PSB et al

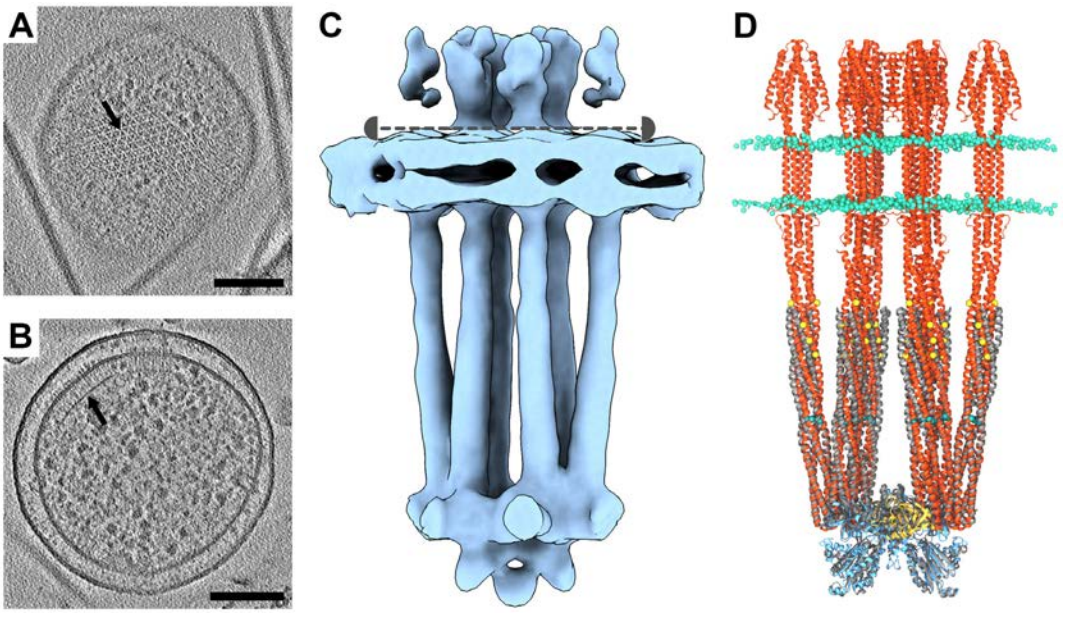
Partnership for Structural Biology Newsletter

Scientific highlights

Moving Structural Biology into the Cell

Structural biology techniques such as X-ray crystallography and cryo-electron microscopy (cryo-EM) require purified protein samples, removing the macromolecule of interest from its native biological context. A reasonable question to ask is thus - "If my purified protein looks like this… what does it look like in the cell?"
Cryo-electron tomography (cryo-ET) can help us to answer this question. Using the same microscopes employed by single particle cryo-EM, we collect multiple projection images, rotating the sample between exposures. These images are then reconstructed computationally to produce a 3D image, a tomogram, of a vitrified biological sample. In order to obtain higher-resolution insights into objects present in the tomogram, we locate, align and average objects together in an iterative process called subtomogram averaging.

Samples for cryo-ET must be thinner than ~500nm. In collaboration with William Margolin (UTH, Texas, USA) our group developed and optimised the production of *E. coli* minicells, resulting from aberrant cell division close to the bacterial cell poles. We then imaged the minicells, focusing on obtaining images of chemotaxis receptor arrays. These arrays allow bacteria to sense and adapt to environmental change, biasing movement towards more favourable external conditions. The large hexagonal arrays of signalling complexes are embedded in the inner membrane of the cells. Once we imaged the cells with a dedicated microscope at eBIC (Diamond, UK), we implemented a pipeline which eventually allowed us to reconstruct the core signalling unit of these arrays *in situ*.
Our 3D map allowed us to visualise, for the first time, the entire complex embedded in its native environment [1]. The map showcases the periplasmic ligand-binding domains, full-length receptor structures linked by a baseplate of kinase proteins ultimately responsible for the direction of flagellar motor rotation. Armed with our reconstruction, Keith Cassidy (University of Oxford, UK) was able to produce the first molecular model of the entire core signalling unit by combination of previously published experimental data and molecular dynamics flexible fitting methods. This model allows interpretation of the reconstruction and paves the way for further investigations into the ultrastructure of this dynamic macromolecular machine. Finally, Sandy Parkinson (University of Utah, USA) and his group set out to biochemically characterise our minicell strain, verifying that the cells were indeed functioning correctly in terms of their chemotactic signalling.
Being able to look at macromolecular machines directly in the cell like this is just now becoming possible. The possibilities that are open to us now that we understand this technique are really exciting, to quote Richard Feynmann…
"It is very easy to answer many of these fundamental biological questions, you just look at the thing!"



Structure of the chemosensory array core signaling unit. **A.** Slice through a 3D image of a minicell showing the hexagonal packing of a chemoreceptor array (scale 100 nm). **B.** Slice through a 3D image of a minicell showing a side view of a chemoreceptor array embedded in the inner membrane (scale 100 nm). **C.** The reconstructed structure of the chemoreceptor array core-signalling unit. Separator marks different isosurface thresholds. **D.** Atomic model of the entire core signalling unit. The extent of previous models is shown in grey.

A. Burt & I. Gutsche (IBS)

[1] A. Burt, C. K. Cassidy, P. Ames, M. Bacia-Verloop, *et al.* (2020). Nature Communications **11** (1) p.743.

CONTENTS

Visualizing the entire transcription cycle of influenza polymerase

Influenza (Flu) virus causes seasonal epidemics of respiratory disease that have enormous socio-economic impact worldwide. Due to rapid evolution of the virus in the large bird and animal reservoir, novel highly pathogenic viruses with pandemic potential occasionally emerge, as last happened in 2009. Despite the existence of moderately effective vaccines and drugs, the development of alternative therapies is key in preparedness for the next pandemic, which could be at least as devastating as the current COVID-19 crisis.

Influenza virus has a negative-sense RNA genome (vRNA) and the first step in infection is to transcribe this into viral mRNA. Transcription is performed by the virally encoded RNA-dependent RNA polymerase (FluPol). It starts with the interaction of FluPol with the cellular RNA polymerase II enabling FluPol to pirate a capped RNA primer in a mechanism called "cap-snatching". The primer is then rotated into the FluPol active site cavity and mRNA synthesis commences with the vRNA as template. Post elongation, when almost the entire template has been copied and translocated through the active site, a 5' terminal RNA secondary structure in the template (known as the 5' hook) remains tightly bound to FluPol, leading to poly(A) tail formation by a stuttering mechanism, the details of which remain elusive. By these unique mechanisms the virus produces translation-competent 5' capped and 3' polyadenylated mRNA for subsequent production of viral proteins.

Building on previous results that focused on early transcription [1], we set out to visualize the entire transcription cycle in our most recent work [2]. To stall actively transcribing FluPol at distinct steps, we optimized transcription reactions with model vRNA and a combination of natural and chain-terminating modified nucleotides. In vitro transcription products were confirmed by next-generation RNA-sequencing in collaboration with the GeneCore Facility at EMBL Heidelberg. To obtain structures, we used X-ray crystallographic data from ID30-A3 at ESRF and cryo-electron microscopy (cryo-EM) images collected at EMBL Heidelberg and processed on the EMBL-IBS computer cluster. This yielded nine high-resolution (up to 2.4 Å) cryo EM and one crystal structure corresponding to snapshots of different points along the pathway. We were then able to deduce a 'molecular movie' of the complete transcription cycle from pre-initiation through elongation and termination/poly-adenylation until product dissociation and polymerase recycling (Figure 1).

