DIFFRACTION METHODS IN STRUCTURAL BIOLOGY EARLY CAREER SEMINAR 2026

This meeting is supported by the DFG – Project Number 585870448

GROWING THE FUTURE OF DIFFRACTION METHODS

A heartfelt welcome from the chairs

Welcome to the 2026 Diffraction Methods in Structural Biology Early Career Seminar!

We are delighted to return to the Harnack Haus in Berlin, Germany, to build on the success of the 2024 meeting. This seminar is built by and for the early-career community, with a program that highlights the latest advances taking place in our field. You can expect a stellar lineup of talks, beginning with a keynote from Montserrat Soler Lopez (ESRF). Dr. Soler Lopez will share not only her research, but also the evolution of her career in biology, a perspective we hope will be especially beneficial for this early-career community. We are similarly pleased to host a career panel, offering a chance to reflect on the many paths open to early career scientists and to learn from those who have walked them.

The research presentations and poster session are the heart of the Early Career Seminar. We encourage you to read the abstracts collected in this booklet, to get a sense for the breadth of science gathered under the Diffraction Methods umbrella – but then let that be your starting point to seek out the researchers around you, ask questions, and dive into discussion. When it's your turn to present, whether a talk or a poster, bring your best work and share your research with enthusiasm!

We titled the ECS "Growing the Future of Diffraction Methods" in the hopes that it would speak to not only the cutting-edge science on offer, but also the early-career attendees who will become the strength of this field. To that end, even with a program overflowing with research presentations, we have reserved 14:00-16:00 on Monday afternoon for unstructured discussion. This is a hallmark of the Diffraction Methods conference, and we think it's just as important to the ECS! We hope you'll take advantage of this time to get to know your fellow attendees, some of whom may be your colleagues in structural biology for years and decades to come.

Neither the ECS nor the main Diffraction Methods meeting could take place without the collective commitment of the chairs, local organizers, discussion leaders, contributors, and the entire diffraction methods community. A huge thank you to everyone who made this event possible.

We wish you an inspiring and productive seminar!

Warm regards,

Maggie Klureza (Chair) & **Anaïs Chretien** (Co-Chair)

KEYNOTE SESSION

Keynote Speaker:

Montserrat Soler Lopez (European Synchrotron Radiation Facility, France)

“Understanding Alzheimer’s Disease Across Scales: My Journey Through Integrative Biology”

My scientific journey has led me from structural studies to a broader integrative view of Alzheimer’s disease as a multiscale problem, spanning molecular assemblies, cellular organization, and biological function. In this talk, I will discuss how combining systems biology, crystallography, cryo-EM, tomography, solution scattering, and complementary biochemical and cellular approaches can uncover disease mechanisms that remain inaccessible to any single method alone. I will highlight our recent work on mitochondrial assembly defects, which provides new insight into pathways underlying neurodegeneration.

TWO METHODS ARE BETTER THAN ONE: PAIRED TECHNIQUES FOR BIOLOGICAL INVESTIGATION

Discussion Leader: Diana Gaman (University of Leeds, UK)

Swati Aggarwal (European Spallation Source ERIC, Sweden)

“Beyond Cryo: Room-Temperature X-ray Insights into UrdA”

Urocanate reductase (UrdA) is an enzyme found in various bacteria, including the species in human microbiota. It was first characterized in 2012, where the substrate of the enzyme was found to be urocanic acid, which is then reduced to imidazole propionate (ImP). In this way bacteria can use urocanic acid as a final electron acceptor and be able to grow in anaerobic conditions. Its catalytic residue Arg411 undergoes a large conformational change in the substrate vs product bound states. In contrast to previously studied cryo-conditions, our studies of UrdA’ using RT crystallography provide further insight into the complexity of active site dynamics showing substrate-like and product-like conformations of Arg411. We further provide evidence that a phosphate ion stabilizes the substrate complex and can bias the Arg411 conformation. Overall, our study suggests when possible both cryogenic and RT X-ray data should be collected for an improved biological interpretation of structural results

Johan Glerup (University of Gothenburg, Sweden)

“Time-Resolved Studies of Nitric Oxide Turnover in Cytochrome C Oxidase”

Cytochrome c oxidase (CcO) is a key enzyme in oxidative phosphorylation, where it couples the reduction of oxygen to water with proton pumping. Some CcOs can also reduce nitric oxide (NO) to nitrous oxide (N_2O). Its mechanism is disputed, particularly whether a key intermediate takes a cis or trans-conformation in the active site. We study this reaction in the ba3-type CcO from Thermus thermophilus using photolabile and pH-dependent NO donors to deliver NO directly into the active site. We follow changes after NO-release by serial crystallography and UV-Vis spectroscopy. I will present the first crystallographic models of NO bound ba3-type CcO and discuss the reaction intermediates. In accordance with previous studies, we observe that NO turnover proceeds far slower than oxygen turnover, presenting both advantages and challenges for time-resolved crystallography. The work highlights current technical limitations when working under anaerobic conditions and triggering reactions with photocages for experiments at synchrotron and XFEL facilities.

