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Content

DAY 1 - Thursday 4th July 2019

9.15-10.15	Registration	
10:00-12:00	Tours of PSB facilities - **Optional**	
11.30-14.00	Registration	
11.30-13:45	Lunch in the canteen	
13.45-14:00	**Stephen Cusack** European Molecular Biology Laboratory Grenoble (FR)	*Conference Welcome*
14:00-15:00	**Opening keynote lecture** Chair: Stephen Cusack (EMBL)	
	Judy Hirst MRC- Mitochondrial Biology Unit (UK)	*CryoEM Structure of Mammalian Respiratory Complex I, an Asymmetric 1 MDa Energy-Converting Enzyme*
15:00-16:25	**Session 1: Molecular machines** Chair: Florent Bernaudat (PSB)	
15:00	**Frank Gabel** Institut de Biologie Structurale / Institut Laue-Langevin (FR)	*SANS as a unique tool for structural biology of biomacromolecular assemblies*
15:35	**Sigrid Milles** Institut de Biologie Structurale (FR)	*Studying intrinsically disordered proteins by integrated NMR and fluorescence spectroscopies*
16:10	**Gang Dong** University of Vienna (AT)	*Assembly and function of BILBO1, a multidomain cytoskeletal protein from the human parasite Trypanosoma brucei*
16:25-16:55	Coffee break (30 min)	
16:55-19:10	**Session 2: Machines on genes** Chair: Marc Jamin (IBS)	
16:55	**Xiaodong Zhang** Imperial College London (UK)	*Structures and Mechanisms of Transcription Initiation and Its Regulation*
17:30	**Andrea Musacchio** Max Planck Institute Dortmund (DE)	*The kinetochore: the ultimate divisive machine*
18:05	**Wei Yang** National Institutes of Health (US)	*Structure, assembly and reaction chemistry of the DNA replisome*
18:40	**Thomas Miller** The Francis Crick Institute (UK)	*Mechanism of head-to-head MCM double-hexamer formation revealed by cryo-EM*
18:55	**Susanne Kassube** Friedrich Miescher Institute (CH)	*Structural basis of Fe-S cluster biogenesis in DNA replication and repair proteins*
19:10	**Clip session for selected abstracts**	
19:25-21:30	Cocktail reception Buffet dinner + poster session	

DAY 2 - Friday 5th July 2019

9:00-11:55	**Session 3: Machines on RNA** Chair: Eva Kowalinski (EMBL)	
09:00	**Michael Sattler** Technical University of Munich (DE)	*NMR and integrative structural biology to study dynamics in regulatory biomolecular interactions*
09:35	**Kathleen Collins** University of California, Berkeley, (US)	*Biogenesis and Structure of Human Telomerase*
10:10	Group photo + Coffee break (40 minutes)	
10:50	**Martin Jinek** Zurich University (CH) *(EMBO Young Investigator Lecture)*	*CRISPR-Cas genome editors: structures, mechanisms and applications*
11:25	**Moamen Abdelkareem** Institute of Genetics and Molecular and Cellular Biology (FR)	*Structural Basis of Transcription: RNA Polymerase backtracking and its reactivation*
11:40	**Sebastian Fica** MRC - Laboratory of Molecular Biology (UK)	*A human post-catalytic spliceosome structure reveals essential roles of metazoan factors for exon ligation*
11:55	**Poster awards**	
12:00-13:00	Lunch in the canteen	
13:00-15:15	**Session 4: Cross membrane talk** Chair: Eaazhisai Kandiah (ESRF)	
13:00	**Henning Tidow** Hamburg University (DE)	*Hybrid approaches for structural studies of integral membrane proteins*
13:35	**Elena Seiradake** University of Oxford (UK) *(EMBO Young Investigator Lecture)*	*A combinatorial code in brain development: how do cell surface receptors guide neurons by forming different structures?*
14:10	**Poul Nissen** Aarhus University (DK)	*Structure and Dynamics of Membrane Transport Proteins*
14:45	**Valentina Palmerini** European Institute of Oncology (IT)	*Structural insights into the TNFR Grindelwald, coupling loss of cell polarity with neoplastic growth*
15:00	**Ian White** Stanford University (US)	*Structural analysis of the NSF reaction coordinate by single-particle cryo-EM*
15:15-15:45	Coffee break (30 min)	
15:45-18:45	**Session 5: Technology advancements** Chair: Trevor Forsyth (ILL)	
15:45	**Julia Mahamid** European Molecular Biology Laboratory Heidelberg (DE)	*Molecular Views into Cellular Functions by in situ Cryo-Electron Tomography*
16:20	**Petra Fromme** Arizona State University (US)	*Time-Resolved Femtosecond Crystallography with XFELs*
16:55	**Gaël McGill** Harvard Medical School (US)	*Visualizing Molecular Choreography: Tools, Techniques and Design*

17:30	**Matthew Blakeley** Institut Laue-Langevin (FR)	*Neutron crystallography*
17:45-18:45	**Closing keynote lecture**	
	James Naismith University of Oxford (UK)	*Putting enzymes to work; making macrocyclic peptide hybrids*
18:45	Closing remarks	
	Transfer from EPN to Conference Dinner venue by tram and cable car	
20:00:00	Reception and Conference Dinner at Le Per'Gras	

Abstracts of invited and selected talks

(by order of presentation)

CryoEM Structure of Mammalian Respiratory Complex I, an Asymmetric 1 MDa Energy-Converting Enzyme

J. Hirst[1]

[1]MRC Mitochondrial Biology Unit, University of Cambridge, UK
jh@mrc-mbu.cam.ac.uk

Respiratory complex I (NADH:ubiquinone oxidoreductase) is one of the largest membrane-bound enzymes in the mammalian cell. It powers ATP synthesis in mitochondria by capturing the free energy produced by electron transfer from NADH to ubiquinone and using it to drive protons across the inner membrane. Mammalian complex I contains 45 subunits. 14 core subunits house the catalytic machinery and are conserved from bacteria to humans, whereas the cohort of 31 supernumerary subunits forms an exoskeleton around the core and is specific to mammalian species. Structures of mammalian complex I, determined by single-particle electron microscopy (cryoEM) have now been determined for several species and have been steadily improving in resolution. In this talk I will discuss recent data on complex I from mouse heart mitochondria, a biomedically relevant model system, including the structures of mechanistically critical elements such as differences in helical geometry in the membrane domain that occur upon activation, or that alter the positions of catalytically important charged residues. The results demonstrate the capability of cryo-EM analyses to challenge and develop mechanistic models for mammalian complex I.

SANS as a unique tool for structural biology of biomacromolecular assemblies

F. Gabel[1]

[1]Institut de Biologie Structurale, CEA-UGA-CNRS, 71 avenue des Martyrs, 38044 Grenoble, France.
frank.gabel@ibs.fr

Small angle neutron scattering (SANS) provides unique insight into biomacromolecular complexes by combining solvent contrast variation (H2O:D2O exchange) with either natural contrast between different classes of biomolecules (proteins, RNA/DNA, lipids/detergents) and/or by applying artificial contrast, i.e. deuteration of specific biomolecules.
Here, I present results from different biological projects where SANS has played a crucial role by providing unique restraints for structural refinement and interpretation, complementary to other techniques (NMR, EM, crystallography).

As a first example, I will show how distance and shape restraints from SANS have helped to improve the uniqueness of structural models for a multi-protein-RNA complexes, in combination with NMR restraints and building blocks from crystallography [1]. In a second example, the stoichiometry and internal topology of a highly symmetric, hetero-dodecameric aminopeptidase enzyme complex is revealed by SANS, and conclusions on the assembling process can be drawn in combination with EM data [2]. In a third example combining time-resolved (TR) SANS with online fluorescence, the active unfolding of GFP by an unfoldase could be monitored at a time resolution of 30 seconds, as well as the concomitant conformational changes of the unfoldase [3]. In a fourth example of supercharged proteins, I will show that SAXS/SANS provides not only shape information but also very valuable insight into the hydration shell properties of biomacromolecules [4].

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Studying intrinsically disordered proteins by integrated NMR and fluorescence spectroscopies

S. Milles[1], S. Guseva[1], M.R. Jensen[1], A. Desfosses[1], G. Communie[1], S. Ivashchenko[1], D. Maurin[1], C. Lazert[2], D. Gerlier[2], G. Schoehn[1], I. Gutsche[1], R.W.H. Ruigrok[1], M. Blackledge[1]

[1]Université Grenoble Alpes, CNRS, Commissariat à l'Energie Atomique et aux Energies Alternatives, Institut de Biologie Structurale, 38000 Grenoble, France,
[2]International Center for Infectiology Research, INSERM, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, Ecole Normale Supérieure de Lyon, Université de Lyon, Lyon, France
sigrid.milles@ibs.fr

Intrinsically disordered proteins (IDPs) lack clearly defined structure and are therefore highly flexible and easily adaptable to different binding partners. This makes them important players in many biological processes, often with vital regulatory functions. Their dynamic features and broad range of interaction modes, however, render them difficult to study and their complexes often require integrated approaches. Exploiting the complementarity of nuclear magnetic resonance (NMR) and fluorescence approaches to investigate IDPs in complex with various interaction partners allowed us to study the dynamic nature of interactions covering affinities ranging from Kd values of only millimolar to few nanomolar, and the associated binding dynamics. In particular, we demonstrate how the measles virus replication machinery exploits disorder on two of its major proteins to regulate the formation of viral nucleocapsids [1,2], and we describe the structure and dynamics of a 90-kDa viral protein complex comprising a total of 450 disordered amino acids, at atomic resolution. Within this complex, NMR reveals the existence of an ultraweak interaction motif that is essential for viral transcription/replication [3]. This essential interaction appears to be conserved across Paramyxoviridae, opening unique new perspectives for drug development against this family of pathogens.

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Assembly and function of BILBO1, a multidomain cytoskeletal protein from the human parasite *Trypanosoma brucei*

K. Vidilaseris, Y. Pivovarova, P. Majneri, J. Lesigang, G. Dong[1]

[1]Max Perutz Lab Vienna, Vienna Bio-center, Medical University of Vienna,
Dr. Bohr-Gasse 9, 1030 Vienna, Austria.
gang.dong@univie.ac.at

Trypanosoma brucei is a protozoan parasite causing sleeping sickness in Africa. At the base of its single flagellum is a bulb-like structure called the flagellar pocket (FP), which is responsible for all endo-/exocytosis of the cell and thus essential for the survival of the parasite. At the neck of the FP is an electron-dense structure called the flagellar pocket collar (FPC), which is essential for FP biogenesis and thus the survival of the parasite. BILBO1 was the first reported protein component of the FPC. We have characterized the structure and assembly of this multidomain cytoskeletal protein. Briefly, our initial bioinformatics analysis suggests that the 67-kDa BILBO1 consists of four structural domains, including a globular N-terminal domain, two central EF-hand motifs followed by a long coiled-coil domain, and a C-terminal leucine zipper. We found that recombinant BILBO1 forms enormously large oligomers *in vitro*, which makes it intractable by any single conventional structural study method. Therefore, we carried out structural dissection of *T. brucei* BILBO1 using integrative structural biology approaches including NMR, crystallography, EM, and various biophysical methods. The high-resolution structure of its N-terminal domain (by both NMR and X-ray) reveals a variant ubiquitin-like fold with a unique conserved surface patch; mutagenesis of this patch causes cell death *in vivo*. We further found that the EF-hand motifs change their conformation upon calcium binding, whereas the coiled-coil domain forms an anti-parallel homodimer. Employing a series of truncation analyses and EM experiments, we revealed that intermolecular interactions between adjacent leucine zippers allow BILBO1 to form extended filaments. These filaments were additionally shown to condense into fibrous bundles through lateral interactions. Based on all these experimental data, we propose a mechanism for BILBO1 assembly at the flagellar pocket collar, which enables it to assume the role as a scaffold of the FPC.

Structures and Mechanisms of Transcription Initiation and Its Regulation

X. Zhang

Section of Structural Biology, Department of Medicine, Imperial College London, UK.
xiaodong.zhang@imperial.ac.uk

Gene transcription is a fundamental cellular process that is carried out by the multi-subunit RNA polymerase (RNAP), which is conserved from bacteria to human. Transcription is highly controlled and many regulatory factors/strategies act on initiation, which involves the opening up the double strand DNA into single strands and the delivery of the DNA template into the RNAP active centre. Transcription initiation is a highly dynamic process, and has thus been difficult to be studied structurally. We use a special form of bacterial RNAP, which allows us to trap intermediate states, to study the transcription initiation process. In the last few years, using cryoEM and X-ray crystallography combined with biochemical studies, we have started to unravel the detailed molecular mechanism of transcription initiation process. I will discuss our work in bacterial transcription initiation, how RNAP and transcription can be regulated and exploited for antimicrobial purposes.

The kinetochore: the ultimate divisive machine

A. Musacchio

Max Planck Institute of Molecular Physiology, Dortmund, Germany
andrea.musacchio@mpi-dortmund.mpg.de

During mitotic cell division, each daughter cell receives from its mother cell an exact, full copy of the genome. For this to happen, the sister chromatids in the mother cell must bi-orient on the mitotic spindle. Sister chromatid separation at the metaphase-to-anaphase transition then leads to equal segregation of the genome to the two daughters.

Chromosome attachment to spindle microtubules takes place at complex protein structures named kinetochores, which contain multiple copies of as many as ~30 individual core subunits. This stable protein core emerges from a specialized region of the chromosome known as the centromere. Microtubule binding by kinetochores is subject to a feedback control mechanism known as error correction (ER), and whose purpose is to detect improper configurations of the attachments and allow their regression. This mechanism is believed to require a force sensor capable of monitoring differences in the action of forces acting on kinetochores when they are bi-oriented (correct attachments) or not (incorrect attachment). The molecular nature of this force sensor remains unclear.

In addition, kinetochores determine the timing of mitotic exit by exercising control over the cell cycle machinery through the spindle assembly checkpoint (SAC). The SAC coordinates completion of bi-orientation with the transition to anaphase, preventing premature mitotic exit in the presence of incompletely attached sister chromatid pairs. All SAC components are recruited to kinetochores and regulated there in a way that reflects attachment status but that remains poorly understood.

In the last several years, our laboratory engaged in the *in vitro* reconstitution and in the structural and functional characterization of several kinetochore sub-complexes that operate at the interface between chromatin and microtubules. We also reconstituted crucial aspects of SAC signalling, identifying a rate-limiting step in the pathway, as well as a set of catalysts that accelerate the accumulation of the checkpoint effector, the mitotic checkpoint complex (MCC).

Our current efforts aim to unravel the role of kinetochores in SAC signalling, using reconstituted material as our entry point in the investigation. I will report on the conceptual challenges associated with this idea, as well as on our recent experimental progress.

Structure assembly and reaction chemistry of DNA replisome

W. Yang[1] and Y. Gao[1]

Laboratory of Molecular Biology, NIDDK, National Institutes of Health, USA
weiy@niddk.nih.gov

The replisome that performs concerted leading and lagging DNA strand synthesis at a replication fork has never been visualized in atomic detail. Using bacteriophage T7 as a model system, we determined cryo-EM structures up to 3.2 Å of helicase translocating along DNA, and of helicase-polymerase-primase complexes engaging in synthesis of both DNA strands. Each domain of the spiral-shaped hexameric helicase translocates hand-over-hand sequentially along a ssDNA coil, akin to the way AAA+ ATPases unfold peptides. Two lagging-strand polymerases are attached to the primase ready for Okazaki-fragment synthesis in tandem. A β-hairpin from the leading-strand polymerase separates two parental DNA strands into a T-shaped fork, thus enabling the closely coupled helicase to advance perpendicular to the downstream DNA duplex. These structures reveal the molecular organization and operating principles of a replisome.

By combining in crystallo catalysis with X-ray diffraction analysis of reaction intermediates, we have observed the processes of DNA synthesis and RNA degradation at unprecedented atomic details. Contrary to the 80-year old transition-state theory and the dogma that enzyme-substrate, transition state, and enzyme-product states have identical atomic composition and catalysis occurs by enzyme stabilizing the transition state, we have discovered that it is essential for enzyme-substrate complexes to capture a third Mg^{2+} ion en route to product formation. We find that cation trafficking propels both DNA synthesis and RNA degradation.

