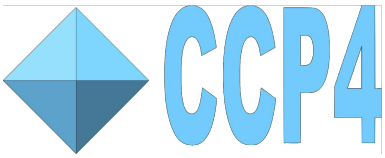
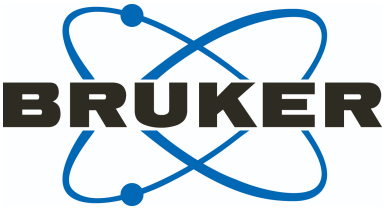
EARLY CAREER DIFFRACTION METHODS SEMINAR 2024





Collecting Data and Solving Structures: the Good, the Bragg, and the Analysis!

A warm welcome from the chairs

Welcome to the Early Career Diffraction Methods Seminar 2024!

We are delighted to be here at the Harnack Haus in Berlin, Germany, with the topic of the meeting "Collecting Data and Solving Structures: the Good, the Bragg, and the Analysis!". Our program is designed to provide a comprehensive overview of the latest advancements and achievements in the early career diffraction methods community. We are excited for a weekend of insightful presentations, engaging posters, and collaborative discussions.

In keeping with the Diffraction Methods Gordon Research Conferences of yesteryear, the free afternoons for networking and discussion remain, with a strong emphasis on being able to talk to everyone. We have a stellar seminar lineup and would encourage you to read the abstracts contained in this booklet. As we delve into these topics, we also encourage you to actively participate, share your perspectives, and seize the opportunity to network with fellow attendees in an open environment free from judgement.

In 2022 the GRC committee unfortunately decided that they would no longer support Gordon Research Conference/Seminar meetings on Diffraction Methods in Structural Biology. Undeterred, the current and prior elected chairs believed that these meetings were too important for the community to lose, and they should continue to happen even without the support of the GRC. It is the collective commitment and enthusiasm of the chairs, local organizers, contributors, and the entire diffraction methods community that has made this event possible, ensuring that we can continue to share knowledge and foster ongoing collaboration. So from all of us, a huge thank you for making this event possible.

We wish you an inspiring and productive seminar!

Warm regards,

Helena Taberman (chair)

Ali Ebrahim (co-chair)

Keynote session

Discussion Leader:
Louise Dunnett (Diamond Light Source, UK)

"I04-1, XChem and Diamond"

Louise is a Beamline Scientist for I04-1 at Diamond Light Source, in her presentation she will discuss the developments and projects that she has been directly involved in since she began working at Diamond following the completion of her PhD in 2018. This includes increases in beamline throughput, research into data collection strategies for the associated XChem Drug Screening platform, upgrade of the detector, implementation of cloud-based data processing and Project Management of an Automated XChem Laboratory (The AXL) as part of the MX Flagship Beamline, K04, for Diamond-II.

Keynote Speaker:
Marcus Fischer (St. Jude Children's Research Hospital, USA)

"Protein Bikram Yoga"

Protein flexibility and hydration are essential for protein function but still underexplored in ligand discovery. To gain a deeper understanding into targetable protein conformational landscapes, we are using a perturbation approach that monitors changes in variables such as temperature and ligands. To facilitate a better understanding of conformational excursions, we have implemented several tools including *FLEXR*, to build multi-conformer models, *Flipper*, to derive conformational barcodes, and most recently *ColdBrew*, to predict water probabilities across temperatures. By tracking changes in protein conformations and water networks we aim to explore new surfaces that drive ligand binding and inform ligand discovery.

Serial and Time-Resolved Crystallography

Discussion Leader:
Yelyzaveta Pulnova (ELI ERIC, Czechia)

Nicolas Caramello (ESRF, France)

"The in crystallo optical spectroscopy toolbox: easy correction and analysis of spectroscopic data recorded in crystals"

The first experiments in time-resolved crystallography (TRMX) were conducted on protein systems activated by visible light. This sparked an interest in optical spectroscopy as a method for biophysical characterization both in crystallo (icOS) and in solution. Since then, it has extended to the study of radiation damage, rhodopsins, metalloproteins and flavoproteins. icOS helps bridge the gap between captured structures and known spectroscopic intermediates. However, a number of optical phenomena can alter the baseline depending on the crystal, its orientation, shape, or position. Fortunately, they can be modelled and corrected, then the quality of a spectrum can be assessed. This communication presents a set of utilities encased in a graphical interface for the easy correction and analysis of spectroscopic data gathered in crystals, aiming to make this sort of analysis more accessible to groups interested in TR-MX and, or coloured proteins.