Juneina Omeiri (Institute of Structural Biology-DYNAMOP, France)

“Studying Photoswitching in a Novel Photochromic Flavoenzyme–Inhibitor Complex by Time-Resolved Crystallography”

Photochromic proteins (PPs) reversibly switch between states with distinct absorption spectra , making them valuable tools for optogenetics and super-resolution microscopy. However, many systems suffer from low quantum yields and limited red-light absorption . Monomeric sarcosine oxidase (MSOX) forms a charge-transfer complex with methylthioacetate (MTA, a substrate inhibitor), and exhibiting ultrafast (~300 fs) photoswitching with near-unity quantum yield. Using cryo-crystallography and microspectrophotometry, we identified light-induced structural changes and MTA rearrangement. We then performed time-resolved serial femtosecond crystallography (TR-SFX) at XFELs, obtaining a 1.6 Å room-temperature structure. These data reveal conformational dynamics of MTA and its interaction with flavin on the nanosecond, confirming the previously proposed photoswitching mechanism and MTA isomerization.

David Buckley (University of Missouri – Columbia, USA)

“Visualization of Covalent Intermediates and Conformational States of Proline Utilization A by X-Ray Crystallography and Molecular Dynamics Simulations”

The bifunctional enzyme proline utilization A (PutA) catalyzes the two-step oxidation of L-proline to L-glutamate using proline dehydrogenase (PRODH) and L-glutamate-γ-semialdehyde dehydrogenase (GSALDH) domains. The two active sites are 42 Å apart and connected by a buried tunnel that is hypothesized to channel the intermediates Δ1-pyrroline-5-carboxylate (P5C) and/or L-glutamate-γ-semialdehyde (GSAL). Kinetic and conventional X-ray crystallography of PutA from Sinorhizobium meliloti (SmPutA) were used to capture high resolution (1.47–1.88 Å) structures of states along the catalytic cycle, including a novel FADH−-proline covalent adduct in the PRODH site, the intermediate P5C bound noncovalently in the reduced PRODH active site, the covalent acyl-enzyme intermediate of the GSALDH reaction, and noncovalent complexes of GSAL and the final product L-glutamate in the GSALDH active site. The FADH−-proline covalent adduct resembles a stable species predicted from quantum mechanical electronic structure calculations of the PRODH reaction. The GSALDH domain complexes are consistent with conservation of substrate recognition and catalytic mechanism by the aldehyde dehydrogenase superfamily. The structure of reduced SmPutA with the P5C bound in the PRODH active site was used as the starting point for molecular dynamics simulations (21 × 2 μs trajectories). P5C diffuses from the PRODH active site into the tunnel in most of the trajectories, but rarely dissociates completely from the enzyme, consistent with previous kinetic evidence of a substrate channeling mechanism. The simulations also provide insight into protein conformational changes associated with substrate channeling, including the opening and closing of a conserved ion pair gate.

Gabriela Schröder (MAX IV Laboratory/Lund University, Sweden)

“Neutron Protein Crystallography of the Macrophage Migration Inhibitory Factor Reveals a Key Water in the Tautomerase Mechanism”

Macrophage migration inhibitory factor (MIF) is a pro-inflammatory, pro-tumorigenic protein and an attractive therapeutic target. MIF catalyzes the interconversion of the keto and enol forms of 3-(4-hydroxyphenyl)-pyruvate (HPP) via a tautomerase reaction. Joint X-ray and neutron protein crystallography was used to determine a 2.5-Å structure of MIF bound to HPP. The neutron scattering length density (NSLD) maps reveal the position of a water molecule involved in the tautomerization reaction and confirm the charged state of Lys32 in the active site. The structure supports the proposed catalytic mechanism of MIF, in which the N-terminal Pro1 nitrogen abstracts a proton from C3 of the keto form of HPP to generate an enolate intermediate which is subsequently protonated by this newly identified water molecule to form the enol product. The detailed information of proton positioning elucidated here offers insight into previously unresolved questions regarding the mechanistic details of MIF.

PROCESSING & EVALUATING CRYSTALLOGRAPHIC DATA

Discussion Leader: Matteo Magri (European Synchrotron Radiation Facility, France)

Robert Bosman (University Medical Center Hamburg-Eppendorf, Germany)

“Anisotropic Multiplicity: Understanding Structure Factor Sampling in Serial Crystallography”

Since the advent of serial crystallography most old school crystallographers have looked on with mild horror that people have tried to recreate structure factors by throwing lots of partial reflections into a monte-carlo simulation wrapped in a reasonbly well crafted error-model. Despite this the field has been able to reproduce electron density for several protein targets and the methods has been used prominently in time-resolved crystallography. Given the reliance on repeated sampling, structure factor accuracy is related to multiplicity in a fundementally different way then conventional rotation crystallography. Due to the stochastic nature of the method, uniform mulitiplicity cannot be ensured. Despite this no metric considers the relationship between reflection sampling and structure factor information contant. Here we show how uneven sampling of the ewald-sphere is required to significantly distort the merged data and propose a alternative metric to assess this issue.

