Structure

Voices

Introducing: Ulrich J. Lorenz, Radoslav I. Enchev, Clare F. Megarity, Helen Ginn, Patrick Rabe, Alice R. Walker, and Kartik Ayyer

In this Voices article we introduce seven impressive young group leaders who spoke at the recent Ringberg Workshop on Structural Biology organized by Ilme Schlichting from the Max Planck Institute for Medical Research, Heidelberg. We asked them to tell us more about their careers and their exciting research.

Ulrich J. Lorenz
Ecole Polytechnique Fédérale de Lausanne

Blazing fast observations of protein dynamics with cryo-EM

My research group is best known for the development of microsecond time-resolved cryo-EM, a novel technique that improves the time resolution of cryo-EM by about three orders of magnitude. This makes it fast enough to observe the motions of proteins that are typically associated with function and that have been largely inaccessible to direct observation.

My research trajectory into time-resolved structural biology started in molecular physics. During my doctoral studies with Tom Rizzo at EPFL, we used a combination of laser spectroscopy and mass spectrometry to gain a fundamental picture of how intramolecular interactions govern the self-organization of small peptides. For my postdoc, I wanted to switch to a method that provides a more direct probe of structure. At Caltech, I was given this opportunity by Ahmed Zewail, who at the time was pioneering ultrafast electron microscopy, a technique that uses short electron pulses to image structural dynamics of materials at high temporal and spatial resolution. His advice to young scientists was to "always shoot high" and tackle big questions. One such question was whether such observations could be extended to the dynamics of proteins.

When I started my own group, I decided to work on this problem as part of an ERC starting grant. But instead of imaging with short electron pulses, I proposed a different strategy for achieving high time resolution. In our experiments, we melt a cryo sample with a laser beam in order to briefly allow protein dynamics to occur while the sample is liquid. When the laser is switched off, the sample cools rapidly and revitrifies, trapping the particles in their transient configurations, which we subsequently image with conventional cryo-EM techniques.

This idea was initially met with quite some skepticism. However, we have since demonstrated not only that it is possible to melt and revitrify cryo samples, but also that this process leaves proteins intact and that near-atomic resolution reconstructions can be obtained from revitrified cryo samples. Moreover, the process is so fast that it allows for observations with microsecond time resolution and potentially even faster. Recently, we have for the first time used our method to observe a process that occurs in nature. We observe the microsecond motions of the capsid of CCMV that play an important role during the life cycle of this plant virus. We predict that our technique will be quite general and will make it possible to elucidate a vast range of protein dynamics that were previously inaccessible—some of which we are currently exploring as part of a consolidator grant from the SNSF. Moreover, we have recently shown that our experiments can also be performed with a correlative light-electron microscopy approach instead of inside a modified electron microscope. We believe that this will make our technology more accessible to other research groups, and we are encouraged that many have started to adopt it.

Radoslav I. Enchev
The Francis Crick Institute, London

Structural dynamics in a blink of an eye

Throughout my academic journey—from undergraduate studies in Heidelberg to doctoral training at the Institute of Cancer Research in London to postdoctoral work at ETH Zurich—I have developed a profound interest in quantitative biophysics and structural biology, specifically the study of dynamic molecular machines that regulate ubiquitin signaling. My focus has been on maximizing the potential of cryo-EM and single-particle analysis to visualize and understand the functional implications of conformational dynamics in biochemical processes.

For much too long, research in the field has been limited to the studies of deliberately stabilized and thus mostly static structures, despite a clear appreciation that life at the molecular level is always far from equilibrium. A turning point for me came with the realization that most of these limitations can be overcome by adopting engineering and automation advances. Consequently, I have been corralling and leading multidisciplinary teams comprising biochemists, structural biologists, engineers, and data scientists to develop a versatile and user-friendly time-resolved sample preparation method for cryo-EM. Our setup features a miniaturized mixer and bioreactor that rapidly initiates and synchronizes biochemical reactions, which process is followed by sample application onto a cryo-EM grid without the need for manual operation. Iterating the procedure at increasing incubation times allows the visualization of a biochemical binding and/or enzymatic reaction as a time-lapse "movie" at near-atomic spatial resolution and millisecond time resolution.