Anaïs Chretien (XFEL, Germany)

"Time-resolved structural analysis of the BLUF photoreceptor OaPAC"

Reactions of biological macromolecules can be studied by time-resolved crystallography (TRX), as it provides high special and temporal resolution. Naturally light-sensitive signaling proteins such as photoreceptors are ideal target to study fast biochemical processes using pump-probe TRX. The photoreceptor PAC is of interest in this study. PAC contains a Blue-Light sensor Using Flavin (BLUF) coupled to an adenylyl cyclase effector domain, converting ATP into cAMP. Pump-probe TRX using XFELs and synchrotrons in combination with FTIR spectroscopy was performed. The data shows the rotation of Gln48 in the flavin chromophore pocket, which initiates a change in the hydrogen bonds network. Cryo-trapping experiment also enabled to capture a late reaction time-point, where PAC adopts a Trp90-in conformation in the light activated state. The performed experiments help to better understand the signaling process of PAC photoreceptors, which can serve as a basis to design novel optogenetic tools.

Caitlin Hatton (University of Hamburg, Germany)

"Using ultrahigh-resolution and time-resolved crystallography to investigate allostery in enzymes"

Allosteric regulation, a pivotal aspect of enzyme function, involves conformational changes in response to ligand binding at sites distinct from the active site. In the context of FAcD, a homodimeric enzyme with observed half-site reactivity (Mehrabi et al. Science. 2019, Mehrabi et al. J. Am. Chem. Soc. 2019.) Substrate in one active site impacts the conformation and turnover of the second subunit. Our research focuses on investigating intrasubunit allostery within FAcD, specifically examining conformational changes in one subunit and the influence on the other promoter. Using a combinational approach of ultrahigh-resolution structures at P14 EMBL-Hamburg, allowing us to observe alternative states, and time-resolved structures, at TREXX EMBL-Hamburg, we begin to reveal exciting new insights into allosteric regulation and communication in FAcD. A mutation at the dimer interface alters the binding of substrate in the active site, this impact on activity will also be discussed.

Jake Hill (University of Leeds, UK)

"Investigating UV damage in cataract formation with serial crystallography"

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Maria Spiliopoulou (UKE, Germany)

"T4 lysozyme, a model system for ligand binding using integrative temperature-dependent, time-resolved crystallography"

While state of the art approaches in time-resolved X-ray crystallography permit addressing ever shorter-lived reaction intermediates along the reaction coordinate pathway, an unresolved question remains the formation of protein-ligand encounter complexes. However, the latter is fundamentally important to basically any protein ligand interaction and thus almost any process in life. To address this challenging question, we have turned to T4 lysozyme (T4L), as a well-established model system. Interestingly, the T4L L99A mutant generates an internal cavity, capable of binding various ligands. This can be exploited to probe protein-ligand interactions under different environmental conditions. Initially we have mapped out the structural response of T4L-ligand complexes to increasing temperatures by comparing these to ultra-high resolution cryo-data. Currently we are characterizing the time-scales of ligand binding at different temperatures as the foundation for burst-series data-collections.

COMPUTATIONAL ADVANCES IN STRUCTURAL BIOLOGY

Discussion Leader:
Mahmoud Rizk

Virginia Apostolopoulou (CFEL, Germany)

"Mathematically deriving loop mobility for single protein structures"

In recent years, progress in understanding static protein structures has been significant, yet our understanding of protein dynamics remains limited, making it difficult to modify protein behavior effectively. Various tools aim to explore protein dynamics, but describing subtle shifts remains challenging. RoPE, a multi-dataset analysis software, offers a promising solution, using torsion angles to detect subtle conformational changes in biomacromolecules. Integrating RoPE with Kinematic Flexibility Analysis (KFA) allows for the identification of protein loops without multiple structures, crucial for understanding functional dynamics. Our approach predicts dynamic loop movements, helping in the construction of comprehensive conformational space. This knowledge is essential for drug design and understanding protein function.