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Mechanism of head-to-head MCM double-hexamer formation revealed by cryo-EM

T.C.R. Miller[1], J. Locke[1], J.F. Greiwe[1], J.F.X. Diffley[2], A. Costa[1]

[1]Macromolecular Machines Laboratory, The Francis Crick Institute, NW1 1AT, London, UK
[2]Chromosome Replication Laboratory, The Francis Crick Institute, NW1 1AT, London, UK
thomas.miller@crick.ac.uk

In preparation for bidirectional replication, the origin recognition complex (ORC) loads two MCM helicases onto DNA, forming a head-to-head double hexamer (DH). Two contrasting models for DH formation have been proposed. Single-molecule experiments suggest a sequential mechanism whereby ORC-dependent loading of the first hexamer drives second hexamer recruitment [1]. However, biochemical data show that two rings are loaded independently via the same ORC-mediated mechanism, at two inverted DNA sites [2, 3]. We used time-resolved EM, *in silico* reconstitution of complete origins of replication, and high-resolution cryo-EM to describe the entire helicase loading mechanism. We confirm that both hexamers are recruited via the same interaction between the MCM and ORC C-terminal domains, and identify an unanticipated mechanism for coupled MCM loading. Our results show how an asymmetric origin can initiate the loading of a symmetric DH, reconciling two apparently conflicting models.

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Structural basis of Fe-S cluster biogenesis in DNA replication and repair proteins

S. A. Kassube[1,2], N. H. Thomä[1,2]

[1]Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland
[2]University of Basel, Basel, Switzerland
susanne.kassube@fmi.ch

Fe-S clusters are ancient and versatile protein cofactors that are found in all three domains of life [1]. A striking number of proteins involved in DNA metabolism, including DNA polymerases, helicases and primase, contain Fe-S clusters [2-4]. In eukaryotes, the cytosolic iron sulfur assembly (CIA) pathway mediates the insertion of Fe-S clusters into these proteins [5]. A critical component of this pathway is the CIA targeting complex that recognizes client proteins and facilitates Fe-S cluster transfer [6, 7]. To shed light on the molecular mechanism of Fe-S cluster transfer and client protein recognition, we determined crystal structures of a heterodimeric catalytic core of the CIA complex, and of the entire CIA targeting complex that additionally contains MMS19, an adaptor protein required for the recruitment of DNA repair and replication proteins.

The structures reveal evolutionarily conserved surface features of the complex involved in client protein recruitment. Single-particle cryo-EM reconstructions of the CIA targeting complex bound to different client proteins, combined with mutational analysis, in vitro binding studies, and yeast complementation assays, confirm these as bona fide interaction sites. Together, these results highlight the high evolutionary conservation of the CIA pathway from yeast to human and its mechanism of client protein recognition.

Our structural analysis of the CIA targeting complex visualizes a key component of the CIA pathway, providing a framework for the interpretation of biochemical and cell biological data. This work thus contributes towards our mechanistic understanding of Fe-S cluster biogenesis, and sheds light on the critical biological functions of Fe-S clusters in DNA repair and replication factors.

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NMR and integrative structural biology to study dynamics in regulatory biomolecular interactions

M. Sattler [1,2,3]

[1] Institute of Structural Biology, Helmholtz Zentrum München, Neuherberg, Germany, [2]Center for Integrated Protein Science Munich and Biomolecular NMR-spectroscopy, Department of Chemistry, Technical University of Munich, Garching, Germany, [3]Bayerisches NMR-Zentrum

sattler@helmholtz-muenchen.de

Most eukaryotic proteins are comprised of multiple structural domains, which are often connected or flanked by intrinsically disordered regions (IDRs). These IDRs enable regulation by posttranslational modifications and dynamic arrangements of multiple domains. The molecular functions of these multidomain proteins often involve dynamic structural ensembles and can be controlled by population shifts between inactive and inactive conformations.

We employ integrative structural biology combining solution techniques such as NMR, small angle scattering (SAXS/SANS) and FRET with X-ray crystallography and biophysical techniques, to unravel the molecular recognition and dynamics of multidomain proteins linked to RNA-based gene regulation and cellular signaling, for example involving molecular chaperones and peroxisome biogenesis. The talk will highlight the importance of conformational dynamics in the formation and function of regulatory RNP (ribonucleoprotein) complexes in splicing regulation and (mi)RNA processing.

I will also describe integrative approaches for early-state structure-based drug discovery targeting protein-protein interactions and dynamic binding sites.

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Biogenesis and Structure of Human Telomerase

T.H.D. Nguyen[1,2,3], E. Nogales[1,3,4], K. Collins[1]

[1] Department of Molecular and Cell Biology, University of California, Berkeley, California 94720, USA
[2] Miller Institute for Basic Research in Science, University of California, Berkeley, California 94720, USA
[3] Molecular Biophysics and Integrative Bio-Imaging Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720, USA
[4] Howard Hughes Medical Institute, University of California, Berkeley, California 94720, USA
kcollins@berkeley.edu

Telomerase adds telomeric repeats to chromosome ends to balance incomplete replication. Telomerase regulation is implicated in cancer, aging and other human diseases, but progress towards telomerase clinical manipulation is hampered by the lack of structural data. We have recently determined a cryo-electron microscopy structure of substrate-bound human telomerase holoenzyme at subnanometer resolution [1]. The cellular holoenzyme has two flexibly tethered lobes bridged by 'ropes' of RNA. One lobe contains the telomerase catalytic core, with telomerase reverse transcriptase (TERT) and conserved motifs of telomerase RNA (hTR). In the catalytic core, RNA encircles TERT, adopting a well-ordered tertiary structure with surprisingly limited protein-RNA interactions. The second lobe is an intact H/ACA ribonucleoprotein (RNP), representing a pioneering structure of a large eukaryotic family of ribosome and spliceosome biogenesis factors. The H/ACA RNP lobe comprises two sets of heterotetrameric H/ACA proteins and one Cajal body protein, TCAB1. Our findings provide a structural framework for understanding human telomerase disease mutations and represent an important step towards telomerase-related clinical therapeutics.

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CRISPR-Cas genome editors: structures, mechanisms and applications

M. Jinek

Department of Biochemistry, University of Zurich, Switzerland
jinek@bioc.uzh.ch

In bacteria, the CRISPR-Cas system functions as an adaptive system to provide resistance against molecular parasites such as viruses and other mobile genetic elements. RNA-guided effector nucleases associated with CRISPR-Cas systems have been repurposed as powerful tools for precision genome editing in eukaryotic cells and organisms. My prior work demonstrated that the CRISPR-associated protein Cas9 functions as a programmable DNA-cutting enzyme whose sequence specificity isdetermined by a short guide RNA molecule, and subsequently demonstrated that the enzyme can be programmed to induce double-strand DNA breaks in cultured human cells, paving the way for CRISPR-based genetic engineering. In my research group at the University of Zurich, our current work focuses on studying the molecular mechanisms of Cas9 and other CRISPR-associated nucleases using a combination of structural, biochemical and biophysical approaches. To this end, we initially determined the three-dimensional structures of Cas9 in complex to a guide RNA and target DNA, revealing the atomic interactions underpinning the recognition of a short motif in the substrate DNA (the protospacer adjacent motif, PAM), which is necessary to facilitate strand separation in the DNA and guide RNA hybridization. These studies have established a structural framework for engineering novel Cas9 variants with altered PAM specificities. More recently, we have focused on Cas12a (Cpf1), another RNA-guided nuclease enzyme that has emerged as a complementary genome editing tool to Cas9. The crystal structure of Cas12a bound to a guide RNA shows that, like Cas9, Cas12a structurally preorganizes the seed sequence of the guide RNA to facilitate target DNA recognition. In turn, structures of Cas12a bound to a guide RNA and a double-stranded DNA target capture nuclease in a pre-cleavage state, revealing the mechanism of R-loop formation. Together with supporting biochemical experiments, the structures reveal that Cas12a contains a single nuclease active site that sequentially cleaves both strands of the target DNA in a defined sequential order. Collectively, our studies provide a mechanistic foundation for understanding the molecular function of CRISPR-based genome editor nucleases and for the on-going development of CRISPR-Cas genetic engineering for biotechnological and therapeutic applications.

Structural Basis of Transcription: RNA Polymerase backtracking and its reactivation

M. Abdelkareem[1], C. Saint-André[1], M. Takacs[1], G. Papai[1], C. Crucifix[1], X. Guo[1], J. Ortiz[1,2], A. Weixlbaumer[1,3]

[1]Department of Integrated Structural Biology, Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC)/Université de Strasbourg/CNRS/INSERM, 67404 Illkirch Cedex, France ;

[2]present address: Forschungszentrum Jülich, Ernst Ruska-Centre for Microscopy and Spectroscopy with Electrons;

[3]Correspondence to: **albert.weixlbaumer@igbmc.fr**

abdelkam@igbmc.fr

To express genetic information inside a cell, several machineries are involved. The first step is the transcription of DNA into RNA and carried out by multi-subunit RNA polymerases (RNAPs). Transcription needs to be highly regulated to ensure an accurate and timely flow of information and to minimize errors. One important regulatory mechanism is called backtracking, which describes the reverse translocation of RNAP along the DNA. It is a universal phenomenon in all kingdoms of life and occurs upon the encounter of regulatory sequences, or erroneous incorporations during DNA transcription [1, 2, 3]. Backtracking switches active transcribing RNAPs into inactive (arrested) ones, by extruding the RNA 3' end from the active site. Reactivation of arrested RNAPs requires the cleavage of RNA and aligning the newly generated RNA 3' end with the active site. In *E. coli*, RNAP requires the help of an accessory factor called GreB, which increases the efficiency of RNA cleavage [4, 5]. Here, we report four cryo-EM reconstructions of *Escherichia coli* RNA polymerase representing the entire reaction pathway: A backtracked complex (i); a backtracked complex with GreB before (ii), and after (iii) RNA cleavage; and a reactivated, substrate bound complex with GreB before RNA extension (iv). The backtracked complex provides new insights regarding the backtracked RNA, which assumes a new and distinct conformation different from its counterpart in eukaryotes. GreB binds RNAP through the coiled-coil helices and the insertion domain (SI3) at the rim of the secondary channel. RNA polymerase conformational changes cause distinct GreB states: i) a fully engaged GreB before cleavage; ii) a disengaged GreB after cleavage; and iii) a dislodged, loosely bound GreB removed from the active site to allow RNA extension. The substrate bound complex shows new features regarding the dynamics of the secondary channel during RNA extension and trigger loop folding and stabilization by another element called the F loop. These reconstructions give insights on the catalytic mechanism and dynamics of RNA cleavage and extension, and suggest how GreB targets backtracked complexes without interfering with canonical transcription.

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A human post-catalytic spliceosome structure reveals essential roles of metazoan factors for exon ligation

S. M. Fica, C. Oubridge, M. E. Wilkinson, A. J. Newman, K. Nagai

MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH, UK
sfica@mrc-lmb.cam.ac.uk

During exon ligation, the *S. cerevisiae* spliceosome recognizes the 3´-splice site (3´SS) of precursor mRNA through non-Watson-Crick pairing with the 5´SS and the branch adenosine, in a conformation stabilised by Prp18 and Prp8. Here we present the 3.3 Å cryoEM structure of a human post-catalytic spliceosome just after exon ligation. The 3´SS docks at the active site through conserved RNA interactions in the absence of Prp18. Unexpectedly, the metazoan-specific FAM32A directly bridges the 5´-exon and intron 3´SS of pre-mRNA and promotes exon-ligation, as shown by functional assays. CACTIN, SDE2, and NKAP – factors implicated in alternative splicing – further stabilize the catalytic conformation of the spliceosome during exon ligation. Together these four proteins act as exon ligation factors. Our study reveals how the human spliceosome has co-opted additional proteins to modulate a conserved RNA-based mechanism for 3´-splice site selection and to potentially fine-tune alternative splicing at the exon ligation stage.

Hybrid approaches for structural studies of integral membrane proteins

H. Tidow[1]

The Hamburg Centre for Ultrafast Imaging (CUI), Department of Chemistry, Institute of Biochemistry and Molecular Biology, University of Hamburg
tidow@chemie.uni-hamburg.de

Structural studies of integral membrane proteins (IMPs) are often hampered by the fact that most membrane proteins suffer loss of stability, activity and function outside of the lipid environment. This is a significant drawback in common approaches where detergents are used to solubilize IMPs from the native lipid bilayer for structural studies. Nanodisc lipid bilayer scaffolds help overcome numerous challenges associated with the handling and stability of membrane proteins since they offer a way of studying the reconstituted membrane proteins in a native-like lipid environments. For this reason they and have become an invaluable tool for biochemical and structural characterization of IMPs.
I will present recent data that made use of the "stealth nanodisc" technology to structurally characterize two different IMPs (the ABC transporter MsbA and the P-type ATPase ACA8) using a combination of small-angle X-ray and neutron scattering (SAXS/SANS). Using fractionally deuterated MSP1D1 belt protein and phosphatidylcholine lipids, we could render the carrier system invisible to neutron radiation and thus probe the solution conformation of the incorporated IMPs. This approach is highly advantageous for studying dynamic and flexible membrane proteins in a native-like lipid environment.
I will further present recent time-resolved SAXS data investigating the conformational cycle of the ABC transporter MsbA. Using light-activation and rapid-mixing, respectively, we could follow the dimerization and subsequent dissociation reactions of MsbA upon ATP-binding and hydrolysis with ms resolution.

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A combinatorial code in brain development: how do cell surface receptors guide neurons by forming different structures?

D. del Toro[1], M. Carrasquero-Ordaz[2], M. Shahin[2], V.A. Jackson[2], M. Chavent[3], M. Berbeira-Santana[2], G. Seyit-Bremer[1], S. Brignani[1], R. Klein[1], E. Seiradake[2]

[1] Max Planck Institute of Neurobiology, [2] University of Oxford, [3] CNRS/Paul Sabatier University
email: elena.seiradake@bioch.ox.ac.uk

Advances in structural biology and microscopy have led to a new level of understanding of molecular processes in neurobiology that were intractable until recently. New structural knowledge of axon guidance cue systems and adhesion molecules can now be combined with live cell and super-resolution microscopy and sophisticated *in vivo* expression techniques. This talk will showcase our recent progress in understanding how a combinatorial code of receptor and ligand interactions directs adhesive and repulsive cellular responses in the developing brain cortex. Depending on the context, these interactions give rise to distinct molecular assemblies with unique functional properties. Understanding the rules governing the assembly of receptor-ligand complexes requires detailed knowledge of their structures and cellular functions. We combine structural biology with *in vitro* and *in vivo* neurobiology to understand how cell guidance receptors function on the molecular, cellular and tissue levels.

