated to protein crystallography: I set-up drops, grew crystals, collected data on inhouse sources at first and later at ESRF and solved structures. The success of these projects allowed us to obtain EU BIOTEC funding on hydrogenases twice and thus purchase our first glove box to manipulate metalloproteins under anaerobic conditions. This in turn led to a number of instrument developments. Metalloproteins are very interesting and intriguing, and with time, we developed a real interest and expertise (at the frontier between Biology and Chemistry) in handling these proteins. It was a real pleasure to decipher the active sites and enzymatic mechanisms of ancestral, 3-billion-year-old proteins!

Did you interact with the neighboring PSB institutes?

Yes, of course! Actually, I was first involved in the national Genomics project and then in the initial discussions about the creation of the PSB, before Eva (Pebay-Peyroula) took over. I also performed some experiments at ILL on a project in collaboration with my then student David Pignol who is now "Chef de Department" at CEA/Cadarache – we used neutron crystallography to visualize a lipid micelle involved in the activation of a pancreatic enzyme. And of course, many of our projects would never have worked without the ESRF! For many years, I was the expert user there and was in charge of fishing the crystals! ESRF was a blessing for my group!

The PSB will celebrate its 20th anniversary this year (15 November 2022). What advice would you give our new generation of scientists for the next 20 years?

I think protein crystallography still has a future alongside cryo-EM and the new AI programs such as AlphaFold, because of the details you can get from high-resolution crystallographic structures – I'm not sure EM will reach such resolutions as easily. I observe that nowadays groups try to master a wide range of techniques, but the problem with that is that each expert is isolated. I think it is a real benefit to bring together several experts in a given field so they can discuss and find solutions to challenging projects. I would also recommend to choose a technique according to your interest. At the start, the LCCP was composed of 25 people; after the reorganization of the IBS, the METALLO unit now includes 8 permanent staff members with a focus on structural studies (by X-ray crystallography, cryo-EM and the help of AlphaFold-generated models) of metalloproteins under anaerobic conditions. Several ex-members of LCCP are involved in other IBS groups, such as IRPAS or GSY. Creating smaller teams gives rise to new problems: more ambition to become a team/group leader, too many people and not enough space or funding. It is never easy to find the right balance.

What are your plans for your retirement?

Now that I am not allowed to do lab work, I spend a lot of time reading scientific articles, thinking about their contents and writing about them in the form of articles or books. I have recently written and published two books, one in French and another in Spanish addressed to interested non-specialists. I write in all three languages. I am now particularly interested in the origins of life, primordial bioenergy, etc.

You gave many enlightening scientific talks for the broader public; will you continue with these after your retirement?

I have been very interested in disseminating science to the general public. I have given talks in schools, small towns and at Minatec… Starting Jan. 2023 I will teach at the "Unversité Inter-âges du Dauphiné" on themes including the origins of life, the universe, earth or the solar system. This long-standing interest in astrophysics led me to interact with young astrophysicists with whom I played football – we used to have heated discussions in the locker room after the matches!!

We know you're a musician, do you have any other retirement plans you'd like to share with us?

I am not really a musician – I just play the guitar for fun to accompany Patricia, who sings very well. We played and sang at ILL a few years ago for the PSB 10th Anniversary. I'm a Bob Dylan fan and like to combine harmonica and guitar. I also very much enjoy doing crosswords (in Spanish) and watching lots of football! For the time being, I'm enjoying the freedom of being able to come and go to IBS whenever I want… I read a lot of articles… This situation suits me just fine.

J. Timmins (IBS), F. Bernaudat (PSB), and A. McCarthy (EMBL)

DATES FOR YOUR DIARY

6th July 2022 – Grenoble Host-Pathogen Interaction Club meeting
This meeting will take place online and will include presentations by invited speakers Olivier Epaulard (University of Grenoble-Alpes Hospital Center, FR), Raphael Carapito (University of Strasbourg, FR), Christiane Schaffitzel (University of Bristol, UK), Christopher Chevillard (IBS Grenoble), and Helen Ginn (Diamond Light Source, UK). For the full programme and to register to the webinar: https://hostpathogen.fr

22nd July 2022 – "The contribution of structural biology to the understanding of the mechanism of metalloproteins"
Symposium in the honour of Dr Juan C. Fontecilla-Camps' retirement. More information at: https://www.ibs.fr/research/research-groups/metalloproteins-group/symposium2022/

13th to 16th September 2022 – Advanced Isotopic Labeling Methods for Integrated Structural Biology International Workshop (AILM)
The AILM workshop will focus on the development of isotopic labelling techniques and their application to the study of biomolecular structure and dynamics. Conference sessions will cover developments in isotopic labelling strategies for NMR spectroscopy, as well as methodological approaches, such as co-expression, segmental labelling, specific labelling, labelling with paramagnetic tags, and sample production *in vitro* or in eukaryotic cells. The program will also include elegant examples of the application of these methods to challenging systems such as high molecular weight proteins and nucleic acids complexes or direct studies of biomacromolecules in cells or tissues. For information and registration: https://www.ailm2022.org

11th October 2022 – PSB Spotlight on "Opportunities and Challenges using artificial intelligence in Structural Biology"
This meeting will take place on the EPN Campus and will focus on applications of the use of artificial intelligence in structural biology. Invited speakers will include Randy Read (University of Cambridge). Further information and the updated programme will be distributed by email and on: https://www.psb-grenoble.eu

25th November 2022 – Grenoble Drug Discovery Club 4th meeting
This meeting will take place on the EPN Campus and will include presentations by invited speakers Alice Doangamath (Diamond Light Source, UK), Jordi Mestres (IMIM Hospital del Mar Research Institute Barcelona, ES), Florence Mahuteau (Université PSL, FR), and Didier Leroy (Medecine for Malaria Venture, CH). For more information about the club and the updated programme of the meeting: https://grenobledrugdiscovery.fr

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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.