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PSB et al

Partnership for Structural Biology Newsletter

Scientific highlights

Neutrons bring insights into protein-carbohydrate interactions

Lectins are carbohydrate-binding proteins whose diverse functions are implicated in many physiological and pathological processes. Every human cell is covered by a thick layer of glycans in the form of glycoproteins and glycolipids, which present the first information about a cell to the outer environment. Lectins can read this complex "glycocode" by specifically targeting different sugars and mediating molecular recognition. Lectins from pathogens can thus be directly involved in cell adhesion at early stages of infection. Increasing antibiotic resistance is a major health problem and preventing bacteria from binding to human cells is the basis of antiadhesive therapy, which is beneficial since it avoids killing bacteria and promoting antibiotic resistance. Detailed knowledge about lectin-glycan interactions involved in host-pathogen associations is required for the design of high-affinity glycomimetic compounds.

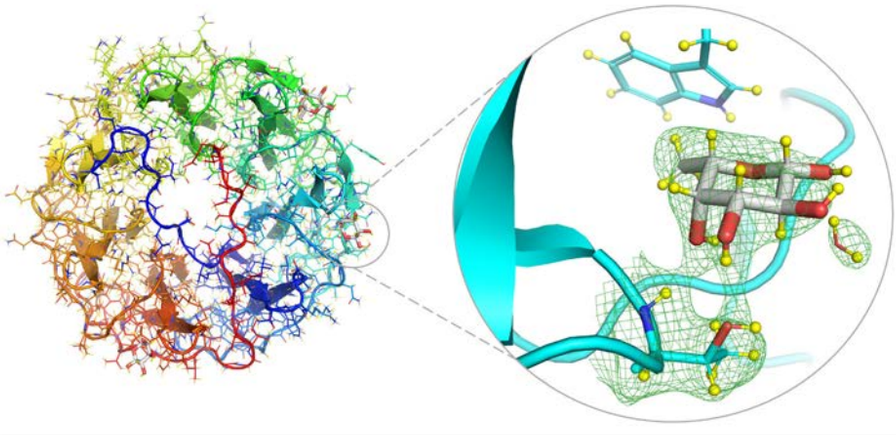
Researchers from the Institut Laue-Langevin (ILL) in collaboration with the Centre de Recherches sur les Macromolécules Végétales (CERMAV, CNRS) and the Central European Institute of Technology (CEITEC) have used neutron macromolecular crystallography (NMX) to provide novel insights into sugar recognition by lectins from pathogenic bacteria. NMX provides details on the position of hydrogen atoms, protonation state of charged amino acids as well as directionality of water molecules that play crucial role in sugar-binding by lectins. The work was focused on a fucose-specific lectin from an insect pathogen *Photorhabdus luminescens* that can also bind to human cells.

Perdeuteration is a process that involves complete replacement of all hydrogen atoms by deuterium atoms in a molecule. Deuterium atoms offer improved properties in neutron diffraction experiments and while perdeuteration of proteins is well established, this is not the case for carbohydrate molecules. Here, we used for the first time, *in vivo* perdeuteration of a monosaccharide, L-fucose, using a glyco-engineered strain of *Escherichia coli* bacteria that was designed by collaborators at CERMAV [1]. The production of both perdeuterated protein and sugar was carried out at the Deuteration Laboratory (D-Lab) at ILL. Perdeuterated fucose was crystallized with perdeuterated lectin and the single-crystal neutron diffraction data were collected on the LADI-III diffractometer at ILL. The X-ray data were collected at the ESRF and IBS for the joint X-ray/neutron refinement of the structures.

The results highlighted the advantages of using perdeuterated molecules in NMX experiments as well as the use of ambient temperature for the data collection that avoided artifacts caused during cryo-protection and cryo-cooling of protein crystals. Complete hydrogen-bonding network between lectin and sugar could be described including fine details on hydrophobic contacts [2]. Our work can be further applied to more complex systems such as oligosaccharides present on human cells that are natural ligands of lectins.

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, A. Kumar, M. Wimmerová *et al.* (2021). Structure, **29**, doi: 10.1016/j.str.2021.03.003.



Fully deuterated fucose in the binding pocket of perdeuterated PLL lectin. Neutron density (green mesh) is contoured at 1σ. Deuterium atoms are represented by yellow spheres. Direct hydrogen bonds can be visualized by the continuous neutron density between fucose and amino acid residues.

CONTENTS

Integrative biology to unveil a unique molecular mechanism that regulates mitochondrial energy pathways

Fatty acid βoxidation (FAO) and oxidative phosphorylation (OXPHOS) are mitochondrial redox processes that generate energy in the form of ATP necessary for life processes. Respiratory complexes are part of the OXPHOS system. Located in the internal membranes of our mitochondria, they are true macromolecular batteries: they couple the flow of electrons through clusters of metals and cofactors with a transfer of protons to create a gradient that ultimately leads to ATP synthesis. The first complex in the respiratory chain, Complex I (CI), is one of the largest membrane protein complexes made up of 45 subunits. The assembly and its sophisticated regulation are still poorly understood, although it is known that their disruption leads to neurodegenerative diseases [1].

The ESRF has joined forces with the IBS, the EMBL and the Grenoble Institute of Neurosciences to study the “helper” complex involved in CI biogenesis, the so-called Mitochondrial CI Assembly (MCIA) complex. By using a combination of biochemical, biophysical and structural approaches, we have discovered that ECSIT, a protein involved in cytoplasmic and nuclear signalling pathways, plays a major role in assembling the MCIA complex [2] (Fig. 1). The modular nature of ECSIT, which is organised into N- and C-terminal binding domains, enables recruitment of its MCIA partners NDUFAF1 and ACAD9 using independent interaction interfaces. Interestingly, the C-terminal domain of ECSIT directly binds to the vestigial dehydrogenase domain of ACAD9 and ejects the FAD cofactor from the catalytic site necessary for FAO, shutting down ACAD9 enzymatic activity and redirecting it towards the action on the assembly of CI (Fig. 2).

Taken together, these results reveal a unique molecular mechanism of deflavination to regulate the dual functions of ACAD9 and suggest an efficient metabolic switch at the crossroads of assembly and activation of FAO and OXPHOS superstructures to ensure efficient energy production. Understanding how FAO and OXPHOS proteins interact and how defects in these two metabolic pathways contribute to mitochondrial disease pathogenesis is thus of potential importance for the development of new tailored therapeutic strategies.