Structure and Dynamics of Membrane Transport Proteins

P. Nissen [1, 2]

[1]Danish Research Institute of Translational Neuroscience – DANDRITE,
Nordic-EMBL Partnership for Molecular Medicine.
[2]Aarhus University, Dept. Molecular Biology and Genetics, Gustav Wieds Vej 10C, DK – 8000 Aarhus C
pn@mbg.au.dk

Using primarily membrane protein crystallography and cryo-EM, and a range of biochemical and biophysical methods such as electrophysiology, single-molecule FRET, and molecular dynamics simulations, we have obtained deep insight into the functional cycle of primary active transporters of the P-type ATPase family.
The transmembrane gradients for the key cations Na+, K+, and Ca2+ are generated by Na,K-ATPase and Ca2+-ATPases of the P-type ATPase family. In brain, Na,K-ATPase activity accounts for an estimated 40-70% of total ATP hydrolysis and potentiates e.g. Na+ and K+ channels for their activity in action potentials, membrane potential, Na+ coupled transport of e.g. glucose, metabolite, neurotransmitters, Ca2+ efflux, pH and Cl- control. Ca2+-ATPases of the same P-type ATPase family maintain steep calcium gradients, internal Ca2+ stores, and cytoplasmic free calcium at accurate levels that define and potentiate calcium signalling pathways. These activities are fundamental to physiology, and malfunctions are linked to diseases such as neurological and cardiovascular disorders.
Lipid flippases, also of the P-type ATPase family maintain asymmetric lipid distributions in biomembranes. Their activity potentiate membrane dynamics, but the structure and function of lipid flippases have so far remained enigmatic. We have determined the first structures using cryo-EM

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Structural insights into the TNFR Grindelwald, coupling loss of cell polarity with neoplastic growth

V. Palmerini[1], J. Colombani[2], S. Monzani[1], V. Cecatiello[1], S Mari[1], S. Pasqualato[1], D. S. Andersen[2], M. Mapelli[1]

[1]Istituto Europeo di Oncologia, Milan, Italy, [2]University of Copenhagen, Copenhagen, Denmark
valentina.palmerini@ieo.it

Disruption of polarity and impairment of programmed cell death are hallmarks of advanced epithelial tumors, whose progression often involves JNK signaling. However, the link between loss of polarity and JNK signaling still remains elusive. We recently characterized a novel *Drosophila* TNF receptor, named Grindelwald (Grnd) [1], that triggers apoptosis by binding the unique fly TNF Eiger via its extracellular domain, and by recruiting to the plasma membrane Traf2, the most upstream component of the JNK pathway. Intriguingly, in $Ras^{V12};scrib^{-/-}$ polarity-perturbed epithelia, Grnd interacts with the polarity protein Veli to promote hyper-proliferation and invasiveness in an Eiger-independent manner. These observations depict Grnd as the first TNFR able to couple cell polarity with tumor overgrowth via the JNK signaling. To shed light on the mechanism of Grnd activation, we determined the crystal structure of Grnd, alone and in complex with Eiger. The structures showed that extracellular Grnd comprises a single cysteine-rich domain, organized in a β-hairpin followed by two α-helices. All cysteines are involved in disulfide bridges, showing a unique organization pattern for Grnd compared to other TNFRs. Grnd forms hetero-hexamers with the TNF domain of Eiger, whose TNF domain folds in a peculiar "jelly-roll" antiparallel β-sheet. Binding assays with interface mutants of Grnd and Eiger designed on the basis of the structure, revealed that the β-hairpin tip and the first α-helix of Grnd, and the loops between β-strands of Eiger are the major determinants of the nanomolar interaction between the two proteins, as measured by ITC. To address the relevance of the Eiger:Grnd interaction for the proliferative and apoptotic activities of Grnd, we engineered Grnd mutants flies and we are currently setting up *in vivo* experiments.

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Structural analysis of the NSF reaction coordinate by single-particle cryo-EM

K. I. White[1,2,3,4,5], L. Esquivies[1,2,3,4,5], R. Pfuetzner[1,2,3,4,5] and A. Brunger[1,2,3,4,5]

Department of Molecular and Cellular Physiology, Stanford University, Stanford, USA[1]
Department of Neurology and Neurological Sciences, Stanford University, Stanford, USA[2]
Department of Structural Biology, Stanford University, Stanford, USA[3]
Department of Photon Science, Stanford University, Stanford, USA[4]
Howard Hughes Medical Institute, Stanford University, Stanford, USA[5]
kiwhite@stanford.edu

The AAA+ proteins (ATPases Associated with various cellular Activities) are essential factors in a variety of critical cellular processes found throughout the cell. Members of this protein family often serve as protein remodeling machines, where chemical energy released upon ATP hydrolysis is used to drive mechanical work. One such protein is N-ethylmaleimide-sensitive-factor (NSF), an essential secretory system component involved in the disassembly of the SNARE complex following membrane fusion. Our recent structures of NSF engaged with the neuronal SNARE complex via the adaptor protein aSNAP reveal a supercomplex gripping one of the three SNARE N-termini [1], but they offer limited insight into the dynamic processes underlying subsequent disassembly. Here, we report progress on the study of the NSF reaction coordinate by single-particle cryo-EM. Given the conserved nature of the interactions between related AAA+ proteins and their substrates, this study is relevant to variety of different cellular contexts as well.

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Molecular Views into Cellular Functions by *in situ* Cryo-Electron Tomography

J. Mahamid[1,2], M. Pöge[2], A. Martinez Sanchez[2], S. Sakami[3], J. Plitzko[2], K. Palczewski[3] & W. Baumeister[2]

[1] Structural and Computational Biology Unit, European Molecular Biology Laboratory, 69117 Heidelberg, Germany, [2] Department of Molecular Structural Biology, Max Planck Institute of Biochemistry, 82152 Martinsried, Germany, [3] Department of Pharmacology, School of Medicine and Cleveland Center for Membrane and Structural Biology, Case Western Reserve University, Cleveland, OH 44106, USA
julia.mahamid@embl.de

Most structural biology focuses on the structure and function of individual macromolecular complexes, but falls short of revealing how they come together to give rise to cellular functions. As a consequence, structural and cell biology have traditionally been separate disciplines and employed techniques that were well defined within the realm of either one or the other. Here, cryo-electron tomography (cryo-ET) provides a unique opportunity for obtaining *in situ* structural information across a wide range of scales - from whole cells to individual macromolecules. There has been a major leap forward in cryo-ET of biological specimens owing to the introduction of direct detection cameras with their unsurpassed signal-to-noise ratio, contrast enhancing phase plates and computational image processing. These developments allow assignment of molecular structures directly from three-dimensional stills of intact cells. To image the dense, crowded interior of mammalian cells is furthermore complicated by the 'immense' sample thickness as seen from a nanoscopic perspective. Cryo-focused ion beam (FIB) micromachining literally opened 'electron-transparent windows' into cells, making large areas of unperturbed cells accessible for cryo-ET at molecular resolution. We demonstrate that the synergistic application of these recent methodological developments provide insight into the nanoscale architecture of mammalian photoreceptor cells and reveals the structural framework giving rise to the functional properties of highly specialized light-sensing cellular compartments. These findings, as well as a handful of recent studies, highlight the enormous discovery potential of structural cell biology in elucidating cellular functions.

Time-Resolved Femtosecond Crystallography with XFELs

P. Fromme

School of Molecular Sciences and Biodesign Center for Applied Structural,
Arizona State University, Tempe, Arizona 85287-1604 USA
pfromme@asu.edu

Serial Femtosecond Crystallography (SFX) provides a novel concept for structure determination, where X-ray diffraction "snapshots" are collected from a fully hydrated stream of nanocrystals, using femtosecond pulses from high energy X-ray free-electron lasers (XFELs) [1-4]. The XFEL pulses are so strong that they destroy any solid material, but a femtosecond is so short (1 fs =10 -15 s) that X-ray damage is diminished and diffraction from the crystals is observed before destruction takes effect [3]. Study of the dynamics biomolecules is one of the grand challenges of Structural Biology as most structures determined so far only provide a static picture of the molecule. Time-resolved femtosecond crystallography opens new avenues to determine molecular movies of molecules "in action" [6-10]. In this talk we will present results from recent experiments to study the dynamic processes in Biology.

The talk will close with a progress report on the development of compact femto and attosecond X-ray Sources at Arizona State University (CXLS and CXFEL) and DESY (AXSIS) [11], which will provide unique new opportunities to study the ultrafast dynamics of reactions with a combination of X-ray diffraction, X-ray spectroscopy and ultrafast optical spectroscopy.

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Visualizing Molecular Choreography: Tools, Techniques and Design

G. McGill[1,2]

[1]Harvard Medical School, [2]Digizyme Inc.
mcgill@digizyme.com

Biovisualization is a field that combines the complexities of science, the technical rigor of programming, the challenges of effective teaching and the creative possibilities of art and design. It is often used in one of two ways: 1) to explore and extract meaningful patterns for data analysis and 2) to communicate and engage various audiences. One of the most powerful yet little-recognized benefits of visualization, however, is the way it synthesizes our knowledge, externalizes our mental models of the science and thereby makes our assumptions explicit. Despite cognitive research that informs us on how visualizations impact target audiences like students, little attention is given to the thought process behind crafting visualizations and how it impacts those involved in planning and production. Many designers and animators report anecdotally that scientists with whom they collaborate gain new insights into their science as a result of navigating this process: "visual thinking" triggered during the planning of a visualization is thought to put familiar data into a new light. This presentation will draw on a range of example projects for scientists, science museums, public broadcasting, publishers, software developers and students. I will focus on the challenges of molecular visualization and provide an overview of tools, techniques, cognitive research and design practices that maximize impact in research and education.

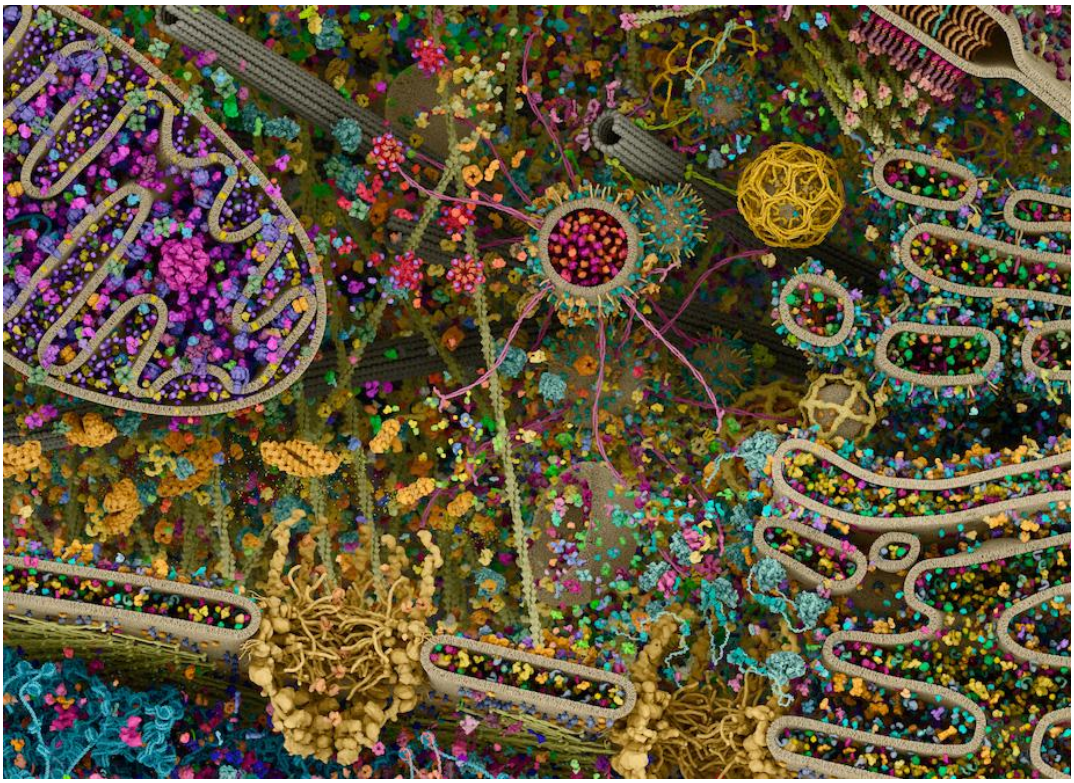

Figure 1: Molecular landscape integrating numerous structural datasets.

Neutron Crystallography

M. P. Blakeley[1]

[1]Institut Laue-Langevin, Large-Scale Structures Group
71 avenue des Martyrs, Grenoble, France
blakeleym@ill.fr

Neutron crystallography provides the positions of the hydrogen isotopes, hydrogen (^{1}H) and deuterium (^{2}H/D), in radiation damage-free crystal structures of biological macromolecules, allowing details of protonation and hydration to be revealed that are necessary for understanding many biological processes. Here I will describe (i) the current status of the field using examples of recent studies performed and (ii) on-going instrument developments, aimed at further extending the limits for neutron macromolecular crystallography.

Putting enzymes to work; making macrocyclic peptide hybrids

J.H. Naismith

Division of Structural Biology Oxford, Rosalind Franklin Institute
naismith@strubi.ox.ac.uk

Bacteria make much better chemists than humans. Peptides are nature's open source chemicals, they connect via a common standard (peptide bond). As chemists we find it hard to target modification of an individual or specific amino acids within a peptide. These modifications may be very desirable in turning amino acids into cell preamble potent biomolecules. In the lecture I will discuss our insights into how very unusual and complex transformations are carried out by enzymes. I will give examples of their use and how we have harnessed structural biology to make libraries of new molecules[1-12].

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PSB Symposium - List of Posters

P1 **Areej Abuhammad** - *Structural studies on carbonic anhydrase III complexed with pyrrole carboxylic acid derivatives: a crystal story from Jordan*

P2 **Michael Adams** - *Opposing Forces: How Legionella Modulates its own Toxicity*

P3 **Elisabetta Boeri erba** - *Native MS complements structural biology for investigating dynamics of macromolecular complexes*

P4 **Jérôme Boisbouvier** - *Structural Investigation of a 1 MDa Chaperonin in Action Reveals How Nucleotide Binding Regulates the Functional Cycle*

P5 **Eva Crosas** - *P11 at PETRA III: A Versatile Beamline for High-Throughput and Serial Crystallography*

P6 **Hadrien Depernet** - *Structural characterisation of near infrared and GFP-like fluorescent proteins*

P7 **Amedee des Georges** - *Functional Pathways of Biomolecules Retrieved from Single-particle cryo-EM Snapshots*

P8 **Neha Dhimole** - *Interplay of the chaperon Trigger Factor and targeting factor SecA inregulating the ribosome nascent chain complex*

P9 **Kamel El-Omari** - *Crystallography at wavelengths longer than 2.7 Å*

P10 **David Fernandez-Martinez** - *Structural studies of multispecific Antibody/Antigen complexes by cryo-EM*

P11 **Herman Fung** - *Visualising the nucleolus by correlative cryo-electron tomography*

P12 **Isabel García-Sáez** - *Structural insights into the conformational plasticity of chromatin*

P13 **Samuel Gérard** - *Cryo-electron microscopy studies of HIV-1 capsid protein assembly*

P14 **Sergei Grudinin** - *Novel computational tools for integrative structural biology: structure predictions using small-angle scattering, cross-links, symmetry, protein docking, normal mode analysis, and artificial intelligence*

P15 **Johanna Hakanpää** - *Applications of serial synchrotron crystallography for user community at DESY/PETRAIII beamline P11*

P16 **Zuzana Hodakova** - *Structural characterisation of sister chromatid cohesion*

P17 **Anne Houdusse** - *Force Generation by Myosin Motors: Tuning force production for new therapeutical treatments against human diseases*

P18 **Ahmad Jomaa** - *Cryo-electron Microscopy Provides Insights into Bacterial and Mammalian Co-translational Targeting of Proteins to the Membrane*

P19 **Pauline Juyoux** - *Structural studies of the interaction between the Toxoplasma gondii protein GRA24 and MAPKs*

P20 **Michelangelo Marasco** - *Molecular mechanism of SHP2 activation by PD-1*

P21 **Julie Ménétrey** - *Characterization of the JIP1-binding surface on KLC1-TPR using Isothermal Titration Calorimetry*

P22 **Ignacio Mir-Sanchis** - *Structures of two replication related proteins encoded by SCCmec elements: the mobile elements responsible of MRSA*

p23 **Inayathulla Mohammed** - *How human lon protease clears the damaged protein from the mitochondrial matrix*

P24 **Andrés Palencia** - *Metal-Captured Inhibition of pre-mRNA Processing Activity by CPSF3 Eliminates Cryptosporidium Infection*

P25 **Valentina Palmerini** - *Structural insights into the TNFR Grindelwald, coupling loss of cell polarity with neoplastic growth*

P26 **Samuel Pazicky** - *Structure of a subcomplex of the apicomplexan invasion machinery*

P27 **Fanomezana Moutse Ranaivoson** - *Structural specificities of the mitotic kinesin MKlp2 and implications to its mechanism and function*

P28 **Finaritra Raoelijaona** - *Understanding cytoplasmic maturation of the eukaryotic ribosomal small subunit (pre-40S)*

P29 **Ludovic Sauguet** - *Structure of the DP1-DP2 PolD complex bound with DNA and its implications for the evolutionary history of DNA and RNA polymerases*

P30 **Montserrat Soler-Lopez** - *ECSIT plays an essential role in ACAD9 activity and the formation of the Mitochondrial Complex I Assembly (MCIA) Complex*

P31 **Henrik Vinther Sørensen** - *Study of bacterial colonization with X-ray and neutron scattering Techniques*

P32 **Philipp Throll** - *Structural and Functional Characterization of the APOBEC1-Editosome*

P33 **Mark Tully** - *The rise of BioSAXS at the ESRF: BM29 beamline for SAXS on proteins in solution*

P34 **Sander Van Der Verren** - *Cryo-electron microscopy structure of the curli secretion apparatus CsgF:CsgG reveals a dual-constriction pore with properties amenable to nanopore sequencing*

P35 **Joanna Wandzik** - *Structure of actively transcribing influenza virus polymerase in the late elongation state*

P36 **Jiangfeng Zhao** - *Crystal structure of a novel multidrug and toxic compound extrusion transporter from Aquifex aeolicus*

P37 **Celine Zheng** - *Structural basis of NetrinG2-dependent neurodevelopmental disease*

Structural studies on carbonic anhydrase III complexed with pyrrole carboxylic acid derivatives: a crystal story from Jordan

A. Abuhammad[1], M. Samman[1], R. Ahamad[1], Y.M. Al-Hiari[1]

[1]School of Pharmacy, University of Jordan
a.abuhammad@ju.edu.jo

Carbonic anhydrases (CAs) are ubiquitous metalloenzymes that present in both prokaryotes and eukaryotes. There are sixteen human CA isozymes that display varieties in their tissue distribution, subcellular localizations, catalytic activities, and sensitivity to inhibitors. These enzymes are involved in many essential physiological processes related to: respiration, electrolyte secretion, CO2 and pH homeostasis, biosynthetic reactions (e.g. ureagenesis, lipogenesis and gluconeogenesis) as well as bone resorption and calcification. The involvement of CAs in a wide variety of physiological processes made them attractive drug targets for many diseases and conditions such as glaucoma, epilepsy, osteoporosis, and obesity.

