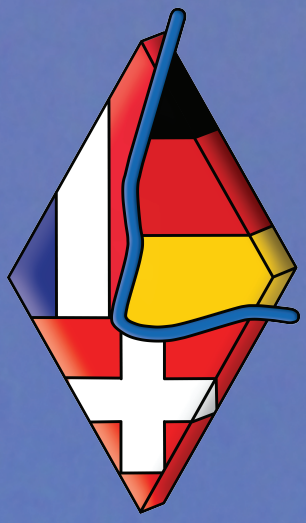

32nd Rhine-Knee Regional Meeting on Structural Biology

26 - 28 September 2018
Emmetten, Switzerland

Book of Abstracts

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Organiser Dr. Vincent Olieric

Secretariat Sonia Reber
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Keynotes
Prof. Phil Willmott, Paul Scherrer Institute, Switzerland
Dr. Arjen Jakobi, Kavli Institute, The Netherlands
Dr. Andy Doré, Sosei Heptares, UK

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08:45 - 09:00	**Patrick Ernst,** *University of Zurich* Rigid Fusions of DARPins and ArmRPs to facilitate crystallography
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Abstracts

Synchrotron radiation – the basics and the newest developments

Philip Willmott

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Synchrotron radiation is a multidisciplinary tool used by a broad spectrum of experimentalists from all walks of the natural sciences[1]. In this lecture, I will explain the basics of the generation and properties of synchrotron radiation, and its application in the biological sciences, in particular with respect to macromolecular crystallography (MX).

In the last decade, two highly significant technological advances have been made in x-ray sciences, both of which have had a revolutionary impact on the biological sciences. The first, x-ray free-electron lasers, have caused a paradigm shift in time-resolved MX studies. The second, namely the fourth-generation of synchrotron facilities, the so-called “diffraction-limited storage rings” (DLSRs), promise to probe the structures of biomolecular structures previously excluded to investigation using MX. Finally, the role of synchrotron and XFEL studies and that of cryo-electron microscopy will be briefly reviewed.

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Sample exchange for high-throughput protein crystallography: TELL at SwissMX and SLS

Isabelle Martiel, Wayne Glettig, Dominik Buntschu, Nathalie Meier, Alexandre Gobbo, Roman Schneider, David Müller, Peter Heimgartner, Jan Hora, Bill Pedrini, Claude Pradervand, Ezequiel Panepucci, Vincent Olieric, Meitian Wang

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With the rise of fast detectors and advanced sources of synchrotron X-rays, sample exchange has become a bottleneck in protein crystallography (PX) data collection [1]. We present here the new sample exchange system developed at the Paul Scherrer Institute for the SwissMX fixed target PX instrument at SwissFEL and for the PX beamlines at the Swiss Light Source (SLS), the TELL system. TELL will provide a large sample storage capacity, fast exchange times and an improved reliability to cover the needs of next generation PX at SwissFEL and SLS. The broader context of the fixed target instrument SwissMX at SwissFEL will also be presented. At the SLS, TELL will be deployed at the SLS X06SA (PXI) beamline in January 2019; from then on, only Unipucks will be supported for automatic mounting at this beamline.

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Dozor-MeshBest software for the analysis of X-ray mesh scans in macromolecular crystallography

Igor Melnikov[1], Gleb Bourenkov[2], Olof Svensson[1], Gordon Leonard[1], Alexander Popov[1]

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Pre-characterisation of the sample under study is a crucial step towards obtaining better data quality in macromolecular crystallography (MX). Tiny crystals, often immersed in opaque environments, are particularly problematic to properly centre in the X-ray beam. In this context, low-dose X-ray mesh (or grid) scans, which are now available on most synchrotron MX beamlines are an ideal technique for sample pre-characterisation in MX. A major advantage of an X-ray mesh scan is that they operate directly with diffraction images and so their quality can be assessed and a map of crystal quality be constructed.

In the work presented here, we feature two software programs *Dozor* [1] and *MeshBest* [2], which can be used for the complex analysis of X-ray mesh scans. *Dozor* contains built-in algorithms which can both detect and score diffraction from protein crystals. *MeshBest* extends this analysis: based on the output of *Dozor*, it determines which of the diffraction images obtained during the scan are from the same crystal and groups them together. Finally, a crystal map is created, depicting by colour separation both all regions containing single crystal diffraction and all regions where diffraction arises from several crystals stacked upon each other. Further analysis includes crystal shape/size estimation and the final result is then used to determine the most appropriate data collection strategy.

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In situ serial crystallography for rapid de novo membrane protein structure determination

Chia-Ying Huang[1], Vincent Olieric[1], Nicole Howe[2], Rangana Warshamanage[1], Tobias Weinert[1], Ezequiel Panepucci[1], Lutz Vogeley[2], Shibom Basu[1], Kay Diederichs[3], Martin Caffrey[2], Meitian Wang[1]

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De novo membrane protein structure determination is often limited by the availability of large crystals and the difficulties in obtaining accurate diffraction data for experimental phasing. Here we used four membrane proteins, including a de novo structure BacA [1], to demonstrate the *in meso in situ* serial crystallography – experimental phasing (IMISX-EP) method [2]. The method enables systematic diffraction screening and rapid data collection from hundreds of microcrystals in IMISX wells without the need for direct crystal harvesting. The requisite data quality for experimental phasing is achieved by accumulating diffraction signals from isomorphous crystals identified post-data collection. The method works in all experimental phasing scenarios and is particularly attractive with fragile, weakly diffracting microcrystals. The automated serial data collection approach can be readily adopted at most microfocus macromolecular crystallography beamlines.

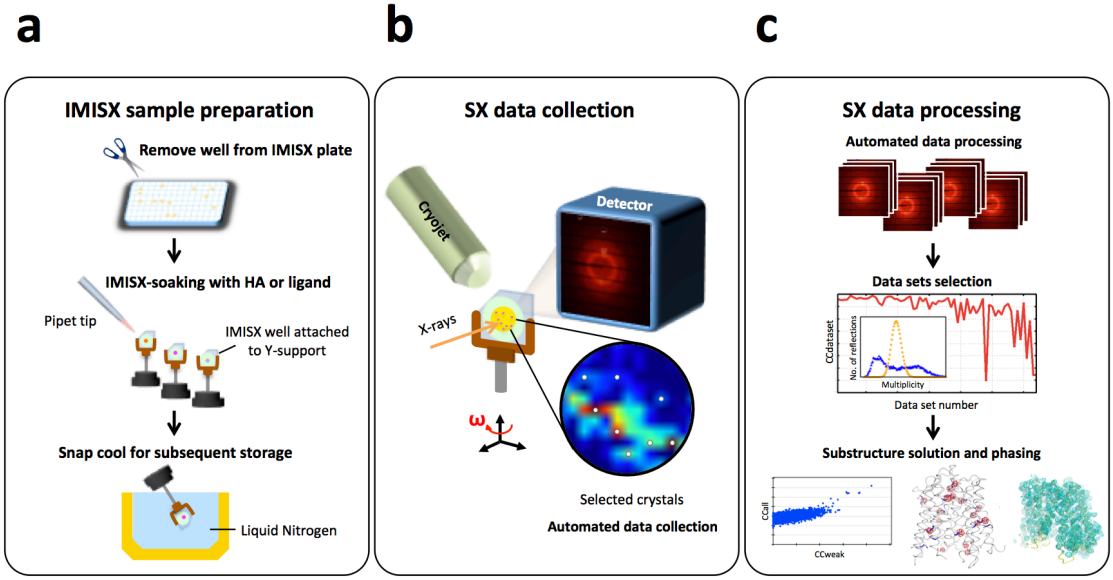

Figure 1. Overview of the IMISX-EP workflow for high-throughput *in situ* de novo phasing of *in meso* grown microcrystals of membrane proteins.