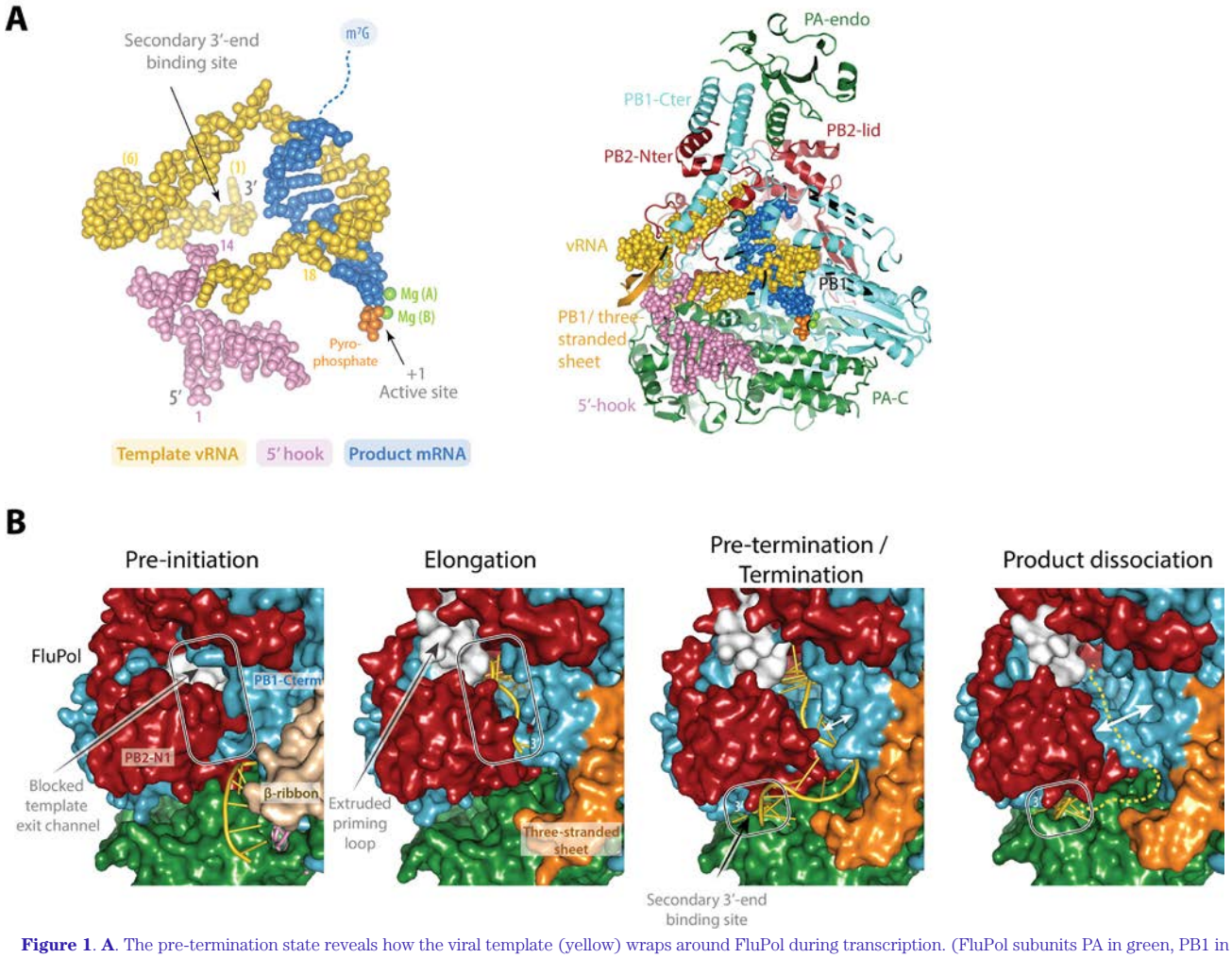


Figure 1. **A**. The pre-termination state reveals how the viral template (yellow) wraps around FluPol during transcription. (FluPol subunits PA in green, PB1 in blue and PB2 in red).
B. Conformational changes underlying subsequent steps of viral transcription. FluPol is represented as surface (colored as in panel A, except for PB1/priming loop in grey) and viral RNA template as cartoon in yellow.

As FluPol transitions from initiation to processive elongation, a number of conformational changes occur as the template 3' end translocates through and out of the active site. These include extrusion of the priming loop, which initially blocks active site cavity, and a rotation of the PB2-N domain to open the template exit groove on the FluPol surface (Figure 1B & 2). The translocating vRNA template then navigates along a positively-charged channel wrapping around the polymerase core to finally dock in a 'secondary binding site' that accommodates specifically the conserved sequence of the template 3' end. The template extremity stays bound here during all subsequent steps. At termination, when uridine 17 (U17) from the 5' end of the template is in the active site, further template translocation can no longer occur due to the tightly bound 5' hook. The polymerase repeatedly copies U17 by flipping it in and out of the active site through concerted template backtracking and product slippage leading to poly(A) tail formation. Upon product release, the template strand is still threaded through the protein and a complex conformational rearrangement has to occur to release it. Upon template release, the pre-initiation RNA promoter can re-establish by flipping of the template 3' end from the secondary binding site to the active site cavity and the next round of transcription can commence.

During the whole cycle FluPol remains bound tightly to both 3' and 5' extremities of the template. The model explains how multiple mRNA transcripts are synthesized from a single incoming copy of template bound FluPol without protein-template dissociation. This is critical very early in infection when there a very few viral genome copies.

Overall our results highlight the template trajectory and dynamic protein changes throughout transcription and provide a mechanistic understanding of polymerase stuttering. Moreover, we provide an understanding of the role of 3' end secondary binding site in assuring efficient multi-cycle transcription which is likely to be a common feature of segmented negative-sense RNA viruses as analogous binding sites were also observed in bunya virus polymerase [3]. Finally our results will be useful for novel anti-viral drug development.

J. Wandzik, T. Kouba and S. Cusack (EMBL)

[1] T. Kouba, P. Drncova, S. Cusack (2019). Nat. Struct. Mol. Biol. **26**, 460-470.
[2] J. M. Wandzik, T. Kouba, M. Karuppasamy et al. (2020). Cell **181**, 877-893.
[3] P. Gerlach, H. Malet, S. Cusack and J. Reguera (2015). Cell, **161**, 1267-1279.