Selectivity is considered a major obstacle in targeting CAs. Interestingly, none of the available CAIs selectively inhibits a particular CA isoform. Therefore, structure-based drug design is considered a method of choice for the design of selective and potent CA inhibitors. Protein crystallography is highly sensitive compared to biochemical assays and can typically identify the binding of very weak ligands (in the mM affinities). The availability of structural information about a ligand-target interaction early in the lead identification stage can significantly accelerate the optimisation process.

This project aims at the determination of the 3D structure of CAIII co-crystallised with pyrrole carboxylic acid derivatives as a tool to guide new synthetic approaches and help identify selective CA inhibitors.

This is the first protein X-ray crystallography research project to be carried out in Jordan.

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Opposing Forces: How Legionella Modulates its own Toxicity

S. Bhogaraju[1,2,3], F. Bonn[1], R. Mukherjee[1,2], M. Adams[3], M. M. Pfleiderer[3], W. P. Galej[3], V. Matkovic[1,2], S. Kalayil[1,2], D. Shin[1,2], I. Dikic[1,2]

[1]Institute of Biochemistry II, Faculty of Medicine, Goethe University, [2]Buchmann Institute for Molecular Life Sciences, Goethe University, [3]European Molecular Biology Laboratory France

miadams@embl.fr

Legionella pneumophila is a gram-negative bacterium responsible for Legionnaire's disease, a form of acute pneumonia. Infection is caused by inhalation of aerosols from stagnant water sources, where they are taken up by the lung's macrophages. Legionella uses a strategy of evading its host's various defense systems and hijacking a variety of regulatory pathways and cellular machinery by injecting over 300 effector proteins into the host's cytoplasm to establish its intracellular replicative niche [1]. One family of the effector proteins that was shown to be extremely cytotoxic to Eukaryotes is the SidE family, of which the most well-studied is SdeA. Interestingly, it was also found that another effector protein named SidJ is able to inhibit the cytotoxic effect of SidJ through an unknown mechanism [2]. While SdeA's unique catalytic activity has since been well-characterized, the mechanism through which SidJ is able to inhibit SdeA specifically, and consequently the SidE family at large, has been unknown to date. Here, we demonstrate that SidJ is able to inhibit SdeA through glutamylation of catalytic residue E860. Furthermore, we demonstrate that SidJ is in an autoinhibited state and is activated through binding with the host-specific cofactor Calmodulin. Lastly, we solved the Cryo-EM structure of the SidJ-Calmodulin complex to reveal the architecture of the complex.

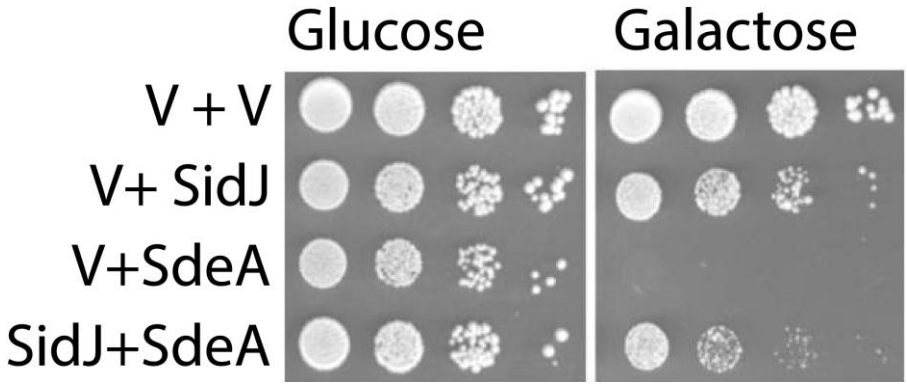

Figure 1: Adapted from Jeong et al. (2015) [3]. Tenfold serial dilution of yeast strains containing SdeA or SidJ grown on selective plates. Expression vectors were transformed into yeast and induced using Galactose. SidJ expression alone was not toxic to the cells. SdeA expression was lethal, and SidJ/SdeA co-expression mitigated the toxic effect of SdeA.

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Native MS complements structural biology for investigating dynamics of macromolecular complexes

E. Boeri Erba[1], A.W. Yee [2,3], G. Aldeghi [4], M. Haertlein[2], T.V. Forsyth [2,3]

[1] Univ. Grenoble Alpes, CEA, CNRS, IBS, Grenoble,[2] Institut Laue-Langevin, Grenoble, 3 Faculty of Natural Sciences, Keele University, Staffordshire UK, .[4]Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

elisabetta.boeri-erba@ibs.fr

Native Mass spectrometry (MS) is used to investigate biomolecules that associate noncovalently [1-2]. One can determine the accurate stoichiometry of intact assemblies and the direct interactions between subunits. By mixing subunits in a stepwise manner, a hierarchy in the assembly pathway can be determined. By incubating light and heavy isoforms of a protein (e.g., labeled with ^{13}C and ^{15}N) the subunit composition of intact complexes can be varied and monitored as a function of time. This type of experiments can be used to monitor the kinetics of subunit exchange, revealing distinct pathways for wild-type and mutant proteins (e.g., involved in amyloidosis, Fig. 1) [3].

I will illustrate the application of native MS to the study of the structure of macromolecular assemblies, including protein complexes involved in fatal forms of amyloidosis. I will underline the role of native MS to complement data obtained using structural biology approaches such as neutron crystallography.

Overall, native MS represents a key tool for gaining important insights into the composition, structure and dynamics of macromolecular complexes.

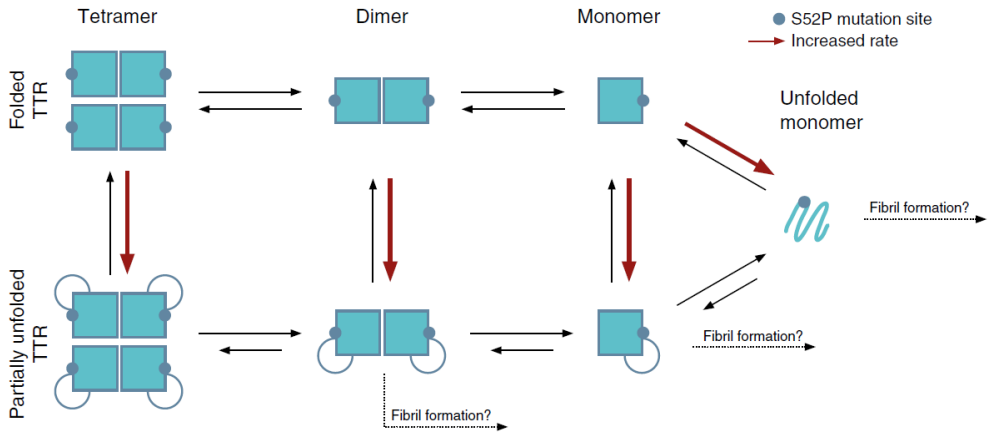

Figure 1: Proposed model of mutational effect on TTR stability. The S52P mutation increases the likelihood of partial and full unfolding of TTR monomeric units. This leads to a lower stability of TTR multimeric assemblies. Partially unfolded dimers and monomers, and fully unfolded monomers, are generated at a higher rate. Some or all of these species are then removed from solution via the formation of amyloid fibrils [3].

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Structural Investigation of a 1 MDa Chaperonin in Action Reveals How Nucleotide Binding Regulates the Functional Cycle

G. Mas, J.-Y. Guan, E. Crublet, E. Colas Debled, C. Moriscot, P. Gans, G. Schoehn, P. Macek, P. Schanda, J. Boisbouvier[1]

[1]University of Grenoble Alpes, CNRS, CEA, IBS, F-38000 Grenoble, France
jerome.boisbouvier@ibs.fr

Chaperonins are ubiquitous protein assemblies present in bacteria, eukaryota, and archaea, facilitating the folding of proteins, preventing protein aggregation, and thus participating in maintaining protein homeostasis in the cell. During their functional cycle, they bind unfolded client proteins inside their double ring structure and promote protein folding by closing the ring chamber in an adenosine 5′-triphosphate (ATP)–dependent manner. Although the static structures of fully open and closed forms of chaperonins were solved by x-ray crystallography or electron microscopy, elucidating the mechanisms of such ATP-driven molecular events requires studying the proteins at the structural level under working conditions. We introduce an approach that combines site-specific nuclear magnetic resonance observation of very large proteins, enabled by advanced isotope labeling methods [1, 2], with an in situ ATP regeneration system. Using this method, we provide functional insight into the 1-MDa large hsp60 chaperonin while processing client proteins and reveal how nucleotide binding, hydrolysis, and release control switching between closed and open states [3]. While the open conformation stabilizes the unfolded state of client proteins, the internalization of the client protein inside the chaperonin cavity speeds up its functional cycle. This approach opens new perspectives to study structures and mechanisms of various ATP-driven biological machineries in the heat of action.

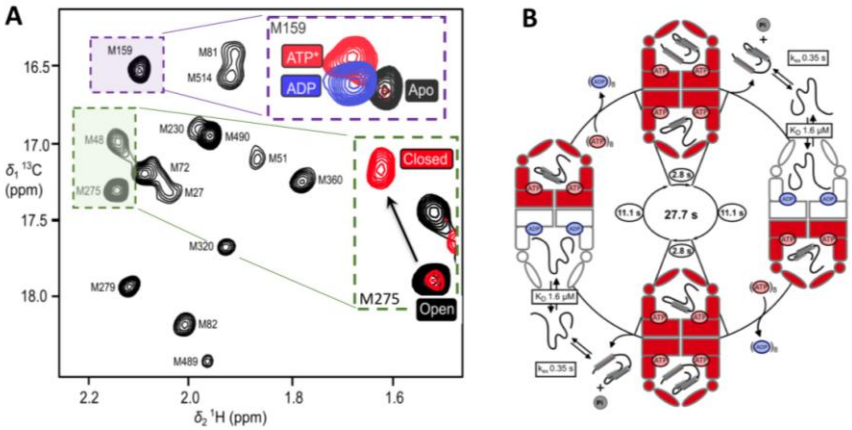

Figure 1: NMR Studies of a 1 MDa Chaperonin in Action. (A) Methionine-directed methyl NMR allows the identification of the nucleotide binding state and the different chaperonin conformational states. (B) Model of the functional cycle of chaperonin derived from real-time NMR study of active Hsp60 chaperonin in presence of unfolded substrate protein.

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P11 at PETRA III: A Versatile Beamline for High-Throughput and Serial Crystallography

E. Crosas[1], S. Saouane[1], J. Hakanpää[1], J. Meyer[1], J. Urbschat[1], P. Fischer[2], B. Reime[1], T. Pakendorf[2], A. Meents[2], A. Burkhardt[1]

[1]Photon Science, DESY, Notkestrasse 85, 22607 Hamburg, Germany, [2]Center for FreeElectron Laser Science,DESY, Notkestrasse 85, 22607 Hamburg, Germany

eva.crosas@desy.de

The Bio-imaging and Diffraction Beamline P11at PETRA III in Hamburg is dedicated to structural investigations of biological samples from atomic to micrometerlength scales. The beamlineprovides two experimental endstations: an X-ray crystallography experiment open to users since 2013 [1] and a scanning X-ray microscopecurrently under construction.The flexible X-rayoptics allow for tailoring the beam properties to the experimental requirements. A first mirror system located in the optics hutch is used for generating a secondary source at 65.5. m downstream from the X-ray source. With this, a parallel beam can be generated which is ideally suited for structure determinations from large unit cell systems, such as large molecular complexes [2]. The KB system installed in the experimental hutch can be used for refocussing the secondary source in order to generate a highly intense microbeam with more than 1.3 × 1013ph/s in a 4 × 9 μm2(v × h, FWHM) focal spot at the crystallography experiment. This allows for the investigation of microcrystals and the application of novel data collection routines, such as serial crystallography [3-6]. The P11 crystallography endstation can be operated between 5.5 and 28 keV and provides full SAD/MAD capability. Energy and beam size changes can be easily realized by the userswithin a few minutes. The endstation is equipped with a high precision single axis goniostat. Crystals can be rapidly exchanged in less than 20 s using an automaticsample changer equipped with an in-house designed cryogenic sample gripper and a large capacity storage Dewar,providing space for 23 uni-pucks (368 samples). Together with thePilatus 6M detector in place, P11 allows for high-throughput crystallography and is ideally suited for industrial applications, such ase.g. fragment screening. For spring 2020 an upgrade of the crystallography endstationis planned.It willinclude the implementation of a new Roadrunner goniometer [6] which willallow for conventional (rotation series) and serial crystallography (fixed targets). In addition, the Pilatus 6M detector will be replaced by a fasterEIGER216M and a new on-axis microscope for better sample visualization of micrometer-sized crystals will be installed.

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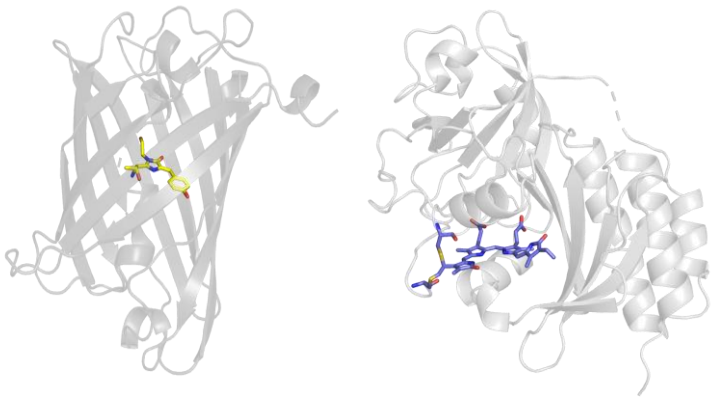
Structural characterisation of near infrared and GFP-like fluorescent proteins

H. Depernet[1], G. Gotthard[1], S. Aumonier[1], G. Leonard[1], A. Royant[1,2]

[1]European Synchrotron Radiation Facility, Grenoble, F-38043, France
[2]Institut de Biologie Structurale (IBS), University of Grenoble Alpes, CNRS, CEA, Grenoble, 38044, France
hadrien.depernet@esrf.fr

Since the cloning of Green Fluorescent Protein (GFP) in 1992 [1] fluorescent proteins (FPs) have become widely used in cell and whole-body imaging. GFP was discovered in a jellyfish called *Aequorea Victoria*, but homologues were then found in other marine organisms such as corals, sea-anemones, lancelets or small crustaceans [2]. I will present the structure of novel GFP homologues discovered in jellyfish species. The first one is a (non-fluorescent) chromoprotein that possess a chromophore never observed before and the second appears to be one of the brightest FPs ever found in Nature.

New types of fluorescent proteins have emerged at the end of the 2000's. For instance, near-infrared fluorescent proteins (NIR-FPs) have been evolved from the red and far-red light photoreceptor phytochrome. The NIR part of the light spectrum matches the optical window of tissues (that is the wavelength range for which light is scattered the least) and is thus most suitable for whole-body imaging. I will present structures of NIR-FPs which have their chromophore biliverdin bound at two different locations of the protein, mIFP [3] and iBlueberry [4], which has a profound effect on the position of the excitation and emission maxima.



