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PSB et al

Partnership for Structural Biology Newsletter

SCIENTIFIC HIGHLIGHTS

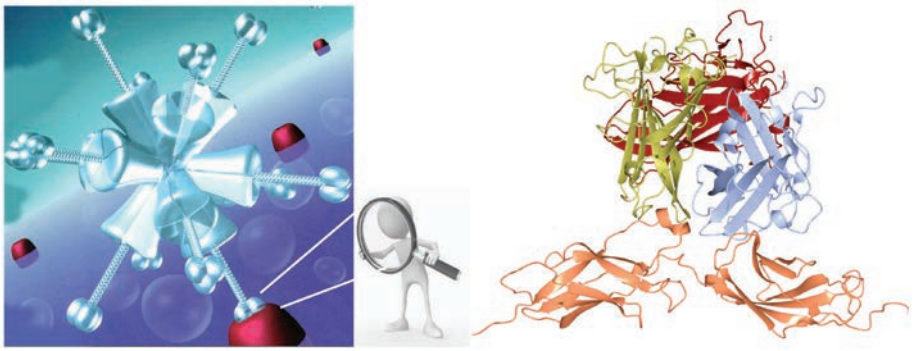
Adenovirus binding to its receptor visualized by cryo-EM

Adenoviruses cause diseases that can sometimes be fatal. By modifying them, they can also become formidable cancer cell killers. Adenoviruses are to date the most commonly used vectors in human clinical trials. PSB researchers have just elucidated by cryo-electron microscopy the mechanism by which adenoviruses bind to the cell surface. These results, published in the journal Nature Communications, could pave the way to the development of new generation anti-tumor vectors.

More than 60 adenovirus (Ad) serotypes are known in humans. While they are able to cause different types of diseases such as gastroenteritis or conjunctivitis, most of them have respiratory tropism. Although not strictly speaking a major public health problem, several serotypes such as Ad3, Ad7 Ad11 and Ad14 (subgroup B2) investigated in this study, were recently reported in an outbreak. Eleven out of the 35 young patients died of Adenovirus infection in New Jersey in November 2018. Besides this, adenoviruses are the most commonly used vectors in human clinical trials (http://www.abedia.com/wiley/vectors.php). This craze relies essentially in their use as oncolytic vectors. To this goal, adenoviruses are modified to replicate only in cancer cells. Numerous clinical trials are underway in the United States and Europe, offering great hope for new anti-tumor strategies.

In this context, the adenovirus team at IBS has collaborated with Prof. Lieber in Seattle to identify the long elusive subgroup B2 receptor leading to the successful identification of desmoglein 2 (DSG2) [1]. A comprehensive biochemical study using the PSB facilities (MALLS, AUC, Mass Spec and Electron Microscopy) was then undertaken by Emilie Stermann-Vassal to map the minimal domain required for this interaction [2]. This led to a 96 kDa complex made of one trimeric adenoviral fiber in complex with two out of the four cadherin domains of the DSG2 ectodomain.

Until recently, solving the atomic structure of such a small complex by cryo-EM seemed inconceivable. The latest technological developments of the Krios microscope (ESRF) coupled to the expertise of Grégory Effantin in the electron microscopy group (IBS) have shown that this barrier can be furthered. The structure showed an unusual mode of binding in which two monomers of the trimeric fiber head binds with two independent cadherin domains of DSG2 thus forming a non-symmetrical complex. Moreover, key amino acid players of the interaction were identified and we showed that a single amino acid substitution in the adenovirus fiber head was sufficient to completely abolish receptor binding [3]. Such a discovery opens the way to the rational design of adenoviral inhibitors on the one hand and to a retargeting of oncolytic adenoviral vectors to tumors on the other hand. This project is already ongoing thanks to the 'Ad-Cadh' funding (ANR-189-CE11-0001).



Artist view (left) of an adenoviral particle binding to a cellular receptor in red (adapted from Fender *et al.*, Nat Biotech 1997). Cryo-EM structure (right) revealed the mechanism by which two out of the three trimeric fiber monomers (gold and blue) bind to two cadherin domains of the DSG2 receptor (in orange).

P. Fender (IBS)

[1] Wang, H. Li, ZY, Liu, Y, Persson, J. *et al.* Nat. Med. **17**, 96–104 (2011).

[2] Vassal-Stermann, E. Mottet, Ducournau, C. Iseni, F *et al.* Scientific Reports **8**, (2018).

[3] Vassal-Stermann, E*. Effantin, G*. Zubieta, C. Burmeister, W. *et al.* Nature Communications **10**, (2019).

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A molecular mechanism for transthyretin amyloidogenesis uncovered using neutron and X-ray crystallography, mass spectrometry, and molecular dynamics

Transthyretin amyloidosis is a progressive condition in which abnormally folded forms of an important hormone transporter protein accumulate as amyloid fibrils/plaques in various parts of the body – resulting in conditions such as familial amyloid cardiomyopathy (FAC) and familial amyloid polyneuropathy (FAP). The most affected tissues include the heart, the kidneys, and the gastrointestinal tract; the condition may also damage the peripheral and autonomic nervous systems.

In this study (published in Nature Communications [1]), normal human transthyretin was studied alongside two mutants of the protein. One of these (the T119M or 'stable' mutant) imparts remarkable stability to transthyretin and is strongly protective against the formation of amyloid fibrils. The other (the S52P or 'unstable' mutant) results in a very aggressive form of hereditary amyloidosis.

The project made extensive use of many PSB platforms. It was driven through the Faculty of Science at Keele University (UK), and involved co-authors from all of ILL, IBS, ESRF, and EMBL partner institutes. All of the transthyretin mutants were studied using neutron and X-ray crystallography, mass spectrometry, and computer modelling. The project also exploited the technologies available within the Deuteration Laboratory (D-Lab) platform of ILL's Life Sciences Group [2]. These studies have resulted in the proposal of a molecular mechanism by which transthyretin forms amyloid fibrils through a parallel equilibrium of partially unfolded protein forms.

Transthyretin has a complex hierarchical structure of four subunits (monomers). The results suggest that the disease progression arises from instability in the C-D loop of the individual monomers. This instability renders the whole protein more susceptible to degradation effects, enhancing the rate of amyloid fibril formation. Furthermore, the study suggests that the binding of small molecule drugs to transthyretin stabilises the folded state of transthyretin in the same way as happens in the highly stable protein mutants.