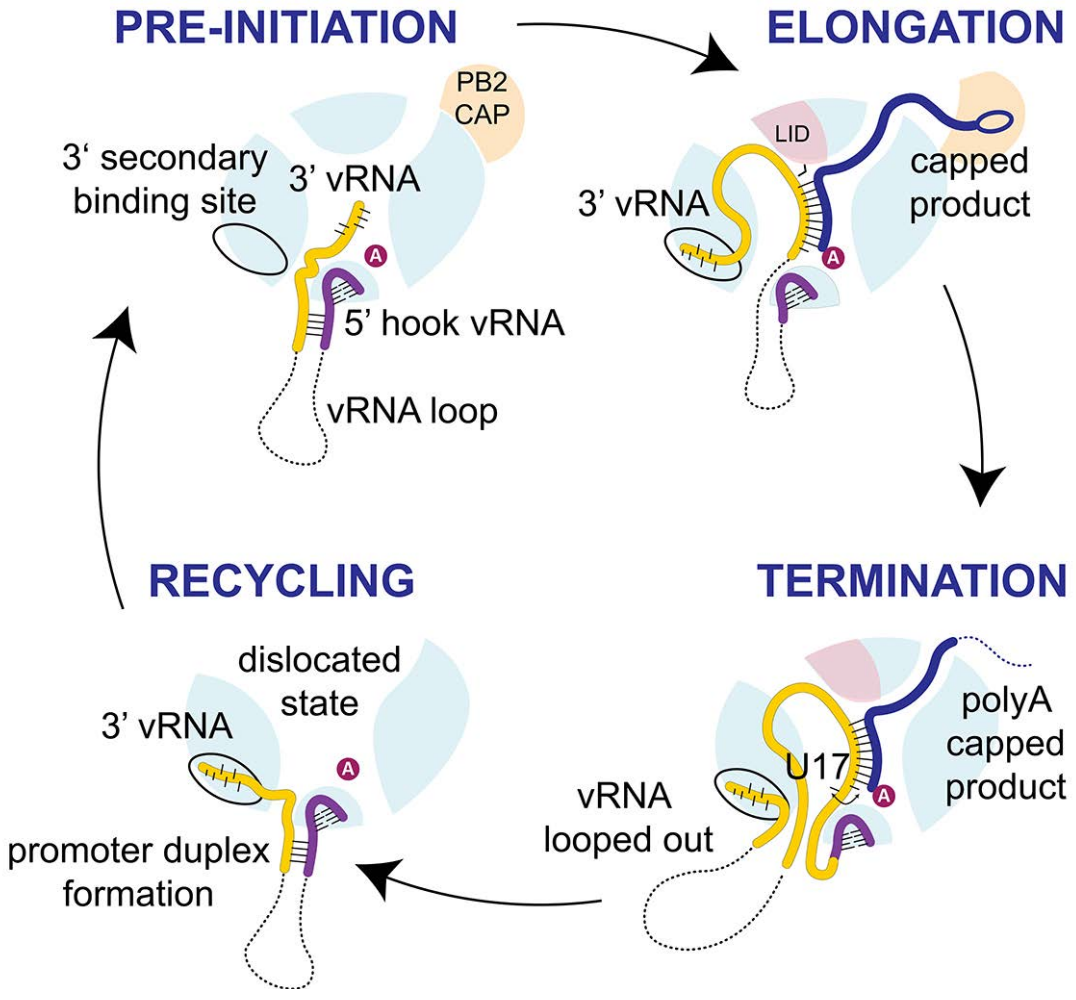


Figure 2. Schematic representation of the complete transcription cycle by FluPol. (vRNA: viral RNA; U17: uridine 17).

Splicing in the movies

A team of EMBL researchers in the Marcia group – in collaboration with computational biologists at the Italian Institute of Technology (IIT, Genova, Italy) – deciphered new key dynamic steps in the mechanism of splicing [1].

Splicing is a vital cellular process that ensures RNA maturation and the correct production of proteins. According to the central dogma of biology, DNA is transcribed to messenger RNA, which is then translated into proteins. Via splicing, protein-coding segments of the messenger RNA – known as the exons – are cut away from non-coding segments – known as the introns – and are ligated together to form the mature template for protein synthesis.

Splicing is performed by the nuclear spliceosome in humans, and by the evolutionary ancestor of the spliceosome – called the group II intron – in bacteria, archaea, fungal mitochondria and plant chloroplasts. Both machineries are RNA enzymes – or ribozymes – because they possess a homologous RNA catalytic core for exon cutting and ligation. Precision is vital: a single nucleotide error in the resulting messenger RNA and proteins would be synthesized incorrectly, leading to severe diseases. The extremely well-organized 3D structure of the spliceosomal and group II intron catalytic cores ensures high splicing fidelity. Studying the 3D structure of these machineries is thus crucial to understand the mechanistic details of splicing.

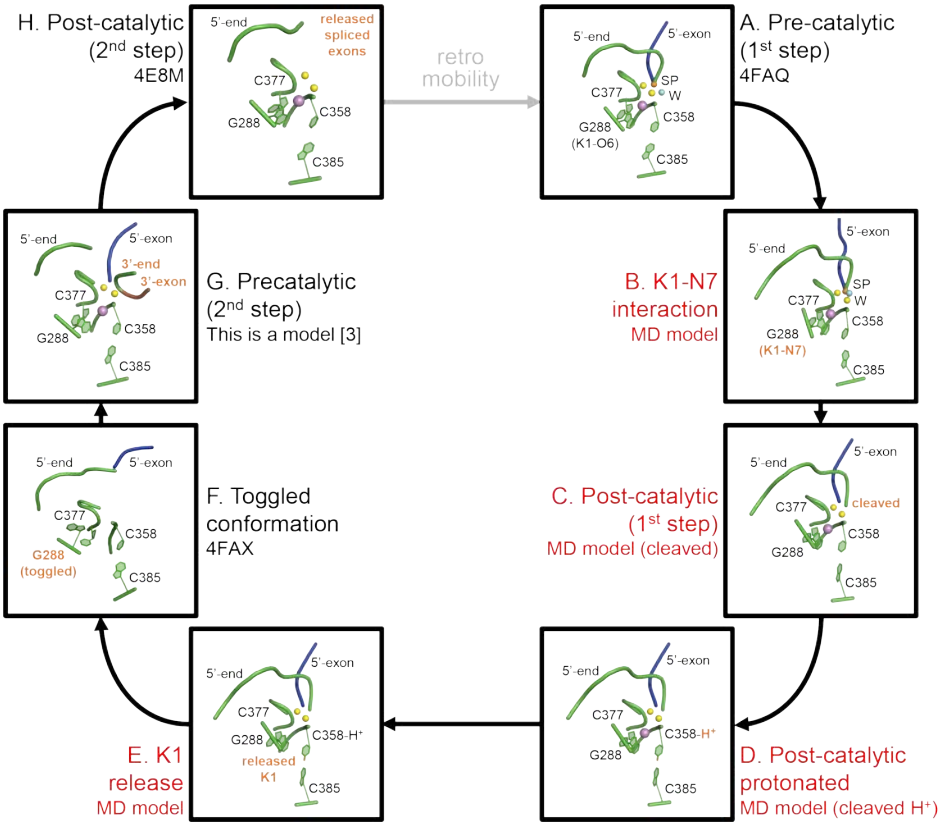
In this work, by studying the structure and function of a group II intron, the authors derive a detailed framework for the splicing reaction, which agrees with available experimental data on the splicesome and thus reinforces the notion that these evolutionarily-related molecular machines share the same enzymatic strategy. Group II intron splicing is a two-step reaction. In the first step, the intron cuts its own junction with the exon at the 5' end. In the second step, it cuts the junction with the 3'-exon, ligates 5'- and 3'-exons together, and liberates the mature messenger RNA. The self-excised intron is still an active ribozyme, and can invade the DNA of the host. This latter reaction – known as retrotransposition – has created genomic diversity during evolution and is nowadays a potentially useful strategy for gene engineering.