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Demonstration of femtosecond time-resolved X-ray diffraction on protein crystals

Nadia Linda Opara[1,4,5], Istvan Mohacsi[1,2], Mikako Makita[1], Daniel Castano-Diez[4], Ana Diaz[1], Pavle Juranić[1], May Marsh[1], Alke Meents[2], Christopher J Milne[1], Aldo Mozzanica[1], Celestino Padeste[1], Valérie Panneels[1], Marcin Sikorski[3], Sanghoon Song[3], Henning Stahlberg[4,5], Ismo Vartiainen[1], Laura Vera[1], Meitian Wang[1], Philip R Willmott[1] & Christian David[1]

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The development of X-ray free-electron lasers (XFELs) has opened the possibility to investigate the ultrafast dynamics of biomacromolecules using X-ray diffraction [1]. Whereas an increasing number of structures solved by means of serial femtosecond crystallography at XFELs is available [2-10], the effect of radiation damage on protein crystals during ultrafast exposures has remained an open question [11-12]. We used a split-and-delay line based on diffractive X-ray optics [13] at the LCLS XFEL to investigate the time dependence of X-ray radiation damage to lysozyme crystals. For these tests, crystals were delivered to the X-ray beam using a fixed target approach. The presented experiments provide probe signals at eight different delay times between 19 and 213 femtoseconds after a single pump event, thereby covering the time scales relevant for femtosecond serial crystallography [14]. Even though significant impact on the crystals was observed at long time scales after exposure with a single X-ray pulse, the collected diffraction data did not show significant signal reduction that could be assigned to beam damage on the crystals in the sampled time window and resolution range. This observation is in agreement with estimations of the applied radiation dose, which in our experiment was clearly below the values expected to cause damage on the femtosecond time scale. The experiments presented here demonstrate the feasibility of time-resolved pump-multiprobe X-ray diffraction experiments on protein crystals.

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First experimental results from serial protein crystallography experiments at the SwissFEL X-ray free-electron laser source

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Serial femtosecond crystallography (SFX) is an emerging technique for structure determination of radiation sensitive systems such as micro-crystals in general and metalloproteins in particular that has been successfully applied at X-ray free-electron laser (XFEL) sources. SFX brought an opportunity to break the nexus between radiation dose and spatial resolution in macromolecular crystallography (MX) by outrunning most radiation damage processes when using ultra-short X-ray pulses. Moreover, the ultra-short pulse duration of an XFEL allows accessing reaction time scales previously not reachable by conventional time-resolved crystallography. SwissFEL, a new XFEL source has been built at the Paul Scherrer Institute in Switzerland. It is currently operating in a commissioning mode with a couple of early pilot user experiments. They aim to demonstrate the feasibility of different types of experiments that can be performed at SwissFEL and to commission the beamlines and various instruments. Normal user operation at SwissFEL will begin in 2019. Recently, a collaborative group of scientists from the Paul Scherrer Institute and members of the LeadXpro and Heptares pharmaceutical companies performed the first SFX pilot user experiment at the SwissFEL. The results from the first SFX pilot user experiment confirm that accurate, high-resolution data from protein micro-crystals can be recorded in an efficient manner at SwissFEL using the SFX technique. A summary of SwissFEL's technical parameters and instruments relevant for MX and results from the first SFX pilot user experiment at SwissFEL will be presented.

NMR-Based Determination of the 3D Structure of the Ligand-Protein Interaction Site without Protein Resonance Assignment

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X-ray crystallography molecular replacement (MR) is a highly versatile tool for the detailed characterization of lead compound and binding modes in the pharmaceutical industry. The two major limitations of its application to drug research are (i) the availability of a similar protein structure, and (ii) obtaining well-diffracting crystals of the ligand-protein complexes of interest. While nowadays the first point is often not a limitation anymore, obtaining well-diffracting crystals might be difficult. In such situations structure determination of protein-ligand complexes by liquid-state NMR is a good option. Unfortunately, the established standard structure determination protocol is in general time-consuming, and a shortcut using available structural data as in the case of MR in X-ray crystallography is not available.

Here, we present *N*MR^2 (**NMR M**olecular **R**eplacement), a MR-like approach in NMR to determine the structures of the binding pockets of ligands at atomic resolution. The calculation of structures of protein-ligand complexes relies on the collection of unassigned semi-quantitative inter-molecular NOE distance restraints and on previously solved structures.[1] The *N*MR^2 method uses a high throughput structure calculation protocol, rather than a docking-scoring simulation. It is fast since it requires only a few days of measuring time and bypasses the time-consuming sequential assignment steps for the protein.

We will present multiple *N*MR^2 applications covering several ligand topologies ranging from peptidomimetic to small molecules that bind strongly or weakly to protein receptors. We also report how *N*MR^2 can make use of partially labelled protein using methyl-specific isotope labelling. Our findings demonstrate that *N*MR^2 may open an avenue for the fast and robust determination of the binding pocket structure of ligand-protein complexes at atomic resolution.

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Structural characterization of the anti-apoptotic CARP/titin-N2A complex reveals a mimetic binding

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The cardiac ankyrin repeat protein, CARP, is overexpressed in the heart upon disease, mechanical or toxic stress, protecting the myocardium against damage. Stress-induced CARP locates primarily to the N2A spring region of the titin filament in the sarcomere, but the functional and molecular basis of this interaction are unclear.

Our previous studies [1] have shown that dimerized CARP can robustly co-segregate with dual domain titin UN2A-Ig81 with a 1:1 molar ratio. The elongated unique titin motif of UN2A has a stable α-helical fold and Ig81 has a unique structure among other immunoglobulin domains from titin. In our latest work, we have investigated the CARP/titin-N2A complex using NMR, H/D exchange mass spectrometry (MS) and NMR-guided modelling (CS-ROSETTA). The results show that UN2A contains a highly structured, helical core region spanning its middle sequence, but that large N- and C-terminal fragments are disordered. Mapping of interacting residues on the NMR-guided 3D-model of UN2A revealed that the UN2A interface resembles an ankyrin repeat, mimicking the structural building units of CARP. Interestingly, the titin-N2A interaction region was identified as being located within the self-dimerization area of CARP. This indicates that titin-N2A interacts with CARP in a manner that mimics CARP dimerization. This explains why the binding breaks the CARP dimer and the complex is formed at a 1:1 molar ratio. Our current data portray a CARP/titin-N2A binding model that permits now analysing the potential disease-linkage of known mutations in cardiomyopathy patients and the role of CARP in providing mechanical resilience to the sarcomere through its binding of titin.

References

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Rigid Fusions of DARPins and ArmRPs to facilitate crystallography

Patrick Ernst[1], Yufan Wu[1,2], Alexander Batyuk[1,3], Christina Ewald[1,4], Floor van der Valk[1,5] Peer R. E. Mittl[1], Annemarie Honegger[1] and Andreas Plückthun[1]

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The demand for binding proteins which recognize specific targets and epitopes is increasing in both research and diagnostics. Although they are widely used, classical binding proteins like monoclonal antibodies are facing validation problems and are rather expensive to produce. Novel binding scaffolds have the potential to overcome these problems. In our group we developed Designed Ankyrin Repeat Proteins (DARPins) which are highly stable and can be selected in a high-throughput manner to bind almost any target molecule [1].

Over the past years we have selected DARPins against more than 400 different target proteins, but the crystallographic validation of these complexes is a major bottleneck. Currently, only 40 DARPin complex structures are deposited in the PDB, and despite their favorable crystallization properties, each complex is still a significant project. We state that this is also due to the small size of DARPins, which leaves only a small number of potential crystallization contacts. Here we present a new approach which links two DARPins via a rigid helix fusion to increase the size of our target binding DARPins and provide more possibilities for forming crystal contacts [2]. These fusions span a wide range of possible geometries and have been successful in obtaining new crystal structures of complexes which previously failed to crystallize.

Furthermore, these fusions cannot only be made between two DARPins but also to other helical proteins. I will in the last part expand the concept of rigid helical fusions to designed Armadillo Repeat Proteins (dArmRPs), which are modular peptide binders. I will show examples highlighting the huge influence of crystal contacts on peptide binding and explain how we want to solve this problem. By constructing a DARPin-dArmRP-DARPin fusion we were able to obtain the first structure showing full modularity with peptide binding not being influenced by crystal contacts.