Additionally, this study has emphasised the potential exploitability of a new neutron approach that can be used to provide important information on the dynamics and stability of particular regions of a protein. Neutron crystallography is usually carried out with all of the 'normal' water (H_2O) replaced by heavy water (D_2O) – this is done for technical reasons and helps to optimise the quality of the data recorded. However, controlled 'back-exchange' of D_2O by H_2O can be carried out [3]. This solvent exchange occurs more readily in regions of the protein that have greater flexibility and movement - regions that are more susceptible to denaturation, misfolding, and amyloid fibril formation. This type of approach has been successfully used in NMR and mass spectrometry work but until now has never been seriously applied in neutron crystallographic studies.

In the case of transthyretin, the back-exchange data recorded using LADI's neutron diffractometer from fully deuterated transthyretin crystals demonstrate very clear differences in the patterns of stability amongst the transthyretin mutants – with the S52P mutant showing further evidence in support of a highly unstable fold, consistent with the computer modelling work.

T. Forsyth (ILL, Keele University)

[1] A.W. Yee *et al.* (2019). Nature Communications **10**, 925
[2] M. Haertlein *et al.* (2016). Meth. Enzym. **566**, 113–157
[3] A.W. Yee *et al.* (2017). J. Appl. Cryst **50**, 660-664

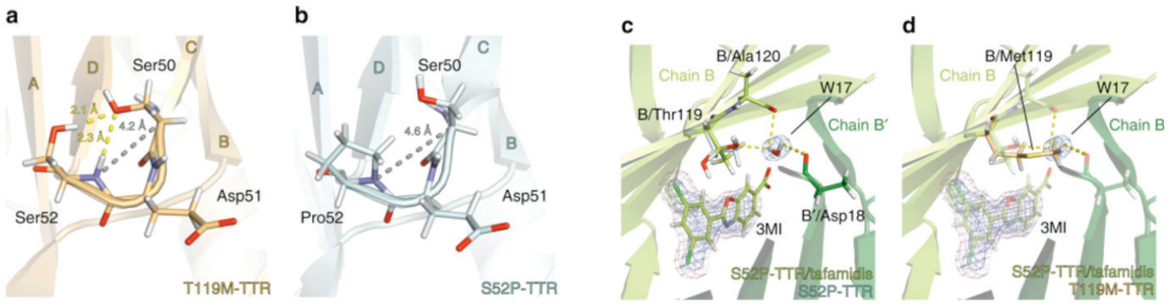


Figure 1. Neutron structures for the transthyretin mutants. **a-** In the T119M mutant the Ser52 residue forms two hydrogen bonds (yellow dash lines) with the Ser50 residue. The distance between Ser50-Cα and the amide-N of Ser52 is 4.2 Å (grey). **b-** In the S52P mutant, the absence of the hydrogen bonds between Pro52 and Ser50 causes a loosening of CD loop. **c-** Binding of the tafamidis drug results in a change of orientation for residue Thr119 (cyan, before binding; green, after binding). In the S52P/tafamidis complex, the hydroxyl side chain of Thr119 forms a hydrogen bond to a water molecule. **d-** The water molecule in the S52P/tafamidis complex occupies the same position as the side chain of Met119 residue (light orange) in the non-pathogenic T119M mutant.

Neutrons probe the effect of polydispersity on protein diffusion in crowded cellular environments

The liquid interior of living cells is marked by the presence of various macromolecules, notably proteins, at high concentrations. Combining high-resolution neutron spectroscopy with computer simulations, the effect of the polydispersity within such a naturally crowded environment can be quantitatively understood on nanometer time and nanometer length scales. This knowledge will contribute to understanding intracellular transport and self-assembly.

In the pursuit to predict biological dynamics on the molecular level, physical concepts can greatly aid a quantitative modeling. Notably, concepts derived from colloid physics can help to understand the diffusion of biological macromolecules on the nanometer length scale commensurate with molecular dimensions. Neutrons provide an ideal tool to probe this diffusion, not causing any damage to the fragile biological samples and circumventing the need for any labels on the proteins.

A team with members from the Universities of Tübingen (Germany), Lund (Sweden), and Grenoble (France) - including the ESRF, as well as the ILL's Spectroscopy, Life sciences, and Theory groups - has combined high-resolution neutron spectroscopy experiments with computer simulations on samples closely mimicking the natural crowded macromolecular environment, but allowing for a systematic control of the system parameters such as the tracer protein and crowder concentrations. This systematic control constitutes a novel approach, combining the benefit of adjustable degrees of freedom with the advantages of studying natural systems. In addition, small-angle x-ray and neutron scattering experiments have helped to further understand the samples.

The ILL Life Sciences Group produced fully deuterated so-called cellular lysate that was mixed with natural, i.e. protonated Immunoglobulin (Ig) tracer proteins, and deuterated water (D_2O). The thus achieved difference in the scattering cross sections from the deuterated and protonated components of the samples allowed to focus on the neutron scattering signal from the Ig. Different samples with various ratios of these components were systematically measured.

Subsequently, the experimental results were analyzed by theoretical concepts from colloid physics as well as, importantly, by so-called Stokesian dynamics computer simulations. In this way, the protein diffusion was accessed on nanosecond time scales where hydrodynamic interactions dominate over negligible protein collisions. Combined with these coarse-grained simulations, the experimental results on the complex, flexible molecules provide a consistent understanding by colloid theories. The experiments as well as the simulations show that tracer proteins in polydisperse solutions close to the effective particle radius diffuse approximately as in a monodisperse suspension. The simulations further show that macromolecules of sizes that differ from this effective radius are slowed differently even at nanosecond time scales. This result is highly relevant for a quantitative understanding of cellular processes.

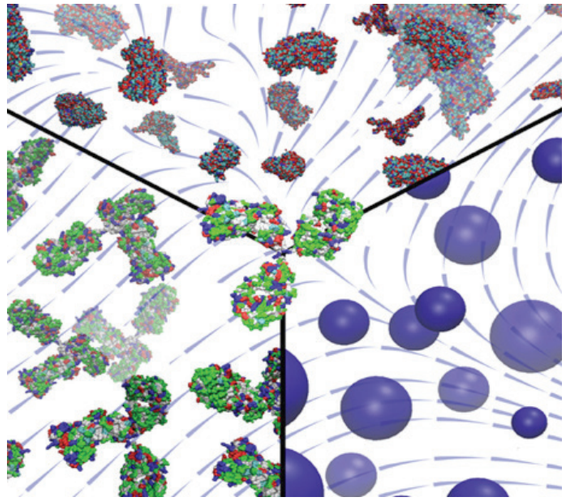


Figure 1. Artistic rendering illustrating the experimental systems of pure Immunoglobulin (Ig) proteins in water (bottom left), the Ig-lysate mixture (top), and the simulated polydisperse hard-sphere suspension (bottom right), highlighting the importance of hydrodynamic interactions by the indicated flow field (blue lines).