Crystal structures of the group II intron before and after the first and second steps of splicing were already available [2, 3]. However, the mechanistic link between the first and the second step was so far unclear. The current work unveils this important missing link by integrating systematic mutagenesis, experimental measurement of splicing kinetics, and determination of new high-resolution crystal structures. The new results show that the scission of the 5'-exon is coupled to protonation of a highly-conserved group II intron nucleotide, and this in turn promotes a reversible structural rearrangement of the active site facilitating the progression to the second step (Figure). Remarkably, connecting the new and previously-determined crystal structures via multi-microsecond molecular dynamics simulations results in a molecular movie of the splicing cycle, which is currently the highest-resolution visualization available for any splicing reaction.

These findings may facilitate the development of group II intron inhibitors by structure-based drug design. Since group II introns are essential for the survival of pathogenic fungi but are not present in humans, such inhibitors would be powerful and highly-selective antibiotics. More broadly, the exquisite level of understanding achieved on the reaction chemistry and conformational dynamics of group II introns may help in future engineering of complex, RNA-based enzymes for use as biotechnological tools and gene-specific therapeutics.

M. Marcia (EMBL)

[1] J. Manigrasso, I. Chillon, V. Genna *et al.* (2020). Nature Communications, **11**, 2837
[2] M. Marcia and A.M. Pyle (2012). Cell, **151**, 497-507.
[3] M. Marcia, S. Somarowthu, A.M. Pyle (2013). Mobile DNA, **4**, 14-26.



The group II intron splicing cycle, structure by structure. Snapshots of the intron active site derived from crystal structures and MD simulations. Relevant intron motifs are in green cartoons, the 5'-exon is in blue, the 3'-exon in brown. Catalytic potassium ions are shown as violet spheres, catalytic magnesium ions as yellow spheres. New states described in [1] are labelled in red. Differences between consecutive panels are indicated as bold orange labels.

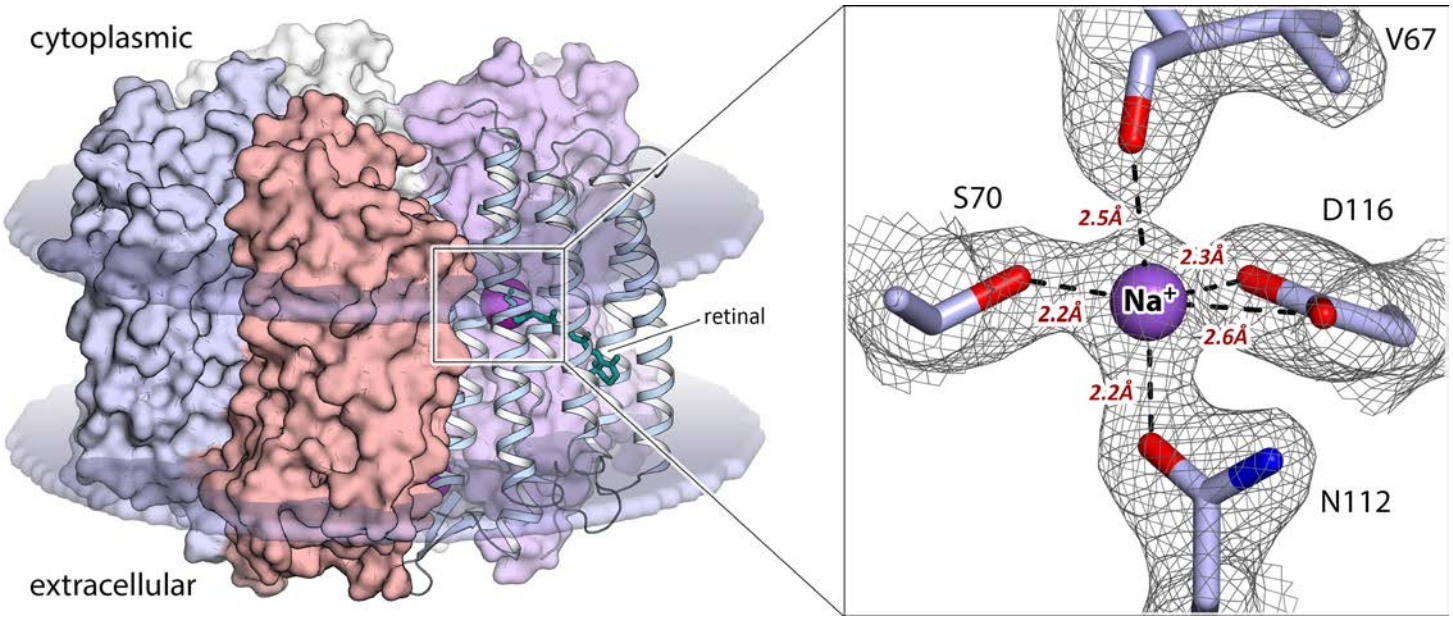
Molecular Mechanism of Light-Driven Sodium Pumping

Light-driven Na^+ pump KR2 is is a microbial rhodopsin that was discovered in marine bacteria in 2013. Its unique ability to actively transport Na^+, but not K^+, Ca^{2+}, and H^+, makes the protein a perspective tool for optogenetics – the biotechnological method for precise and minimally-invasive optical control of the living matter. The molecular mechanism of light-driven Na^+ pumping was unknown so far, with the existing 3D structures of the KR2 limited to its inactive, ground state.

Researchers of IBS, with support from ESRF, EMBL, Research Center Juelich, Moscow Institute of Physics and Technology, and ALBA synchrotron, have solved the crystal structure of the pentameric KR2 in its active, key intermediate state. For that, extensive crystallization of KR2 was performed using the HTX platform at the EMBL Grenoble. Numerous protein crystals were then investigated at the icOS facility from the ESRF to optimize the protocols for the accumulation and freeze-trapping of the intermediate state directly in the crystals [1]. Afterwards, the crystals were transported to PETRAIII, EMBL Hamburg, Germany, for crystallography experiments. The structure of the active state of KR2 revealed a transient Na^+ ion-binding site inside the rhodopsin (Figure). Structure-based molecular dynamics simulations predicted Na^+ pathway through the protein, which was further validated by the mutational analysis. The findings allowed the researchers to show that the active light-driven Na^+ transport proceeds via a combination of relay mechanism and passive diffusion of the ions through the polar cavities inside KR2 [2]. The mechanism of light-driven non-proton cation transport is principally different from the Grotthuss mechanism of proton transport in a classical representative of microbial rhodopsins, bacteriorhodopsin. Furthermore, the description of the Na^+ binding site and the ion pathway in the protein facilitates the rational engineering of the enhanced KR2-based optogenetic tools.

K. Kovalev (IBS), R. Astashkin (IBS), M. Rulev (ESRF), A. Royant (IBS/ESRF), P. Carpentier (ESRF/CEA) and V. Gordeliy (IBS)

[1] D. Von Stetten *et al.*, (2015) Acta Crystallogr. D Biol. Crystallogr., 71(Pt 1),15-26
[2] K. Kovalev *et al.*, (2020) Nat. Commun., 11(1), 2137.