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Structural basis of cell wall peptidoglycan amidation by the GatD/MurT complex of *Staphylococcus aureus*

Erik R. Nöldeke[1], Lena M. Muckenfuss[1,4], Volker Niemann[1,5], Anna Müller[2], Elena Störk[1], Georg Zocher[1], Tanja Schneider[2] & Thilo Stehle[1,3]

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The peptidoglycan of *Staphylococcus aureus* is highly amidated. Amidation of α-D-isoglutamic acid in position 2 of the stem peptide plays a decisive role in the polymerization of cell wall building blocks. *S. aureus* mutants with a reduced degree of amidation are less viable and show increased susceptibility to methicillin, indicating that targeting the amidation reaction could be a useful strategy to combat this pathogen. The enzyme complex that catalyzes the formation of α-D-isoglutamine in the Lipid II stem peptide was identified recently and shown to consist of two subunits, the glutamine amidotransferase- like protein GatD and the Mur ligase homolog MurT. We have solved the crystal structure of the GatD/MurT complex at high resolution, revealing an open, boomerang-shaped conformation in which GatD is docked onto one end of MurT. Putative active site residues cluster at the interface between GatD and MurT and are contributed by both proteins, thus explaining the requirement for the assembled complex to carry out the reaction. Site-directed mutagenesis experiments confirm the validity of the observed interactions. Small-angle X-ray scattering data show that the complex has a similar conformation in solution, although some movement at domain interfaces can occur, allowing the two proteins to approach each other during catalysis. Several other Gram-positive pathogens, including *Streptococcus pneumoniae, Clostridium perfringens* and *Mycobacterium tuberculosis* have homologous enzyme complexes. Combined with established biochemical assays, the structure of the GatD/MurT complex provides a solid basis for inhibitor screening in *S. aureus* and other pathogens.

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Structural basis of GFAT-1 feedback inhibition and gain-of-function mechanism

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Glutamine fructose-6-phosphate aminotransferase-1 (GFAT-1) catalyzes the first and rate-limiting step of the hexosamine biosynthesis pathway [1]. GFAT is organized into two domains, the glutaminase domain responsible for hydrolysis of L-Gln into L-Glu and isomerase/transferase domain, which catalyzes the isomerization of Frc6P to Glc6P as well as the transfer of ammonia to Glc6P to build GlcN6P [2]. The activity of eukaryotic GFAT is regulated by the pathway's end product uridine 5'-diphospho-N-acetyl-D-glucosamine (UDP-GlcNAc) [3]. Although UDP-GlcNAc binds to the isomerase domain, the glutaminase domain is inhibited [4], but the inhibitory mechanism remained unclear. Eight gain-of-function mutations, all single amino acid substitutions, in the *gfat−1* gene of *Caenorhabditis elegans* were previously identified in unbiased genetic screens. These mutations lead to increased hexosamine pathway flux, resulting in improved protein quality control and consequently in reduced toxic protein aggregation and lifespan extension in the worm [5]. This project aims to decipher the underlying mechanism of the gain-of-function mutations and to understand GFAT-1 regulation by UDP-GlcNAc. Kinetic studies indicate that at least one gain-of-function mutant (G451E) does not react to UDP-GlcNAc feedback inhibition. Recently, we solved the first eukaryotic full-length structure of human wild type GFAT-1. Currently, the structural and functional consequences of UDP-GlcNAc binding and gain-of-function mutations are under investigation.

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Screening Protein Stability with Prometheus – before you run other costly methods

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Prometheus uses an advanced Differential Scanning Fluorimetry (nanoDSF) technology. It detects smallest changes in the fluorescence of tryptophan or tyrosine present in virtually all proteins. The optical readout is used to measure unfolding profiles and aggregation of proteins. The technique is truly label-free and works under any buffer condition. Unfolding and aggregation can be induced by a temperature ramp or chemical denaturants.

Facts on nanoDSF:

- Only quantities in the range of nanograms of protein are required.
- Protein concentration can vary from 350 mg/ml down to 5 µg/ml.
- The method is highly reproducible with a relative standard deviation (RSD) of 0.07% between melting temperatures (T_m) of 48 equivalent samples.
- 48 different conditions can be screened in less than 30 minutes.

Typical application examples are:

- Improve sample homogeneity prior to structural studies
- Screen for best protein condition for Cryo-EM
- Check for proper folding in highly concentrated samples
- Label-free Thermal Shift Assays to identify binders
- Ensure quality of purified proteins
- Find optimal conditions for enzyme assays
- Detergent screen for membrane proteins

New biophysical approaches to study IRES/ribosome interactions

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Translation initiation, in both eukaryotes and bacteria, requires essential elements such as mRNA, ribosome, initiator tRNA and finally initiation factors. For each domain of life, canonical mechanisms and signals are observed to initiate protein synthesis. However, in some cases other ways of initiation can be used, as for viral mRNAs. Viruses hijack cellular machinery to translate some of their mRNAs through a non-canonical initiation pathway using Internal Ribosome Entry Site (IRES), a highly structured RNAs which can directly recruit the ribosome.

Here we took advantage of innovative biophysical approaches to study interactions between the intergenic IRES from the cricket paralysis virus (CrPV) and the eukaryotic yeast ribosome. Isothermal Titration Calorimetry (ITC) and kinetic ITC (kinITC) provided thermodynamic and kinetic data [1]. A comparison is made with data collected on a biosensor using the new switchSENSE technology, based on electroswitchable DNA chips [2].

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How RNA-guided endonucleases cut specific regions of the Genome?

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Since ancestral time mankind has tried to modify plants and animals to obtain an offspring that could adapt to our needs. In this way farmers have selected more efficient animals to produce more meat, more resistant plants, faster and resistant horses or cows that produce a larger amount of milk. Our ability to modify organisms has changed dramatically with the introduction of genome editing, til a point where we can start to redesign an organism. Thanks to the discovery of new genome modifying enzymes we can perform a safer and faster manipulation to edit a genome. I will address the molecular mechanism that control specificity and cleavage of one of these tools, Cpf1, a single RNA-guided endonuclease of class 2 type V CRISPR-Cas system, which is emerging as a powerful genome editing tool. To provide insight into its DNA targeting mechanism, we have determined the crystal structure of Francisella novicida Cpf1(FnCpf1) in complex with the triple strand R-loop formed after target DNA cleavage. The structure reveals a unique machinery for target DNA unwinding to form a crRNA-DNA hybrid and a displaced DNA strand inside FnCpf1. Our study reveals a singular working model of RNA-guided DNA cleavage by Cpf1, opening up new avenues for engineering this genome modification system.

Single crystal timelapse measurement using ultrasonic acoustic levitation

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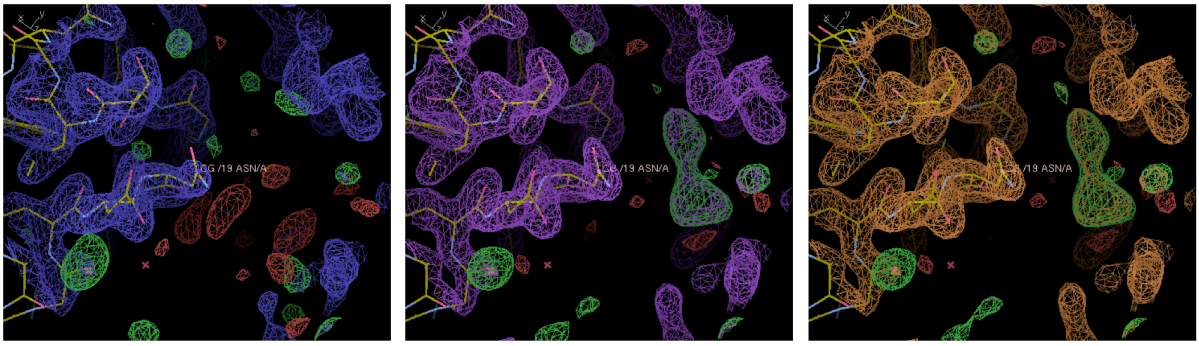
We are developing the acoustic levitation diffractometer, a new contact-free diffractometer at the Paul Scherrer Institut [1]. By rotating single crystals in an acoustically levitated droplet, data collection can be completed within a few hundred milliseconds or shorter at room temperature. Here we report first examples of timelapse measurements of ligand soaking using a single crystal. This was achieved by collecting a series of datasets after soaking ligand solution into single lysozyme crystals in a levitated droplet in the acoustic cavity of the diffractometer. Electron density maps of the lysozyme crystals obtained every 30 seconds after the ligand soaking showed meaningful conformational changes around the binding site of the ligand. Details of the diffractometer and the diffraction experiments, possible applications with the methods will be presented.