Sebastian Bielfeldt (DESY, Germany)

“Vacuum Matching: Calculating Extrapolation Factors in Low-Occupancy Time-Resolved Crystallography”

Time-resolved crystallography captures proteins in action but usually yields a mixture of ground and triggered states. If the time-delayed state is only populated at low occupancy, one must extract it from the mixture. This is commonly done by calculating the difference map of the mixed and reference states, multiplying this difference by an extrapolation factor, and adding the result to the reference state. We introduce Vacuum Matching, which estimates the extrapolation factor from the constraint that electron density cannot fall below vacuum. It avoids the repeated refinement required by existing approaches, supplies quality-control metrics, and offers an intuitive visual representation of the underlying data. On simulated data, Vacuum Matching outperforms existing methods across occupancies and noise levels. On real TRX datasets it produces consistent predictions and correctly flags cases where the input difference map does not support a reliable estimate.

Bhawna Chaudhary (University of Warsaw, Poland)

“Improving MicroED Structure Refinement with Advanced Scattering Models”

MicroED, determines atomic structures of proteins from sub-micron-sized, three-dimensional crystals. This study investigates advanced atomic scattering models to improve MicroED structure refinement. We use high-potential iron–sulfur protein (HiPIP), a [4Fe–4S] electron-transfer protein, as a model system to probe subtle electronic and structural features. Protein purification was performed, and crystals suitable for both MicroED and X-ray data collection were obtained. We compare the Independent Atom Model (IAM) with the Transferable Aspherical Atom Model (TAAM) using PHENIX refinement, which provides a more accurate description of atomic electron density. We anticipate that TAAM-based refinement will improve electron density maps and better represent the iron–sulfur cluster environment, demonstrating improved structural accuracy over conventional methods. This work was supported by the National Science Centre (NCN), Poland, under project number UMO-2024/53/B/ST4/02777.

Gayathri Yuvaraj (Lund University, Sweden)

“Quantum-Refinement for Time-Resolved Crystallography”

In standard crystallographic refinement of proteins, the experimental data are normally not enough to unambiguously decide the positions of all atoms. Therefore, the crystallographic data are supplemented by a set of empirical restraints that ensure that bond lengths and angles make chemical sense. To obtain more accurate results, we have suggested that this potential can be replaced by more accurate quantum-mechanical calculations for a small, but interesting part of the protein, giving the method of quantum refinement. Our group has shown that quantum refinement can locally improve crystal structures, decide protonation state of metal-bound ligands, oxidation state of metal sites, detect photoreduction of metal ions and solve scientific problems regarding what is really is seen in crystal structures. We investigate how quantum refinement can be used for time-resolved crystallography.

Dimitris Triandafillidis (University of Hamburg, Germany)

“From clusters to clusters”

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, since they can be applied to crystallization-native conditions. Using a combination of Small Angle X-ray Scattering (SAXS) and Fluctuation X-ray Scattering (FXS), we are able to determine the average size and shape of the growing protein particles, as well as their underlying symmetry, organization and dynamics. We will discuss effective ways for sample delivery, as well as approaches to process and model the complex data resulting from the interplay of protein molecules at varying levels of organization, from monomers and oligomers to clusters and crystals.

PROBING THE SMALL-SCALE INTERACTIONS THAT DRIVE PROTEIN FUNCTION

Discussion Leader: Virginia Apostolopoulou (University of Hamburg, Germany)

Helen Ginn (University of Hamburg/DESY, Germany)

“Allosteric effects on catalysis through the lens of proton network communication”

The catalytic activity of fluoroacetate dehalogenase (FAcD) depends on a half-of-sites reactivity between two sides of a homodimer. These two sides are linked by a bridging proton and water network. Ultra-high resolution studies reveal a myriad of interconnected alternate conformers and possible hydrogen-bonding patterns. Subtle changes in populations and conformations accompany two mutants of a key residue in this bridging proton network which either increase or obliterate activity. However, by eye, no one feature stands out as an explanation for changes in catalytic activity. By modelling the full chemical logic of hydrogen bonding in a constrained network, we can computationally examine the behaviour of this network to formulate a hypothesis of why catalytic activity changes. This shows substantial changes in communication behaviour in the proton network between the variants. The wild-type has too many disjointed subnetworks, preventing long-range communication, whereas the inactive mutant has too few, easily getting trapped in one state. In contrast, the most active mutant occupies a “Goldilocks” optimum between these two extremes. This talk/poster focuses on the computational modelling behind these results.

Dilip Narayanan (University of Copenhagen, Denmark)

“Frag2Drug - UCPH's Fragment-Based Screening and Drug Discovery Facility”

Early-stage drug discovery often suffers from disconnection between screening, structural biology, and medicinal chemistry, slowing progression from initial hits to viable leads. To address this, we are establishing Frag2Drug (F2D)—an open-access, integrated platform designed to accelerate fragment-to-lead (F2L) development through tightly coupled biophysical, structural, and computational workflows. Frag2Drug combines surface plasmon resonance (SPR) for high-confidence hit identification and affinity profiling with high-throughput X-ray crystallography to deliver rapid structural insights into ligand–protein interactions. Built on proven expertise in fragment-based drug discovery, Frag2Drug is designed to deliver actionable data that directly informs lead optimization strategies. Frag2Drug aims to serve as a central hub for early drug discovery, accelerating the conversion of fragment hits into high-quality lead compounds and enabling more efficient, data-driven medicinal chemistry.