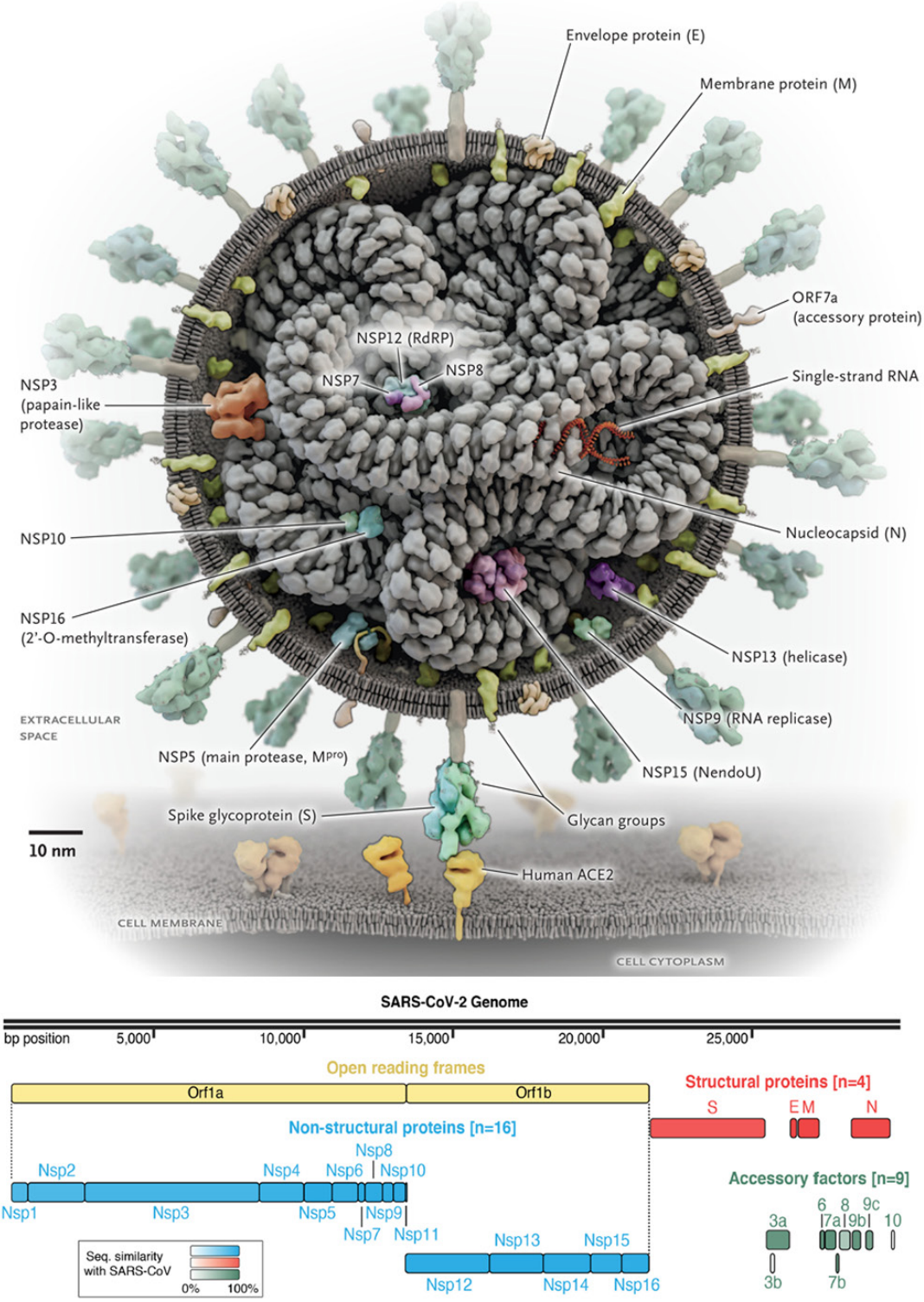


The overall structure of the O intermediate state of pentameric KR2 (left part) and transient Na^+ binding site inside the KR2 protomer (right part). $2F_o$-F_c electron density maps are shown at the level of 1.5σ.

PSB research contributions to fighting the SARS-CoV-2 Coronavirus

The current COVID-19 pandemic is caused by a novel coronavirus, SARS-CoV-2. Deepening our knowledge and understanding of the fundamental pathogenesis and infection of the virus is urgent, as are efforts to develop therapeutic agents.

Researchers at the PSB are using structural and cell biology methods to provide mechanistic insights into SARS-CoV-2 and develop potential drug and vaccine candidates.



The SARS-CoV-2 virion and its proteins. Source: JM. Parks & JC. Smith, (2020) *N. Engl. J. Med.*, **382**, 2261-2264; D. Gordon *et al.* (2020) Nature, doi: 10.1038/s41586-020-2286-9.

The SARS-CoV-2 RNA genome codes for 29 proteins, which allow the virus to attach to host cells, make RNA copies of the genome, or suppress the cell's antiviral defences. While global research on coronaviruses has shed light on the function of many SARS-CoV-2 proteins, the role of some crucial components remains unknown. By studying the detailed molecular structure of SARS-CoV-2 proteins and RNA, scientists can learn the mechanisms that control how the virus infects cells, replicates, spreads – and how to stop it.

Researchers at EMBL Grenoble are combining methods such as X-ray crystallography, cryo-electron microscopy, and RNA structure probing to try to solve some of the puzzles of the novel coronavirus's molecular mechanics. EMBL Grenoble research groups are focused on several key targets. Scientists in the Cusack group aim to determine the structure of the SARS-CoV-2 replication machinery, together with the RNA template that the virus uses to make copies of its genome. Inhibitors of this machinery, such as remdesivir, are potential anti-SARS-CoV-2 drugs. The Bhogaraju group is focused on a key component of the coronavirus's largest protein, which acts like a pair of molecular scissors to set other viral proteins free to carry out their roles and also disturbs the balance of proteins in the infected cell. Researchers in the Marcia group are studying regions of the viral RNA genome that are not directly translated into proteins but can nevertheless form complex structures that contribute to the translation of genomic information into viral proteins. These RNA elements could represent important molecular targets for drug development. Working together with these research teams, the Marquez group is applying fragment screening pipelines to a large-scale search for molecules that might inhibit these key viral components. These synergistic, complementary research efforts – deployed within the unique environment of the Partnership for Structural Biology (PSB) on the EPN campus – aim to dissect key mechanistic aspects of coronavirus molecular machines and potentially accelerate the development of new antivirals to contain the pandemic.

At the ESRF, E. Kandiah from the Structural Biology Group leads a project on the non-structural polyprotein 3 (Nsp3) of SARS-CoV-2, an important therapeutic target as it plays a vital role in viral replication. Nsp3 is a polyprotein precursor on which 15 individual proteins, crucial to the function and life cycle of the virus, are linked together and auto-cleaved by viral proteases to produce functional proteins. This project aims at structural studies of Nsp3 polyprotein precursor of SARS-CoV-2 by using integrated structural biology techniques. The ANR-Flash funded 'COVNSP3' project, is set up as a multi-collaborative approach with tight connections to the IBS and extensive use of the PSB's technical platforms.