Maggie Klureza (Harvard University, USA)

"Data processing methods for Hadamard time-resolved X-ray crystallography"

While state of the art approaches in time-resolved X-ray crystallography permit addressing ever shorter-lived reaction intermediates along the reaction coordinate pathway, an unresolved question remains the formation of protein-ligand encounter complexes. However, the latter is fundamentally important to basically any protein ligand interaction and thus almost any process in life. To address this challenging question, we have turned to T4 lysozyme (T4L), as a well-established model system. Interestingly, the T4L L99A mutant generates an internal cavity, capable of binding various ligands. This can be exploited to probe protein-ligand interactions under different environmental conditions. Initially we have mapped out the structural response of T4L-ligand complexes to increasing temperatures by comparing these to ultra-high resolution cryo-data. Currently we are characterizing the time-scales of ligand binding at different temperatures as the foundation for burst-series data-collections.

Katie O'Flynn (DESY, Germany)

"Mapping protein conformational changes using torsion angles"

At the Protein Machinists Lab, we apply our Representation of Protein Entities (RoPE) software to the dynamics of proteins by extracting the conformational space from their torsion angles, rather than the traditional atomic coordinates. This allows us to see the effect of significant subtle conformational changes associated with nevertheless important functional consequences, such as those associated with protein misfolding diseases.

Amy Thompson (Diamond Light Source, UK)

"New clustering methods to identify dynamics in multi-crystal datasets"

Multi-crystal strategies have long been used in macromolecular crystallography to combat challenges associated with room temperature collection and radiation damage. Advances in computational capability allow hundreds of datasets to be automatically processed within a matter of hours, allowing data processing to approach the pace of rapid high-throughput beamlines such as VMXi (Diamond Light Source). This provides exciting new opportunities to expand this methodology in ways previously too computationally daunting to consider. Recent developments in processing multi-crystal data will be presented within the framework of xia2.multiplex. Current clustering methods available within multiplex can be used to not only improve data quality, but also to visualise distinct conformations within multi-crystal datasets. New clustering methods within this software have also been developed with the aim of separating differences which are otherwise too subtle to identify with previous algorithms.

Conor Wild (Diamond Light Source, UK)

"PanDDA 2: leveraging large scale diffraction data to accelerate fragment screens"

XChem has collected over 100.000 high quality diffraction datasets in fragment screens for over 150 fragment screens, however the automation required for the 10x throughput increases that will be available over the next 5 years requires automated methods for partial occupancy fragment identification in electron density maps. PanDDA 2 combines a new statistical model that can handle heterogeneous ground states with ML to make this possible.

CRYSTALLOGRAPHIC METHODS AND ANALYSIS

Discussion Leader:
Mikael Londen (Åbo Akademi University, Finland)

Liliana Guerrero Porras (CUNY ASRC, USA)

"Unveiling the dynamic world of STEP: structural analysis in response to temperature, pressure, ligand binding, and dehydration"

Protein tyrosine phosphatases (PTPs) regulate cellular processes by countering protein tyrosine kinases. Striatal-Enriched Protein Tyrosine Phosphatase (STEP, PTPN5) is crucial for synaptic function, neuronal plasticity, and cognitive processes. This study explores STEP's structural responses to temperature, pressure, ligand binding, and dehydration. Comparative analysis reveals unique effects on solvent patterns, backbone, and side-chain conformations under different conditions. Novel crystal structures unveil conformational heterogeneity, an inhibitory citrate molecule, allosteric changes, and an intramolecular disulfide bond. Surface-exposed cysteine suggests new regulatory mechanisms and drug targets. These findings highlight crystallography's power in probing macromolecular responses to diverse perturbations, including temperature, pressure, and ligand interactions.

Laura Pacoste (Stockholm University, Sweden)

"Refinement of 3D ED data reveals organic ligands, not metal ions, as primary influencers in iron(III) acetyl acetonate charge distribution"

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Dimitrios Triantafyllidis (University of Hamburg, Germany)

"Emergence of order from proteins under nucleation"

Protein crystallization plays a crucial role in protein structure determination, but also in the efficient delivery of crystalline therapeutic pharmaceuticals. Despite its tremendous importance, the underlying mechanisms that govern protein nucleation are still not completely understood. Solution scattering techniques are an excellent tool for nucleation studies, as they can be applied to crystallization-native conditions. By employing a combination of Small Angle X-ray Scattering (SAXS) and X-ray Cross-Correlation Analysis (XCCA), we are able to determine the average size and shape of the growing protein particles, as well as their underlying symmetry and organization. Additionally, the symmetry information from XCCA is crucial for understanding polymorph selection. This combinatorial approach enables characterization of the various steps underlying protein nucleation, acting as a real-time probe for identifying potential off-pathways that prevent the desired crystallization outcome.