T. Seydel (ILL)

[1] M. Grimaldo *et al.*, (2019), Journal of Physical Chemistry Letters,**10**, 1709.

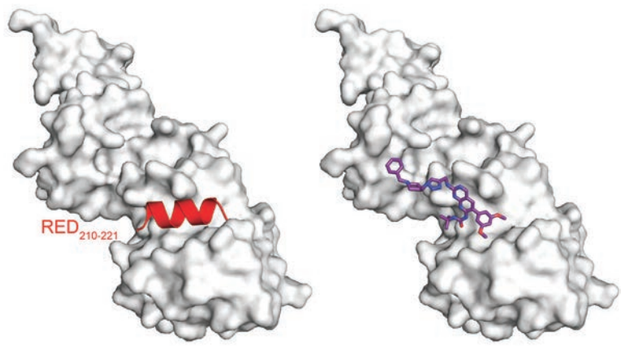
Host-directed therapy to fight against flu

New therapeutic strategies targeting influenza are actively sought due to limitations in current drugs available. Host-directed therapy is an emerging concept to target host functions involved in pathogen life cycle and/or pathogenesis, rather than pathogen components themselves. Therefore, scientists from the IBS and EMBL, in collaboration with colleagues from Institut Pasteur and the Universities Paris Diderot and Paris Descartes, focused on an essential host partner of influenza viruses, the RED–SMU1 splicing complex.

By combining structural biology and molecular dynamics studies, they identified two synthetic molecules targeting an interface essential for the RED–SMU1 complex assembly. They solved the structure of the SMU1 N-terminal domain in complex with RED or bound to one inhibitor identified to disrupt this complex. They showed that these compounds also decrease endogenous RED-SMU1 levels and inhibit viral mRNA splicing and viral multiplication, while preserving cell viability. Overall, their data demonstrate the potential of RED-SMU1 destabilizing molecules as an antiviral therapy that could be active against a wide range of influenza viruses and be less prone to drug resistance. Structural data were collected on the ESRF ID29 beamline, and this work also benefited from access to the PSB Biophysics platform.

T. Crepin

Ashraf U, Tengo L, Le Corre L *et al* (2019) Proc Natl Acad Sci U S A. doi: 10.1073/pnas.1901214116.



Destabilization of the human RED–SMU1 splicing complex as a basis for host-directed antiinfluenza strategy.

Structural snapshots of actively transcribing influenza polymerase

Influenza viruses cause widespread mild to severe respiratory disease. Due to their rapid mutation rate and ability to jump between different animal hosts, new strains are continually emerging that cause seasonal epidemics or occasional pandemics. The viral RNA genome (vRNA) is transcribed and replicated in the nucleus of infected cells by a virally encoded RNA-dependent RNA polymerase. Influenza polymerase (FluPol) employs unique mechanisms to synthesize 5' capped and 3' polyadenylated viral messenger RNA. The polymerase is initially bound to a promoter comprising the partially base-paired 3′ and 5′ regions of the vRNA [1]. A short, capped primer, 'cap-snatched' from a nascent host polymerase II transcript, is directed towards the polymerase active site to hybridize with the 3' extremity of the template to initiate RNA synthesis. After processive elongation, polyadenylation is achieved by stuttering of the polymerase on an oligo(U) stretch proximal to the template 5' end [2]. Attempts to structurally characterize the initial steps of actively transcribing influenza polymerase have hitherto been unsuccessful. This is because of the intrinsic flexibility of the 3' end of the template, the instability of the initiation state, and steric clash of the primer with a priming loop, a specific feature of the FluPol that is required for the vRNA replication [3]. However, the molecular details of this process are crucially important in order to develop compounds that directly inhibit viral RNA synthesis.

In our work [4], we employed a modified template that stabilizes the initial primer-template interaction to enable visualization by X-ray crystallography of a transcription initiation-like state (Figure 1A) using data collected on MASSIF-1 and ID29 at the ESRF. Subsequent addition of only ATP and GTP leads to product elongation by five nucleotides with concomitant template translocation before the polymerase stalls due to lack of additional NTPs. Trapping this stalled state enabled us to visualize by cryo-electron microscopy (cryo-EM) the initiation to elongation transition (Figure 1B) from data collected on CM01 at the ESRF.

Our results, summarized in (Figure 2), show that in the early stages of transcription by FluPol, the initiation to elongation transition involves progressive extrusion of the priming loop coupled to widening of the active site cavity. The latter accommodates growth of the product-template duplex to a steady state of nine base pairs. Subsequently, the PB2 helical lid enforces strand separation, directing the template into the newly opened template exit channel, whereas the increasingly bulged capped mRNA transcript eventually forces its release from the cap binding domain. Concomitantly, template translocation causes promoter disruption, resulting in collapse of the PB1 β-ribbon onto the vRNA 5' end and template entry channel remodelling.

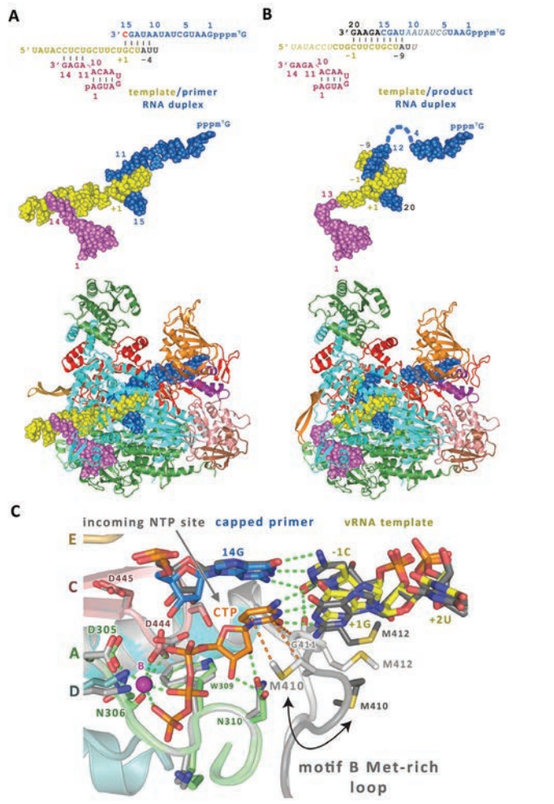


Figure 1. Structural snapshots of (A), mixed initiation (B), elongation state complexes of influenza polymerase. Top. Sequence and secondary structure of nucleic acid moieties in the complex. Middle: Structure of the RNA moieties, represented as spheres. Bottom: Ribbon diagrams of the mixed initiation (crystal) and elongation (cryo-EM) state complexes coloured according to domain structure (C). PB1 catalytic site showing the conserved RNA-dependent RNA polymerase functional motifs A, C, D and E. Template (yellow), primer (blue), metal B (magenta sphere) are shown as observed in the pre-translocation initiation state. (C) When incoming CTP (orange) is present, the motif B loop remodels to stabilize the base by stacking with Met410.