Tim Gitzinger (University of Hamburg, Germany)

“Linking Structure to Energetics: Characterising the Stress-Strain Relationship of Proteins”’

Computationally designed enzymes consistently underperform their natural counterparts by orders of magnitude, suggesting that current protein engineering approaches lack a fundamental understanding of the internal force landscape governing function. We are establishing how to apply static force analysis (SFA), a classical structural engineering technique, to experimentally determined protein crystal structures. Treating an equilibrium structure as a system at rest allows us to construct a system of linear equations for each covalent bond and solve for Newtonian reaction forces, termed "proxy forces". Where our force model is incomplete, these proxy forces represent the net contribution of unmodelled interactions, providing a sensitive readout of missing physics. Looking ahead, applying this framework to systems such as human gamma-D crystallin and kemp eliminase may open a path toward a quantitative link between active-site stress localisation and catalytic efficiency, and a new physical foundation for enzyme design.

Maria Spiliopoulou (University Medical Center Hamburg-Eppendorf, Germany)

“Unravelling Ligand-Binding Dynamics: Advancing Structural Biology with Time-Resolved Crystallography”

Ligand binding is a fundamental process that can reshape the conformational landscape of proteins. Traditional crystallography approaches have provided valuable input about end states in ligand-binding reactions. However, dynamical relationships between ligand binding, environmental triggers, and protein backbone/side chain rearrangement often remain obscured. In this study, we use time-resolved serial synchrotron crystallography to visualize ligand binding in protein microcrystals under controlled conditions. By integrating fixed-target crystallography with LAMA-based ligand delivery, we capture progression of ligand binding and associated structural changes. This approach also enables investigation of binding events induced by external factors such as pH changes, allowing comparison of distinct mechanisms. Using an occupancy refinement protocol, we quantify evolving structural populations and show ligand binding proceeds via diffusion-limited or environment-triggered mechanisms.

Lei Wang (Stockholm University, Sweden)

“Microcrystal-Based Flash Ligand Soaking and Complex Structure Determination Using Electron Diffraction”

Macromolecular crystallography provides high-resolution structures for drug discovery, while electron diffraction methods such as MicroED and SerialED enable structure determination from nanocrystals. However, their application to proteins remains limited due to challenges in crystal optimization, sample preparation, radiation damage, and data processing. Here, we present an integrated workflow for rapid ligand soaking and protein–ligand structure determination. Using MTH1 as a model, microcrystals prepared by vortex were soaked with ligands on a TEM grid within 3 seconds. MicroED enabled rapid structure determination (~2.5 Å in 25–50 min), while continuous SerialED achieved higher resolution (~1.7 Å). This workflow demonstrates the potential of electron diffraction for future drug discovery.

POSTERS

Group 1

Swati Aggarwal

“Beyond Cryo: Room-Temperature X-ray Insights into UrdA”

Urocanate reductase (UrdA) is an enzyme found in various bacteria, including the species in human microbiota. It was first characterized in 2012, where the substrate of the enzyme was found to be urocanic acid, which is then reduced to imidazole propionate (ImP). In this way bacteria can use urocanic acid as a final electron acceptor and be able to grow in anaerobic conditions. Its catalytic residue Arg411 undergoes a large conformational change in the substrate vs product bound states. In contrast to previously studied cryo-conditions, our studies of UrdA’ using RT crystallography provide further insight into the complexity of active site dynamics showing substrate-like and product-like conformations of Arg411. We further provide evidence that a phosphate ion stabilizes the substrate complex and can bias the Arg411 conformation. Overall, our study suggests when possible both cryogenic and RT X-ray data should be collected for an improved biological interpretation of structural results

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Diana Gaman

“Time-Resolved Investigation of Photoredox Mechanisms”

We aim to understand how lens clarity is maintained despite daily exposure to UV radiation (UVR), and what mechanisms are involved in the earliest stages of protein aggregation that contribute to cataract formation. Here we specifically focus on the human γ-D crystallin protein, a major structural component of the lens and implication in cataracts. To achieve these objectives, we employ a range of time-resolved spectroscopic and diffraction techniques to monitor protein behaviour under various conditions, including in response to UVR. We have generated a library of cysteine and tryptophan mutants to investigate photoredox mechanisms and how they affect protein stability and aggregation tendencies. To investigate early structural events associated with UVR exposure we apply time-resolved serial crystallography and X-ray absorption spectroscopy across a broad range (fs - s) timescales utilising synchrotron and XFEL facilities.