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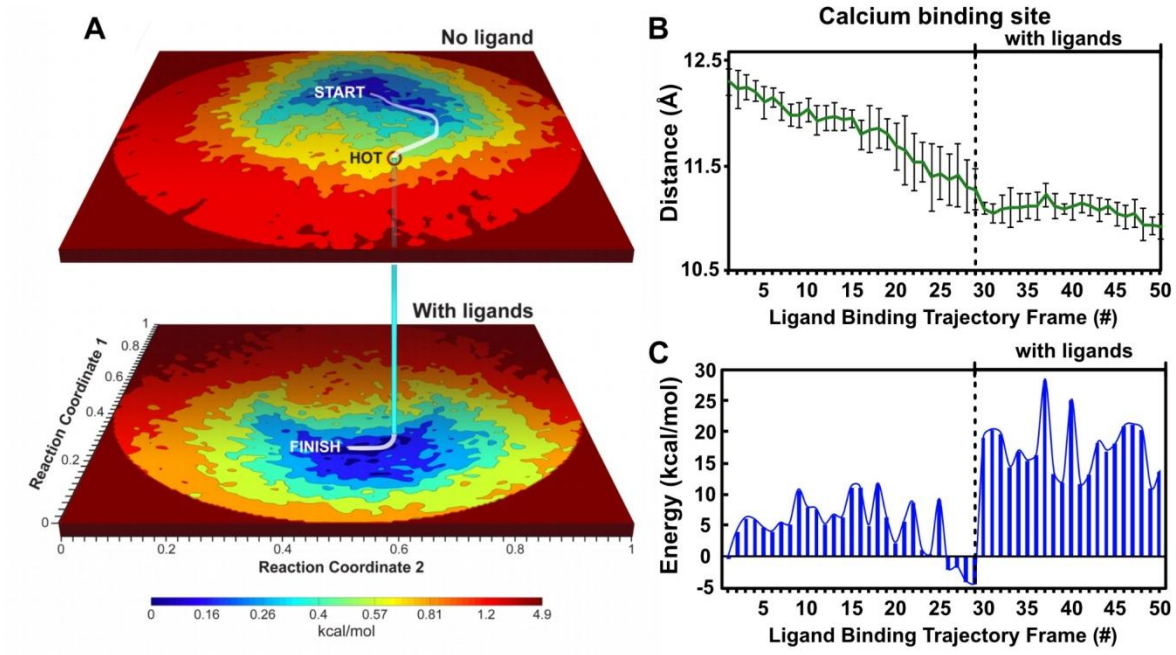
Functional Pathways of Biomolecules Retrieved from Single-particle cryo-EM Snapshots

A. Dashti, M. Shekhar, D.B. Hail, G. Mashayekhi, P. Schwander, A. des Georges[1], J. Frank, A. Singharoy, A. Ourmazd

[1]Structural Biology Initiative, CUNY Advanced Science Research Center, USA
Amedee.desGeorges@asrc.cuny.edu

We present a new approach to determining the conformational changes associated with biological function, and demonstrate its capabilities in the context of experimental single-particle cryo-EM snapshots of ryanodine receptor (RyR1), a Ca2+-channel involved in skeletal muscle excitation/contraction coupling. These results include the detailed conformational motions associated with functional paths including transitions between energy landscapes. The functional motions differ substantially from those inferred from discrete structures, shedding new light on the gating mechanism in RyR1. The differences include the conformationally active structural domains, the nature, sequence, extent of conformational motions involved in function, and the way allosteric signals are transduced within and between domains. It also provides meaningful starting points for MD simulations along the experimental trajectory. The approach is general, and applicable to a wide range of systems and processes.



Interplay of the chaperon Trigger Factor and targeting factor SecA in regulating the ribosome nascent chain complex

N. Dhimole[1], A. Schedlbauer[2], P. Fucini[2]

[1]Leibniz University Hannover, Institute of Organic Chemistry and Center for Biomolecular Drug Research, Schneiderberg 38, D-30167 Hannover, Germany
[2]Structural Biology Unit, CIC bioGUNE, Parque Tecnológico de Bizkaia, Derio 48160, Spain
neha.dhimole@oci.uni-hannover.de

The ribosome is a 2.3MDa rRNA-protein complex that translates the mRNA into a protein. The newly synthesized polypeptide nascent chain travels through the 80-100Å long ribosomal tunnel located in the large ribosomal subunit and exits the ribosome. In concert with several other factors the ribosome brings about the correct folding and localization of the protein, which are the prerequisites for its proper functioning. Evidence suggests that the rate of protein synthesis and the interaction of the newly synthesized nascent chain with the chaperons and the ribosomal tunnel can direct the folding and localization processes. The effect of the chaperons and targeting factor on ribosome and nascent chain folding has been studied biochemically but a structural understanding of nascent chain conformations, folding and its regulation in response to the cellular chaperon and targeting factors remain sparse. This is largely due to the highly dynamic nature of the translation process, making it inaccessible to conventional structural biology methods.

To this end, we explored the interactions and consequences of ribosome-chaperon interaction on the nascent chain conformations by liquid state NMR and PURE in vitro translation systems. We were able to differentiate the effect of cellular chaperon Trigger factor and translocation factor ATPase SecA on the conformation of the nascent chain segment that is outside the tunnel and inside the tunnel.

We found that SecA and TF can modulate the interaction of nascent chain with the ribosome and alter its conformation. Moreover, the effect of SecA and TF on the nascent chain dynamics is not limited by the physical proximity of the nascent chain but can be propagated into the ribosomal tunnel to nascent chain, which is buried deep inside the ribosome and inaccessible to any protein factor for direct communication. This is the first report to shed light on regulation of translation by SecA and TF through a novel signal transduction mechanism from ribosomal surface to the peptidyltransferase center (peptide bond formation center in the ribosome) for efficient NC folding and targeting.

Crystallography at wavelengths longer than 2.7 Å

K. El Omari , R. Duman, V. B. Mykhaylyk, A. Wagner

Diamond Light Source, Harwell Science and Innovation Campus, Chilton, Didcot OX11 15 0DE, United Kingdom. **kamel.el-omari@diamond.ac.uk**

The I23 Long wavelength MX beamline at the Diamond Light source is a unique instrument dedicated to experimental phasing and optimised to operate at wavelengths between 1.5 to 4 Å [1]. The beamline enables users to exploit the increased anomalous signal specific to scatterers naturally present in proteins such as S, P, Cl, Ca and K. This renders obsolete the need for selenomethionine incorporation or heavy-atom derivatisation of protein crystals, which represents a significant barrier in the process of experimental phasing.
In addition to the long wavelength phasing experiments, I23 is the sole beamline where identification and localisation of biologically important ions, such as K, Ca, Cl is feasible by generation of anomalous difference Fourier maps below and above the element absorption edge [2]. Recent results of native phasing and ion identification/localisation demonstrating the capability and potential of the beamline will be presented.

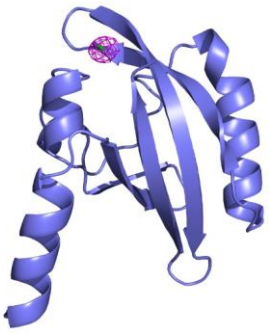


Figure 1: Sulphur anomalous difference Fourier maps of a 116 residue protein domain solved by S-SAD with a single cysteine.

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Structural studies of multispecific Antibody/Antigen complexes by cryo-EM

D. Fernandez-Martinez[1,2], E. Kandiah[1], M. Mathieu[2], G. Leonard[1]

[1]Structural Biology Group, European Synchrotron Radiation Facility, Grenoble, France
[2]Sanofi R&D, LGCR, Center de Recherche Vitry-sur-Seine, Vitry-sur-Seine Cedex, France
david.fernandez@esrf.fr

Multispecific antibodies are artificially engineered molecules designed to bind simultaneously to several (different) antigens [1]. Potential advantages of generating viable multispecific antibodies include the identification of malignant cells coupled with the concurrent recruitment of immune cells and the blocking of complex viral escape mechanisms [2-3]. The cross-over dual-variable immunoglobulin (CODV-Ig) has been proposed as a universal bispecific therapeutic format [4]. Its unique antigen-binding fragment (Fab) architecture provides pM affinities for ligands, no positional effect in target binding and a stable self-supporting structure. However, a disparity between *in vitro* and *in vivo* effects suggests that the three-dimensional arrangement of the constant and antigen-binding fragments in the CODV-Ig format is not ideal. To further understand the structure and function of multispecific antibodies based on the CODV-Ig format high-resolution structural information is required. Towards this, we use cryo-electron microscopy (cryo-EM).

We purified CODV-Ig both in an unbound state and in complex with a single antigen and validated sample quality using SDS-PAGE, Small Angle X-Ray Scattering (SAXS) and negative-stain electron microscopy (NSEM; Tecnai T12 microscope at IBS, Grenoble). NSEM resulted in low-resolution structural models and suggested a preferential orientation of CODV-Ig under negative-stain conditions. Close-to-optimal vitrification conditions for CODV-Ig have been identified. Efforts are in progress to reduce the antibody's propensity to aggregation and aversion to conventional cryo-EM supports. Nevertheless, data of sufficient quality for image analysis was obtained using a Titan Krios microscope (ESRF, Grenoble) equipped with a Quantum LS energy filter and K2 Summit direct electron detector. Image processing of both CODV-Ig alone and in complex with antigens suggests high flexibility and conformational heterogeneity.

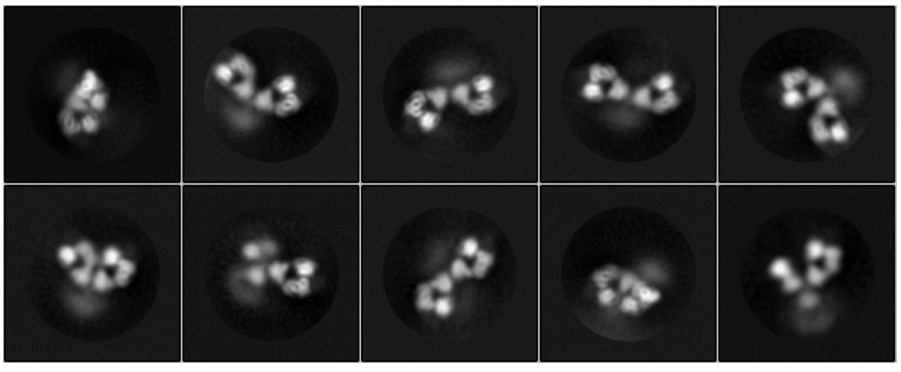

Figure 1. Representative classes from a 2D classification of an antigen-bound CODV-Ig cryo-EM sample.

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Visualising the nucleolus by correlative cryo-electron tomography

H.K.H. Fung, H. Grötsch, X. Zhang, J. Mahamid, C.W. Müller

European Molecular Biology Laboratory, Heidelberg, Germany
herman.fung@embl.de

Ribosome biogenesis in eukaryotes begins in the nucleolus with the transcription of ribosomal DNA into pre-ribosomal RNA. To understand the molecular environment of the transcription process, we have taken a cryo-correlative light/electron-microscopy approach to image the nucleoli of budding yeast *in situ*. First, yeast cells fluorescently labelled for the nucleolus were frozen and imaged on a cryo-confocal microscope. Then, by targeted FIB milling, the cells were thinned into 100–250 nm sections that would contain the fluorescence signal which were then imaged by cryo-electron tomography. Here, we present a description of our workflow and initial results from our analysis of the yeast nucleolus.

Structural insights into the conformational plasticity of chromatin

García-Sáez I[1], Menoni H[2,3], Boopathi R[2,3], Noirclerc-Savoye M[1], Le Roy A[1], Papai G[4], Tonchev O[2,3], Syed SH[2,3], Crucifix C[4], Shukla MS[2,3], Soueidan L[2,3], Skoufias DA[1], Hamiche A[4], Schultz P[4], Bednar J[2], Angelov D[3], Dimitrov S[2], Petosa C[1].

[1]Institut de Biologie Structurale, U. Grenoble Alpes/CNRS/CEA, 38000 Grenoble, France; [2]Institute for Advanced Biosciences, U. Grenoble Alpes/CNRS/INSERM, 38700, La Tronche, France; [3]Ecole Normale Supérieure de Lyon/U. de Lyon/CNRS, 69007, Lyon, France; [4]Institut de Génétique et Biologie Moléculaire et Cellulaire, U. de Strasbourg/CNRS/INSERM, 67404 Illkirch Cedex, France

isabel.garcia@ibs.fr

Chromatin adopts a diversity of regular and irregular fiber structures in vitro and in vivo, including a compact 30-nm fiber associated with the inactivation of gene expression. Linker histones bind to the linker DNA connecting consecutive nucleosomes and play a key role in determining chromatin structure. However, how an array of nucleosomes folds into, and switches between, different fiber conformations remains poorly understood. We determined crystal and cryoEM structures of a 197-base pair nucleosome bound to linker histone H1 [1]. The H1 globular domain binds on the nucleosome pseudodyad axis in contact with both DNA linker arms, while the H1 C-terminal domain associates predominantly with a single DNA linker, conferring significant asymmetry to the nucleosome. More recently, we determined the crystal structure of a 6-nucleosome array bound to linker histone H1 [2]. The structure reveals a flat two-start helix with uniform nucleosomal stacking interfaces and a nucleosome packing density which is only half that of a twisted 30-nm fiber. Hydroxyl-radical footprinting showed that H1 binds the array in an on-dyad configuration resembling that observed for the mononucleosome. Strikingly, cryo-EM, SAXS and AUC data revealed that a minor change in ionic environment shifts the conformational landscape to a more compact, twisted form, which has previously been associated with an off-dyad binding mode for H1. These findings provide insights into the structural plasticity of chromatin and suggest a possible assembly pathway for a 30-nm fiber.

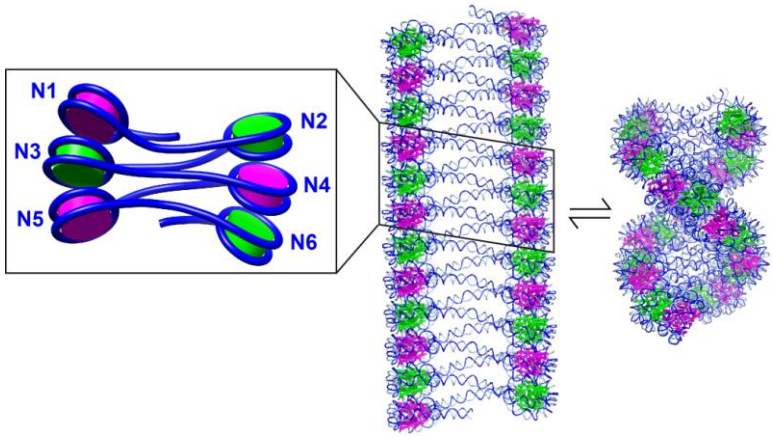

Figure 1: Structure of a 6-nucleosome array suggests an assembly pathway for a twisted 30-nm chromatin fiber.

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Cryo-electron microscopy studies of HIV-1 capsid protein assembly

S. F. Gérard[1], T. Ni[1], J. Ning[2], J. Zhou[3], C. Aiken[3] and P. Zhang[1]

[1]The Division of Structural Biology, The Henry Wellcome Building for Genomic Medicine, Roosevelt Drive, Oxford, OX3 7BN
[2]Department of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260, USA
[3]Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical, 13 Center, Nashville, TN, USA
samuel.gerard@merton.ox.ac.uk

The capsid of the human immunodeficiency virus type 1 (HIV-1) is a RNA-encapsulating fullerene cone, composed of a surface array of about 200 hexamers and 12 pentamers of the same capsid protein (CA) [1,2]. Though it is highly organized, efforts to solve the molecular structure of the HIV-1 capsid have been undermined by its pleomorphic and asymmetric nature [3].
In vitro tubular assemblies of CA are analogues of the native HIV-1 capsid, therefore they are relevant to elucidate its molecular organization and assembly mechanism. Here, we present the cryo-EM structures of several *in vitro* HIV-1 CA tubular assemblies, and a 3.7A structure of the CA hexamer using localized reconstruction. In particular, we show that hexamers are intrinsically curved and asymmetric, as opposed to the previously described planar crystal structure. We propose that the plasticity of CA domain arrangement accounts for the various curvatures of the mature capsid.