Furthermore, we previously showed that ECSIT interacts with enzymes producing β-amyloid (Aβ) involved in AD pathogenesis [3]. Our recent results suggest that a potential sequestering of ECSIT by Aβ enzymes would thus compromise both the assembly of CI and also the regulation of FAO, a reprogramming of pathways that could lead to altered metabolism in brain mitochondria and ultimately to neurodegeneration [2].

M. Soler López (ESRF) and I. Gutsche (IBS)

[1] G. Giachin, R. Bouverot, S. Acajjaoui, S. Pantalone *et al.* (2016). Front. Mol. Biosci., **3**, 43.
[2] G. Giachin, M. Jessop, R. Bouverot, S. Acajjaoui *et al.* (2021). Angew. Chem. Int. Ed., **60**(9), 4689.
[3] M. Soler-Lopez M, A. Zanzoni, R. Lluis, U. Stelzl *et al.* (2011) Genome Res., **21**(3), 364.

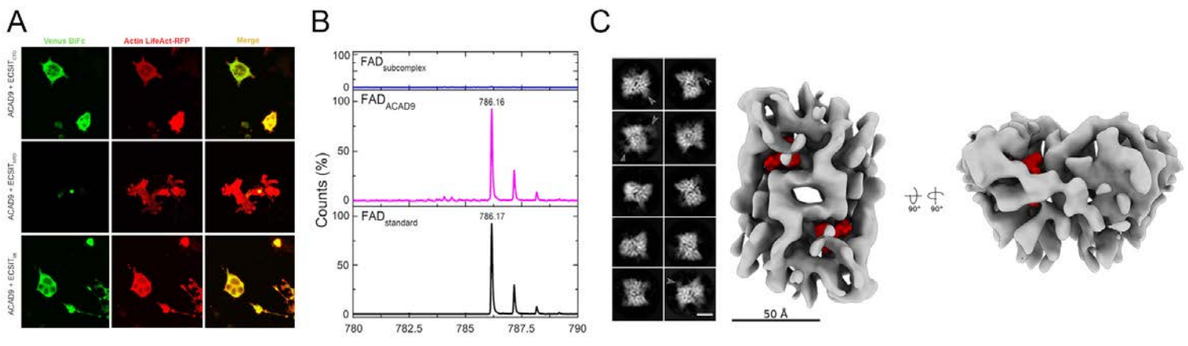


Figure 1. A. BiFC assays in HEK293 human cells. Active reassembly of Venus (green signal) indicates that mitochondrial and C-terminal ECSIT ($ECSIT_{M}$, $ECSIT_{CTD}$) interact with ACAD9. Red immunofluorescence from actin (LifeAct-RFP) as a cell marker. Overlapping signals (yellow) confirm positive interacting partners. **B.** ESI MS spectra showing free FAD standard (black), FAD released from ACAD9 (in magenta) and absence of FAD signal in the ACAD9-$ECSIT_{CTD}$ complex (in blue). **C.** selected cryo-EM 2D class averages of the complex reveal a compact rectangular core with visible secondary structural features. Diffuse densities at defined locations on the ACAD9 core (potential ECSIT binding sites) are indicated with arrowheads. Scale bar = 50 Å. *Right*, cryo-EM map of the complex as an isosurface illustrates the lack of bound FAD (FAD binding sites are indicated in red).

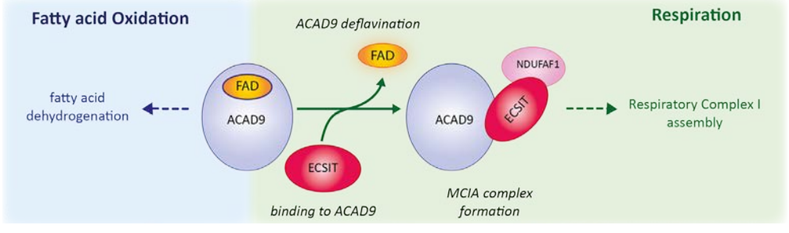


Figure 2. Proposed model of how deflavination of ACAD9 by ECSIT permits the coordinated regulation of the mitochondrial energy metabolism pathways FAO and respiratory chain.

Metabolic biorthogonal labeling and fluorescence nanoscopy reveal secrets of bacterial cell wall synthesis

The cell wall is a three-dimensional sugar and peptide network that surrounds the bacteria (Fig. 1A). It confers a cell shape adapted to the ecological niche of the bacterium, allowing it to feed, move and multiply in an optimal way. It also protects the bacteria against mechanical stress exerted by the internal turgor pressure. Cell wall synthesis and integrity are thus essential for bacterial proliferation and survival. Despite the importance of these fundamental processes, which also represent major antibiotic targets (such as proteins targeted by the penicillin antibiotics), we still only poorly understand how the cell wall is assembled and remodeled in space and time to maintain cell morphology and prevent lethal lesions. This is particularly true for ovoid-shaped bacteria such as streptococci and enterococci pathogens, in which two different modes of cell wall synthesis, dedicated to cell division and elongation, are confined to a nanometer-scale region at midcell.