(a) (b) (c)

FIGURE. parts of 2Fo - Fc electron density maps of a lysozyme crystal (a) before ligand soaking (b) after 30 and (c) 120 seconds of ligand soaking. The sigma level of electron density of the ligand (in green) seems to be the same as (b) and (c). The electron density of the side chain, ASN 19 (at the centre) was not clear before the ligand soaking. After 30 second of ligand soaking, the density is distinctly visible potentially due to the formation of hydrogen bonds between the side chain and the ligand.

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DIY - How an EIGER detector turns a TEM into an electron diffractometer

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As part of the nanoArgovia project A3EDPI, Applicability of 3D electron diffraction for the Pharmaceutical Industry, we mounted an EIGER X 1M detector (DECTRIS Ltd.) onto a transmission electron microscope. This turned the imaging instrument into an electron diffractometer. The installation took only half a day, plus an overnight pumping to bring up the system vacuum. We could solve the structure of a new methylene blue derivative in a couple of hours. This makes our electron diffractometers as efficient and as affordable as an inhouse X-ray diffractometer. We also extracted the structure of paracetamol from a pill that we bought in the pharmacy next door (Fig. 1): Electrons turn microcrystalline powder into ``huge'' single crystals and open structure determination from crystals much less than 1 micrometer thickness. Currently, TEMs are not optimised for diffraction, and seemingly simple parameters like oscillation width and the direction of the rotation axis are not directly accessible. Until manufacturers properly integrate hybrid pixel detectors, workarounds are necessary to achieve the efficiency that we reached with our ``prototype electron diffractometer''. I will present the do-it-yourself solutions for thos who are too impatient to wait for the market to catch up with state of the art crystallography.

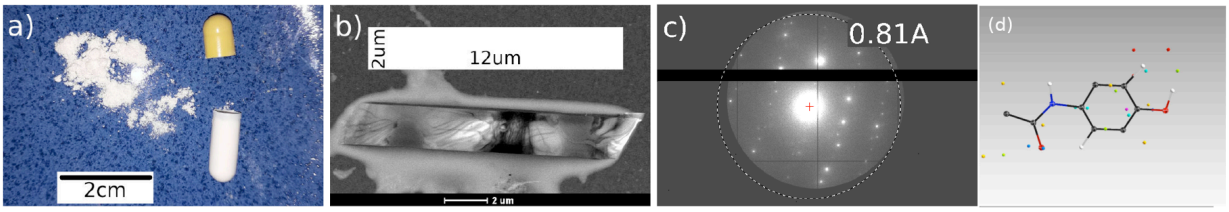

Figure 1: Single Crystal Structure from the powder of the Pill of Grippostad.

DECTRIS Detectors

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Advances in automated data analysis and processing within autoPROC, combined with improved characterisation, mitigation and visualisation of the anisotropy of diffraction limits using STARANISO

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Current trends towards ever higher levels of automation require not only improvements in the efficiency and speed of data processing programs and pipelines, but also new and improved approaches to data analysis and to the visual presentation of its results. This is not just a matter of producing ever more "pretty pictures", but of helping automated processes - as well as ordinary users - make use of the most appropriate quality metrics in evaluating experimental protocols and in designing optimal experiments for given instrument capabilities and crystal characteristics. In the context of serial crystallography this provides improved diagnostics and criteria for guiding the handling of individual datasets and producing optimal groupings among them.

Well designed visualisation tools are essential in enabling the rapid assessment of the characteristics and quality of conventional (single crystal/sweep) datasets, as well as of combinations of partial datasets originating from multi-sweep and/or multi-crystal datasets and/or serial experiments. The proper perception of the relationships between partial datasets requires a full 3D visualisation of local geometric properties such as redundancy and of statistical properties such as local averages of I/sig(I). To that end we have developed the STARANISO program [1] for analysing and mitigating anisotropy in diffraction data. This capability is available both via autoPROC [2] (our package for automated data processing and analysis) and the public STARANISO webserver [3] (processing, analysis and visualisation of user-provided datasets, either as merged or unmerged intensity data). A recent additional capability has been provided by the "PDBpeep" server, allowing, with extreme simplicity, the analysis and 3D visual examination of diffraction datasets deposited with PDB entries [4].

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New tools for cryo-EM density interpretation

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Improvements in detector technology and image processing algorithms have rendered single-particle cryo-EM capable of routinely delivering structures of macromolecular assemblies at near-atomic resolution, permitting building and refinement of atomic models. Yet, the interpretation of cryo-EM density maps often remains challenging due to specific properties such as inherent contrast loss and spatial variation in map resolution. Variations in map resolution, if combined with global contrast restoration procedures, may result in density maps with locally inappropriate sharpening levels that are prone to hamper or even misguide atomic model building. I will illustrate pitfalls in atomic model building resulting from map sharpening artifacts and outline practical issues affecting optimal sharpening results. I will present new tools that facilitate more faithful restoration of density contrast along with procedures that permit the assignment of significance to structural features in cryo-EM density maps. The presented procedures should help overcome common difficulties in map interpretation using high- resolution cryo-EM density maps.

Aminoglycoside interactions and impacts on the eukaryotic ribosome

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Aminoglycosides are chemically diverse, broad-spectrum antibiotics that target functional centers within the bacterial ribosome to impact all four principle stages (initiation, elongation, termination, and recycling) of the translation mechanism. The propensity of aminoglycosides to induce miscoding errors that suppress the termination of protein synthesis supports their potential as therapeutic interventions in human diseases associated with premature termination codons (PTCs). However, the sites of interaction of aminoglycosides with the eukaryotic ribosome and their modes of action in eukaryotic translation remain largely unexplored. Here, we use the combination of X-ray crystallography and single-molecule FRET analysis to reveal the interactions of distinct classes of aminoglycosides with the 80S eukaryotic ribosome. Crystal structures of the 80S ribosome in complex with paromomycin, geneticin (G418), gentamicin, and TC007, solved at 3.3- to 3.7-Å resolution, reveal multiple aminoglycoside-binding sites within the large and small subunits, wherein the 6'-hydroxyl substituent in ring I serves as a key determinant of binding to the canonical eukaryotic ribosomal decoding center. Multivalent binding interactions with the human ribosome are also evidenced through their capacity to affect large-scale conformational dynamics within the pretranslocation complex that contribute to multiple aspects of the translation mechanism. The distinct impacts of the aminoglycosides examined suggest that their chemical composition and distinct modes of interaction with the ribosome influence PTC read-through efficiency. These findings provide structural and functional insights into aminoglycoside-induced impacts on the eukaryotic ribosome and implicate pleiotropic mechanisms of action beyond decoding.

Heterogeneity in cryo-EM datasets and variance analysis

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The recent advances in cryo-electron microscopy and more particularly in single particle analysis have made it possible to routinely determine structures of large biological macromolecules to near atomic resolution. For many samples, structural heterogeneity still limits the achievable overall and local resolutions, resulting in a “blurring” of the reconstruction around the variable regions. The most commonly used method to deal with this problem combines maximum likelihood statistics with masks around the homogeneous or heterogeneous areas [1]. Here, we show the importance of mask design exemplified by a recent work from our group [2] and present a new program, currently in development, to help with this task.