Our structures also reveal how the six conserved characteristic structural motifs (A–F) of the catalytic core are responsible, together with two divalent cations, for controlling the nucleotide addition cycle (Figure 1C). We show how these motifs, and other parts of the influenza polymerase core, interact with the primer, template and incoming nucleotide during transcription initiation and elongation in both pre- and post-translocation states. The comparison of the two highlights the role of the flexible, methionine-rich motif B loop (PB1/407-GMMMGMF-413, highly conserved in all orthomyxoviruses) in adapting to the presence of a base at the incoming nucleotide position +1.

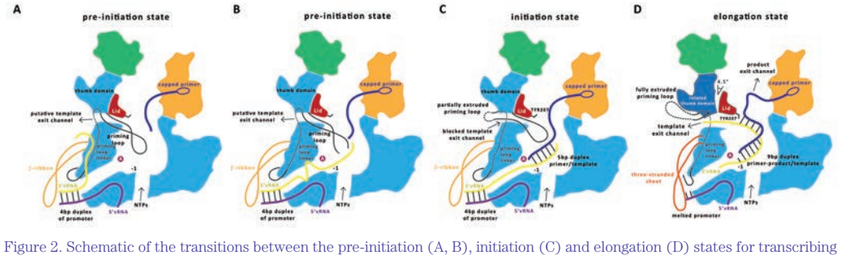


Figure 2. Schematic of the transitions between the pre-initiation (A, B), initiation (C) and elongation (D) states for transcribing influenza polymerase.

Knowledge of the different configurations of the catalytic core will be of particular help in optimizing specific inhibitors, such as nucleoside analogues, that target directly the influenza polymerase RNA synthesis activity.

T. Kouba, P. Drncová and S. Cusack (EMBL)

[1] S. Reich *et al.* (2014) Nature 516, 361-366
[2] M. Lukarska *et al.* (2017) Nature 541, 117-121
[3] S. Reich, D. Guilligay & S. Cusack (2017) Nucleic Acids Res 45, 3353-3368
[4] T. Kouba, P. Drncová & S. Cusack (2019) Nat Struct Mol Biol. 26, 460-470

News from the platforms

CM01 ESRF Platform Upgrades. Structural insights into RNA polymerase backtracking and its reactivation

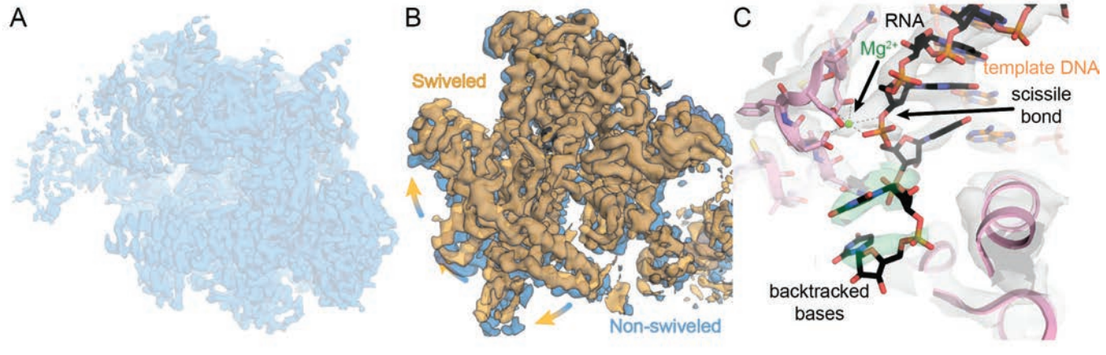
The Titan Krios electron microscope located on CM01 at the ESRF has recently been upgraded with a K2 sensor with better characteristics including a higher detector quantum efficiency or DQE. The new K2 sensor is fully commissioned and in use since January 2019. At the moment, the automatic data collection for single-particle experiments is controlled through the FEI software EPU, yielding a maximum throughput of around 1000 movies a day; this corresponds roughly to 2.4 Tb/day. We will progressively shift the data collection towards an open source software for rapid data collection, SerialEM, with the expectation of a minimum 2-fold increase in data throughput. The software package has been installed in March 2019 and is since then being commissioned and benchmarked. Ultimately, this will allow us to increase the number of experiments performed using the Titan Krios. Parallelised image pre-processing using several GPU-equipped computing clusters is automatically launched on collected movies, using the SCIPION wrapper. CM01 staff has also taken part in several European courses (ESONN, HERCULES) and continues to organise recurring practical courses on sample preparation (see PSB Event page 8).

The first year of operation has been very successful with the vast majority of users from academic institutions located in the 13 different ESRF member states. Some of the key figures achieved so far using CM01 are 2.2 Å for the highest resolution obtained and 65 kDa for the smallest protein structure solved. At the time of writing, 10 publications reporting data collected on CM01 are already available, most of them in high-impact journals. The Volta Phase Plate and its potential to study small macromolecules (<150 kDa) has been exploited successfully as in the structure of adenovirus type 3 fiber head with its receptor desmoglein 2 [1] (see Cover page article).