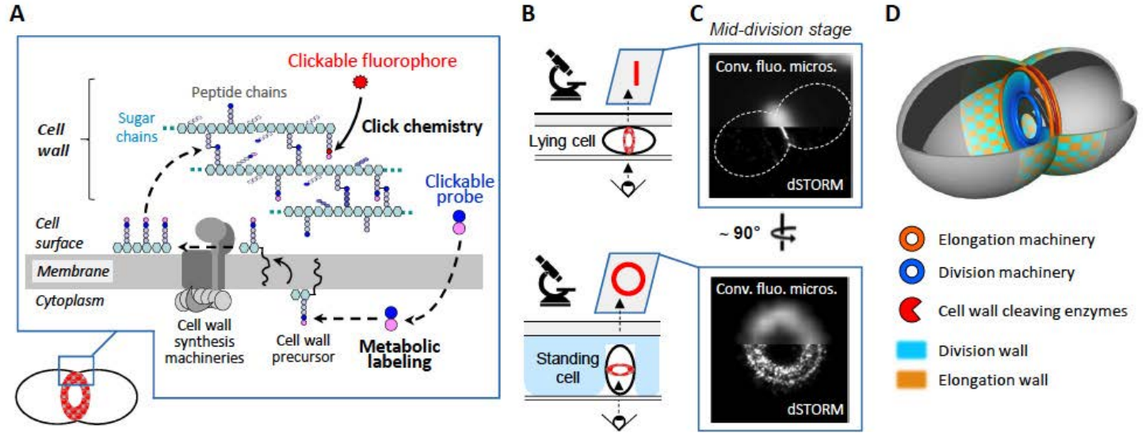
Fluorescence microscopy is a method of choice to investigate cell wall assembly but it suffers from two major drawbacks. First, the newly synthesized material must be labeled with a probe that will not perturb the physiological process. Second, the physical properties of light limit the resolution to about 250 nm, which approximates the dimensions of the cell wall synthesis region. In collaboration with Yung-Sing Wong (DPM, UGA) and Dominique Bourgeois (IBS), we have met these two challenges by implementing a novel combination of metabolic cell wall labeling and super-resolution fluorescence microscopy in the ovoid-shaped human pathogen *Streptococcus pneumoniae* [1]. We have used a chemical trick known as "biorthogonal click chemistry" to introduce a fluorescent dye into the newly synthesized cell wall [2] (Fig. 1A), and have observed cells using a method that allows visualizing and localizing individual fluorescent molecules. This method is known as "direct STochastic Optical Reconstruction Microscopy" (dSTORM) [3] and provides a spatial resolution of about 30 nm (Fig. 1B and C). We obtained geometrical and kinetic parameters of cell wall synthesis and remodeling from nanoscale-resolution data collected on the PALM/STORM microscope of the M4D cellular imaging platform of the PSB. We further used these data to simulate the morphogenesis of the ovoid cell *in silico*. Altogether, experimental and modeling analyses revealed how cell wall assembly and remodeling correlate with cell morphogenesis [1].

In particular, our work shows that cell wall synthesis dedicated to cell division and elongation progress separately along the cell cycle and that elongation persists after division is completed. It also provides evidence that division wall is synthesized and remodeled from the start of the cell cycle, through cleavage and insertion of elongation wall at its periphery (Fig. 1D).

This work not only opens new concepts regarding cell wall synthesis and morphogenesis in bacteria, but also provides a new methodological approach that can be exploited in different ways. For example, it can be used on mutant cells to understand the function of elongation and division proteins, or in combination with cell wall-targeting antibiotics to understand their mode of action. More generally, this approach can be adapted through the development of specific biorthogonal probes to study other cellular processes in all fields of life.

J. Trouvé and C. Morlot (IBS)

[1] J. Trouvé, A. Zapun, C. Arthaud, C. Durmort *et al.* (2021). Current Biology, in press.
[2] M.S. Siegrist, B.M. Swarts, D.M. Fox, S.A. Lim *et al.* (2015). FEMS Microbiology Reviews 39, 184 202.
[3] S. van de Linde, A. Löschberger, T. Klein, M. Heidbreder *et al.* (2011). Nature Protocols, 6, 991 1009.



A. Schematics of metabolic cell wall labeling using a clickable probe and fluorescent dye. **B.** Cell mounting between a glass slide and a coverslip allows a side view of the labeling (top). Cell trapping in a microhole allows an annular top view of the cell wall synthesis region (bottom). **C.** Composite images of *S. pneumoniae* cells labeled at mid-division, showing the cell wall synthesis region at low-resolution using conventional fluorescence microscopy (top) and at high-resolution using dSTORM (bottom). **D.** Illustration of one of the outcomes deduced from this work: division wall synthesized at midcell (blue) is remodeled through cleavage and insertion of elongation wall (orange) at its periphery.

New insights into the catalytic core of the Integrator complex

The Integrator is a multi-subunit protein complex, which was discovered as the specialized 3'-end processing machinery required for maturation of small nuclear RNAs (snRNAs) [1]. However, recent studies highlight its role as a general transcription regulator of promoter-proximal RNA Polymerase II (RNAPII). Integrator recognizes specific states of the C-terminal domain of RNAPII and modulates its activity by dephosphorylation of RNAPII and its associated factors. A second catalytic activity is found in its nuclease module, which can release nascent transcripts.

In order to gain insights into Integrator regulation and how its nuclease specificity is achieved we characterized its general architecture and determined a cryo-EM structure of its nuclease module. Due to its large size (16 subunits – INTS 1-14 and PPP2CA, PPP2R1A), and lack of information about potential subcomplexes we set out on a "divide-and-conquer" approach that would allow studying the Integrator in smaller pieces. For this we established a protocol to analyse the interactome of each Integrator subunit and discovered multiple modules in vivo. INTS4/9/11, INTS5/8 and INTS10/13/14 were confirmed as stable subcomplexes *in vitro* by recombinant expression in insect cells with support from the Eukaryotic Expression Facility. Further biochemical characterization revealed additional contacts between INTS4/9/11 and INTS10/13/14, and showed that all identified modules are capable of binding putative RNA substrates.

Next, we determined the cryo-EM structure of the Integrator nuclease module consisting of the MBL/β-CASP nucleases INTS9 and INTS11, and the mostly helical scaffold INTS4. Structure determination proved to be difficult due to missing angles in the reconstruction, and was only possible with help from ESRF CM01 and EMBL Cryo-EM core facility. In the end, we obtained a 3.5 Å reconstruction that allowed *de-novo* modelling. INTS9 and INTS11 form a heterodimer with a pseudo-two-fold symmetry axis along its interface (Fig. 1). Despite the close proximity of the nuclease domains no direct interactions could be observed. However, their respective C-terminal extensions are strongly intertwined and form two extensive dimerization interfaces (CTD1 and CTD2). In addition, we were able to identify a novel domain in INTS9 which we named INTS9 Accessory Domain (NAD). INTS9^{NAD} is formed by two non-canonical inserts into the MBL domain, which are separate in sequence and conserved from fruit fly to man. INTS4 interacts strongly with the nuclease domains of INTS9 and INTS11, and their composite region CTD1. By performing point mutations that disrupt the barrel-like tertiary structure of CTD1 but do not interfere with its contacts to INTS4 we could show that proper folding of CTD1 is required for binding of INTS4. Likewise, we could confirm that CTD1 is not required for dimerization of the nuclease heterodimer but relies solely on CTD2. Therefore, we proposed a mechanism of sequential dimerization of INTS9/11, where INTS4 binds only to a fully assembled nuclease heterodimer, highlighting a key step in the regulation of Integrator nuclease activity.