Tim Gitzinger

“Linking Structure to Energetics: Characterising the Stress-Strain Relationship of Proteins”

Computationally designed enzymes consistently underperform their natural counterparts by orders of magnitude, suggesting that current protein engineering approaches lack a fundamental understanding of the internal force landscape governing function. We are establishing how to apply static force analysis (SFA), a classical structural engineering technique, to experimentally determined protein crystal structures. Treating an equilibrium structure as a system at rest allows us to construct a system of linear equations for each covalent bond and solve for Newtonian reaction forces, termed "proxy forces". Where our force model is incomplete, these proxy forces represent the net contribution of unmodelled interactions, providing a sensitive readout of missing physics. Looking ahead, applying this framework to systems such as human gamma-D crystallin and kemp eliminase may open a path toward a quantitative link between active-site stress localisation and catalytic efficiency, and a new physical foundation for enzyme design.

Emma Newby

“Structural Studies of Cofactor Activation in the Pyruvate Dehydrogenase E1 Enzyme”

Thiamine diphosphate (ThDP) is an essential cofactor across archaea, bacteria and eukaryotes, with a mechanistic role in several central metabolic enzymes. One example is pyruvate dehydrogenase, the E1 component of the pyruvate dehydrogenase complex (PDHc). The E1 reaction requires initial ThDP activation, achieved by deprotonation at the C2 position of the central thiazole ring, followed by pyruvate decarboxylation and acetyl transfer. Whilst the E1 has a pH optimum around 7, the calculated solution pKa of the C2 proton is between 17 and 19, and this discrepancy would suggest that the reaction should not be feasible. A degree of pre-organisation within the enzyme active site is therefore required to reduce the energetic penalty of deprotonation. Sub-atomic resolution crystal structures have identified a significant distortion of the ThDP thiazole ring from the expected planarity of an aromatic system, which may modulate cofactor activation. These structures have also allowed visualisation of quantum mechanical phenomena, such as deformation densities, highlighting their potential roles in enzyme catalysis.

Gabriela Schröder

“Neutron Protein Crystallography of the Macrophage Migration Inhibitory Factor Reveals a Key Water in the Tautomerase Mechanism”

Macrophage migration inhibitory factor (MIF) is a pro-inflammatory, pro-tumorigenic protein and an attractive therapeutic target. MIF catalyzes the interconversion of the keto and enol forms of 3-(4-hydroxyphenyl)-pyruvate (HPP) via a tautomerase reaction. Joint X-ray and neutron protein crystallography was used to determine a 2.5-Å structure of MIF bound to HPP. The neutron scattering length density (NSLD) maps reveal the position of a water molecule involved in the tautomerization reaction and confirm the charged state of Lys32 in the active site. The structure supports the proposed catalytic mechanism of MIF, in which the N-terminal Pro1 nitrogen abstracts a proton from C3 of the keto form of HPP to generate an enolate intermediate which is subsequently protonated by this newly identified water molecule to form the enol product. The detailed information of proton positioning elucidated here offers insight into previously unresolved questions regarding the mechanistic details of MIF.

Dilip Narayanan

“Frag2Drug - UCPH's Fragment-Based Screening and Drug Discovery Facility”

Early-stage drug discovery often suffers from disconnection between screening, structural biology, and medicinal chemistry, slowing progression from initial hits to viable leads. To address this, we are establishing Frag2Drug (F2D)—an open-access, integrated platform designed to accelerate fragment-to-lead (F2L) development through tightly coupled biophysical, structural, and computational workflows. Frag2Drug combines surface plasmon resonance (SPR) for high-confidence hit identification and affinity profiling with high-throughput X-ray crystallography to deliver rapid structural insights into ligand–protein interactions. Built on proven expertise in fragment-based drug discovery, Frag2Drug is designed to deliver actionable data that directly informs lead optimization strategies. Frag2Drug aims to serve as a central hub for early drug discovery, accelerating the conversion of fragment hits into high-quality lead compounds and enabling more efficient, data-driven medicinal chemistry.

Group 2

Justin Bergmann

“FragHAR: Bringing Tailor-Made Aspherical Scattering Factors to Macromolecular Structures”

Hirshfeld atom refinement (HAR) improves crystallographic models over the independent atom model by using aspherical atomic form factors derived from quantum mechanical calculations, but its application to macromolecules is limited by high computational cost. We address this limitation with fragHAR, which computes aspherical scattering factors from small overlapping molecular fragments. We present a new implementation of fragHAR in Olex2 via the NoSpherA2 interface, including an improved treatment of hydrogen bonding through automatic fragment extension. Tests on oligopeptides show results indistinguishable from full HAR, while applications to the proteins crambin and rubredoxin demonstrate improved refinement quality compared with IAM and near-linear scaling, achieving a 46-fold speedup over HAR. The successful refinement of rubredoxin represents the first HAR-based study of a metalloprotein.