Recently, high-resolution reconstructions with datasets collected at CM01 have unveiled structural insights into RNA polymerase backtracking and its reactivation [2]. The reconstructions obtained allowed users from IGBMC Strasbourg to build atomic models of the RNA polymerase trapped in distinct states and propose a model for the mechanisms of RNA cleavage and reactivation (Figure): (1) backtracked RNA induces an active site conformation that is incompatible with further RNA extension and opens a channel to allow cleavage factor access; (2) binding of transcription factor GreB further stabilizes this inactive state, favours binding of ions involved in the cleavage reaction, and the backtracked RNA substrate itself stabilizes GreB; (3) after the cleavage occurred, GreB can still access the active site but is less ordered; (4) binding of a substrate to the active site induces a conformation, which is no longer accessible for GreB and thus ensures that cleavage factors do not interfere with RNA extension. Taken together, Albert Weixlbaumer, principal investigator of the study, claims that single particle cryo-EM has allowed them to observe multiple protein complex conformations collected in a single dataset, and also to trap distinct states at a resolution comparable to X-ray crystallography.

C.-M Dieckmann (ESRF), G. Schoehn (IBS) and E. Kandiah (ESRF)

[1] E. Vassal-Stermann, G. Effantin, C. Zubieta, W. Burmeister, *et al* (2019). Nat. Commun. 10, 1181.
[2] M. Abdelkareem, C. Saint-Andre, M. Takacs, G. Papai, *et al* (2019). Mol. Cell, in press (https://doi.org/10.1016/j.molcel.2019.04.029)



(A) Single particle cryo-EM reconstruction of a functional, backtracked RNA polymerase elongation complex refined to a nominal resolution of about 3.4 Å. (B) 3D classification allows to refine different RNA polymerase conformations (compare blue vs. orange map). (C) In the active site, side chains, ions, and nucleic acid bases are resolved. The backtracked portion of the RNA transcript becomes visible at a lower contour level (green density).

The Polara electron microscope has been replaced by a new latest generation microscope: the Talos Glacios.


The IBS/ISBG Talos Glacios electron microscope

It was sad for us to have to say goodbye to the Polara due to the discontinuation of its service contract. It was a very good and very stable electron microscope and is currently being reassembled in MIPT, Russia. On the bright side, it offered us a good opportunity to update our ISBG platform and to increase our efficiency. Thanks to the CEA, the ESRF, the CNRS and FRISBI, we have been able to purchase a new electron microscope, the Talos Glacios, for the ISBG electron microscopy platform. The microscope is located in the IBS building and is a last generation Thermo Fisher 200 kV FEG electron microscope. It not only enables us to screen grids efficiently but also to collect high-resolution data. The Glacios is currently equipped with a Falcon II direct electron detector (controlled by the FEI EPU automated data collection software). The K2 summit electron counting detector retrieved from the Polara will also be installed on the Glacios soon. Up to 12 cryo grids mounted on Krios compatible autogrids can be loaded into the Glacios simultaneously and be kept safely at liquid nitrogen temperature for several days inside the microscope. The loaded autogrids can also be recovered later for storage or for use on another microscope (e.g. ESRF Krios). The new microscope will be used for single particle cryo experiments as well as for cryo electron tomography. Since April 15th, the Glacios is open to all EPN electron microscopists (ESRF is guaranteed 20% of its time) as well as French and international users via direct or subsidized access (INSTRUCT or FRISBI applications). First results have been very encouraging: the resolution of a 3D structure obtained on a test sample (beta galactosidase) has reached 2.7 Å! If you have samples for the Glacios or need more information, please contact us (ibs-plateforme-em.contact@ibs.fr)! Last but not least, thank you to all the people who made the very fast replacement of our high-end microscope possible and to all the others involved in all the steps of buying it, refurbishing the room, installing the electron microscope…

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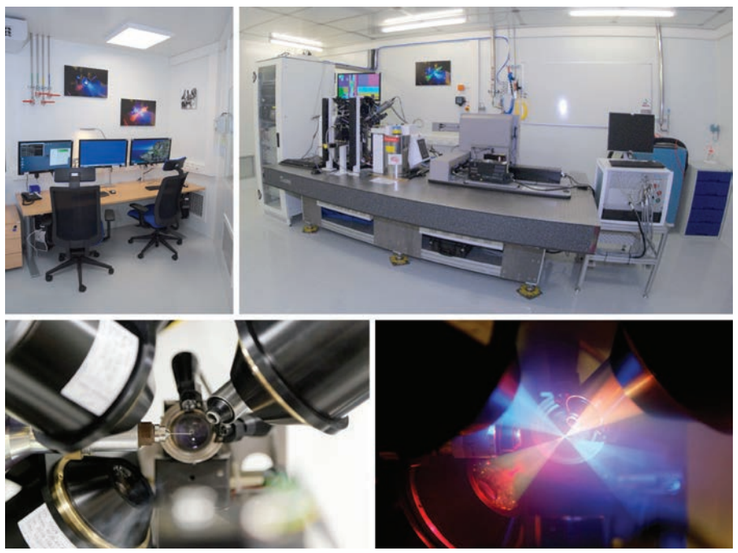
Opening of the *in crystallo* Optical Spectroscopy (*ic*OS) Laboratory

The Cryobench Laboratory project started in 1999 with the goal of recording UV-visible light absorption spectra from cryo-cooled (hence the name 'Cryobench') protein crystals in order to prepare kinetic crystallography experiments. The fluorescence emission modality was added to probe physico-chemical parameters of protein crystals [1], and later, to probe the mechanisms of fluorescent proteins. In 2003, a more robust setup was developed [2] and the Raman functionality was added [3] to probe, for instance, the progress of covalent bond breakage or build-up. Over the last 20 years, more than 100 publications have reported experimental results recorded at the Cryobench, many of which are reviewed in [4]. The experimental setup is continually being refurbished and, following the progressive automation of sample mounting, crystal centring and data collection at the ESRF macromolecular crystallography beamlines, the sample environment is now based on a modified MD2M mini-diffractometer equipped with motorized objectives. This greatly facilitates the centring of the focal volume of each objective onto the crystal and thus the optimization of the recorded spectra quality. The installation of a HC1 humidity controller allows experiments to be routinely performed at ambient temperatures, paving the way for time-resolved experiments.


Top: photos of the control cabin and experimental hutch of the icOS Laboratory. Bottom left: A sample mounted on the modified MD2M which incorporates the three magnifying objectives used to record fluorescence emission and UV-visible absorption spectra. Bottom right: Intersecting light cones coming from each of the three objectives connected via optical fibres to blue, green and red LEDs, respectively.