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Building, refinement and validation of atomic models derived from cryo-EM maps

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Due to the recent technological advances in cryo-electron microscopy, an increasing number of EM structures are resolved at atomic or near-atomic resolution. For interpretation of the calculated EM-maps at atomic detail it is possible to use model building and refinement tools originally established for X-ray crystallography. Nevertheless, the unique characteristics of EM maps, such as varying local resolution and altered map features for amino acids and nucleotides, pose specific challenges for model building. Using recently determined ribosomal structures in our group as an example [1,2] practical aspects of EM model building, refinement and validation will be presented.

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The molecular architecture of a volume-regulated anion channel of the LRRC8 family

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Membrane proteins are important drug targets and their high-resolution structures are essential for the development of therapeutics. However, poor expression yields and high instability of these proteins together with the tendency to aggregate limit the availability of structural information and hence hinder our understanding of their molecular mechanisms. Recent advances in cryo-EM provide new opportunities to study these challenging proteins and to determine their architectures at atomic details.

The volume-regulated anion channel (VRAC) primarily regulates an increase in the cell volume upon exposure to extracellular hypotonic stress. As part of the volume regulatory decrease, VRAC facilitates the efflux of anions and small organic osmolytes such as amino acids and taurine. Apart from volume regulation, VRAC is also thought to be involved in apoptotic volume decrease and the uptake of platinum-based anti-cancer drugs. These channels are composed of members of the leucine-rich repeat-containing protein 8 (LRRC8) family. In vertebrates, this family comprises five paralogs (A-E), which co-assemble into hexameric complexes with various stoichiometries. While LRRC8A was shown to be indispensable for channel activity and localization to the plasma membrane - the ion selectivity, conductance and inactivation kinetics depend on the composition of the channel subunits.

Here, we present the first high-resolution cryo-EM structure of a homomeric channel of the obligatory subunit LRRC8A [1]. The protein modular architecture comprises a transmembrane domain followed by a highly flexible cytoplasmic leucine-rich repeat domain. We first performed extensive 3D classification to identify a homogeneous subset of the population, which resulted in a reconstruction of the full-length protein at 4.25 Å. We subsequently applied focused refinement to *in silico* modified particles to determine the transmembrane pore structure at 3.66 Å. As the poorer quality of the density of the cytoplasmic entity prevented us from an unambiguous interpretation, we crystallized the isolated monomeric domain. Our cryo-EM reconstructions combined with the 1.8 Å crystal structure of the cytoplasmic domain reveal the detailed architecture of the channel and elucidate the molecular basis of selective anion conductance.

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Unique features of mammalian mitochondrial translation initiation revealed by cryo-EM

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Mitochondria maintain their own specialized protein synthesis machinery, which in mammals is used exclusively for the synthesis of the membrane proteins responsible for oxidative phosphorylation. The initiation of protein synthesis in mitochondria differs substantially from bacterial or cytosolic translation systems. Mitochondrial translation initiation lacks initiation factor 1, which is essential in all other translation systems from bacteria to mammals. Furthermore, only one type of methionyl transfer RNA (tRNAMet) is used for both initiation and elongation, necessitating that the initiation factor specifically recognizes the formylated version of tRNAMet (fMet-tRNAMet). Lastly, most mitochondrial mRNAs do not possess 5' leader sequences to promote mRNA binding to the ribosome. There is currently little mechanistic insight into mammalian mitochondrial translation initiation, and it is not clear how mRNA engagement, initiator-tRNA recruitment and start-codon selection occur. Here we determine the cryo-EM structure of the complete translation initiation complex from mammalian mitochondria at 3.2 Å. We describe the function of an additional domain insertion that is present in the mammalian mitochondrial initiation factor 2 (mtIF2). By closing the decoding centre, this insertion stabilizes the binding of leaderless mRNAs and induces conformational changes in the rRNA nucleotides involved in decoding. We identify unique features of mtIF2 that are required for specific recognition of fMet-tRNAMet and regulation of its GTPase activity. Finally, we observe that the ribosomal tunnel in the initiating ribosome is blocked by insertion of the N-terminal portion of mitochondrial protein mL45, which becomes exposed as the ribosome switches to elongation mode and may have an additional role in targeting of mitochondrial ribosomes to the protein-conducting pore in the inner mitochondrial membrane.

Mechanism of diguanylate cyclase activation by phosphorylation.

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Diguanylate cyclases (DGCs) synthesize the bacterial second messenger c-di-GMP. Thereby, two substrates complexed GGDEF domains have to align antiparallelly, resulting in a symmetrical cyclic product. DGCs almost invariably contain accessory domains to control activity in a signal dependent manner. Conspicuously, typical accessory domains show an amphipathic helix that connects to the catalytical GGDEF domain. It was proposed that, in the context of dimeric DGCs, this helix forms a coiled-coil that is crucial for linking signal induced changes in the input domain to the arrangement and dynamics of the catalytic GGDEF domains, ultimately promoting the formation of a catalytically competent GGDEF dimer. Although there is a vast number of GGDEF domain structures available, only few full-length DGC structures have been determined and their activation mechanism unraveled. Here, we work on Lpd1, a Rec-GGDEF from *Leptospira interrogans*. Biophysical data provide strong evidence that Lpd1 is a constitutive dimer and is activated *in vitro* by beryllium fluoride modification that mimics phosphorylation. Crystal structures of Lpd1 in the native and in the activated state have been obtained. Both structures are dimeric with, as anticipated, the C-terminal α5 helix forming a dimeric coiled-coil. Comparison of the structures shows that activation induces a 16 degrees rotation of the α3 to β5 part of the Rec domain with respect to the rest, leading to structural differences mainly located in the dimer interface. Strikingly, the α5 coiled-coil exhibits a distinct register in the two states probably allowing symmetric and productive encounter of the GGDEF domains only in the activated state. In summary, we have started to elucidate the mechanism of phosphorylation induced activation of a prototypic and simple DGC.

Binding stoichiometry of HSP47 to collagen depends on the collagen sequence

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HSP47 – also known as SERPINH1 – is of outmost importance for the proper folding of collagen molecules and mutations in HSP47 can lead to defective collagen secretion and *Osteogenesis imperfecta* [1]. On the other side in cases of excessive collagen secretion (e.g. fibrotic diseases) HSP47 was always found to be upregulated [2]. HSP47 was previously reported to recognise a specific motif on collagen helices (Gx[S/T]GxRGxx). Structural analysis of the collagen-HSP47 complex showed the importance of Asp_{385} which forms a salt bridge with the arginine on the collagen chain [3]. We identified an previously unknown high affinity binding site for HSP47 which introduces an additional phenylalanine in the binding motif (Gx[S/T]GxRG**F**x) [unpublished]; however, the exact mode of binding to this altered binding site was unknown. Binding assays showed an increased affinity for peptides having a GxRG**F** motif, and slight improvement for GxRG**L** motifs. Interestingly, modelling phenylalanine in our existing crystals structure lead to server steric clashes. Therefore, we successfully crystallised HSP47 in complex with synthetic, homotrimeric collagen peptides containing RGF and RGL sequences. Interestingly, both complexes crystallised in different crystals forms but as a 1:1 complex (collagen triple helix:HSP47), contrasting to our earlier structures showing a 1:2 complex. Preliminary analysis of the structures showed that HSP47 undergoes some minor rearrangements to form a pocket for the hydrophobic phenylalanine residue. The increased affinity most likely results from a gain of water entropy upon shielding of the aromatic residues. Interestingly, due to the staggered nature of the collagen helix, the bulky phenylalanine only allows a single HSP47 to bind. At the second binding site the phenylalanine residue is positioned differently and cannot be accommodated in the above-mentioned pocket. This demonstrates that although HSP47 is in principle able to bind on two sides, the actual stoichiometry greatly depends on the collagen sequence.