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Novel computational tools for integrative structural biology: structure predictions using small-angle scattering, cross-links, symmetry, protein docking, normal mode analysis, and artificial intelligence

S. Grudinin[1], G. Pages[1], M. Kadukova[1], M. Karasikov[1], A. Martel[2], S. Prevost[2]

[1]UGA, CNRS, Inria, Grenoble INP, LJK, 38000 Grenoble, [2]ILL Grenoble
sergei.grudinin@inria.fr

While crystallography has been providing atomic-resolution structures of biomolecules for over half a century, the real challenge of today's biophysics is to correlate molecules' structure and dynamics in solution with their function. Owing to the complexity of the problem, the answer can only be found if multiple sources of information are used simultaneously. I will present a set of tools developed for this purpose in our group [1].

Recently, we introduced SAXS and SANS packages called Pepsi-SAXS, and Pepsi-SANS, correspondingly [1,2]. Pepsi-SAXS is a very efficient method that calculates small angle X-ray scattering profiles from atomistic models. It is based on the multipole expansion scheme and is significantly faster than other methods with the same level of precision. One of the challenges in the field is, however, flexible fitting of atomistic models into small-angle scattering profiles. We designed a computational scheme that uses the nonlinear normal modes [1,3] as a low-dimensional representation of the protein motion subspace and optimizes protein structures guided by the SAXS and SANS profiles. For example, this scheme was ranked first in the recent CASP13 structure prediction challenge. Another challenge is data-assisted protein docking. We have designed a scheme for SAXS-assisted rescoring of docking predictions. This was made possible due to the polynomial representation of partial scattering amplitudes for each of the docking partners.
We are also interested in protein symmetry, as many protein complexes are symmetric homooligomers. We have designed a novel free-docking symmetry-assisted method [4] and also a dual analytical technique for identification of symmetry in a protein assembly [5-7]. This allowed us to study principles of protein organizations on the PDB scale.

Finally, artificial intelligence has made a big leap forward and found many applications in structural bioinformatics. On our side, we have been using it for multiple tasks of protein structure prediction, including protein-protein [8] and protein-ligand [9] docking, shape analysis [7] and structure prediction [10-11].

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Applications of serial synchrotron crystallography for user community at DESY/PETRAIII beamline P11

J. Hakanpää, E. Crosas, S. Saouane, J. Lieske, S. Günther, D. Oberthür, J. Meyer, J. Urbschat, P. Fischer, B. Reime, T. Pakendorf, A. Meents and A. Burkhardt

Deutsches Elektronen Synchrotron (DESY), Notkestraße 85, 22607 Hamburg, Germany
johanna.hakanpaa@desy.de

In serial synchrotron crystallography (SSX), still images (snapshots) from hundreds or thousands of small, randomly oriented crystals are recorded and merged into a complete data set. As in conventional crystallography, it is also possible to collect small rotation wedges from many small crystals and merge the partial data sets together. SSX requires a high-brilliance synchrotron source with a beam size similar to the sample size, an appropriate sample delivery method and a detector with a sufficient frame rate.

At the microfocus beamline P11 at PETRA III in Hamburg, several pioneering experiments have been carried out in the field of SSX since 2013 [1]. Using the two optical mirror systems in place, a highly intense microbeam of 4×9 μm^2 (v × h, FWHM) with 1.3 x 10^{13} ph/s in the focal spot can be generated. Smaller beam sizes down to 1×1 μm^2 with more than 2×10^{11} ph/s can be obtained by slitting down the secondary source. In addition, the beamline is equipped with a Pilatus 6M detector which allows for data collection with frame rates of up to 25 Hz.

Several sample delivery systems for SSX are available to the P11 users: An inhouse-developed tape-drive system allows for dynamic studies of biological macromolecules by mix-and-diffuse experiments at room temperature [2]. Ligand or substrate can be introduced by a microfluidic mixer with variable mixing times. A rotating chopper wheel is used to realize X-ray short exposure times between 5 and 20 ms. With this sample heating caused by the intense X-ray beam, which leads to crystal deterioration, can be avoided.

Micropatterned silicon chips are available as sample holders for fixed-target crystallography at cryogenic and room temperature [3]. With this, only micrograms of sample are required for a high-resolution structure determination. The crystals can be either directly grown on the chips [4] or crystallized off-line by standard techniques (e.g. hanging drop vapor diffusion or batch crystallization) and then pipetted onto the chip. The chips are commercially available with pore sizes of 2-50 μm and are mounted on standard magnetic caps. The sample holder is manufactured from single crystalline silicon and optimized for data collection at low background levels. In addition, the air scattering on the detector can be significantly reduced by using a so-called “capillary beamstop”, which encloses most of the direct X-ray beam path in capillary shields. This makes P11 ideally suited for data collection from weakly diffracting and radiation sensitive samples.

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Structural characterisation of sister chromatid cohesion

Z. Hodakova[1,2], W. Chao[2], P. Cherepanov[1], M. Singleton[2]

[1]The Francis Crick Institute, Chromatin Structure and Mobile DNA Laboratory, London, UK
[2]The Francis Crick Institute, Structural Biology of Chromosome Segregation Laboratory, London, UK
zuzana.hodakova@crick.ac.uk

Cohesin is a protein complex essential for correct chromosome segregation, functioning by entrapping sister chromatids and releasing them at the correct cell cycle stage. Whereas DNA exit has been well defined, DNA entrapment is poorly understood. Key component in cohesin loading onto DNA is the cohesin loader complex, but given its considerably smaller size when compared to cohesin, precise interaction and mechanisms of loader enveloping cohesin have not been elucidated.

In order to gain a better understanding of this fundamental cellular process, we have applied a structural approach to study the interaction between cohesin and its loader complex using cryo-electron microscopy. Given the high internal flexibility of both complexes, our aim was to reduce this flexibility by protein engineering and crosslinking to create rigid constructs more suitable for single particle analysis.

Force Generation by Myosin Motors: Tuning force production for new therapeutical treatments against human diseases

A. Houdusse

Institut Curie, UMR144 CNRS, Structural Motility Laboratory, Paris, France.
anne.houdusse@curie.fr

Force production by myosin motors plays major roles in muscle contraction, intra-cellular trafficking and maintenance of critical cellular structures such as microvilli, stereocilia and invadopodia. Deficit in different myosin motors can lead to a number of serious diseases, thus myosins are important potential targets for therapeutical treatment. Structures of myosins in complexes with small molecules reveal unsuspected allosteric sites and provide valuable insights for the design of specific modulators. These reveal the mechanistic control of motor transitions by inhibitors and activators and provide novel understanding of the rearrangements controlling the force producing lever arm swing. Current progress and outstanding questions regarding the important sequential rearrangements that lead to force production by myosins will be presented in light of recently solved X-ray structures of myosin/drug complexes. New insights into the mechanism of allosteric tuning of myosin force generation is thus anticipated to lead the way in the development of new myosin-directed therapeutics.

Cryo-electron Microscopy Provides Insights into Bacterial and Mammalian Co-translational Targeting of Proteins to the Membrane

A. Jomaa[1], K. Kobayashi[1], J. H. Lee[2], Y.H. Hwang-Fu[2], S. Chandrasekar[2], D. Boehringer[1], M. Leibundgut[1], S.O. Shan[2], N. Ban[1]

[1]Department of Biology, Institute of Molecular Biology and Biophysics, Otto-Stern-Weg 5, ETH Zurich, CH-8093, Switzerland, [2]Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125, USA.

jomaaa@ethz.ch

Targeting of proteins to the membrane while being synthesized by the translational machinery is a universal process in all forms of life. The key players in this process are the translating ribosome, the signal recognition particle (SRP), SRP receptor (SR), and the Sec translocon. Co-translational protein targeting to the membrane involves two central events: recognition on the translating ribosome by SRP, and cargo handover from SRP to the Sec translocon, where the latter event requires interactions between all components of the targeting machinery. We recently isolated and visualized a quaternary complex between the bacterial translating ribosome, SRP, SRP receptor, and the Sec translocon. In particular, we observed that the SRP receptor positions the Sec translocon in the vicinity of the ribosomal tunnel exit where nascent proteins emerge from the ribosome tunnel. On the other hand, the eukaryotic targeting machinery is considerably more complex than its bacterial counterpart and contains several additional eukaryotic-specific SRP and SR components. Although these components are essential for the SRP targeting process in eukaryotes, their precise role remains elusive. Here, we determined the cryo-EM structure of the complete eukaryotic protein-targeting complex on the translating ribosome in a cargo pre-handover state. Our structure reveals the role of eukaryotic-specific components of the SRP and SR proteins in the mammalian targeting process and allows us to propose a model for cargo handover to the Sec translocon, which is distinct from the mechanism employed by the bacterial system. Our study underscores an evolutionary divergence in the SRP targeting pathway despite it being a universal and central process in all forms of life.

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Structural studies of the interaction between the *Toxoplasma gondii* protein GRA24 and MAPKs

P. Juyoux [1,2] , E. Pellegrini[3], M. Bowler[1]

[1]Synchrotron Crystallography team, European Molecular Biology Laboratory - Grenoble, France, [2]Université Grenoble Alpes, France, [3]Structural biology of protein-RNA complexes and viral proteins, European Molecular Biology Laboratory - Grenoble, France
pjuyoux@embl.fr

The intracellular parasite *Toxoplasma gondii* is the causative agent of toxoplasmosis. During host cell invasion, *T. gondii* secretes a number of effectors to control its host's immune response that directly alter host gene expression [1]. In particular, *T. gondii* seizes control of intracellular signalling pathways, including the MAPKs (mitogen-activated protein kinases) network.
The MAPKs are a family of intracellular kinases that control fundamental mechanisms such as proliferation, differentiation, inflammation and cell death. They are activated through specific phosphorylation by MAPK kinases (MAPKKs). The docking affinity and specificity of binding partners (activating MAPKKs, inactivating phosphatases, substrates, etc) towards MAPKs is mediated by short linear motifs called Kinase Interacting Motifs (KIMs) [2].
GRA24, one of the *T. gondii* effectors, is mainly intrinsically disordered and contains two KIM repeats [3]. GRA24 triggers the MAPKs signalling pathway by interacting with p38α as previously described in [4]. In this way, it triggers inflammation to repress parasite over-proliferation that would kill the host and thus facilitate long-term parasitism.
Collaborators recently showed that GRA24 also interacts with ERK1, another MAPK, during host invasion. Our work describes here the characterization of the docking interaction between the GRA24 KIM1 peptide and ERK1. Isothermal calorimetry (ITC) shows high affinity of the GRA24 KIM1 peptide for ERK1 and also no affinity for the third member of the MAPK JNK1. The structure of this docking interaction was determined at 2.4 Å. The GRA24 KIM1 peptide binds in a similar manner to the docking site of both p38α and ERK1 and induces an allosteric conformational change to a ready to be activated conformation of the MAPKs. This work gives insights into the mechanism of action of GRA24 protein on key signalling MAPKs during *T. gondii* host cell invasion.

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Molecular mechanism of SHP2 activation by PD-1

M. Marasco[1], A. Berteotti[2], P. Rios[3], J. Weyershaeuser[3], J. Kirkpatrick[1,4], J. Krausze[1], J. Sikorska[4], N. Thorausch[3], H. Brandt[3], W. Schamel[3], M. Köhn[2,3], T. Carlomagno[1,4]

[1]Leibniz University Hannover, Institute of Organic Chemistry and Center for Biomolecular Drug Research, Schneiderberg 38, D-30167 Hannover, Germany, [2]European Molecular Biology Laboratory, Meyerhofstrasse 1, 69117, Heidelberg, Germany, [3]BIOSS-Centre for Biological Signalling Studies, Albert-Ludwigs-University of Freiburg, Schänzlestrasse 18, 79104, Freiburg, Germany, [4]Helmholtz Centre for Infection Research, Group of Structural Chemistry, Inhoffenstrasse 7, D-38124 Braunschweig, Germany
michelangelo.marasco@oci.uni-hannover.de

SHP2 is a 70-kDa protein tyrosine phosphatase composed of two regulatory SH2 domains arranged in tandem (nSH2 and cSH2) and a catalytic PTP domain. It exists in an auto-inhibited state, with the nSH2 occluding the catalytic site on the PTP. This inhibition is relieved when phosphopeptides bind to the SH2 domains, triggering a conformational change that activates the protein [1]. However, the structural details of the activation mechanism of SHP2 have remained obscure so far.

Additionally, SHP2 propagates downstream signals from immune checkpoint receptor PD-1. This interaction is mediated by two motifs on PD-1, the Immune Tyrosine Inhibitory Motif (ITIM) and the Immune Tyrosine Switch Motif (ITSM). Upon engagement of PD-1 by its ligands, Y223 of ITIM and Y248 of ITSM become phosphorylated and can be recognized by the SH2 domains of SHP2. The result of this signalling cascade is suppression of T cell functionality. Cancer cells exploit this pathway to avoid killing by effector T cells, therefore there has been a huge interest in understanding how PD-1 functions [2]. Unfortunately, the details of the interaction between SHP2 and PD-1 have not been elucidated yet, nor is it known what the exact role of each motif is in the propagation of the signal.

In this work, we elucidate the molecular basis of the activation of SHP2 by PD-1 using a combination of NMR, X-ray crystallography, small-angle scattering, biochemistry and *in vivo* experiments. We find that the immune motifs of PD-1 bind the SH2 domains of SHP2 with distinct affinities and solve the structures of the corresponding complexes. We demonstrate that a bidentate peptide, containing both ITIM and ITSM, activates SHP2 much more efficiently than each either motif in isolation. Finally, our structural data reveal that the concurrent binding of the two phosphorylated sites of the divalent bidentate peptide to the tandem SH2 domain (nSH2-cSH2) occurs at low concentrations in a specific arrangement, with ITIM binding to nSH2 and ITSM to cSH2, respectively. The formation of the 1:1 complex between the bidentate peptide and the tandem SH2 requires a conformational change of the protein, which alters the nSH2:cSH2 interface and may represent a mechanism to regulate SHP2 activation.

Altogether, our data provide a comprehensive characterization of the interaction between SHP2 and PD-1, which has tremendous consequences for the development of new immunotherapeutic approaches against cancer. Furthermore, we shed light on the intriguing activation mechanism of this phosphatase, clarifying the role of steric restraints imposed by bidentate activators.

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Characterization of the JIP1-binding surface on KLC1-TPR using Isothermal Titration Calorimetry

T. Quyen Nguyen[1,2] , M. Aumont-Nicaise[2], J. Andreani[2], C. Velours[2], M. Chenon[1,2], F. Vilela[1,2], C. Geneste[2], P.F. Varela[1,2], P. Llinas[1,2*] and J. Ménétrey[1,2*]

[1] Laboratoire d'Enzymologie et Biochimie Structurales (LEBS), CNRS, Université Paris-Sud, 1 avenue de la Terrasse, 91190 Gif-sur-Yvette, France.
[2] Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris-Sud, Université Paris- Saclay, 91198, Gif-sur-Yvette cedex, France.
julie.menetrey@i2bc.paris-saclay.fr

JNK-Interacting Protein 1 (JIP1) which was first identified as scaffold protein for JNK MAP kinase, is a cargo protein for the microtubule-based molecular motor, kinesin1. JIP1 plays significant and broad roles in neurons, mainly as a regulator of kinesin1-dependent transport which can be associated to human pathologies, as cancer or Alzheimer disease. JIP1 is specifically recruited by the Kinesin-Light Chain 1 (KLC1) of kinesin1, but the details of this interaction are not yet fully elucidated. Here, we present an extensive biochemical characterization of the interaction between KLC1 and JIP1 using Isothermal Titration Calorimetry (ITC). The JIP1-binding region of KLC1 was narrowed down using various truncated fragments of the TPR domain of KLC1 and seven critical residues of KLC1 were identified for JIP1 binding. Altogether, these ITC binding data allowed to footprint the JIP1-binding site on KLC1-TPR. This JIP1-binding site footprint was used to discuss the structural basis for the marginal inhibition of JIP1 binding by the auto-inhibitory LFP-acidic motif of KLC1, as well as for the competition between JIP1 and another cargo protein of kinesin1, the W-acidic motif cargo ALCa. Finally, the role of each of these critical residues of KLC1 for JIP1 binding was examined in the light of the crystal structure of KLC1-TPR:JIP1 complex recently released. Thus, this extensive biochemical characterization of the KLC:JIP1 interaction in solution provides important data to better understand the mode of recruitment of JIP1 by KLC1.