Several IBS research groups tackle a number of projects to fight the SARS-CoV-2 Coronavirus. Since the beginning of April, a concerted action by Fieschi, Lortat-Jacob, Poignard, Thielens and Weissenhorn groups has started to produce and characterize the Spike (S) glycoprotein located in the membrane of the SARS-CoV-2 virus. This collective action aims to study the immune response against S, its attachment to secondary cellular receptors and to develop different entry inhibitors. The Adenovirus team led by P. Fender and the electron microscopy platform led by G. Schoehn work on the ANR-Flash funded 'CoV-Mime' project, which aims to employ their adenoviral pseudo-particle technology to mimic the SARS-CoV-2 surface and therefore accelerate both fundamental and applied research against COVID-19. The Poignard group participates in the ANR-Flash funded 'AM-Cov-Path' project, which focuses on the study of the pathogenesis of the SARS-CoV-2 infection and the role of antibodies in protection, in a pre-clinical model. This study could contribute to understanding whether convalescent COVID-19 patients are protected against re-infection and to the development of vaccine candidates and monoclonal antibody therapies. The Blackledge group is participating in an international Covid19-NMR consortium, which uses NMR to identify novel interactions involving viral proteins and RNA in order to define new drug targets. The Blackledge group will also focus on the nucleoprotein and its role in replication machinery.

At the ILL, a COVID-19 task force was formed in the early stages of the lockdown period to investigate approaches by which neutron scattering methods could be used to study the SARS-CoV-2 virus structure and dynamics. These discussions involved staff from the Life Sciences, Large Scale Structures and Spectroscopy groups and focused on methods whereby purpose-designed samples could be produced to highlight specific aspects of the viral structure & dynamics that are of interest in regulating its pathological activity. The sample constructs were conceived to exploit specific deuteration regimes in a manner that allows neutron small-angle scattering, neutron crystallography, neutron reflection and neutron spectroscopy methods to probe particular aspects of the virus. Current projects include structural studies of the main (Mpro) protease and ligand interactions by SANS, crystallography and spectroscopy, comparative neutron crystallographic studies of a SARS-CoV and SARS-CoV-2 nucleoprotein domain, neutron reflection studies of the E protein interaction with membranes, and a range of structural studies of spike protein-lipid and receptor interactions. These projects, many of which are being carried out in collaboration with scientists from ILL's user community, are being prioritised through Director's Discretion Time (DDT) applications for ILL beamtime and have started a completely new generation of project activity both within ILL and between ILL and PSB partner institutes.

News from the platforms

Mail-in protein-to-structure services at EMBL Grenoble to support research on COVID-19

The High throughput Crystallization Facility at EMBL Grenoble (HTX Lab) provides access to a fully automated protein-to-structure pipeline based on the CrystalDirect technology and the CRIMS software, coupled to the high-brilliance X-ray beamlines at the ESRF. This pipeline starts with mail-in samples and integrates crystallization screening, crystal optimization and automated harvesting in a continuous and fully automated workflow. These capabilities are uniquely suited to support structural biology projects. The HTX Lab also provides access to dedicated pipelines for the rapid analysis of protein-small molecule interactions as well as for large-scale fragment screening to support development of inhibitors and chemical tools. This includes access to two fragment libraries (1200 and 700 fragments respectively) and automated data processing for hit identification. This approach can be applied to support the development of tool compounds for proteins with high biomedical or biotechnological potential, which can be used both to investigate function as well as to establish their biomedical relevance and potential as drug targets. These pipelines have been made available to over a hundred scientists through the E.C. funded iNEXT and iNEXT-Discovery projects. These pipelines were also in high demand from the pharma industry that use them to support structure-based-drug development programmes. This led to the creation, in 2019 of ALPX, the fist EMBL Start-Up company based in France dedicated to provide structure guided drug design services based on EMBL technologies to the pharma and biotech sectors.

The HTX lab is accepting projects on COVID-19 related research as part of a global effort to zoom in on the biomolecules of SARS-CoV-2 to develop drugs that inactivate the virus. If you want to benefit from access to the HTX lab for COVID-19 research, please send an e-mail to htx@embl.fr indicating the project goals and a description of the samples you would be shipping. To avail of seamless integration of the EMBL HTX Lab with the highly automated JSBG MX beamlines at the ESRF, users should send an e-mail to jsbg-covid-19@embl.fr indicating interest, whereupon a short application form will be sent for completion.

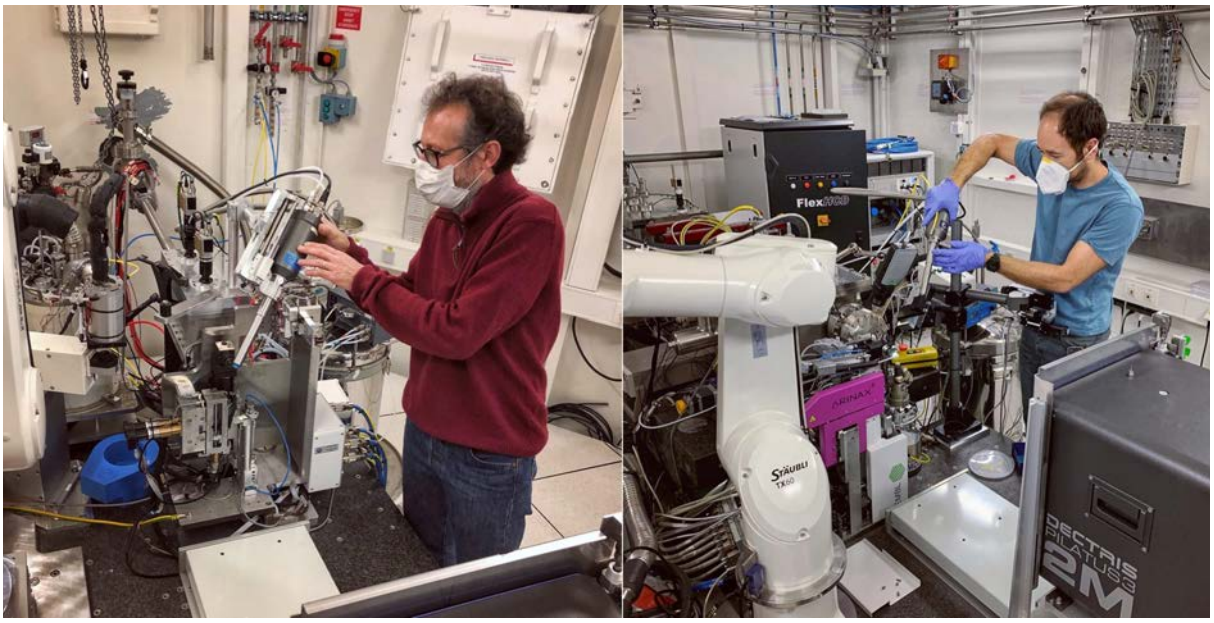
Status of the ESRF beamlines for Structural Biology

Following the required post-EBS safety certification of all of the ESRF's macromolecular crystallography beamlines, two of these, ID30A-1 (MASSIF-1) and ID23-2, as well as the high-end Titan Krios cryo-electron microscope (cryo-EM) CM01 were made available for experiments following an initiative of the ESRF (http://www.esrf.eu/home/news/general/content-news/general/covid-19-scientific-research.html) to support Covid-19 SARS-CoV-2 related research. In parallel, ESRF's biochemical laboratory located at the CIBB (https://www.psb-grenoble.eu/spip.php?rubrique22) is resuming its work to support research carried out at the ESRF.