My Visual Biochemistry Laboratory at the Francis Crick Institute in London is advancing further technology developments and consolidating them into an innovative biological research program with focal points around the molecular mechanisms of Cullin-RING ubiquitin E3 ligases, DNA damage repair by homologous recombination, and protein folding by the chaperonin GroEL. Ultimately, any method is as good as its applications, and a significant portion of my time is dedicated to fostering partnerships that broaden the scope of our time-resolved structural studies across a diverse range of biological processes. An equally exciting opportunity lies in exploring the method's utility for structure-guided drug discovery. For example, observing transient allosteric pockets could enable the design of conformationally selective drugs. Moreover, the field of targeted protein degradation relies on chemical modalities that stabilize otherwise very short-lived protein-protein interaction interfaces. Time-resolved cryo-EM offers a unique opportunity to kinetically trap and visualize such interactions, enabling computational chemistry and protein design approaches to fine-tune interaction affinities for therapeutic benefits. It is an immense privilege to work with a talented team and outstanding collaborators and to contribute to the convergence of technological innovation and basic biology and translational research topics.

Clare F. Megarity
University of Manchester

Enzyme cascades entrapped, crowded, and electrified

My journey to become a scientist was unconventional. At school, I was drawn to science and art; I chose art and obtained a degree in Fine Art painting from the University of Ulster. My interest in science did not leave me, though, and so I returned to university—this time, Queen's University Belfast for a degree in Biochemistry immediately followed by a PhD.

I carried out my PhD in the laboratory of Professor David Timson, a classical biochemist/enzymologist. Using rational enzyme engineering, I unraveled a mechanism of negative cooperativity (the sequential decrease in ligand/drug affinity to an enzyme) and showed that it involved motions propagated through a pathway traversing the enzyme connecting two active sites.

I first encountered protein film electrochemistry—studying enzymes by direct electrochemistry—in a conference lecture by Professor Fraser Armstrong (University of Oxford) and, as a PhD student in enzymology, was amazed at how redox enzymes could be driven directly by electricity! I joined Professor Armstrong's laboratory in my first postdoc position and studied terminal enzymes of photosynthetic electron-transfer chains—two hydrogenases and ferredoxin $NADP^+$ reductase (FNR).

Electrochemistry connects electricity to the natural world. It is powerful because it brings the dimension of thermodynamic driving force (electrical potential)—and, importantly, its control—to redox enzymes and measures their rate directly as electric current. As I watched my first cyclic voltammogram trace out the potential-dependent rate of a hydrogenase, I was hooked. By investigating active site H-cluster assembly in di-iron hydrogenases, I revealed an intermediate state trapped at highly reducing potentials, which led to a mechanistic model.

A novel platform called the Electrochemical Leaf (e-Leaf) resulted from my work on FNR, the photosynthetic enzyme that converts light-energised electrons to hydride on NADPH, biology's reducing cofactor. In the e-Leaf, enzymes (one of which must be FNR) are entrapped and crowded into a porous metal oxide electrode, where, under potential control, electrons tunnel bidirectionally between the electrode and the flavin in FNR, driving it to catalyze the interconversion of NADP(H). This crucial step is the gateway to electrochemically drive myriad enzyme cascades through the recycling of NADP(H) between FNR and one of the cascade enzymes. Multi-enzyme pathways previously inaccessible to electrochemistry become amenable to electrochemical control while functioning under crowded and entrapped conditions similar to those in nature. This powerful combination makes the e-Leaf interactive and has led to an exponential trajectory of discovery. Examples include reversibly driving a five-enzyme cascade (that incorporated CO_2 into a complex molecule) with CO_2 produced *in situ* by co-entrapped carbonic anhydrase, exploitation of directional control to create a deracemiser, measurement of drug binding kinetics to a human enzyme driven continuously under steady state, and extension to kinases for confocal recycling of NADP(H) and ATP.