M. Pfleiderer and W. Galej (EMBL)

[1] D. Bailat *et al.*, (2005) Cell, **123**, 265-276.
[2] M.M. Pfleiderer and W.P. Galej (2021) Molecular Cell, **81**, 1246-1259.

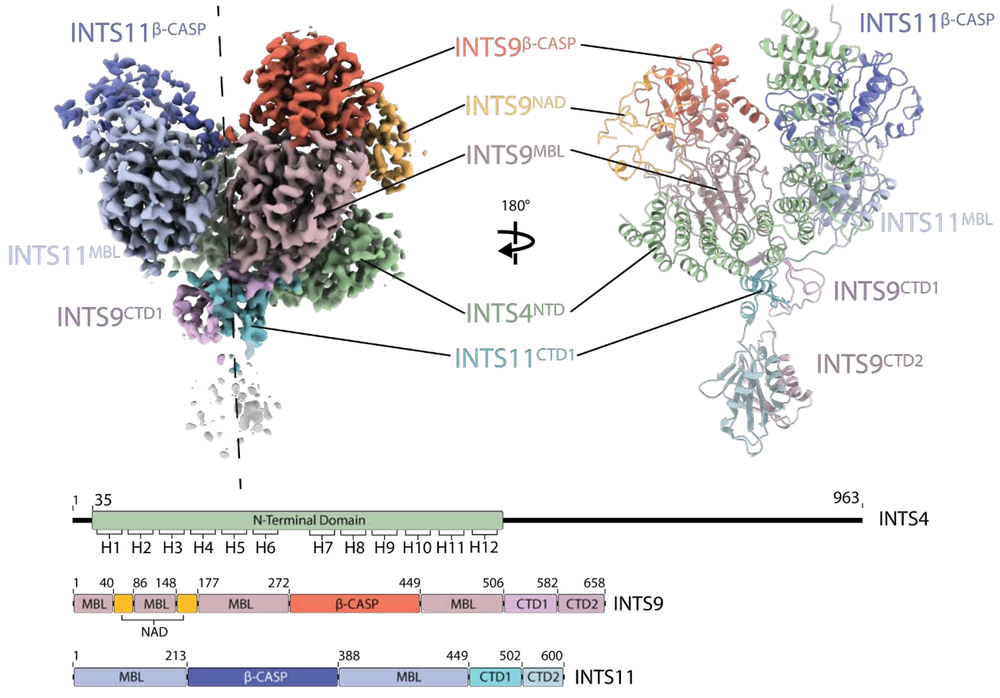


Figure 1. Cryo-EM map and atomic model of the INTS4/9/11 complex colored by subunit and domain identity. The pseudo two-fold axis is indicated as a dotted line.

The coronavirus structural taskforce

Methods developers in structural biology usually work far from the spotlight. When the COVID-19 pandemic began, we asked ourselves: How can we contribute to the fight against the virus? The Coronavirus structural taskforce [1] was born!

As early as February 2020, we started evaluating the structures of macromolecules in SARS-CoV and later SARS-CoV-2 available from the Protein Data Bank and found ample room for improvement. We set up a website and a database containing our evaluations and revised models. We have established an automatic structure evaluation and revise individual structures on a weekly basis. We also engage in outreach activities, writing blog posts about the structural biology of SARS-CoV-2 aimed at both the scientific community and the general public, refining structures live on Twitch and offering a 3D-printable virus model for schools.

In the beginning, there were no tenured academics in the Coronavirus Structural Task Force; we were an ad hoc collaboration of mostly junior researchers across nine time zones, lacking management experience, computing facilities and administrative infrastructure. Still, we were able to rapidly establish a large network of COVID-19-related research, forge friendships and collaborations across national boundaries, spread knowledge about the structural biology of the virus and provide improved models for in-silico drug discovery projects. More information about our contribution to fight the pandemic on our website, https://insidecorona.net!

G. Santoni (ESRF)

[1] T.I. Croll, K. Diederichs, F. Fischer *et al.* (2021) Making the invisible enemy visible. *Nat. Struct. Mol. Biol.*, **28**, 404.

News from the platforms

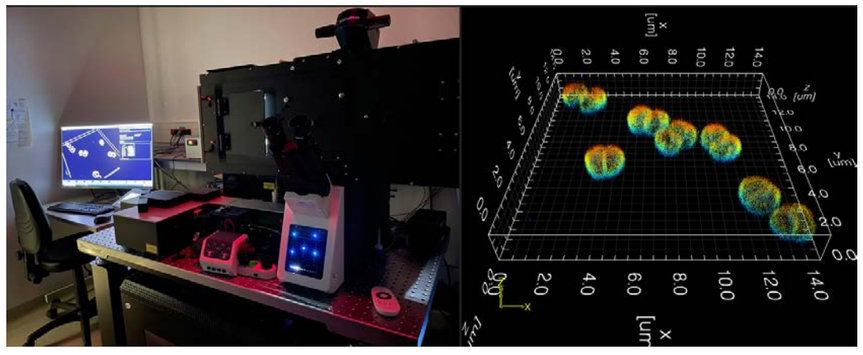
New super-resolution microscope on the M4D platform

Thanks to the support of GRAL, a new super-resolution microscope, with stunning capabilities for nanoscale imaging of biological samples, is now available at the M4D cell imaging platform to satisfy a high users' demand. Located at the IBS, the platform is part of the ISBG facilities. This state-of-the-art instrument completes the conventional time-lapse video fluorescence microscopy, confocal imaging, and flow cytometry analysis offerings, and reinforces the super-resolution capabilities of the platform, beside our current home-made PALM microscope that will still be open for specific applications and future developments.

The new instrument, a Safe360 from Abbelight™ and Olympus, offers exclusive multicolor 3D super-resolution in STORM/ PALM and PAINT modalities, with single-particle tracking capabilities. The system is driven by a 6 high power lasers combiner and an EPI/HiLo/TIRF illumination module. Two high speed and high sensitivity sCMOS cameras can acquire more than 100 frames per second to drastically reduce the acquisition time.

This instrument is accessible to users from the PSB and CEA laboratories, but is also accessible to industrial partners and all academic users as part of the large European INSTRUCT-Eric network. Oleksandr Glushonkov recently joined the platform as an engineer (see Newcomers). With his help, we will be able to better support the platform users in their imaging, and in particular, super-resolution projects.