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Structural characterization of neutralizing, cross-reactive antibodies against JC and BK Polyomavirus

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The human JC Polyomavirus (JCPyV) causes the demyelinating disease Progressive Multifocal Leukoencephalopathy (PML) in immunocompromised individuals. PML is a mostly fatal, opportunistic disease for which currently no effective treatment is available. Mutations at specific locations in the JCPyV capsid protein frequently accompany infections, and these mutations are thought to help the virus escape the host immune response. A recently isolated panel of monoclonal antibodies binds with high affinity to wild-type and mutant JCPyV VP1 pentamers. Many of these antibodies neutralize JCPyV, and some display cross-reactivity with the closely related BK polyomavirus (BKPyV). These properties make them promising candidates for therapy.

For structural studies and the investigation of the binding properties, our goal was to generate functional single chain variable fragments (scFvs) from an antibody that can bind JCPyV and also cross-react with BKPyV. Functional scFvs were designed, cloned, expressed and purified to enable structural analyses. The scFv was then crystallized in complex with both JCPyV VP1 and BKPyV VP1 pentamers, and the structures were solved using X-ray crystallography at 3.1 Å and 2.6 Å, respectively. Previously solved structures of Fab-VP1 pentamer complexes all revealed binding epitopes near the carbohydrate receptor binding site at the top of the pentamer, in regions that differ between JCPyV and BKPyV. The newly-determined structures show that the scFv engages an epitope on the side of the pentamer that is largely conserved between JCPyV and BKPyV. The structures therefore explain the recognition of VP1 variants as well as the cross-reactivity with BKPyV.

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Structural characterization of a new glycosyltransferase from Methicillin-resistant *Staphylococcus aureus*

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Staphylococcus aureus is a Gram-positive bacterium representing a leading cause of bacterial infections in humans and cause a variety of severe diseases. Methicillin-resistant *S. aureus* strains (MRSAs) show multiple drug resistance and have spread through hospitals and communities[1]. One key bacterial factor for such infections is wall teichoic acid (WTA), a poly-ribitol-phosphate (RboP) surface polymer modified with N-acetylglucosamine (GlcNAc) at the 4-position with -configuration by the standard enzyme TarS or with an α-configuration by TarM. The presence of β-O-GlcNAc on WTA is essential for full-level β-lactam resistance in MRSA strains[2].

We demonstrate that a considerable proportion of the healthcare-associated (HA) and livestock-associated (LA) MRSA clones CC5 and CC398 contain prophages that encode an alternative WTA glycosyltransferase. This enzyme, named TarP, transfers GlcNAc to a different hydroxyl group of the WTA RboP than the standard enzyme TarS, with major consequences for immune recognition. We verified that GlcNAc in the product of TarP-catalyzed reaction is at 3-position with Nuclear magnetic resonance (NMR) and solved the TarP structure with high resolution. WTA polymers composed of three or six RboP repeating units (3RboP or 6RboP-$(CH_2)_6NH_2$, respectively) were synthesized and used for soaking TarP crystals, yielding the first protein structure visualizing the binding of a WTA-based polymer. The ternary structure of TarP-UDP-GlcNAc-3RboP allows for a prediction of how polyRboP binds to the homologous TarS enzyme and explained why TarS glycosylates at C4 position. Our structural characterization of TarP will instruct the development of specific TarP inhibitors that could become important in combination with anti-WTA vaccines or antibiotic therapies.

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Protein engineering of the fungal prenyltransferase TyrPT

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Prenylations are important reactions in the secondary metabolism of diverse organisms as they contribute to the chemical diversity of natural compounds [1]. In fungi and bacteria this reaction is catalyzed by ABBA prenyltransferases. They belong to a subclass of the aromatic prenyltransferases and utilize diverse aromatic acceptors and numerous isoprenyl diphosphate donors for catalysis in a Friedel-Crafts alkylation [2]. Depending on the prenyltransferase the electrophilic aromatic substitution on the aromatic ring proceeds with a different regioselectivity. The diversity of the possible products render ABBA-prenyltransferases interesting for biotechnology. In order to manipulate them in a way that will yield to the desired reaction a profound understanding of the catalytic mechanism is necessary. A structural characterization of different ABBA- prenyltransferases is therefore beneficial.

Here we report on the crystal structures of the O-prenylating tyrosine prenyltransferase TyrPT at a resolution of 1.35 Å. The structure was solved by experimental SAD phasing. TyrPT possess a typical ABBA-fold with differences in the conformation of the aromatic acceptor binding loops. Unexpectedly, the protein binds tightly to its natural substrate L-tyr already upon purification, which causes problems obtaining a ternary complex structure. The architecture of the active center and the catalytic mechanism of other known prenyltransferases allowed us to propose a reaction mechanism. On the basis of our structural data, we designed variants to redirect the regioselectivity.

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Structure-based protein engineering of a bacterial prenyltransferase

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Plants, bacteria and fungi produce a large diversity of secondary metabolites for their defence, communication and adaptation to the environment. Prenylated indole derivatives are a subgroup of these metabolites and usually show enhanced biological and pharmacological activity compared to non-prenylated variants [1]. The transfer of diverse prenyl moieties (n x C_5) onto the indole ring system is mediated by ABBA-barrel fold prenyltranferases in a Friedel-Craft alkylation. The DMATS (dimethylallyl tryptophane synthase) family uses mainly DMAPP (dimethylallyl pyrophosphate) for prenylation. A particular member of this family is the bacterial prenyltranferase 6-DMATS from *Micromonospora olivasterospora*, which prenylates selectively at the C6 position of L-Trp [2].

Here, we present the native and ligand bound structure of the 6-DMATS. Based on the structural information, we introduced mutants to identify residues crucial to the reaction mechanism. Additional variants were designed to achieve a changed regioselectivity, as well as to enhance the acceptance of a lager prenyl moiety. We successfully switched the substrate preference from DMAPP to the larger GPP (C10) and redirected the prenylation from C6 to C5 of the indole ring system.

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Structural insights into the allosteric control of GPCR activity

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Chemokines and their G protein-coupled receptors play a diverse role in immune defence by controlling the migration, activation and survival of immune cells1. They are also involved in viral entry, tumour growth and metastasis and hence are important drug targets in a wide range of diseases2,3. Despite very significant efforts by the pharmaceutical industry to develop drugs, with over 50 small molecule drugs directed at the family entering clinical development, only two compounds have reached the market – maraviroc (CCR5) for HIV infection and plerixafor (CXCR4) for stem cell mobilisation4. The high failure rate may in part be due to limited understanding of the mechanism of action of chemokine antagonists and an inability to optimise compounds in the absence of structural information5. CCR9 activation by CCL25 plays a key role in leucocyte recruitment to the gut and represents a therapeutic target in inflammatory bowel disease6. The selective CCR9 antagonist vercirnon progressed to Phase 3 clinical trials in Crohn's disease but efficacy was limited, due in part to poor drug-like properties and the need for very high doses to block receptor activation6. Here we present the crystal structure of the CCR9 receptor in complex with vercirnon at 2.8 Å resolution. Remarkably, vercirnon binds to the intracellular side of the receptor, exerting allosteric antagonism and preventing G protein coupling. This binding site explains the need for relatively lipophilic ligands and describes another example of an allosteric site on GPCRs that can be targeted for drug design, not only at CCR9, but potentially extending to other chemokine and class A receptors. In light of other recent structures of GPCRs with small molecules bound to intracellular and extra-helical allosteric sites, our view of allosteric control of GPCRs is rapidly evolving.

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X-ray Crystallography-based Fragment Screen targeting Tubulin

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Microtubules (MT) are major components of the cytoskeleton of cells and are involved in various fundamental tasks like cell division, cell motility, and intracellular transport. In the course of these processes, MTs undergo periods of growth and shrinkage. This equilibrium is called dynamic instability and is due to the property of the MT-building block tubulin, which can reversely polymerize by switching from a curved conformation in solution to a straight conformation in MTs. This polymerization is naturally regulated by a large number of proteins and can also be influenced by drugs. Anticancer drugs, such as the taxanes and vinca alcaloids, suppress MT dynamicity thereby disrupting cell division of the highly proliferating tumor cells.