After the EBS upgrade of the ESRF, the current ESRF ID29 beamline will be reconstructed as a beamline for (time-resolved) serial crystallography experiments. As the experimental hutch of the new ID29 will be in the Chartreuse Extension of the ESRF experimental hall, this project has involved the moving of the Cryobench to new premises. It has also meant the development of a dedicated time-resolved spectroscopy setup to support experiments at ID29 and (potentially) elsewhere. Thus, at the end of 2018 the Cryobench was moved to the Chartreuse Extension and its experimental hutch reconstructed in order to accommodate the planned time-resolved setup. Moreover, as experiments are no longer restricted to cryogenic temperatures, the facility has been renamed the 'in crystallo Optical Spectroscopy (icOS) Laboratory'. First users were welcomed in February 2019. Importantly, the laboratory will remain operational during the EBS shutdown for cryogenic/room temperature experiments based on UV-vis absorption, fluorescence emission or Raman spectroscopy. Full details are available on the icOS website.

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https://www.esrf.eu/home/UsersAndScience/Experiments/MX/icOS.html

[1] D. Bourgeois *et al.* (2002) *J. Appl. Crystallor.* 35, 319-326.
[2] A. Royant *et al.* (2007) *J. Appl. Crystallor.* 40, 1105-1112.
[3] P. Carpentier, A. Royant, J. Ohana, D. Bourgeois (2007) *J. Appl. Crystallor.* 40, 1113-1122.
[4] D. von Stetten *et al.* (2015) *Acta Crystallogr.* D71, 15-26.

'Orbitrap Velos Pro': novel capabilities for mass spectrometry at the PSB

Overview of the Orbitrap Velos Pro instrument installed at the IBS.

Mass spectrometry (MS) can assess the mass of biomolecules with high accuracy, sensitivity and speed. In April 2019, an electrospray-Orbitrap Velos Pro (Thermo Fisher) coupled with liquid chromatography, owned by the EMBL, was transferred from Heidelberg and installed at the IBS MS platform, benefiting from facility support, space and the existing installation fittings (e.g., helium and nitrogen lines).
This instrument has three distinct analysers, a key component of a mass spectrometer where the ions are separated according to their mass and charge. There are two linear ion traps and one Orbitrap [1]. In the latter, ions are electrostatically trapped, while rotating around the central electrode and performing axial oscillations. The oscillating ions induce an image current that is measured and converted by Fourier transformation to the individual frequencies and intensities, yielding mass spectra. Compared to time-of-flight (TOF) analysers, Orbitraps are better in terms of mass accuracy, mass resolution and dynamic range of concentrations that can be analysed. The Orbitrap Velos Pro is an excellent instrument for analysing peptides and may be applied to intact proteins and their post-translational modifications (PTMs).
The instrument is currently being tested to understand better its capacities. To conclude, the instrument is the first Orbitrap mass spectrometer installed at the PSB and represents an excellent opportunity to start exploiting this type of technology.

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ibs-plateforme-ms.contact@ibs.fr
http://www.isbg.fr/samples-preparation/mass-spectrometry/

[1] Makarov A. Anal. Chem. 2000, 72, 1156-1162.

ESRF's Extremely Brilliant Source upgrade advances

On 10th December 2018, the ESRF shut down its iconic third-generation synchrotron for a 20-month upgrade to a new, fourth-generation machine – named Extremely Brilliant Source (EBS) – that will boost the brilliance and coherence of its X-ray beams by a factor of 100.
Now a quarter of the way through the shutdown period, the project is making impressive headway. The previous storage ring has been dismantled, with around 1720 tonnes of equipment removed from the 844-metre-circumference storage ring tunnel in less than three months. Following a period of civil work to prepare the existing infrastructure for the new storage ring, the installation of 129 girders, pre-assembled with over 10,000 cutting-edge components that comprise the EBS, began in March 2019.
Representing something of a logistical challenge for teams, the 12-tonne girders had to be lifted over the storage ring tunnel roof via a gantry and rolled into position in the tunnel with a specially designed transport module. Once in place, engineers connected and precisely aligned them to within 50 microns. Today, all 129 girders are in place – an important and visible achievement. However, the installation process is far from

complete, with the installation of front-ends, radiofrequency cavities, insertion devices and services such as cabling and piping in progress until the end of the year.

The commissioning of the new machine is set to begin in November 2019, followed by beamline commissioning in March 2020, with this powerful new research tool set to open to users in August 2020. The EBS project includes the creation of a new flagship beamline, EBSL8 – the 'old' ID29, dedicated to the technique of serial crystallography (SX). Combined with a 100,000-factor increase in flux density and new techniques, EBSL8 will also allow crystallographic studies at sub-millisecond time resolution, a timescale on which many conformational changes in biological macromolecules occur. Importantly, the other ESRF beamlines dedicated to structural biology will be optimised to ensure they fully exploit the opportunities provided by the improved properties of EBS X-ray beams. These include faster measurements, improved signal-to-noise ratios and a higher spatial resolution sampling of much smaller crystals.


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A. Joly, G. Leonard (ESRF)

Events

2nd practical workshop on sample preparation for Single Particle cryo-EM

The series of practical hands-on workshops organized by the ESRF, EMBL and the IBS continued for the second time from May 21st to 23rd. 12 participants, selected from over 70 applications, all with own, real world samples, were shortly introduced by a general presentation on single particle cryo-electron microscopy. During the following two and a half days, all participants checked the quality of their samples using the negative staining technique on the T12 microscope at the IBS. Tips and tricks from experienced tutors helped them to prepare own sample grids which were then individually checked using the Glacios microscopes located at the IBS and the EMBL as well the Titan Krios of the ESRF. Nearly half of the participants found starting cryo-conditions for their samples. Three samples were of sufficiently high quality such that automatic data collection for two of them could be set-up using an overnight session.

Special thanks go to Claudine Romero for helping to organize the workshop and to Thermo Fisher for both financially and personally supporting the workshop.