Structures of two replication related proteins encoded by SCCmec elements: the mobile elements responsible of MRSA

I. Mir-Sanchis[1,2], P. Rice[1]

[1]Department of Biochemistry and Molecular Biology, The University of Chicago. [2]Present Affiliation: Department of Medical Biochemistry and Biophysics, Umeå University, and Wallenberg Centre for Molecular Medicine, Sweden.
ignacio.mir-sanchis@umu.se

Methicillin Resistant *Staphylococcus aureus* (MRSA) is a worldwide public threat, killing hundreds of thousands of people annually. The methicillin resistant phenotype is caused by a mobile element called Staphylococcal Cassette Chromosome (SCC). In nature these elements move horizontally from donor to recipient cells, but this horizontal gene transfer between bacteria has not been detected in laboratory conditions. Although we know that SCC elements encode their own site-specific recombinases responsible for the integration/excision of the element, nothing else is known about the biology of these elements. Despite these elements were classified as non-replicative mobile elements we propose that they become replicative at some point during their horizontal transfer. Here we will show that in addition to the site-specific recombinases, SCC elements encode other conserved genes whose products are related to DNA replication. We present the X-ray crystal structure of two conserved proteins: Cch [1], an ATPase with helicase activity (2.9 Å resolution) and LP1413 [2], a single stranded DNA binding protein (2.7 Å resolution). Cch has three domains, forms a three layered ring shaped hexamer and its ATPase domain is structurally related to the MCM replicative helicases from the archaea and eukaryotic cells. LP1413, the single stranded DNA binding protein, adopts a winged helix turn helix motif and has a hydrophobic pocket that might be used presumably to interact to other replication related proteins. These structures allow us to speculate about the role of these proteins *in vivo* during SCC's biology and transfer.

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How human lon protease clears the damaged protein from the mitochondrial matrix

I. Mohammed[1], J.A. Abrahams[1,2]

[1]C-CINA, Biozentrum, Basel University, Mattenstrasse 26 Basel, Switzerland
[2]Paul Scherrer Inst., Forschungsstrasse 111, PSI Villigen, Switzerland
inayathulla.mohammed@unibas.ch

Substantial damage to mitochondrial proteins is an unavoidable side-effect of ATP synthesis by the respiratory cycle. This damage is cleared by the lon protease (lonP, 600 Kilo-Dalton), a hexameric protein complex that recognizes, binds, unfolds and cleaves soluble, damaged proteins. It is unknown how lonP, despite its wide substrate range, only proteolyzes proteins that are damaged. Here we show how binding and hydrolysis of ATP controls the asymmetric, rotationally interchanging catalytic states of human mitochondrial lonP. Four cryo-EM maps (resolved to 4 Å or better) reveal its relevant conformational states. In the absence of damaged protein substrate, the protein subunits adopt a staggered orientation, resulting in a roughly lefthanded helical arrangement. Substrate binding induces a righthanded helical twist. The maps show how the unfolded protein substrate is threaded by conserved tyrosine residues from an accessible compartment formed by the N-terminal domains of the lonP hexamer, through three subsequent pores that ultimately lead into a large central cavity formed by the C-terminal protease domains. The proteolytic sites of lonP are located inside the cavity opposite to the pore entrance. These observations indicate that damage is first recognized by lonP's N-terminal domains, leading to a conformational cascade that is catalyzed by ATP hydrolysis, which unfolds the protein substrate and feeds it to its concealed catalytic sites, thus explaining why only damage proteins are processed by lonP. Our results provide insight into a fundamental process allowing sustainable exploitation of the highly reactive chemistry that provides life with its universal energy carrier ATP.

Metal-Captured Inhibition of pre-mRNA Processing Activity by CPSF3 Eliminates *Cryptosporidium* Infection

A. Palencia[1]

[1] National Institute of Health and Medical Research, Institute for Advanced Biosciences, Grenoble, France
andres.palencia@univ-grenoble-alpes.fr

Caused by the protozoan parasite *Cryptosporidium*, Cryptosporidiosis is a severe diarrheal disease (Shirley et al., 2012) that poses major problems for human and animal health. According to the WHO (WHO report, 2017), it is the leading cause of mortality and malnutrition for children below five years of age in developing countries (~1.7 Billion cases a year) and a life- threatening disease for immunocompromised patients.The most relevant *Cryptosporidium* species to humans, *C. parvum* and *C. hominis*, are extremely resistant to chlorine-based water disinfectants, there is no vaccine, and the only FDA-approved treatment, nitazoxanide (NTZ), lacks efficacy in high risk groups. This highlights the need for new classes of drugs and protein-drug targets to treat *Cryptosporidium* infections. Here we show that inhibition of cleavage and polyadenylation specificity factor 3 (CPSF3), an essential endonuclease responsible for the processing of pre-mRNAs (Mandel et al., 2006), is a promising strategy to control *Cryptosporidium* infection. Remarkably, we find that oxaborole-mediated inhibition of CPSF3 reduces intestinal parasite burden in the infant- and immunocompromised-infection models with better efficacy than the standard of care NTZ. We present crystal structures revealing the unprecedented mechanism of action of these compounds, whereby the mRNA processing activity of CPSF3 is efficiently blocked by the binding of the oxaborole group to the metal-dependent catalytic center. Our data provide new insights to accelerate the development of next-generation anti-*Cryptosporidium* therapeutics.

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*Correspondence to: andres.palencia@univ-grenoble-alpes.fr
Website: www.noveltargets-palencia.fr

Structural insights into the TNFR Grindelwald, coupling loss of cell polarity with neoplastic growth

V. Palmerini[1], J. Colombani[2], S. Monzani[1], V. Cecatiello[1], S Mari[1], S. Pasqualato[1], D. S. Andersen[2], M. Mapelli[1]

[1]Istituto Europeo di Oncologia, Milan, Italy, [2]University of Copenhagen, Copenhagen, Denmark
valentina.palmerini@ieo.it

Disruption of polarity and impairment of programmed cell death are hallmarks of advanced epithelial tumors, whose progression often involves JNK signaling. However, the link between loss of polarity and JNK signaling still remains elusive. We recently characterized a novel *Drosophila* TNF receptor, named Grindelwald (Grnd) [1], that triggers apoptosis by binding the unique fly TNF Eiger via its extracellular domain, and by recruiting to the plasma membrane Traf2, the most upstream component of the JNK pathway. Intriguingly, in $Ras^{V12};scrib^{-/-}$ polarity-perturbed epithelia, Grnd interacts with the polarity protein Veli to promote hyper-proliferation and invasiveness in an Eiger-independent manner. These observations depict Grnd as the first TNFR able to couple cell polarity with tumor overgrowth via the JNK signaling. To shed light on the mechanism of Grnd activation, we determined the crystal structure of Grnd, alone and in complex with Eiger. The structures showed that extracellular Grnd comprises a single cysteine-rich domain, organized in a β-hairpin followed by two α-helices. All cysteines are involved in disulfide bridges, showing a unique organization pattern for Grnd compared to other TNFRs. Grnd forms hetero-hexamers with the TNF domain of Eiger, whose TNF domain folds in a peculiar "jelly-roll" antiparallel β-sheet. Binding assays with interface mutants of Grnd and Eiger designed on the basis of the structure, revealed that the β-hairpin tip and the first α-helix of Grnd, and the loops between β-strands of Eiger are the major determinants of the nanomolar interaction between the two proteins, as measured by ITC. To address the relevance of the Eiger:Grnd interaction for the proliferative and apoptotic activities of Grnd, we engineered Grnd mutants flies and we are currently setting up *in vivo* experiments.

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Structure of a subcomplex of the apicomplexan invasion machinery

S. Pazicky[1], K. Dhamotharan[1], H. Mertens[2], U. Weininger[3], C. Löw[1]

[1]Centre for Structural Systems Biology (CSSB), DESY and European Molecular Biology Laboratory Hamburg, Notkestrasse 85, D-22607 Hamburg, Germany.
[2]European Molecular Biology Laboratory Hamburg, Notkestrasse 85, D-22607 Hamburg, Germany
[3]Institute of Physics, Biophysics, Martin-Luther-University Halle-Wittenberg, 06120, Halle (Saale), Germany
spazicky@embl-hamburg.de

Apicomplexa are a large phylum of intracellular parasites that infect animals and humans. *Toxoplasma gondii*, the most prevalent infectious organism of humans, causes life-threating infection of unborn foetuses. Another Apicomplexan, *Plasmodium falciparum*, causes over 200 million malaria cases a year with almost 500 thousand deaths.

In order to invade and egress the host cells and to move through the tissues, the *Apicomplexa* use a unique type of movement - gliding. The gliding power is mediated by an actin/myosin motor, located between the plasma membrane and the alveoli – a double membrane underlying the plasma membrane. To transfer the gliding power to the entire parasite, actin is attached to the surface of the host cell via the plasma membrane proteins, whereas myosin is anchored in the inner membrane complex – the protein network of alveoli [1].

The protein complex which anchors myosin to the inner membrane complex is called the glideosome and consists of several glideosome associated proteins (GAP40, GAP45, GAP50), two calmodulin-like proteins (ELC and MLC1) and myosin. The structure of the glideosome remains elusive, with only the structures of two subcomponents, the soluble domains of GAP50 and MLC1, solved so far [2].

We have determined the crystal structure of the heterotrimeric complex of TgELC2, TgMLC1 and the C-terminus of myosin A from *Toxoplasma gondii* (Fig. 1). The structure shows that the alpha-helical C-terminus of myosin A is bent in the middle and the two calmodulin-like proteins ELC2 and MLC1 interact via several hydrophilic interactions. ELC2 binds one calcium ion in the N-terminal EF-hand, whereas the other residues of the same EF-hand mediate the interaction with MLC1. An NMR structure of the N-terminal part of PfELC1 from *Plasmodium falciparum* is very similar to TgELC2, however, it does not bind calcium and its C terminus is unfolded in the absence of a binding partner.

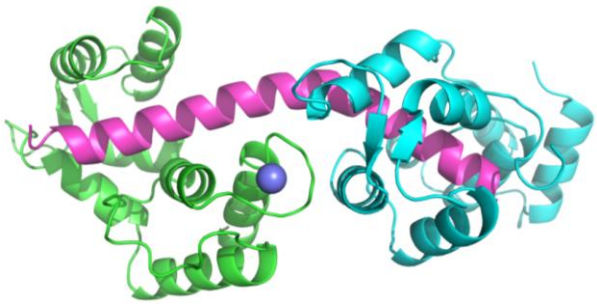

Fig. 1: Structure of *Toxoplasma gondii* C-terminus of MyoA (pink) bound to ELC2 (green) and MLC1 (cyan).

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Structural specificities of the mitotic kinesin MKlp2 and implications to its mechanism and function

F.M. Ranaivoson[1], P. Conan[1], A. Abdalla Mohammed Khalid[2], S. Inagaki[3], C. Schmidt[2], S.S. Rosenfeld[3], A. Houdusse[1]

[1]Institut Curie, Structural Motility team, UMR 144, Paris, France
[2]Georg-August-Universität, Fakultät für Physik, Göttingen, Germany
[3]Mayo Clinic, Department of Neuro-Oncology, Jacksonville, FL, United States
fanomezana-moutse.ranaivoson@curie.fr

During cell division, the kinesin-6 MKlp2 plays a critical role from the metaphase-anaphase transition to the cytokinesis. In particular, in late metaphase, MKlp2 localizes at the antiparallel microtubule (MT) bundles that form at the spindle midzone, where it mediates the translocation of the Chromosome Passenger Complex (CPC) from the centromeres to the mitotic spindle and then to the contractile ring at the equatorial cortex [1]. At this stage, the ensemble {Mklp2-CPC} regulates the furrow ingression process and eventually the abscission to split the two daughter cells apart. Due to this important function in cell division and because several cancer cells overexpress MKlp2, a growing interest in 1) investigating the peculiar MKlp2's molecular mechanism and 2) specifically targeting this mitotic kinesin by anti-tumoral drugs arose during the past decade [2]. Like other kinesin-6s, MKlp2 is characterized by a large and poorly conserved insertion in the loop L6 of its motor domain (MD) and an unusually long neck linker (NL), a fragment that connects the C-terminus of one MD to the dimerization (coiled-coil) domain. Our initial cryo-EM and biochemical data highlighted MKlp2's mechanistic specificities when compared to other kinesins. Importantly, we showed that the ADP state of this kinesin displays an unusually high affinity towards MTs, which led to the first structural characterisation of a MT-bound ADP-kinesin [3].

Here we further decipher the structural and functional properties of MKlp2. Using X-ray crystallography we reached a resolution of 2.1 Å that allowed us to confidently define the conformation of the L6 insert and to establish that its N-terminal part is compactly folded with the core MD, while its C-terminal part is disordered. At the opposite side of the structure, we show that the nucleotide-free state is accompanied by a backward-docked NL, which is a newly observed structural feature of a kinesin motor pre-stroke state. In addition, although deleterious to its velocity, the MKlp2 long NL (~50 residues) would allow one MD of a dimer to explore a wide space while the other binds to a MT, presumably reaching another MT. Indeed, using motility assays, we demonstrate that dimeric MKlp2 constructs move along MT at low rate. Instead, these constructs allow the bundling of MTs and their organisation into a three-dimensional network, a property usually observed with tetrameric kinesins. Altogether, our results provide unprecedented structural and physical exploration into the molecular mechanism of MKlp2 and shed new lights towards a fine understanding of MKlp2 functions at the mitotic spindle.

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Understanding cytoplasmic maturation of the eukaryotic ribosomal small subunit (pre-40S)

F. Raoelijaona[1], S. Lebaron[2], S. Thore[1], S. Fribourg[1]

[1]RNA: Natural and Artificial Regulations (ARNA), INSERM U1212 - CNRS UMR 5320 - University of Bordeaux
2.Dynamique et dysfonctionnement de la synthèse des ribosomes , LBME CBI Toulouse-UMR5099, Université Toulouse III Paul Sabatier
finaritra.raoelijaona@inserm.fr

Ribosomes are translational machineries universally responsible of protein synthesis. In eukaryote, ribosome assembly is a complex and highly regulated process that requires the action of more than 200 biogenesis factors through its entire pathway (from the nucleolus to the cytoplasm).
Cytoplasmic maturation of the small ribosomal subunit is associated with sequential release of the late assembly factors and concomitant maturation of the pre-ribosomal RNA. During final maturation of the small subunit, the pre-18S rRNA is cleaved off by the endonuclease hNob1 which is coordinated by its binding partner Pno1. Detailed information on pre-ribosome architecture has provided structural snapshots of maturation events, however, key functional aspects such as the architecture required for pre-18S rRNA cleavage remains elusive. In order to better understand these late steps of cytoplasmic pre-40S maturation, we first redefine the endonuclease domain then study its binding mode with hPno1 using different tools such as sequence analysis, structure prediction and biochemical experiments. We finally perform functional assay to elucidate the role played by Pno1 during the pre-18S rRNA maturation.
Our results have shown that eukaryotic Nob1 adopts an atypical PIN domain conformation: two fragments separated (res 1-104 and 230-255) by an internal loop which is essential for Pno1 recognition. We also found out that Pno1 inhibits Nob1 activity by masking the cleavage site. Our findings further support the recently published cryo-EM structure of the pre-40S.

Structure of the DP1-DP2 PolD complex bound with DNA and its implications for the evolutionary history of DNA and RNA polymerases

P. Raia, M. Carroni, E. Henry, G. Pehau-Arnaudet, S. Brûlé, P. Béguin, G. Henneke, E. Lindahl, M. Delarue, L. Sauguet[1]

[1]Unité de Dynamique Structurale des Macromolécules, Institut Pasteur, France
ludovic.sauguet@pasteur.fr

PolD is an archaeal replicative DNA polymerase (DNAP) made of a proofreading exonuclease subunit (DP1) and a larger polymerase catalytic subunit (DP2). Recently, we reported the individual crystal structures of the DP1 and DP2 catalytic cores, thereby revealing that PolD is an atypical DNAP that has all functional properties of a replicative DNAP but with the catalytic core of an RNA polymerase (RNAP). We now report the DNA-bound cryo-electron microscopy (cryo-EM) structure of the heterodimeric DP1-DP2 PolD complex from Pyrococcus abyssi, revealing a unique DNA-binding site. Comparison of PolD and RNAPs extends their structural similarities and brings to light the minimal catalytic core shared by all cellular transcriptases. Finally, elucidating the structure of the PolD DP1-DP2 interface, which is conserved in all eukaryotic replicative DNAPs, clarifies their evolutionary relationships with PolD and sheds light on the domain acquisition and exchange mechanism that occurred during the evolution of the eukaryotic replisome.