So far, six different drug-binding sites have been identified on tubulin. While the structures as well as the modes of action between the drugs targeting these sites vary, they share one common trait: they are large and complex molecules. Hence, they are difficult and expensive to synthesize as well as not orally bioavailable, thereby lowering patient comfort. To overcome this limitation, new starting points for drug development have to be found. X-ray crystallography-based fragment based screening represents an optimal first line screening approach to find hits of low molecular weight and to further explore smaller, yet unidentified binding pockets on tubulin.

Here we present our findings from our fragment screening campaign targeting tubulin using our well established crystal system. We were able to identify 59 fragments binding to four known as well as additional six newly identified binding sites. Overall, we found five common binding motifs targeting different pockets showing promising selectivity. Taken together, we now have small molecular starting points to develop either new MT targeting agents or biochemical tools for the better understanding of MT dynamics.

The binding mode of the natural compound ilicicolin H to the Q_i site of the mitochondrial cytochrome bc_1 complex

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The mitochondrial cytochrome bc_1 complex (cyt bc_1) is essential for oxidative phosphorylation, the most efficient energy conversion machinery of eukaryotic organisms. The development of cyt bc_1 inhibitors is of interest for clinical and agricultural applications. Cyt bc_1 catalyzes ubiquinol-dependent cytochrome *c* reduction via the Q cycle mechanism. It has two catalytic centers, the Q_o site at which ubiquinol is oxidized, and the Q_i site at which ubiquinone is reduced. During the enzymatic forward reaction, electrons are transferred from the Q_o to the Q_i site. Blocking either Q_o or Q_i site inhibits the enzyme activity efficiently and cyt bc_1 loses its function in energy conversion. Q_o site inhibitors are widely applied as therapeutics and agrochemicals and are well characterized. For instance, our structural analysis of atovaquone-inhibited cyt bc_1 revealed the molecular basis of antimalarial drug action of atovaquone, which is in clinical use [1]. In contrast, Q_i site inhibitors are less well-known, but are currently attracting the attention of developers as there is an urgent need in combating resistance against Q_o site inhibitors. We determined the X-ray structure of cyt bc_1 from *Saccharomyces cerevisiae* with the bound Q_i site inhibitor ilicicolin H at high resolution. The structural analysis provided a detailed characterization of the binding mode of the inhibitor. Comparison with a high-resolution structure of the same complex with the Q_i site inhibitor antimycin A highlighted the unique features of the ilicicolin binding mode. A comprehensive sequence analysis of cytochrome *b*, the subunit harboring the catalytic sites of cyt bc_1, suggests that Q_i-site inhibitors have a high tendency for species-specific inhibitory action.

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Structural basis for allosteric ligand recognition in the human CC chemokine receptor 7

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A coordinated immune response in humans is orchestrated through the chemokine system with its 20 G protein-coupled receptors (GPCRs) and over 40 chemokines. The homeostatic C-C motif chemokine ligand 19 and 21 (CCL19 and CCL21) are the sole ligands of the CC chemokine receptor 7 (CCR7) which guides B cells, T cells and antigen-presenting dendritic cells to lymph nodes throughout the body [1][2]. In cancer, CCR7-mediated trafficking leads to lymph node metastasis suggesting the receptor as promising therapeutic target. Here we present the crystal structure of human CCR7 fused to the insertion protein Sialidase NanA using data up to 2.1 Å resolution. The structure shows the ligand Cmp2105 from the chemically diverse thiadiazole-dioxide series bound to an intracellular allosteric binding pocket similar as described for binding of Cmpd-15PA to the β2-adrenergic receptor [3], binding of CCR2-RA-[R] to CCR2 [4] and binding of Vercirnon to CCR9 [5]. The intracellular allosteric antagonist spatially overlaps with the intracellular effector binding site, thus locks the receptor in an inactive conformation. A sulfonamide group characteristic for various chemokine receptor ligands binds a patch of conserved residues in the Gi protein binding regions between transmembrane helix 7 (TM7) and helix 8 (H8). The TM7-H8 motif in the allosteric pocket provides a particularly interesting pharmacological hotspot that can be targeted with a large chemical variety of small molecules to prevent binding of intracellular effector proteins. Interestingly, we found a stabilizing compound in Navarixin which has been described as a potent and bioavailable antagonist for CXCR1/CXCR2 [6]. Navarixin is currently tested in phase II clinical trials (clinicaltrials.gov) for its anti-metastatic effect on colorectal and other aggressive cancers [7][8]. Molecular docking into the CCR7 structure confirms similar interactions to TM7-H8 motif as Cmp2105 in CCR7. Our results therefore could indicate that part of the Navarixin anticancer effects might be due to Navarixin silencing CCR7 instead of acting solely via CXCR1/CXCR2.

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X-ray Free Electron Laser: Opportunities for drug discovery

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Past decades have shown the impact of structural information derived from complexes of drug candidates with their protein targets to facilitate the discovery of safe and effective medicines. However, membrane protein drug targets like ion-channels, transporters and GPCRs still represent a significant challenge. Recent developments in single particle cryo-electron microscopy have significantly improved the options to derive structural information for ion-channels and complexes of GPCRs with G protein. LeadXpro is a structure based lead discovery company focusing on challenging membrane protein drug targets, including G-protein coupled receptors (GPCRs), ion channels and transporters.

Advances in serial crystallography are a pre-requisite to use the unique properties of X-ray Free Electron Laser (XFEL) with unmet peak brilliance and beam focus, which allows successful structure determination from smaller and weakly diffracting crystals. Serial crystallography at synchrotron has already been shown to be instrumental for structure determination and here we present an example in which a GPCR structure was solved using such a method. To further capitalize on the XFEL advantage which allows the capturing of dynamic processes of drug molecule binding and associated conformational changes with great impact to the design of candidate drug compounds, innovations in crystal sample delivery for the X-ray experiment, data collection and processing methods are required and some recent developments will be shown.

In August 2018, the SwissFEL facility was used for the very first biostructure experiments. We performed successfully the structure determination of a GPCR at the ALVRA beamline using the LCP jet and the brand-new Jungfrau 16M detector.