Anaïs Chretien

“Controlling Tryptophan Synthase Enzymatic Reaction with Unnatural Amino Acids for Time-Resolved Crystallography Experiments”

Tryptophan synthase (TS) catalyzes the final steps of tryptophan biosynthesis through substrate channeling, where indole traverses a ~25 Å tunnel between subunits. Understanding substrate cleavage, indole translocation, and allosteric changes requires precise temporal control during time-resolved crystallography. We developed light-sensitive TS variants by incorporating caged tyrosine at strategic positions. One variant positioned mid-tunnel showed negligible dark activity and near wild-type activity upon UV exposure. Crystallographic analysis revealed this variant blocks IGP cleavage in the a subunit through inhibition of allosteric communication, while L-serine binds PLP and advances to the aminoacrylate intermediate in the b subunit. This creates an ideal starting state where UV uncaging synchronously initiates the complete catalytic cycle, enabling direct visualization of channeling dynamics and validating unnatural amino acids as powerful tools for controlling enzymatic reactions.

Helen Ginn

“Allosteric Effects on Catalysis through the Lens of Proton Network Communication”

The catalytic activity of fluoroacetate dehalogenase (FAcD) depends on a half-of-sites reactivity between two sides of a homodimer. These two sides are linked by a bridging proton and water network. Ultra-high resolution studies reveal a myriad of interconnected alternate conformers and possible hydrogen-bonding patterns. Subtle changes in populations and conformations accompany two mutants of a key residue in this bridging proton network which either increase or obliterate activity. However, by eye, no one feature stands out as an explanation for changes in catalytic activity. By modelling the full chemical logic of hydrogen bonding in a constrained network, we can computationally examine the behaviour of this network to formulate a hypothesis of why catalytic activity changes. This shows substantial changes in communication behaviour in the proton network between the variants. The wild-type has too many disjointed subnetworks, preventing long-range communication, whereas the inactive mutant has too few, easily getting trapped in one state. In contrast, the most active mutant occupies a “Goldilocks” optimum between these two extremes. This talk/poster focuses on the computational modelling behind these results.

Gina Jackisch

“Electric-Field-Stimulated Protein Mechanics to Study Protein-Based Water Flow”

Aquaporins are membrane channel proteins that maintain water homeostasis by regulating the transport of water and small molecules across biological membranes. Their activity is controlled at many different levels, with rapid changes in water transport activity achieved through gating, where the pore transitions between open and closed states. In this study, we focus on the gated eukaryotic water channel AQY1 of the yeast Pichia pastoris. The hypothesis that static electric fields within the pore prevent proton transport will be tested by applying strong electrical fields (Hekstra et al., 2016) across single crystals of AQY1. The resulting structural response is probed using time-resolved X-ray diffraction, followed by a symmetry-based analysis. By following the rearrangements of the water molecules in response to the applied electric fields, we aim to directly observe conformational changes and isolate electric-field-induced structural differences.

Matteo Magri

“Fast Detector Simulations of Pink Beam Serial Synchrotron Crystallography”

Serial synchrotron crystallography (SSX) combines the low-radiation damage, time-resolved capability of serial crystallography with the high brilliance of microfocus beamlines at storage ring. Fourth-generation synchrotron sources further enhance performance by using broader bandwidths to compensate for the lack of crystal rocking when measuring Bragg spot intensities. However, this also produces elongated diffraction spots that must be accurately modelled for reliable intensity estimation. To quantify how beam and crystal properties shape diffraction intensities, we have developed a novel algorithm that simulates the 2D pixel-detector images from pink beam sources. Using closed-formed approximations of the moments of the spot profile distribution, our method efficiently predicts both integrated intensity and spot shape. These fast algorithms could enable simulation-based inference approaches that exploit full pixel-level detector information to improve SSX data merging.

Yelyzaveta Pulnova

“Hadamard Time-Resolved Crystallography for Radiation Damage Study”

Hadamard Time-Resolved Crystallography (HATRX) is a multiplexing approach in which diffraction from multiple time points is recorded as a combined signal and subsequently separated using the inverse Hadamard transform. This strategy aims to increase temporal resolution in macromolecular crystallography and offers the potential to recover structural information at doses lower than those required for conventional single-frame acquisition. Key challenges lie in data processing, namely ensuring consistent scaling across datasets and rigorous propagation of uncertainties. Our workflow combines shell-wise scaling with Jacobian-based error propagation. We present initial HATRX results on radiation damage in myoglobin. Seven multiplexed timepoints with increasing dose provide a controlled framework to study dose-dependent structural changes. Ongoing work targets optimizing data processing protocols and establishing validation criteria for time-resolved multiplexed crystallography experiments.

Kiyofumi Takaba

“Strategies in 3D Electron Diffraction Development: Insights from Automated and Manual Workflows”

3D ED has become an increasingly accessible for structural analysis of small crystals, yet its implementation often depends strongly on instrument-specific conditions. Optimal strategies differ between conventional 200 keV TEM systems and 300 keV instruments, together with variations in detector technologies such as active-pixel sensor and hybrid-pixel detectors, which impose practical constraints on data collection. Multiple data acquisition can improve overall data quality, particularly for biomolecular samples, but requires carefully designed strategies. I present 3D ED workflows developed under distinct environments: automated acquisition on a 300 keV microscope using established control software, SerialEM, and manually guided acquisition on a 200 keV microscope using a dedicated user interface coupled with a JUNGFRAU detector. By comparing these developments and their applications, essential considerations for achieving efficient and reliable data collection will be discussed.