Currently, all ESRF MX and bioSAXS beamlines operated by the ESRF-EMBL JSBG (ID23-1, ID23-2, BM29, ID30A-1, ID30-3 and ID30B) are being commissioned with the aim of having them ready for post-EBS operation on or, in the cases of ID30A-1 and BM29, soon after August 25th, 2020. In addition to these commissioning activities, the beamlines remain available for SARS-CoV-2 research. CM01, which recently welcomed its first external user experiment following the relaxation of restrictions put in place to combat the spread of Covid-19, is also available for such research. The (re)construction of ID29 as a beamline dedicated to synchrotron serial crystallography (SSX) is underway with the beamline due to open in late 2021.

Due to the current travel restrictions, as well as the safety measurements put in place in response to the COVID-19 pandemic, all experiments performed at the ESRF will until further notice, be either via remote access with or via a mail –in service. For the latter, ESRF staff will both set up and perform experiments.

C. Mueller-Dieckmann and G. Leonard (ESRF)



Didier Nurizzo and Max Nanao setting up the MASSIF-1 and ID23-2 MX beamlines.

EVENTS

HERCULES 2020 - European School

The 2020 edition of Hercules, the European school for PhD students and young researchers on the application of neutron and synchrotron radiation for biology, chemistry, and condensed matter physics, was held from March the 2nd to April the 3rd. The school, coordinated by the Université Grenoble Alpes, hosted 60 participants during the first two weeks in Grenoble, then the fast escalation of the COVID-19 situation imposed to reorganise the last three weeks, when participants were supposed to travel to different large scale facilities in Europe to attend tutorials and practicals.
With all the participants back home, the school continued its program (obviously largely rearranged because of the conditions) remotely. The effort made by the organising committee, with the full collaboration of scientists from different countries and institutions, to replace the experimental activity at the facilities with remote tutorials and practicals was appreciated by the participants who attended the courses until the last day.
The new direction team, who took office in June 2020 and composed of Marc de Boissieu (director, INP), Béatrice Grenier (director of studies, ILL/CEA), Giorgio Schirò (deputy director and responsible for life sciences, CNRS/IBS), is already designing, with the other members of the organizing committee and the collaboration of the former director Vincent Favre-Nicolin, the next Hercules edition for 2021. Whether Hercules 2021 will be held in Grenoble or will be organised fully remotely is currently under discussion.

G. Schirò (IBS)

PSB STUDENT DAY 2020

On the 17th of February, students from ESRF, ILL, IBS, and EMBL got together in the Chadwick amphitheater to celebrate the annual PSB student day, which enabled them to exchange ideas in a laid-back and engaging format. During this event, students from all four institutes were invited to present their research projects to one another in the form of short flash-talks, poster sessions, and in-depth presentations, alongside keynote talks from Sigrid Milles (IBS) who presented her work on “Studying intrinsically disordered proteins by integrating NMR and single molecule fluorescence”, and shared her experience on how she became a team leader, and Benjamin Brocco (Former ILL PhD student) who presented the advantages of “Doing a Masters in Technology and Innovation following the PhD”. Following the assessment of a jury during the poster session, Mathilde Folacci (IBS) was awarded the best poster prize, and the audience also voted for Anas Malki (IBS) as best flash presentation.

The event was organized by student representatives from PSB member institutes: Michael Adams (EMBL), Wiktor Adamski (IBS), Nina Christou (IBS), Hadrien Depernet (ESRF) and Lukas Gajdos (ILL), with help from Florent Bernaudat (PSB).

We are currently looking for new student representatives. If interested, please contact Michael Adams at miadams@embl.fr for more information.

M. Adams (EMBL)

30th ESRF User meeting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The annual ESRF User Meeting 2020 was held on the EPN Campus from 3rd to 5th February 2020, dedicated to the ESRF user science and its future horizons. UM2020 attracted more than 300 participants. On day 1, the participants could choose among a series of beamline tutorials, which included a MX BAG Meeting. A plenary session was held on day 2 in the ESRF Auditorium, with a keynote lecture by Stephen Cusack (EMBL Grenoble) on the molecular mechanisms of the influenza polymerase.
Three User-Dedicated Microsymposia (UDMs) completed the UM2020 activities.
The UDM3 entitled "Multi-crystal and serial data collection in Structural Biology" was organised by Daniele de Sanctis (ESRF), Max Nanao (ESRF), Marina Mapelli (ESRF User Organisation) and Claudine Roméro (ESRF). This symposium brought together participants from interdisciplinary fields to discuss and stimulate exchange among ESRF Structural Biology users on multi-crystal diffraction strategies and serial crystallography. The symposium comprised four keynote lectures and six selected contributions from submitted abstracts on challenges in serial crystallography, serial experimentation and data analysis. Taken together, UDM3 provided an excellent overview of the recent developments and new perspectives in the use of microfocus beamlines that can fully exploit the unprecedented capabilities of the EBS MX beamlines.

M. Soler López (ESRF)

FEBS INSTRUCT MOBIEU Practical course HyThaBio 2020: Hydrodynamic and Thermodynamic analysis of Biological macromolecules and their interactions

The HyThaBio practical course was organized at IBS from January 26th to 31st 2020. It was locally organized by Christine Ebel, Aline Le Roy, Caroline Mas, and Jean-Baptiste Reiser, respectively responsible of the AUC, Biophysics, and SPR/BLI platforms of IBS-ISBG. The workshop benefited from support from FEBS, INSTRUCT and COST MOBIEU. 18 renowned speakers and tutors from USA, Europe and Japan were invited to provide the course to 25 participants.
It allowed theoretical, practical and data analysis training in modern methods for monitoring and quantifying molecular interactions, mainly AUC, ITC, SPR and BLI, but also DLS, MST, DSF, and fluorescence spectroscopy. Complementary SAS, MS, EM, super-resolution microscopy and NMR were presented during the mini-symposium day. The aim was to emphasize the complementarity of these methods.

The course provided lectures on both the theory of the biophysical concepts underlying the methods and on practicalities to set-up experiments. Demonstrations were performed on the state-of-art instruments hosted by the IBS, ISBG and PSB. Following basic and advanced data analysis demonstrations, hands-on practicals, including combined analysis of datasets, were performed with different free softwares developed by Peter Shuck's team and collaborators from NIH, Bethesda, USA. The abstract book and most presentations can be downloaded from https://biomacromoldynamics2020.febsevents.org/.