J.-P. Kleman, F. Lacroix and O. Glushonkov (IBS)



Left: Newly installed Safe360 setup available at the M4D platform. Right: 3D super-resolution imaging of *Deinoccus radiodurans* bacteria using PAINT (Nile Red labeling of the membrane). The image was reconstructed using the ViSP software from 5 stacks (shown in different colors) of 40,000 frames acquired at different Z positions (spaced by 400nm).

Status of the Structural Biology beamlines at the ESRF

The ESRF beamlines operated by the ESRF-EMBL Joint Structural Biology Group (JSBG) are fast approaching the first year of continuous user operation since the ESRF Extremely Brilliant Source upgrade. Since restarting on August 25th, 2020 the JSBG beamlines (ID23-1, ID23-2, MASSIF-3 & ID30B) have supported 598 experiments in mostly remote, but also mail-in (BM29 & CM01) and fully automated (MASSIF-1) operation modes. The significant efforts in developing MxCuBE3, BsxCuBE3, Extended ISPyB and the BES over the years have been particularly important in providing users with access to the JSBG beamlines. In addition, the Cryo-Electron Microscope on CM01, operated as a Partnership for Structural Biology (PSB) endeavour, has continued to provide mail-in user experiment support for more than 60 experiments during the pandemic. All the staff of the JSBG beamlines and of CM01 have provided invaluable support to external users continuing their research, including some COVID-19 projects aimed at helping to fight the pandemic. This is a major achievement in these challenging times and a credit to everyone involved.

Despite these challenges many improvements have also been implemented on the JSBG beamlines in the last 6 months. In particular the EMBL-ESRF co-developed FlexHCD sample changers have all converged to using the same control software developed by the EMBL Instrumentation team. ID23-1 has also undergone a significant instrument and control software upgrade comprising the successful installation of a MD2S diffractometer and the newest X-ray detector technology available, a 16M EIGER2X with CdTe sensor from DECTRIS. Following their successful commissioning these are now available to users, enabling the use of high energy X-rays for data collection that could help alleviate X-ray radiation damage. More information can be found on the ID23-1 webpage or by contacting the beamline team, Gianluca Santoni and Sasha Popov, for more information on the options available. Meanwhile, on MASSIF-1 a CrystalDirect™(CD) harvester has been installed by the EMBL instrumentation team with support from the ESRF and EMBL HTX and Synchrotron Crystallography teams. The integration of the CD on MASSIF-1 is currently underway and, once fully commissioned, the device will facilitate many new types of diffraction experiments. This project will also enable a higher level of integration between the operation of MASSIF 1 and the HTX Facility. Finally, on CM01 there has been a significant upgrade to the Titan electron microscope following the successful installation of a new generation GATAN K3 direct electron detector. The final commissioning for this is currently underway. Together with the recent control software upgrade to EPU2, these upgrades will provide CM01 users with better and faster data collection options.

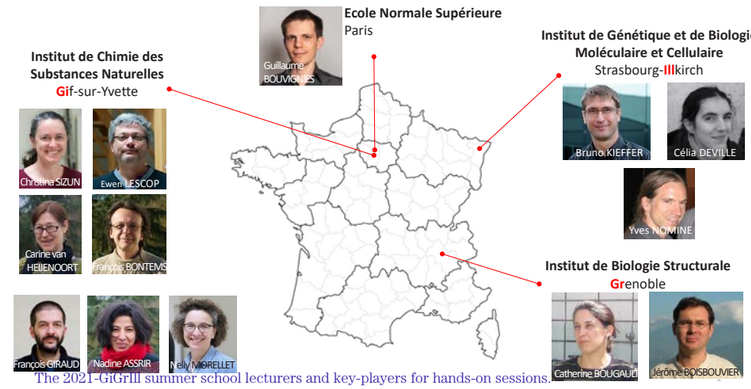
C. Mueller-Dieckmann (ESRF) and A. McCarthy (EMBL)

The recently refurbished ID23-1 experimental setup with new DECTRIS EIGER2 X CdTe 16M detector and Arinax MD2S diffractometer.

EVENTS

FRISBI (GiGrIII) 2021 – NMR and Biological Interaction Summer School

Linked by the National Infrastructure of Research (IR-RMN, FR3050 CNRS) in Very High Field Nuclear Magnetic Resonance (NMR), NMR labs at IBS and ICSN launched in 2009 a set of national summer schools with hands-on sessions in structure and interactions by NMR intended for young scientists and researchers in the industry. The NMR lab at IGBMC in Strasbourg joined in 2013 completing the offer with state-of-the-art methods in labeling strategies and sample preparation.



The 2021-GiGrIII summer school lecturers and key-players for hands-on sessions.

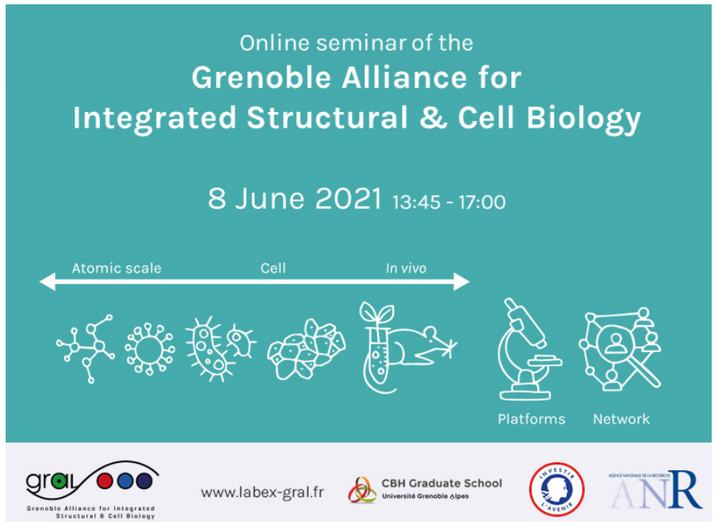
Canceled due to the COVID-19 pandemic in 2020, the 2021-session on "NMR and Biological interaction" is taking place in a new post-COVID format. The first 2.5-days session was offered on-line from May 10th to 12th to 20 PhD-students, 7 researchers (from industry and academia), 3 postdoctoral fellows and 2 M2 trainees. Virtual interactive lectures developed fundamental aspects of NMR spectroscopy before more in-depth discussions of structural and dynamical information on protein-ligand interactions and large protein complexes. The second session is programmed for 18 of the participants in the NMR facility of ICSN from July 5th to 7th. Hands-on data collection on the local spectrometers and data analysis are planned for this session that has just been authorized by CNRS. Organizers hope attendees will be as enthusiastic and inclusive as during the first session. This 2021-edition was supported by the French Infrastructure for Integrated Structural Biology (FRISBI) and is part of the national RéNaFoBiS initiative for an education in structural biology.