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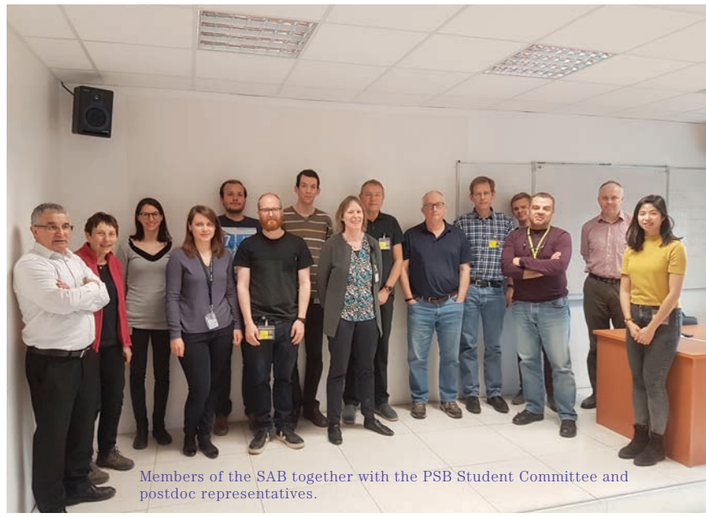
E. Kandiah (ESRF), G. Effantin (IBS), M. Hons (EMBL), G. Schoehn (IBS), C. Mueller-Dieckmann (ESRF)

PSB Scientific Advisory Board review 2019

Excitement was in the air at the PSB on the 2nd and 3rd of April! The Scientific Advisory Board (SAB) visited our campus to evaluate the quality of the PSB. The SAB consists of 8 internationally renowned scientists from all over the world. Their task is to critically revise the functioning of the PSB with an expert "outsider's view". These regular reviews (every 2-3 years) help to maintain the high quality of science and services at the PSB.


Members of the SAB together with the PSB Student Committee and postdoc representatives.

Upon arrival, the review panel got an overview of the activities and actions of the PSB in the last 3-years period, as well as an update on the development of platforms and particularly of cryo-EM on the EPN campus. Next, the SAB members visited the PSB platforms to get a picture of the PSB services on the ground. Later, PSB students presented poster clips followed by a poster session which was combined with the PSB get-together. The panel had the possibility to also informally interact during dinner with the PSB Science Board and PSB Steering Committee scientists as well as with the PSB Student Committee and postdocs during a "working" coffee break the next day. Also on the second day, the scientific directors and heads of EMBL, ESRF, IBS and ILL presented short updates on each institute and their interactions with the PSB, and postdocs from each PSB Partner had the chance to present some scientific highlights in short presentations to the panel.

In a concluding discussion and a written report, the SAB stated high satisfaction with the performance of the PSB in the past review period. Ongoing efforts of the PSB to connect the researchers on campus and provide service platforms were encouraged. Furthermore, the members of the SAB gave insightful ideas how to further improve the fruitful work of the PSB. The PSB researchers wish to thank the SAB members: Jean Cavarelli (IGBMC, FR), Robert Gilbert (Oxford U., UK), Guillermo Montoya (Copenhagen U., DK), Helen Saibil (Birkbeck College, UK), Titia Sixma (NKI, NL. Chair of the SAB), Vladimír Sklenář (CEITEC, CZ), Jeremy C. Smith (U. of Tennessee/ORNL, USA) and Henning Stahlberg (Basel U., CH), for their efforts and their valuable input, and look forward to seeing them again in 3 years!

E. Kowalinski (EMBL)

INTERNATIONAL WORKSHOP ON ADVANCED ISOTOPIC LABELLING METHODS FOR INTEGRATED STRUCTURAL BIOLOGY

From March 26th to 29th, the third edition of the AILM workshop was held on the EPN campus in the IBS and the ESRF buildings. This meeting brought together 160 researchers, half of which were international visitors coming from Europe, USA, Brazil, India and Japan. Four plenary lectures, 24 invited lectures and 19 oral presentations selected from submitted abstracts were programmed together with 70 posters covering the latest advances in isotopic labelling and their applications to the study of complex biological systems by NMR, neutron and mass spectrometry. More than 40 researchers from the EPN campus attended the presentations. AILM2019 was co-organized by IBS, IBPC, IGBMC and York University and was supported by CNRS, Frisbi, CEA, GRAL, University of Grenoble Alps and the French Biophysics Society. 18 young trainees were selected to pursue from March 30th to April 5th with an advanced practical training on isotopic labelling and were offered the opportunity to be trained in advanced techniques using their own protein/RNA constructs. The next edition of the AILM workshop is scheduled for 2021.

J. Boisbouvier (IBS/NMR group)

PSB Spotlight on Imaging

The sixth PSB Spotlight meeting on Imaging took place on 19 March 2019 and over 60 participants attended the morning talks in the IBS seminar room. Three external speakers (Johan Decelle - IRIG Grenoble, Tim Salditt - Göttingen University, DE and Christian Sieben - EPFL, CH) and three local speakers (Alessandro Tengattini - ILL, Peter Cloetens - ESRF and Alister Burt - IBS), presented scientific talks focusing on various types of imaging techniques including electron microscopy, nanoSIM, X-ray fluroscence and holography, super-resolution imaging, neutron tomography, electron cryotomography and nanotomography. These presentations illustrated how different experimental imaging techniques can be combined to perform biological studies at different scales, ranging from single particles to large biological structures.

Speakers of the morning session. From left to right, Top: Johan Decelle, Tim Salditt and Christian Sieben. Bottom: Alessandro Tengattini, Peter Cloetens and Alister Burt.

The afternoon session started with a short introduction of various platforms present on the EPN campus: AFM (Jean-Luc Pellequer, IBS), M4D/Super-resolution microscopy (Jean-Phlippe Kleman, IBS), Platforms of the Partnership for Soft and Condensed Matter (Diego Pontoni, ESRF), and CryoEM and cellular EM (Guy Schoehn, IBS). Following the short talks, 22 persons then participated to one of the various practical sessions that were proposed on AFM, super-resolution microscopy, cryoEM, study of thin cell sections for cellular EM, or X-ray/neutron tomographic reconstruction.

The organizers wish to thank all the speakers and participants for making this day a success.

F. Bernaudat (PSB coordinator)

28th ESRF User meeting

The annual ESRF User Meeting 2019 was held on the EPN Campus from 4th to 6th February 2019, and despite the beginning of the 20-month long shutdown for the ESRF users on 10th December 2018, the meeting was attended by nearly 300 participants. On day 1 the participants could choose between nine "tutorials for users" covering a wide range of different topics, including a lunchtime roundtable debate and discussion on "Gender Parity in Science". The meeting then followed with a plenary session on day 2 which included a keynote lecture by Helena Käck (AstraZeneca, Sweden) on the importance of synchrotron radiation in drug discovery, and on the last day three User-Dedicated Microsymposia (UDMs) were organised. The UDM1 entitled "ID29: Tunable past and time-resolved future", gathered more than 75 participants, and aimed to celebrate the long service of ID29 as a MX beamline dedicated to multiwavelength anomalous scattering experiments and to introduce the future of ID29 as a novel beamline dedicated to time resolved serial crystallography experiments. The first session was dedicated to the past activity of ID29 and included an overall presentation by Sine Larsen (former ESRF Director of Research for Life Sciences in 2003-2009) of the development of ID29 over the last eighteen years beside the evolution of macromolecular crystallography. The second session focused on the future of ID29. The latest developments in sample delivery methods and software for the processing of serial crystallography diffraction data were discussed, and several talks illustrated the future opportunities for serial crystallography and for time resolved studies that will be available on ID29 following the long ESRF shutdown.