Dimitris Triandafillidis

“From Clusters to Clusters”

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, since they can be applied to crystallization-native conditions. Using a combination of Small Angle X-ray Scattering (SAXS) and Fluctuation X-ray Scattering (FXS), we are able to determine the average size and shape of the growing protein particles, as well as their underlying symmetry, organization and dynamics. We will discuss effective ways for sample delivery, as well as approaches to process and model the complex data resulting from the interplay of protein molecules at varying levels of organization, from monomers and oligomers to clusters and crystals.

Group 3

Virginia Apostolopoulou

“How Hydrogen Bonds Shape Protein Flexibility”

Hydrogen bonds are critical structural constraints that stabilise the folded protein and simultaneously permit the dynamic flexibility essential to biological function. The resulting hydrogen bond network enables subtle conformational adjustments that support selective binding and interaction with other molecules across multiple states. Such properties are of growing importance, as de novo protein design increasingly demands precise control over flexibility. Existing computational approaches, such as Normal Mode Analysis, have contributed substantially to our understanding of these dynamics. They do, however, carry well-known limitations including the harmonic approximation and the neglect of solvent effects, leaving room for alternative strategies. While X-ray crystallography remains the gold standard for directly observing conformational changes, its considerable cost and time demands motivate the development of computational tools that can guide experiments more efficiently. Here, we present a fast and computationally inexpensive approach to screen for potentially flexible regions in proteins starting directly from experimental structures, built on two core components: the ROPE software [Ginn, 2023] and kinematic flexibility analysis theory [Budday et al., 2018]. By treating hydrogen bonds as kinematic constraints, the method enables the exploration of how the hydrogen bond network shapes structural flexibility. We demonstrate the approach on three proteins, comparing calculated flexibility profiles against experimental data as a first proof of concept of this framework. Ginn, H. M. (2023). Torsion angles to map and visualize the conformational space of a protein. Protein Science, 32(4), e4608. Budday, D., Leyendecker, S., & van den Bedem, H. (2018). Kinematic flexibility analysis: Hydrogen bonding patterns impart a spatial hierarchy of protein motion. Journal of chemical information and modeling, 58(10), 2108-2122.

Ashiq Ayub

“Deciphering the Chain-Length Control Mechanism during Fatty Acid Biosynthesis”

Fatty acid (FA) biosynthesis is an essential metabolic process occurring in a cyclic multi-step process to produce FAs. Seven or eight iterations involving carbon-carbon bond formation and associated transformations produces a saturated 16- or 18-carbon long FA chain. In yeast, FA biosynthesis is catalyzed by a 2.6 MDa, barrel-shaped Fatty Acid Synthase (FAS) multi-enzyme complex formed by 6 alpha- and 6 beta-subunits. The Acyl-Carrier-Protein domain (ACP), tethered inside FAS is the central player by shuttling substrates and intermediates across reaction sites. Currently the FA chain-length control mechanism remains elusive and was investigated. However, the highly dynamic ACP hinders structural investigation through conformational and compositional heterogeneity inside FAS. To overcome this, mechanism-based crosslinkers were employed, which stalled ACP at Ketosynthase domain (KS). Structural snapshots were then captured using X-ray crystallography rather than Cryo-EM, as internal structural information are lost during image processing in latter. The snapshots, 3 Å resolution and better revealed that two pockets exist for binding FAS intermediates in KS, one allowing transacylation and the other for condensation half-reaction during chain-elongation. Furthermore, they point out that chain-length control is established in the transacylation pocket owing to Van der Waals repulsion by the length of the FAS intermediate in adjacent KSs.

Robert Bosman

“Anisotropic Multiplicity: Understanding Structure Factor Sampling in Serial Crystallography”

Since the advent of serial crystallography most old school crystallographers have looked on with mild horror that people have tried to recreate structure factors by throwing lots of partial reflections into a monte-carlo simulation wrapped in a reasonbly well crafted error-model. Despite this the field has been able to reproduce electron density for several protein targets and the methods has been used prominently in time-resolved crystallography. Given the reliance on repeated sampling, structure factor accuracy is related to multiplicity in a fundementally different way then conventional rotation crystallography. Due to the stochastic nature of the method, uniform mulitiplicity cannot be ensured. Despite this no metric considers the relationship between reflection sampling and structure factor information contant. Here we show how uneven sampling of the ewald-sphere is required to significantly distort the merged data and propose a alternative metric to assess this issue.