C. Bougault (IBS)

GRAL's first online seminar

The Grenoble Alliance for Integrated Structural & Cell Biology (GRAL, www.labex-gral.fr) organized a half-day online seminar on June 8th. The seminar started with a short introduction about GRAL and the Chemistry Biology Health Graduate School (http://grad-chembiohealth.univ-grenoble-alpes.fr/) of Université Grenoble Alpes, which GRAL is part of since 2018.
Anne-Mathilde Thierry (GRAL) and Léa Zambrano (CBH Graduate School) presented GRAL and CBH Graduate School's missions and funding opportunities, including incoming and outgoing mobility grants, Master and PhD scholarships, support for technology transfer, and funding for student projects.

The 12 PhD students currently funded by GRAL presented their projects: Lorenzo Gaifas (IBS), Olivia Garnier (Bio-Santé), Andrea Catacora Grundy (LPCV), Adèle Renier (IBS), Shaghayegh Askarian Amiri (IBS), Jip Wulffele (IBS), Lenette Kjaer (IBS), Vaitson Çumaku (BioSanté), Rory Munro (IBS), Raphaël Dupeyron (IBS), Hussein Daher (IBS), and Mohammad Al Tarass (BioSanté).



The presentations of the PhD students were followed by an invited talk from Barbora Kozlikova from the Faculty of Informatics of Masaryk University (Czech Republic), about her work on the visual approaches to the exploration of molecular dynamics simulations.

Over 80 participants from the 4 GRAL labs (IBS, BioSanté, LPCV and LCBM) attended the seminar. Participant feedback shows that there is a wish for other webinars, which GRAL will gladly organize in the future. GRAL also hopes to soon start planning the organisation of the next 2-day in-person seminar in spring 2022.

A.-M. Thierry (GRAL)

Grenoble Drug Discovery Club kick-off event

Motivated by the growing impact of drug discovery in research, several researchers from Grenoble-based institutes (EMBL Grenoble, IBS, ESRF, IRIG-CEA, DPM, DCM, IAB and CERMAV) recently launched the Grenoble Drug Discovery Club (https://grenobledrugdiscovery.fr), inspired by the successful Grenoble Epigenetics Club (https://epigenetics.fr) and Grenoble Host-pathogen Interactions Club (https://hostpathogen.fr).

This initiative aims to bring together a large community of scientists from the University of Grenoble Alpes and the GIANT campus involved in translational biology, and in platforms and facilities relevant to bioactive molecule discovery and drug design. A kick-off meeting was organized by videoconference on 18 May 2021, and the programme included presentations by three main invited speakers, Nelly Dubarry (Evotec ID SAS, Lyon), Didier Roche (Edelris), and Benoit Deprez (University of Lille), as well as six selected short talks by local scientists. This first meeting was a success and gathered more than 120 participants.

For further information about the club, upcoming events, and to register to the mailing list, please check the club's website mentioned above.

F. Bernaudat (PSB)

Les Houches - TSRC Protein Dynamics Workshop

This workshop is held biennially since 2014 at the Ecole de Physique des Houches. Normally organized as a five-day in-person meeting with a spectacular view on the Mt. Blanc massif, this workshop gathers about 70 people to exchange on hot topics in the field of protein dynamics, including many experimental, numerical and theoretical approaches within a very interactive format.

Postponed from 2020, this edition was finally turned into an online meeting, on May 18th and 19th, with ca. 320 registered participants and 5 "plenary" lectures of 45 minutes and 15 minutes of lively discussions: Dorothee Kern, Benoit Roux, Claus Seidel, James Fraser and Frank Noé presented latest results on evolution of protein dynamics, MD simulations, single-molecule FRET, advanced ensemble-refined X-ray crystallography and machine-learning applied to protein dynamics, respectively. The talks are available on the youtube channel of IST Austria (www.youtube.com/user/ISTAustria), which kindly supported this conference. A particular success were the two one-hour long poster sessions (in online breakout rooms) with intense discussions.

The next Protein Dynamics Workshop will be in-person in May 2022 in Aussois (https://sites.google.com/view/houches2020/workshop-2022).

P. Schanda and M. Weik (IBS)

31st ESRF User meeting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The annual ESRF User Meeting 2021 was held online from 8th to 10th February 2021, dedicated to the ESRF user community: learn about, discuss and experience the science and cutting-edge research made with ESRF synchrotron light. UM2021 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 and the usage of the new MXCuBE3 interface and workflows. A plenary session was held on day 2, with a lecture by Edith Heard (Director General EMBL) on Science of Women.

Three User-Dedicated Microsymposia (UDMs) completed the UM2021 activities.The UDM1 entitled "Emerging viral diseases: How can structural studies help to seek remedies?" was organised by Isai Kandiah, Christoph Mueller-Dieckmann, Narayan Theyencheri (ESRF) and Marina Mapelli (ESRF User Organisation). This symposium brought together 80 participants from interdisciplinary fields to discuss and stimulate exchange among ESRF Structural Biology users on fundamental virus research. The symposium comprised three plenary lectures and four selected contributions from submitted abstracts. Taken together, UDM1 provided an excellent overview of cutting-edge virus research and new perspectives, particularly for SARS-CoV2, in the use of beamlines that can fully exploit the unprecedented capabilities of the EBS.

The winner of the ESRF Young Scientist Award was Valentin Borshchevskiy shown in the accompanying photo, researcher at the Moscow Institute of Physics and Technology and who did his PhD at the IBS, for his outstanding contribution to structure-based functional studies of membrane proteins.