F. Bernaudat (ESRF, PSB Coordinator)

PROFILE

Sagar Bhogaraju

Could you tell us a few words about yourself and your scientific background?

I did my PhD in Esben Lorentzen's lab at the Max-Planck Institute for Biochemistry, Martinsried, Germany. There I worked to elucidate the structure and function of intraflagellar transport protein complexes, getting exposed to protein biochemistry and structural biology. Later, I moved to the lab of Prof. Ivan Dikic at Goethe University in Frankfurt for my postdoc. My postdoc environment was multi-disciplinary, with numerous techniques and topics of science within the lab. The experience I gained during my PhD and Postdoc have greatly helped me to develop my current scientific interests and career.

What is the research topic in your lab?

In my lab we're trying to understand a family of proteins known as melanoma antigens (MAGEs); in other words, proteins expressed by certain tumours, which can trigger an immune response. They're called melanoma antigens because they were first discovered in a melanoma patient. That was one of the first tumour antigens discovered in humans, and since then around 50 other similar proteins have been discovered. Because these antigens elicit an immune response, it's possible to use them to develop vaccines for certain types of cancer. Most of the research is focused in this area, and there are numerous clinical trials currently in progress. What is less well known, however, is what roles these proteins have in cells. Recent papers have reported that these proteins can be oncogenic; in other words, they're not just markers of tumours but actually have a role in the development of many different cancer types, although it's not understood how. My lab will be focusing on gaining a molecular understanding of the cellular role of these proteins.

Was it challenging creating your own lab?

Yes, I would say the transition period from postdoc to group leader was challenging. During this time, you're involved in setting up the lab. Of course, it's very important to design the lab the way you want it to be, but it takes your focus away from science briefly, and I'm not used to that. For the past ten years of my research career, I've thought only of science and not the external factors which are imperative to make it happen. To continuously have to think about these organisational matters is a challenge, although things are quite streamlined at EMBL. I think it's important to realise that it's just a passing phase, and as soon as you have some PhD students and postdocs in the lab the science will take over.

Had you heard about the PSB before coming here and how do you think it will help you in your work?

I was aware of PSB and I think it is a great initiative to bring structural biologists together. I will surely benefit from direct interactions with fellow members as well as having access to the many platforms available in the PSB. The upcoming PSB symposium is also very exciting, and I am very much looking forward to it.

What advice would you give a young researcher wishing to become a group leader one day?

My advice is, to be open minded and learn to appreciate all kinds of science and methods. That will prepare you to venture into new directions as a group leader, and to be aware of not so obvious solutions to your scientific problems.

What do you think are the required qualities, and what would you say your best one is?

Of course all the normal qualities like knowledge, expertise, ability to troubleshoot, are important to carve new directions in your scientific career. An ability to recognize a difficult and important scientific question is also crucial. While being patient, and dedicating time to student training will help. But I would say that concerning this profession my best qualities are probably my mental flexibility and calmness.

Newcomers

Daouda Traore

Has joined the ILL Life Science Group in June 2019, on a joint appointment with Keele University (UK). He will contribute to the operation of CM01 Cryo-Electron Microscope at the ESRF. Daouda returns to Grenoble after a 10-year exile in Australia where he was initially a Post-Doc at Monash University, then remained on independent fellowships. He has on a long-standing interest in studies involving of Nucleic Acids/Proteins interactions. His most recent work utilised biochemistry, biophysics, X-ray crystallography and Cryo-EM for the study of conjugative DNA transfer mechanisms in Gram-positive pathogens.

Contact: traored@ill.fr

DATES FOR YOUR DIARY

September 23th – 27th 2019 - Small angle neutron and X-ray scattering from biomacromolecules in solution
This practical course covers the use of small angle scattering from neutrons and X-rays to study biomolecules in solution. The talks are open for all but practicals are limited to selected applicants. More information can be found here: http://meetings.embo.org/event/19-small-angle-scattering

October 5th – 13th 2019 – Fête de la Science
This year the Fête de la Science in Grenoble will take place from October 5th to 13th. The Science Fair provides a unique opportunity for the general public to meet scientists. It's a chance for children to discover science and get information about careers in research, and for adults to deepen their scientific knowledge and discover technological innovations directly derived from science. More information concerning the various events organized in Isère: http://www.fetedelascience-aura.com/isere/

October 7th – 11th 2019 – 1st French Congress on Integrative Structural Biology
The first French meeting dedicated to integrated structural biology and co-organized by the French Crystallography Association and the French Biophysics Society, will take place from October 7th to 11th in the historical city of Toulouse. This meeting provides a unique opportunity to bring together biochemists, biophysicists and structural biologists from all over France. More information and registration details: http://bsi-2019.ipbs.fr/

October 21st – 25th 2019 – 11th AFM BioMed 2019 Summer School
This summer school offers an introduction to atomic force microscopy in life and health sciences. The school covers both fundamentals and expert questions with the possibility for students to bring their own samples. The school runs for a full week and includes morning classes and practical work in the afternoons. For more information: http://bsi-2019.ipbs.fr/

November 15th 2019 – EMBL Science & Society talk by Nina Dudnik
Nina decided at a very early age to become a scientist, but her interest always had a humanitarian angle. She is the founder and former CEO of Seeding labs, a social enterprise whose mission is to empower scientists everywhere to transform the world. For her talk Nina will present the challenges facing scientists in the developing world, and the efforts needed to accelerate their potentials. More information on Nina can be found here: https://ninadudnik.wordpress.com/

ANNOUNCEMENTS

The European Research Council (ERC) has awarded an "Advanced Grant" to **Martin Blackledge**, head of the 'Flexibility and dynamics of proteins' group at the IBS for his project 'DynamicAssemblies'. This ERC project aims at describing the structural and dynamic behaviour of highly disordered viral replication machines, including pre- and post-nucleocapsid assembly complexes, their interaction kinetics with host and viral partners, the effects of post-translational modifications, their assembly and functional mechanisms.

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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.