Hosni El Zein

“Elucidation of the Singlet Oxygen Quenching Mechanism of the Orange Carotenoid Protein by Use of Time-Resolved Crystallography”

The Orange Carotenoid Protein (OCP) is a soluble photoactive protein that protects cyanobacteria from high light stress. It consists of two domains connected by a flexible linker, with a keto-carotenoid pigment at their interface. Upon absorbing blue-green light, OCP shifts from an inactive orange state (OCPO) to an active red state (OCPR), enabling energy quenching via interaction with the phycobilisome. This prevents overexcitation of photosystems and limits the formation of ROS, such as singlet oxygen $^1O_2$. OCP can also directly quench $^1O_2$ via physical and chemical mechanisms. Physical quenching involves energy transfer between $^1O_2$ and the carotenoid triplet state, restoring ground-state oxygen, whereas in chemical quenching the carotenoid is oxidised. The structural nature of the carotenoid triplet state, and $^1O_2$ access pathways remain unclear. This study aims to apply time-resolved crystallography to investigate the structural features of OCP and its pigment during $^1O_2$ quenching.

Aybeg Nafiz Günenç

“Investigation of Fatty Acid Kinase Systems at Sub-Angstrom Resolution”

Recent advances in Macromolecular X-ray crystallography allow us to push the boundaries of our current understanding of biological macromolecules from mere revelation of folds to exact atomic positions and even minute changes at potentially electron level. However, mainstream processing and refinement strategies fall short to describe certain phenomena observed at this level, which dictates specialized strategies. Here we present the protein structures of the Fatty acid Kinase (FaK) system of the opportunistic pathogen Staphylococcus aureus (FakA, FakB1 and FakB2), at resolutions reaching up to 0.7 Å with considerably low mosaicity and anisotropy. Here we display phenomena like negative densities around electron heavy atoms, stretches of main chain splits and hydrogen densities; and how we interpret them. Lastly, we tried to obtain different states of the proteins via various pre- and post-crystallization treatments to better understand the enzymatic mechanisms of the system.

Maggie Klureza

“Implementing Electric Field-Stimulated X-Ray Crystallography at a Monochromatic Synchrotron Beamline”

Electric field-stimulated X-ray crystallography (EF-X) offers a novel means to perturb proteins and observe the resulting motions. Establishing an electric field across a protein crystal creates a pattern of forces, localized to the charged moieties within each protein molecule. The resulting motions can then be read out through time-resolved diffraction data. However, the sub-microsecond timescale of these motions has typically required use of a polychromatic Laue beam to achieve sufficient temporal resolution. Here, we present recent efforts to implement EF-X at the EMBL monochromatic P14 T-REXX beamline at DESY by instead coupling it to Hadamard time-resolved X-ray crystallography (HATRX).

Arturo Landeros de la Isla

“Time-Resolved Crystallography and Optical Spectroscopy at the I24 Beamline at Diamond Light Source”

I24 is a tuneable microfocus macromolecular crystallography beamline at Diamond Light Source designed for high-quality cryogenic data collection from challenging microcrystals as well as multiple serial crystallography approaches including fixed targets and the LCP extruder. A modular end station enables rapid switching between rotation, serial and time-resolved experiments, using laser excitation or rapid mixing. Fixed-target silicon chips enable fast, low-consumption serial experiments under biologically relevant conditions, including anaerobic data collection. Photocages have attracted large interest for the study of enzymatic reactions that are not naturally photoactivatable. A caged ligand is soaked into or co-crystallised with the sample and released by light-triggering, enabling time-resolved data collection. I will present ongoing work and new results in the application of nitric oxide and oxygen photocages to metalloproteins using serial crystallography.

Jin Siqi

“Tracking Radiation-Induced Degradation in Subatomic-Resolution Rubredoxin: A Serial Dose X-Ray Diffraction Study”

Background: Subatomic-resolution XRD of metalloproteins is limited by dose-dependent radiation damage. Understanding its structural impact is vital for accurate refinement. Methods: We present a serial dose analysis of rubredoxin via subatomic-resolution XRD, tracking real-space and reciprocal-space degradation against absorbed dose. Results: Real-space damage features measurable Fe-S distance elongation and linearly increasing atomic displacement with dose, strongly correlating with the Kabsch matrix. In reciprocal space, we observe a linear loss of effective reflections. These lost reflections consistently exhibit the largest sigma or Z-scores, indicating targeted signal decay mapped across resolution and amplitude bins. Conclusion: Correlating structural distortions with selective high-sigma reflection loss provides a robust framework to quantify radiation damage, offering diagnostic metrics to optimize data collection.

Romain Vincent

“Thermodynamic Studies of Late-Stage Protein Folding: An Outline”

Protein structure prediction has been recently improved, especially with the advent of Alphafold. However, these models predict the final structure without inferring the folding pathway, which would otherwise be valuable information when considering changes to the protein sequence. Protein folding is a complex process: the result of a selection of a linear sequence of amino acids and interaction with solvent that will then fold to form the final structure, and our understanding of the whole process is lagging behind. To understand the folding dynamics fully, it is required to understand the continuous reaction from the linear peptidic chain to the fully folded protein. Here we present a way of studying the rotamers present in the final structure to try to determine the final folding steps, through contacts of sequence-distant amino acid side chains. This will help us study the folding process from a thermodynamic perspective. This will also assist mutagenesis assays, by predicting the impact of planned mutations on the folding pathway.