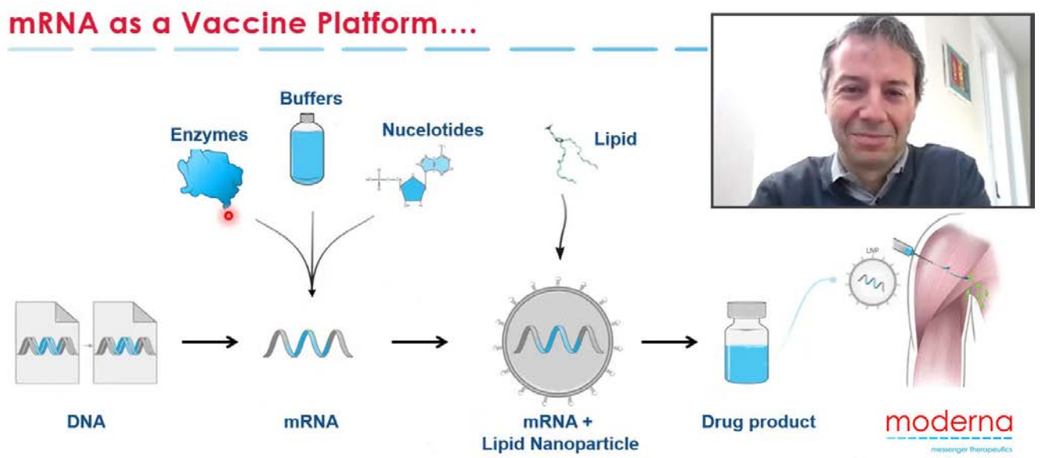
M. Soler López (ESRF)

Grenoble Host-Pathogen Spring Meeting with Guest lecturer Andrea Carfi from Moderna

While the vaccination campaign was ramping up in France last winter, excitement was in the air at the Grenoble Host-Pathogen Club on 30 March 2021, when Andrea Carfi, Vice-President and Head of Research for Infectious Disease at Moderna, Cambridge, MA, USA, gave a webinar entitled "Development of the Moderna mRNA vaccine against COVID-19". The talk attracted over 360 viewers, with the speaker receiving an especially warm welcome as an IBS alumnus. After receiving his doctorate under the direction of (now retired) Otto Dideberg at the IBS in 1997 Andrea completed his training as a postdoctoral fellow at Children's Hospital (Harvard University) in Boston, MA, where he met the current IBS director Winfried Weissenhorn. Andrea then moved to industry, joining Merck in 2002. He returned to Cambridge (USA) in 2010 as a senior manager first at Novartis Vaccines and then at GSK vaccines. He joined Moderna in 2017, where he contributed to the development of Moderna's COVID-19 mRNA vaccine.

If you missed it or would like to watch it again, Andrea's webinar can be viewed here: https://youtu.be/3cyVwS9WyJY

F. Bernaudat (PSB)



Hercules 2021

The 2021 edition of the Hercules European School, organized every year since 1991 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 in a fully online form from February 22 to March 26. The school provided PhD students, postdoctoral and senior scientists coming from European and non-European institutions with lectures, practicals and tutorials on the use of neutrons and synchrotron radiation (integrated by a large number of other experimental techniques) for condensed matter studies, including physics, chemistry, biology, geology and materials science. The traditional five weeks format has been maintained, with the "outside Grenoble" week that students typically spend in different partner institutions (this year ALBA in Barcelona, KIT in Karlsruhe, DESY and European XFEL in Hambourg, ELETTRA and FERMI in Trieste, SOLEIL in Paris-Saclay, and PSI in Villigen) organized as virtual visits with both tutorials and experimental activity.

Whether the 2022 edition will be held online or it will host the students in Grenoble is currently a matter of discussion among the members of the organizing committee, who will continue monitoring the evolution of the Covid pandemic. Stay tuned!

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

Tracy Brown – "Challenging the Misrepresentation of Science and Evidence in Public Life"

Social scientist Tracy Brown OBE from Sense about Science gave an EMBL Science & Society virtual seminar on the 26th of February. Tracey relayed compelling stories about the power of educated public citizens, the inherent limitations of incomplete or distorted scientific information, and how the COVID-19 pandemic has brought the need for publicly shared, evidence-based science to the fore. A recording of the seminar can be found here for those who were unable to make it.

A. McCarthy (EMBL)

PROFILE

Paul Schanda

For those who don't know you so well, could you tell us in a few words who you are and what your scientific interests are?

I would consider that I am a physical chemist by training, with a strong interest in fundamental biological and biophysical questions. I am particularly motivated to understand how protein dynamics is linked to protein function. In that vein, I love deciphering how chaperones work (highly dynamic!) and how enzymes perform their tasks. All these proteins must be dynamic, yet this aspect is a challenging physico-chemical "puzzle".

What brought you to Grenoble and to IBS?

I seem to be attracted again and again by Grenoble. I came the first time as an Erasmus student in 2001. I thought that I would probably not come again. I came the second time to Grenoble in 2004 for 3 years to do my PhD (admittedly, also driven by a person who I hardly knew then and with whom I have been married for over a decade now). I thought that I would probably not come again. I came the third time to Grenoble after my post-doc at ETH in 2011, with an ANR funding (Retour-postdoc) and then an ERC starting grant, until now. So now I don't make predictions any more… will there be another, fourth, Grenoble period? In all cases, what brought me here was an exceptional and outstanding mix of great scientific opportunities and an unbelievably nice region. I will really miss all the nice people, good friends and the beautiful surrounding mountains!

In what way has the PSB contributed to your research projects?

The concentration of techniques and experts in Grenoble is really remarkable. In my view, most structural biology projects nowadays become much stronger when multiple approaches are combined. In my projects, I have had the chance to use SAXS, AUC, X-ray diffraction, cryo-EM, negative staining EM, MS, and the crystallization robot (and probably I forget a few here!). These techniques were essential for many projects. At IBS, I also gained a lot of expertise in NMR thanks to the great engineers that run the NMR facility, who are both highly competent and friendly. Not to forget lots of seminars from international people. It's a unique place, at least in France.

What do you consider to be the major strengths of the PSB? What aspect(s) of the PSB will you most miss?

A major strength of the PSB is the easy access to a wide range of techniques on the same site. I may miss the numerous platforms, although I will have access to a similar system in my future position at the Institute of Science and Technology (IST) in Austria. The PSB also offers plenty of opportunities to interact with other scientists, notably during the PSB Get Together sessions during which members of all partner institutes meet around a few drinks and some posters to discuss their scientific projects. These initiatives are important to spark discussions!

What is your best memory of your time at IBS?