Eta Isiorho (CUNY ASRC, USA)

"Seeding success: practical insights for nucleating a crystallization facility"

The rate-limiting step of crystal diffraction studies is obtaining the well-ordered crystals needed for successful diffraction experiments. Crystallization facilities exist to streamline the arduous process of crystallization, especially biomolecular crystallization. But what if research centers lack a facility for crystallization? This talk will be centered on how to build a functional crystallization facility ‘from scratch’, from resources, infrastructure and implementation of efficient methods in order to facilitate and train scientists in crystallization of biomolecules, data acquisition, structure determination and refinement. Highlights of multiple users’ and collaborators’ output from both Auburn University and the City University of New York will be used to highlight the qualities that are useful in yielding a versatile and robust crystallization facility.

PANEL DISCUSSION: NAVIGATING YOUR CAREER IN STRUCTURAL BIOLOGY

Discussion Leader:
Karin Kühnel (Structure, Cell Press)

"Navigating your career in structural biology - my journey"

I am very excited to be the discussion leader for this panel of impressive scientists and to learn more about their careers and work. During my presentation I will describe how I made the transition from academia to becoming an editor, first at Nature Communications and now as editor-in-chief of Structure. I will also mention the challenges I encountered, how I overcame them and what I learned along the way.

Panel members:

Louise Dunnett (Diamond light source, UK)
Marcus Fischer (St. Jude Children's Research Hospital, USA)
Helen Ginn (DESY, Germany)
Nicholas Pearce (Linköping University, Sweden)

POSTERS

Paul Bond

“Automated Model Building with ModelCraft”

ModelCraft is an automated model-building pipeline for X-ray crystallography and cryo-EM that combines Buccaneer for building protein, Nautilus for building DNA/RNA and Refmac/Servalcat for refinement. The X-ray pipeline also includes shift-field refinement, machine-learned pruning of incorrect protein residues, classical density modification, dummy-atom refinement, addition of water, and final rebuilding of side chains. These extra steps make the pipeline more likely to build a complete model when starting from a poor molecular-replacement solution than the previous Buccaneer or Nautilus pipelines. New developments to ModelCraft make it better at building protein/nucleotide complexes. Additionally, deep neural networks have been trained to predict next and previous residue positions and to predict high resolution calculated backbone density from low-resolution maps.

Victoria Jane Burge

“C. difficile: Spore no more!”

C. difficile is responsible for most cases of antibiotic-associated diarrhoea. C. difficile infections (CDI) occur after disruption of gut microbiota by broad-spectrum antibiotics. Treatment of CDIs is currently a further course of antibiotics, which enhances gut dysbiosis. C. difficile can form resistant spores which allow high transmission, and lead to recurrent infections. Sporulation begins with cell division, followed by forespore engulfment, maturation, and mother cell lysis. Here we focus on peptidoglycan hydrolases SpoIID and SpoIIP, required for remodeling the peptidoglycan during engulfment. Previously determined SpoIID structure and activity showed requirement for zinc, but its role has not been determined. Current work involves crystallisation of SpoIID catalytic and zinc-binding mutants to further investigate protein activity and specificity. Also to identify novel binding sites using fragment screening, to develop novel species-specific CDI therapeutics.

Johan Glerup

“Structural studies of the human drug-metabolising protein CYP3A4, room-temperature vs. cryo-crystallography and cryo-EM”

A highly flexible protein with an active site that changes its volume to fit a wide variety of ligands. A lid made of loops changes conformation based on ligand-size. Inhibition of this enzyme stops metabolism of drugs. This is an automatic disqualification of a drug candidate. Room temperature SSX shows better definition of some flexible loops, even at worse resolution. SSX data collection at tens of kilo Gray produces a similar active-site to our XFEL structure. I present an internal distance matrix analysis of a subset of PDB CYP3A4 structures to determine that crystal form and to some extent ligand-size dominates the clustering of global similarity with little difference caused by temperature. The protein crystalises as a monomer but SAX data shows a homo-tetramer in solution bringing it into the perfect size range for cryo-EM. Ligand binding studies in room-temperature crystals have not been successful, does the emerging field of time-resolved cryo-EM studies hold the answers?