C. Ebel (IBS), J.-B. Reiser (IBS) and C. Mas (ISBG)

OBITUARY — Jean-Luc Ferrer

Jean-Luc Ferrer obtained his Engineer degree from the prestigious École Centrale Paris in 1987.
After getting a Diplôme d'Etudes Approfondies (DEA) in Physical Chemistry from Paris VI University, Jean-Luc Ferrer prepared a thesis in physics on the dynamics of the spectrum of a free electron laser at the center of studies of the CEA, Bruyères-le-Châtel. This work allowed him to obtain a doctorate degree from the Paris XI University in 1990. At the end of the same year, Jean-Luc was recruited by the Direction des Sciences du Vivant (DSV) at the Commissariat à l'Energie Atomique (CEA) Grenoble and joined the Laboratory of Protein Crystallography and Crystallogenesis (LCCP). This laboratory, directed by Juan Fontecilla-Camps, had just been created by Michel Suscillon, Director of DSV, as part of the "Protein 2000" program, to potentiate the development of structural biology in France. This initiative had been taken following the decision, in January 1989, to build the European Synchrotron Radiation Facility (ESRF) in Grenoble, which became operational in 1994. Once at the LCCP, Jean-Luc joined Michel Roth's team, whose mission was to build the "Collaborative Research Group" (CRG) French beamline BM02-D2AM. This beamline, 50% dedicated to protein crystallography was especially designed for X-ray multi- and single-wavelength anomalous dispersion (MAD and SAD) data collection.

In 1992, the LCCP joined the Institute for Structural Biology (IBS), which resulted from the merging of the CEA "Protein 2000" program with its CNRS counterpart "IMABIO".
From the very start, Jean-Luc distinguished himself with his interest and skills in both the technical and the scientific aspects, sometimes very complex, of beamline construction and the resolution of protein structures. Concerning the latter, he kept a very fruitful collaboration with Prof. Joseph P. Noel at the Salk Institute (La Jolla, USA), where he spent sabbatical leaves in 1996 and 2005. Jean-Luc's research work concentrated mainly on the structural biology of phenylpropanoid synthesis in plants: he co-authored 20 papers with Prof. Noel on this and related subjects, which were published in prestigious international journals such as Cell, Nat. Struct. Biol., J. Biol. Chem. and the Proc. Natl. Acad. Sci, USA. At the IBS, he developed with his colleagues from the Cell & Plant Physiology Laboratory at the CEA a strong and fruitful collaboration on the study of enzymes involved in amino-acid biosynthesis in photosynthetic organisms.

After the retirement of Michel Roth in 2001, Jean-Luc became head of the new CRG French protein crystallography beamline, BM30A-FIP (a national platform). Over the years, the FIP beamline has become an essential tool for the French and international communities of protein crystallography. As before, but now with newly acquired responsibilities, Jean-Luc displayed a great deal of creativity, persistence and dynamism to highlight the advantages of synchrotron radiation for protein crystallography.

As an example, he was able to anticipate the automation of crystallography beamlines and helped develop, at the ESRF, the first crystal sample changer based on a 6-axis robot arm: the CATS (Cryogenic Automated Transfer System). This sample changer or one of its derivatives is installed on most of the MX beamlines around the world. The CATS was improved and developed to propose new functionalities to users and become the G-Rob system. To save beamtime, the grip was modified to allow swift mounting and dismounting of crystals. To help users on the beamline, the robot was equipped with tools to center and setup the beam, to wash the crystal to remove ice or to perform annealing. However, the main achievement is probably the use of the robotic arm for the *in situ* data collection coupled to the automation of the experiment with the development of the Crystal Listing. This allowed the G-Rob to screen automatically hundreds of crystals selected by the users, paving the way to the high-throughput ligand screening for drug design. For this purpose, FIP has since then developed, in collaboration with GREINER Bio-One, a new crystallization plate devoted to this application. With these technology innovations, a web-based interface for diffraction experiments and the first automated pipeline for data processing (ADP for Automated Data Processing) connected to the data collection were developed. In 2009, Jean-Luc ensured the technological transfer of these different innovations by founding the start-up biotechnological company NatX-ray. Along with the automation of the data collection and the use of robots, he complemented FIP and FIP2 with other options, such as samples mounted in a capillary, UV-vis absorption spectrophotometry, and high-pressure diffraction. Recently, Jean-Luc was also involved in the study of the automation of the NMX macromolecular diffractometer at the European Spallation Source (ESS) with the integration of different robots positioning the sample and the detectors.

As the head of the FIP-BM30A beamline at the ESRF, Jean-Luc very much enjoyed explaining and teaching recent developments in protein crystallography to students and young scientists. Jean-Luc participated in the organization of the training network in integrative structural biology (RéNaFoBiS) and worked on the programming and implementation of the first school in Oléron in 2014 and subsequent editions. Also, starting 2015, Jean-Luc organized every year an international workshop called MXIS then ADTB, with his colleagues from the CBS of Montpellier and the EPN campus of Grenoble, and with the support of his team. This workshop was dedicated to the latest developments in diffraction in biology.

Following the reorganization of the IBS in 2011, Jean-Luc became head of the Synchrotron Group (GSY). As part of the ESRF upgrade program aimed at obtaining a new extremely bright X-ray source (EBS), Jean-Luc sought and obtained funding for the FIP beamline rebuilding and renovation project in order to continue to provide the best possible service to the community. He carried this beamline project to its end. BM07-FIP2 will open next fall when the ESRF reopens.

We all learned a lot from Jean-Luc. He has left a warm souvenir in the hearts of many researchers and students. We have lost a precious friend and a colleague of great experience.

J. Fontecilla-Camps (IBS) on behalf of Jean-Luc's colleagues from the IBS

DATES FOR YOUR DIARY

8th October 2020 – PSB Spotlight on mass spectrometry

This one-day scientific meeting will comprise introductory talks and practicals to inform local students and researchers on how to exploit mass spectrometry to advance their research projects. However, depending on the evolution of the ongoing situation due to the COVID-19 pandemics, the format of the meeting may be adapted. More information will be sent by mail and on: www.psb-grenoble.eu

26th of February 2021 – EMBL Science & society talk: Challenging the Misrepresentation of Science and Evidence in Public Life by Tracy Brown OBE

More information: https://senseaboutscience.org

29th May to 5th June 2021 - EMBO Practical Course on Characterization of macromolecular complexes by integrative structural biology

This course will take place on the EPN Campus. It aims to teach how to expedite structural biology projects involving macromolecular complexes by combining multiple experimental approaches. The course is primarily intended for advanced PhD students and early-stage postdocs (limited to 20 registered participants), but the lectures will be open to everyone. More information will come soon.

16th to 21st June 2021 – Les Houches TSRC Protein Dynamics Workshop

The TSRC Protein Dynamics Workshop initially planned on 7th to 12th June 2020 has been postponed, due to the COVID-19 pandemics, to take place in 2021

More information: www.tinyurl.com/protdyn2020

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

The AFC congress initially planned on 30th June to 3rd July 2020 has been postponed, due to the COVID-19 pandemics, to take place in 2021.

More information: https://afc2020.afc.asso.fr

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

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

NEWCOMERS

Igor Melnikov

Has been appointed ESRF Beamline Scientist for ID30A-3 (MASSIF-3) in April 2020. Igor graduated from Moscow Institute of Physics and Technology and then spent six years at the ESRF. During these years he did his PhD in Structural Biology working mainly on methods facilitating MX data collection procedures and structure solving. He then switched to combining MX and cryoEM during his post-doc carried out in part in V. Gordeliy's group at IBS.

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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.