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Recent Publications

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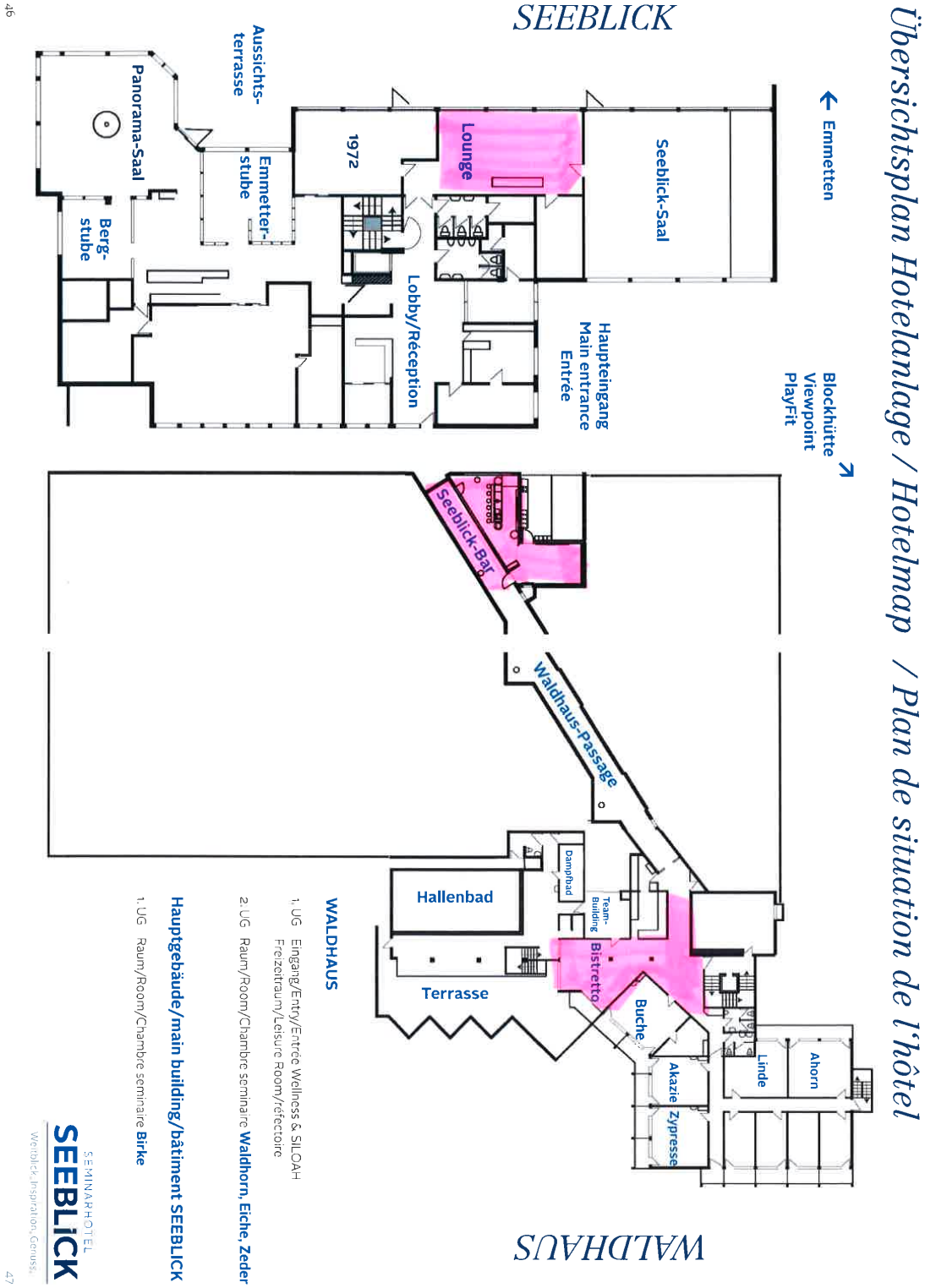
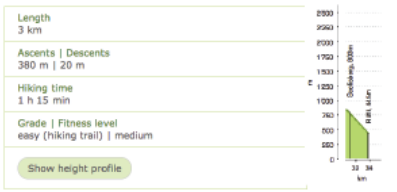


Excursion

Rütli, the heart of Switzerland



13:50 Boarding bus at Hotel Seeblick Emmetten
14:00 Departure to Seelisberg Bergstation
14:25 Funicular: Seelisberg Bergstation - Treib
15:02 Boat: Treib – Brunnen – Rütli
Hikers stop in Rütli
Non-hikers may get off in Brunnen or Rütli
In Brunnen, we recommend the Vitorinox Visitor Centre (https://www.swissknifevalley.ch)
15:21 Hike: Rütli - Seelisberg (1h-1h30)
16:50/17:20 Bus (PostAuto) Seelisberg, Tanzplatz back to Emmetten, Post in ~10 min, then 10 min walk back to Hotel

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History of Rhine-Knee Regional Meetings

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1989 - Schauinsland D - Georg Schulz (University of Freiburg)
1990 - Schwarzenberg CH - Johan Jansonius (University of Basel)
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2016 - Schöntal D - Thilo Stehle (Universität Tübingen)
2017 - Munster F - Claude Sauter, Eric Ennifar (IBMC Strasbourg)
2018 - Emmetten CH - Vincent Olieric (Paul Scherrer Institute)

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32nd Rhine-Knee Regional Meeting on Structural Biology

Day 1: Wednesday, September 26th, 2018

15:00 - 17:30	Registration (Bistretto*)*
17:30 - 17:45	Welcome address (*Waldhorn*) - Vincent Olieric
17:45 - 18:30	**Keynote Lecture - Phil Willmott (Part I), *Paul Scherrer Institut*** (Waldhorn)
18:30 - 20:00	Dinner (*Panorama Saal)*
20:00 - 20:45	**Keynote Lecture - Phil Willmott (Part II), *Paul Scherrer Institut*** (Waldhorn)
	Session 1 – X-ray Facilities / Methods (Waldhorn)
20:45 - 21:00	**Isabelle Martiel**, *Paul Scherrer Institut*
21:00 - 21:15	**Igor Melnikov,** *ESRF*
21:15 - 21:30	**Chia-Ying Huang,** *Paul Scherrer Institut*
21:30 - 21:45	**Nadia Opara,** *University of Basel*
21:45 - 22:00	**Karol Nass,** *Paul Scherrer Institut*
22:00 -	Networking (*Seeblick Bar, then Seeblick Lounge after 00:30)*

Day 2: Thursday, September 27th, 2018

07:00 - 08:15	Breakfast (*Panorama Saal*)
	Session 2 - Biophysical Methods (Waldhorn)
08:15 - 08:30	**Julien Orts,** *ETH Zurich*
08:30 - 08:45	**Tiankun Zhou,** *University of Constance*
08:45 - 09:00	**Patrick Ernst,** *University of Zurich*
09:00 - 09:15	**Erik Noeldeke,** *University of Tuebingen*
09:15 - 09:30	**Sabine Ruegenberg,** *University of Cologne*
09:30 - 09:45	**Tobias Pflueger,** *NanoTemper Technologies GmbH*
09:45 - 10:00	**Eric Ennifar**, *Université de Strasbourg*
10:00 - 10:30	Znüni Break (*Bistretto)*
10:30 - 11:15	**Keynote Lecture - Guillermo Montoya, *University of Copenhagen***
	Session 3 - Diffraction / Methods (Waldhorn)
11:15 - 11:30	**Takashi Tomizaki,** *Paul Scherrer Institut*
11:30 - 11:45	**Tim Gruene,** *Paul Scherrer Institut*
11:45 - 12:00	**Marcus Mueller,** *DECTRIS Ltd.*
12:00 - 12:30	**Clemens Vonrhein,** *Global Phasing Ltd.*
12:30 - 13:50	Lunch box + Group picture
13:50 - 17:30	**Excursion**

18:00 - 19:45	Dinner (*Panorama Saal)*
19:45 - 20:45	**Keynote Lecture - Arjen Jakobi, *Delft University of Technology***
	Session 4 - Larges Complexes (Waldhorn)
20:45 - 21:00	**Muminjon Djumagulov,** *IGBMC Strasbourg*
21:00 - 21:15	**Alain Scaiola,** *ETH Zurich*
21:15 - 21:30	**Marc Leibundgut,** *ETH Zurich*
21:30 - 21:45	**Marta Sawicka**, *University of Zurich*
21:45 - 22:00	**Eva Kummer**, *ETH Zurich*
22:00 -	Networking (*Seeblick Bar, then Seeblick Lounge after 00:30)*

Day 3: Friday, September 28th, 2018

07:00 - 08:30	Breakfast (*Panorama Saal*)
	Session 5 - Enzymes (Waldhorn)
08:30 - 08:45	**Raphael Teixeira,** *University of Basel*
08:45 - 09:00	**Abraham Elena Theres,** *University of Cologne*
09:00 - 09:15	**Christina Harprecht,** *University of Tuebingen*
09:15 - 09:30	**Yinglan Guo,** *University of Tuebingen*
09:30 - 09:45	**Matina-Jasemi Loukeri,** *University of Tuebingen*
09:45 - 10:00	**Elena Störk,** *University of Tuebingen*
10:00 - 10:30	Znüni Break (*Bistretto)*
10:30 - 11:30	**Keynote Lecture - Andy Doré,** *Sosei Heptares*
	Session 6 - Drug Discovery (Waldhorn)
11:30 - 11:45	**Tobias Mühlethaler,** *Paul Scherrer Institut*
11:45 - 12:00	**Kao Wei-Chun,** *University of Freiburg*
12:00 - 12:15	**Steffen Bruenle,** *Paul Scherrer Institut*
12:15 - 12:30	**Robert Chen,** *LeadXpro AG*
12:30 - 12:40	Concluding remarks
12:40 - 14:00	Lunch (Panorama Saal) and Departure