Stephan Kleine-Doepke

“Investigating the catalytic mechanism of Sars-CoV-2 MPro”

In recent years the family of the beta coronaviruses has caused multiple outbreaks like SARS, MERS and COVID. Due to their high mutation rates, it is likely that they cause further outbreaks in the future. Therefore, it’s of great interest to find antiviral drugs that target the family of beta coronaviruses. They share a highly conserved 3C-like main protease (MPro) which is vital for its replication, making it an interesting drug target. Current antiviral drugs are under threat of resistant variants. An increased understanding in the catalytic mechanism could help guide drug design to develop more potent drugs to prevent outbreaks in the future. Therefore, we are ultimately interested in studying the catalytic mechanism of MPro in a time-resolved manner utilizing serial synchrotron crystallography in combination with ligand soaking using the Liquid application method (LAMA). The catalytically inactive C145S mutant can provide information on the substrate binding strength and mode by using ITC and crystallography. The naturally occuring, drug resistant to nirmatrelvir, mutant M165Y has a 40x slower catalysis than the wildtype enabling SSX and cryotrapping experiments via mixing at a synchrotron.

Mikael Londen

“Structural immunology of the VAP-1 - Siglec-9 interaction”

VAP-1 is important for inflammatory signaling and as a ligand for Siglec-9. The interaction between VAP-1 and Siglec-9 is important for leukocyte rolling and extravasation, and a loop of Siglec-9 binds to the active site of VAP-1 in this interaction. The knowledge of the interaction has been used to design a radiolabeled peptide based on the interacting Siglec-9-loop, which is used as a PET-tracer. The binding mode of the peptide is not currently known. I assess the structural basis for the interaction by studying VAP-1 with several different Siglec-derived peptides. STD-NMR is used to identify peptide residues that are important for the interaction, and VAP-1 is crystallized with peptides. The experimental results are complemented with docking studies and molecular dynamics simulations to shed further light on the interaction. Results can be used to understand the protein-protein interaction in vivo, and the precise function of the developed PET-tracer in ongoing human trials.

Yelyzaveta Pulnova

“Application of Hadamard time-resolved crystallography to enzyme catalysis”

Time-resolved X-ray diffraction provides unique insights into the molecular mechanisms that underpin life. We are developing multiplexing methods to improve the signal-to-noise ratio of diffraction measurements at flux limited sources. Hadamard time-resolved crystallography (HATRX) is a technique that modifies pump-probe approach by replacing the single probe with the encoded sequence of pulses to boost SNR/time resolution. With HATRX we studied molecular mechanism of aspartate 1-decarboxylase, which catalyses the conversion of L-aspartate to beta-alanine as part of the pantothenate biosynthetic pathway in M.Tuberculosis. Photoinitiation was achieved through photocaging the subtrate (L-aspartate) and data recorded at ms time-resolution at beamline i24, Diamond Light Source. The upgrade of the plasma X-ray source (PXS) at ELI Beamlines for implementing HATRX is also reported. The PXS is a tabletop laser driven X-ray source that generates ~100 fs pulses at 8 keV with 1kHz repetition rate

Mahmoud Rizk

“Improving serial crystallographic data using a Genetic Algorithm”

In Structure-based Drug Design, determining the structure of ligands in complex with target proteins is critical to the drug design process. X-ray Crystallography is a modern tool in structural biology used to investigate these molecular structures at high resolution. The aim of this project is to improve the electron density maps around these ligands, thereby improving the atomic models. Diffraction data from these complexes are collected using serial X-ray crystallography from many micro crystals. We are developing algorithms such as Genetic algorithms and Hierarchical cluster analysis that can identify isomorphic groups from these large pools of data to improve ligand electron density.

Alexander Mehr

“Structural characterization of the pyruvate dehydrogenase complex E1 decarboxylation reaction”

The pyruvate dehydrogenase complex (PDHc) serves a universally important metabolic function in most organisms connecting glycolysis and the citric acid cycle. PDHc converts pyruvate into the vital cellular cofactor acetyl-CoA. *Bacillus subtilis* PDHc is a large protein assembly, 10 MDa in size, consisting of multiple copies of E1, E2, and E3 subunits. The E1 is a thiamine diphosphate (TPP)- dependent enzyme which catalyzes the decarboxylation of pyruvate as a first step. While TPP-dependent decarboxylation mechanisms have been studied in other enzymes, intermediates of the E1 reaction at high resolution and dynamic aspects of TPP-dependent enzyme function remain elusive. To gain comprehensive insights into E1 TPP-dependent decarboxylation of pyruvate, we have used a combination of cryo-trapping and room temperature serial X-ray crystallography to visualize reaction intermediates at high resolution.