I have taken part in several rewarding projects during the past 10 years. In fact, one of these actually started with a major failure – a denatured membrane protein! But eventually after much struggle, we started to work on membrane protein chaperones to help in the folding of this challenging target, and this has now developed into one of my main research interests, i.e., folding and transport of mitochondrial membrane proteins. I realize that my research interests have progressively moved from method development to biologically relevant projects that are also much more ambitious and complex.

What has been the most important evolution in the NMR field since you started? How do you expect NMR, applied to structural biology, to evolve in the coming years?

I would argue that NMR is the most versatile structural biology technique. This is not to say that it is the best technique, but it is remarkable which different aspects one can look at. Structure determination is probably not its greatest strength (in part due to lack of automation), but NMR has the ability to provide insight into many functional details that the structure alone will not tell you, such as the observation of short-lived transient states. Important evolutions include: (i) techniques that allow to see transient states that have only minute population (few percent) and life times of milliseconds, and even determine their structures, (ii) the development of solid-state NMR, and its maturation to study large protein complexes and (iii) the emergence of NMR on living cells or native membranes. During my post-doc we were proud to determine a structure of a 8 kDa protein in a crystal by this technique. Now we just determined the structure of a 468 kDa oligomeric enzyme complex from solid-state NMR data, and figured out how dynamics of a so-far overlooked loop control its function. And we understand now mechanistic details of the assembly of a tube-forming protein of 50 kDa. It is rewarding to see that continued methods development keeps pushing the frontiers of what we can see (protein size and level of detail). I take this opportunity to say that I truly hope that IBS will continue developing solid-state NMR after my departure. We have established this technique (several collaborations, international visibility and specific solid-state NMR equipment worth about 1 M€!). It would be a real shame if this were simply abandoned. In the future, I expect that NMR will move towards the study of samples in "in vivo" settings such as membrane proteins in native lipid bilayers or NMR of intact cells. A goal in my future lab will be to combine NMR with electron paramagnetic resonance (EPR) and single-molecule FRET to study membrane proteins in isolated mitochondria for example.

How could the visibility of NMR be improved within the PSB and more broadly within the structural biology community?

At the international level, I would say that NMR is fairly visible and at IBS, it has attracted 4 ERCs and has a very strong research output. But, within the PSB, it is still lagging behind X-ray crystallography and cryo-EM. Maybe the more relevant question is: how could NMR be even more useful for the local community? NMR is powerful but complex, and depending on the question, it may be long to reach an answer. As it can do so many different things, it takes time to think of the best approaches, and it takes time to collect and analyze data. Given the French funding system, I generally find myself doing such collaborative projects by myself. This is not very efficient. I expect that a system with a bit more manpower and flexibility to work spontaneously on a "hot" project (rather than having to wait for dedicated funding) would allow us to respond most positively to requests from the scientific community.

What are your future plans? Where will you go now and will you pursue the same research projects?

I will start a group at IST Austria, near Vienna, as a full Professor. This position will involve some teaching, mostly to graduate students from IST. IST is a fundamental research institute covering broad, interdisciplinary fields of research. It was set-up approximately 10 years ago and now brings together 55 groups, including 5 dedicated to structural biology. It's a great challenge, as I have to build the NMR facility from scratch there. Three new high-performance instruments will be delivered in the coming months. In the long run, I intend to widen my topics of research in collaboration with groups at IST Austria and in the fairly large biology community in the Vienna area, but first I will start with projects that I have initiated here, including the mechanisms of mitochondrial protein import and membrane-protein insertion into mitochondrial membranes, as well as enzymatic mechanisms. I have even just started a new collaboration with IBS - a good excuse to come back and visit, by the way!

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

Newcomers

Estelle Mossou joined the ESRF in March 2021 as a Structural Biology Beamline Scientist to develop pipelines for room-temperature data collection with microfluidic crystallization chips. Estelle obtained her PhD on the study of self-assembled filamentous systems by X-ray and neutron diffraction from Keele University (U.K). Followed by a postdoc on the development of common neutron and X-ray methods for crystallography at the ILL, she worked on D19, a monochromatic thermal diffractometer at the ILL, dedicated to small molecule and small protein crystallography as well as fibre diffraction.

Oleksandr (Sasha) Glushonkov recently joined the M4D imaging platform at IBS as an engineer to reinforce the expertise in super-resolution microscopy. As such, he will help the users to perform their experiments and analyse their data, and will contribute to new developments on the platform.

Véronique Mayeux joined EMBL Grenoble in April 2021 as the new joint EMBL and CIBB Safety Engineer. Véronique obtained a PhD in molecular and cellular biology in 1995 at the University of Montpellier, France, and then moved to the UK for a 3.5 years post doc. Back in Montpellier, she first worked on the motor neuron as research fellow for a privately funded research association, before preparing a Masters of Science on Risk Management that included an internship at the regional department of health and social affairs (DRASS) to study the impact of hospital wastewaters on the environment. In 2006, she was hired at ESRF as Biology Safety Engineer until early 2021, but she is already a known face for many as she was also then strongly implicated in the CIBB for the training of newcomers.

ANNOUNCEMENTS

Rebekka Wild started an ATIP-funded Junior team at IBS in January 2021. Her team is part of the Structure and Activity of Glycosaminoglycan Group (SAGAG) and focuses on understanding glycosyltransferases and glycan-modifying enzymes at a molecular level. The aim is to dissect the architecture and catalytic mechanisms of these enzymes by combining structural biology approaches, in particular single-particle cryo-electron microscopy, with *in vitro* functional and biophysical assays and *in cellulo* studies.

The France CryoEM ANR Equipex+ project has been successful in receiving funding which will enable the purchase of three 300 kV microscopes to be installed in Grenoble, Paris-Saclay and Strasbourg. On the EPN campus, the new microscope to be located at ESRF will be operating in CRG mode by the IBS. Expected delivery second half of 2022.

DATES FOR YOUR DIARY

29th November to 3rd December 2021 – 2nd Integrative Structural Biology (BSI) Meeting in Paris-Saclay.

The second French Congress on Integrative Structural Biology (BSI), jointly organized by the Association Française de Cristallographie (AFC) and the Société Française de Biophysique (SFB), will take place this year from November 29th to December 3rd 2021 on the Paris-Saclay campus. The scientific program is available on the congress website (https://bsi-2021.cnrs.fr/). Registration will be open soon.

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