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ECSIT plays an essential role in ACAD9 activity and the formation of the Mitochondrial Complex I Assembly (MCIA) Complex

G. Giachin[1], M. Jessop[2], R. Bouverot[1], S. Acajjaoui[1], M. Saïdi[1], A. Chretien[1], L.Signor[2], P.J. Mas[3], A. Favier[2], E. B. Meneroud[4], E. Kandiah[1], M. Hons[5], D. J. Hart[2], E.B. Erba[2], A. Buisson[4], G. Leonard[1], I. Gutsche[2] & M. Soler-Lopez[1]

[1] Structural Biology Group, European Synchrotron Radiation Facility (ESRF), CS 40220, 38043 Grenoble Cedex 9, France. [2] Institut de Biologie Structurale (IBS), CNRS, CEA, Université Grenoble Alpes, CNRS, 38044 Grenoble, France. [3] Integrated Structural Biology Grenoble (ISBG) CNRS, CEA, Université Grenoble Alpes, EMBL, 71 avenue des Martyrs, F-38042, Grenoble, France. [4] Grenoble Institut des Neurosciences (GIN), Centre Inserm U1216, Equipe Neuropathologies et Dysfonctions Synaptiques, Université Grenoble Alpes, Grenoble, France. [5]European Molecular Biology Laboratory (EMBL), Grenoble Outstation, CS 90181, 38042 Grenoble, Cedex 9, France.
solerlop@esrf.fr

ECSIT (*Evolutionarily Conserved Signaling Intermediate in Toll pathways*) was originally identified as a cytosolic protein implicated in the innate immune system response. ECSIT has also been described as a mitochondrial respiratory complex I (CI) assembly factor. There, ECSIT predominantly functions in concert with ACAD9 (*Acyl-CoA Dehydrogenase family member 9*) and NDUFAF1 (*Complex I intermediate-associated protein 30*) to form the so-called mitochondrial CI assembly (MCIA) complex. The crucial role of the MCIA complex stands by deficiencies in CI - the most commonly observed disorders associated with mitochondrial dysfunctions - in patients harboring mutations in genes encoding components of this complex [1]. How the MCIA factors function to promote CI assembly and stability is not know yet. Here, we present the molecular characterization of the human MCIA factors, their interaction affinity *in vitro* and *in vivo* and the 3D reconstruction of an MCIA subcomplex at near-atomic resolution. Taken together, our results describe for the first time the MCIA complex at the molecular level, its formation and organization, and provide a framework for understanding the underlying mechanisms of CI biogenesis.

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Study of bacterial colonization with X-ray and neutron scattering Techniques

H.V. Sørensen[1], K.L. Browning[2], K. Bjerregaard-Andersen[1], M. Blakeley[3], M. Cárdenas[4], R. Lund[1], U. Krengel[1]

[1]Department of Chemistry, University of Oslo, Norway, [2]Department of Pharmacy, University of Copenhagen, Denmark, [3]Institut Laue-Langevin, France, [4]Malmö University, Sweden
h.v.sorensensen@kjemi.uio.no

Vibrio cholerae is the bacterium responsible for cholera, a severe diarrheal disease [1]. A crucial factor for bacterial colonization is *N*-acetyl glucosamine-binding protein A (GbpA). This protein binds the chitin layers of crustaceans and plankton as well as the mucus layer in the small intestine of humans [2]. GbpA is a four-domain protein, of which domains I and IV have affinity for chitin [3, 4]. In addition, domain I is a lytic polysaccharide monooxygenase that degrades chitin [4]. Domains I-III have previously been structurally characterized by X-ray diffraction by Wong *et al.* [3], however, so far there is no information
as to how GbpA interacts with and degrades chitin. In order to get an in depth understanding on how GbpA carries out its function as a colonization factor and enzyme, we want to apply X-ray and neutron scattering techniques, such as Small-Angle X-ray Scattering, Small-Angle Neutron Scattering and Neutron Reflectometry. Here we will discuss our preliminary results.

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Structural and Functional Characterization of the APOBEC1-Editosome

P. Throll[1], E. Kowalinski[1]

[1]European Molecular Biology Laboratory (EMBL), 71 Avenue des Martyrs, Grenoble, France
pthroll@embl.fr

RNA-editing introduces nucleotide base modifications, insertions or deletions leading to changes in a target RNA sequence. In humans, the deaminase APOBEC1 specifically targets mRNA sequences to deaminate cytosine residues to uracil. This editing can lead to different protein isoforms and can affect mRNA translation, localization and stability. APOBEC1 is the catalytic subunit of a multi-protein complex called the APOBEC1 editosome.

However, the exact sub-unit composition of the APOBEC1 editosome, its regulation and how its target specificity is achieved are still poorly understood. To understand the function and regulation of the editosome, we set out to solve molecular structures of the complex and characterize the interaction of the individual subunits with each other and with substrate RNA. This will help us to understand the roles of the different subunits and how APOBEC1 target selection is moderated.

While correct RNA editing by APOBEC1 is vital for example in human lipid metabolism, misregulated RNA editing is connected to diseases like epilepsy and cancer. In this study, we aim to establish a detailed atomic model for the composition and functioning of the APOBEC1 editosome. Such a model will help to understand why RNA-editing by APOBEC1 is maintaining cells in healthy conditions and how diseases can arrise from defective RNA-editing.

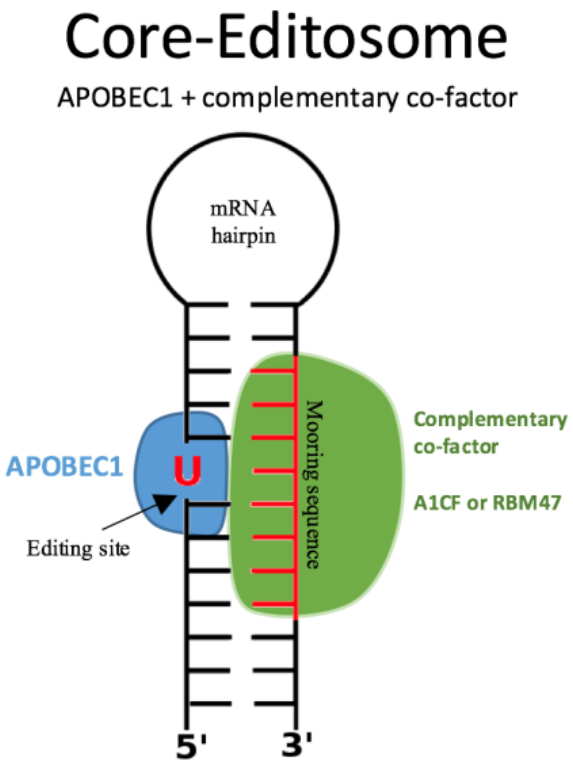

Figure 1: Schematic representation of the putative APOBEC1 core-editosome.

The rise of BioSAXS at the ESRF: BM29 beamline for SAXS on proteins in solution

M. Tully[1], M. Brennich[2] and Petra Pernot[1]

[1]ESRF-The European Synchrotron, 71 avenue des Martyrs, Grenoble, France
[2]European Molecular Biology Laboratory (EMBL), Grenoble Outstation, Grenoble, France
mark.tully@esrf.fr

As the first synchrotron in the world to have a SAXS beamline dedicated exclusively to bio-solutions the ESRF is leading the way. BM29, a bending magnet-based BioSAXS station opened to users in 2008 [1]. The number of BioSAXS publications based on ESRF-collected data has grown near-exponentially and BM29 is now significantly oversubscribed. This is a worldwide trend as in recent years a combination of advances in sample handling, computer modelling and synchrotron sources have made BioSAXS more and more attractive. Moreover, whereas in the past even experienced crystallographers complained that SAXS experiments required too much time and effort, now they consider them indispensable.
BioSAXS experiments at BM29 are highly automated, offering two main modes of operation: BioSAXS with a robotic liquid handling sample changer (SC) and BioSAXS with online size-exclusion chromatography (SEC), purifying samples immediately prior to X-ray exposure. Switching between SC and SEC-SAXS modes is fully automated, maximizing the efficiency of the beamline. In SC operation up to a thousand measurements can be performed per day, with help from dedicated beamline control, data-acquisition software, real-time data display and automatic data processing. In both modes data collection parameters and results are logged and stored in the modified ISPyB database.

After the completion of the ESRF-Extremely Brilliant Source project in 2020, BM29 will have a new source, a 2-pole wiggler, to replace the current bending magnet. This will provide a smaller, more intense X-ray beam at the sample position. This will be coupled with a new, in vacuum detector while the current BioSAXS sample environment will be modified accordingly. These upgrades will generate high quality, low noise data with lower concentrations of sample (0.1mg/ml) necessary.
Here the experimental setups available on BM29 will be described together with the various examples of the BioSAXS data obtained. Recent examples of BM29 research, including the integration of microfluidics systems and online ion-exchange chromatography as well as the possibilities for new experiments in BioSAXS.

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Cryo-electron microscopy structure of the curli secretion apparatus CsgF:CsgG reveals a dual-constriction pore with properties amenable to nanopore sequencing

S. E. Van der Verren[1,2], N. Van Gerven[1,2], R. Efremov[1] and H. Remaut[1,2,*]

[1] Structural Biology Brussels, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium
[2] Structural and Molecular Microbiology, VIB-VUB centre for Structural Biology, Pleinlaan 2, 1050 Brussels, Belgium
Authors for correspondence: Han Remaut (**han.remaut@vub.be**)

Curli are proteinaceous fibers found in a several proteobacteria where they mediate biofilm formation and bacterial adherence to biological and non-biological surfaces. Previously, our lab solved the crystal structure of CsgG, a 36-stranded β-barrel, transporting curli subunits across the outer membrane. CsgF is believed to be a vital coupling factor between curli secretion and assembly on top of the bacterial cell, but this is supported by little biological evidence. Here we present structural insights in CsgF-mediated secretion through cryo-electron microscopy of the CsgF:CsgG complex. The 33 N-terminal residues of mature CsgF engage in a tight interaction inside the β-barrel of the CsgG pore, forming a second constriction formed by asparagine 17. The addition of a second pore to the system offers opportunities to ameliorate DNA sensing of the CsgG pore, which is used in commercial Oxford Nanopore Technologies Devices. Nanopore sequencing offers high-throughput and fast single molecule sequencing with high accuracy and at low cost, in portable devices amenable to field research. Complexes of CsgG and the CsgF constriction peptide (FCP) give rise to nanopores with two reading heads separated in space by 25 Å, corresponding to an approximately 5-nucleotide gap between the two constrictions. We show that both constrictions of CsgG:FCP-based nanopores contribute discriminating signal during ssDNA translocation, resulting in increased resolving signal and higher base calling accuracy in homopolymer regions compared to the CsgG pores alone.

Structure of actively transcribing influenza virus polymerase in the late elongation state

J.M. Wandzik[1], T. Kouba[1], S. Reich[1], S. Cusack[1]

[1]European Molecular Biology Laboratory (EMBL), 71 Avenue des Martyrs, Grenoble, France;
jwandzik@embl.fr

Influenza virus is a pathogen responsible for seasonal influenza outbreaks and occasional pandemics. It represents a serious health risk especially for immunosuppressed patients, young children, pregnant women and the elderly. Therefore, it is of great interest to develop antiviral drugs along with various vaccination strategies. As influenza virus polymerase (FluPol) is a validated drug target [1], studies on its activity and structure are crucial for development of new and potent anti-influenza drugs.

Influenza polymerase associates with both termini of the viral genomic ssRNA (vRNA) while multiple copies of nucleoprotein (NP) decorate the rest of vRNA. This assembly forms a ribonucleoprotein particle (RNP) which is the functional unit for viral replication and transcription during viral infection. [2]

FluPol is an RNA-dependent RNA polymerase that produces fully functional capped and polyadenylated messenger RNA (mRNA). To initiate transcription, FluPol acquires a host-derived capped primer by "cap-snatching". During elongation, FluPol transcribes the genomic RNA until the polyadenylation signal where it stutters, producing a poly(A) tail by reiterative copying of a short uridine-stretch leading to termination. [3], [4]

Our group has determined the first atomic structure of FluPol using X-ray crystallography [5], [6]. We are now investigating the detailed mechanism of transcription by structural and functional studies *in vitro*. We are able to trap different steps of transcription by controlling RNA synthesis reaction conditions, for example by RNA template design, choice of NTPs and incubation time. This approach in combination with the powerful technique of cryo- electron microscopy (cryo-EM) allowed us to gain new insight into the successive functional states of actively transcribing polymerase [7].

Here I present the atomic structure of bat influenza A polymerase trapped in a late elongation state of mRNA transcription, obtained by single particle cryo-EM. We observe a nine base-pair long RNA duplex formed between the template and the newly synthesized product strand in the active site cavity. For the first time, we could describe the template exit channel of the polymerase and we also observed an additional binding site that specifically accommodates the emerging template RNA 3' end. Extensive characterization of the interactions between this highly conserved binding site and the 3' extremity of the RNA template leads us to a new hypothesis on the overall mechanism of viral transcription. We speculate that both ends of the template remain bound to FluPol during mRNA synthesis aiding efficient reinitiation and multiple rounds of transcription from a single RNP.

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Crystal structure of a novel multidrug and toxic compound extrusion transporter from *Aquifex aeolicus*

J. Zhao[1,2], J. Wang[1] and H. Michel[2]

[1] Tianjin University, School of Chemical Engineering and Technology, State Key Laboratory for Chemical Engineering, Collaborative Innovation Center of Chemical Science and Chemical Engineering, 300072 Tianjin, P. R. China
[2] Department of Molecular Membrane Biology, Max Planck Institute of Biophysics, Max-von-Laue-Strasse 3, 60438 Frankfurt am Main, Germany
jizhao@biophys.mpg.de

Multidrug and toxic compound extrusion (MATE) transporters are widespread among all domains of life. Bacterial MATE transporters confer multidrug resistance to cells by utilizing an electrochemical gradient of H^+ or Na^+ to export xenobiotics across the membrane[1,2]. Despite the availability of X-ray structures of several MATE proteins[1,3-8], a detailed understanding of the transport mechanism has remained elusive. Here we report the crystal structure of a MATE transporter from *Aquifex aeolicus* at 2 Å resolution, which is the highest resolution reported for a MATE transporter to date. Due to the lack of conserved acidic residues, this protein may represent a novel subdivision of the prokaryotic MATE transporters. The observation of a crystallographic dimer, combined with the cross-linking analyses, provide the first evidence that MATE transporters may exist as oligomers in the membrane. Furthermore, we identified an uncommon cation-binding site located in the N-bundle, and its importance is demonstrated by structure-based mutation analysis, which suggests a different transport mechanism than previously proposed. Our results provide structural insights into transport mechanism of a novel MATE transporter, which further advance our understanding of this important transporter family.

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Structural basis of NetrinG2-dependent neurodevelopmental disease

C. Zheng[1], R. Parton[1], R. Maroofian[2], E. Seiradake[1]

[1]Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK
[2]Genetics Research Centre, Molecular and Clinical Sciences Institute, St George's, University of London, Cranmer Terrace, London SW17 0RE, UK
celine.zheng@merton.ox.ac.uk

Brain wiring depends on cells making highly localised and selective connections through surface protein-protein interactions. In particular, NetrinG proteins and NetrinG ligands (NGLs) act as trans-synaptic adhesion proteins and are thought to determine synapse specificity [1-4]. NetrinGs are laminin-related proteins and are the only members of the conserved netrin family to be vertebrate specific. Structurally, NetrinG interacts with NGL in a hand-clasp fashion, which is conserved across the cognate pairs NetG1-NGL1 and NetG2-NGL2 [5]. Recent work has shown that mutations in NetrinG2 are linked to hereditary neurodevelopmental disorders in humans. Here, we discuss the structural implications of those disease-linked mutations through cell-surface NetG2 expression assays and NGL2-binding studies. These results reveal first insights into the molecular understanding of specific NetrinG2-dependent neurodevelopmental disorders.

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