I established my laboratory in the University of Manchester in 2022. I am creating new electrochemical platforms to exploit the crowded entrapment of enzyme cascades and their electrochemical control, and I am taking the e-Leaf in new directions for fundamental discovery in antimicrobial resistance, intricate synthesis, and metabolic disease.

Helen Ginn
Deutsches Elektronen-Synchrotron & University of Hamburg

Exploring the symbiosis between structural biology and computation

I stumbled upon my DPhil project in a rather unusual and interesting way. The summer after the launch of the iPad, I began an undergraduate internship in STRUBI at the University of Oxford. During the day I was dipping my toes into crystal structure refinement, and by night I indulged myself in a private side project: developing a functional protein molecular viewer (CMol) for the iPhone and newly released iPad. Somehow, upon release, the app quickly found its way onto my future DPhil supervisor's iPhone. Thanks to the keen eye of his colleagues who put two and two together, we were introduced. Despite loving the colourful landscape of protein structure, I was hesitant to pursue structural biology, as I worried it was somewhat observational and felt it rarely got around to actually solving a problem. Despite these concerns, I found myself starting an immensely enjoyable DPhil in Structural Biology at Oxford with Prof. David Stuart in 2013.

This gave me an opportunity to learn the ropes of scientific algorithm development. More importantly, it gave me the space to discover that the computational side of biochemistry was not a closed book, regardless of what my undergraduate lecturers had led me to believe. I developed algorithms to improve serial femtosecond data reduction techniques for X-ray free electron laser macromolecular structural studies. However, although I was able to tap into programming knowledge I had picked up during childhood, the lack of rigorous training in software development meant that my work was rough and largely inaccessible to others, a lesson I had to learn from.

I was in a very fortunate position after my DPhil to have a post-doctoral research associate position at Diamond Light Source Ltd. developing and exploring new methods to treat macromolecular models in structural biology. My research took an orthogonal turn at the beginning of the pandemic. It turns out that unifying and quantifying many lines of immunological and structural data on SARS-CoV-2 was in itself a full-time job as part of a formidable team of wet lab scientists at the University of

Oxford investigating the interplay between humoral immunity and new variants. This broadened my horizons and underlined the importance of exposure to new techniques and algorithmic ideas, which one can then use to tackle old problems.

After this period, emboldened by the lessons learned from this form of integrated structural biology, I felt there was room for development to overcome the problem that nearly turned me away altogether. This desire to not only observe but manipulate protein behavior came at the time of the release of AlphaFold and RosettaFold, opening up a new frontier of protein science. I am delighted to now pursue this research with the support of a Helmholtz Young Investigator fellowship and a joint W2 professorship position at DESY and the University of Hamburg. I have also tapped into combining art, software, game, and GUI design to communicate scientific ideas in a way I hope will accelerate progress toward a programmable world of proteins.

Patrick Rabe
University of Oxford

Molecules in motion: Understanding catalysis is our inspiration

The intricate chemical mechanisms governing enzyme reactions crucial to biological processes are largely unspecified, and unraveling them is my passion. I am particularly fascinated by how these molecular catalysts are fine-tuned to drive essential biological functions. Throughout my academic journey, my relentless pursuit toward the elucidation of the details of enzymatic reactions has led my research into unexplored territories.

During my early steps in research, I delved into biosynthetic studies of novel terpene synthases sourced from bacteria, fungi, and social ameba. By employing a range of spectroscopic techniques, including NMR and mass spectrometry, I meticulously characterized these enzymes using synthesized isotopically labeled compounds. This interdisciplinary approach—spanning biochemistry, synthetic organic chemistry, and spectroscopy—laid the foundation for my subsequent postdoctoral work.

During my postdoctoral studies at the University of Oxford, for which I was awarded a Leopoldina fellowship, my focus shifted to the elucidation of mechanistic details of metallo-enzymes, including microbial and human oxygenases. Notably, my investigations included mechanistic inquiries of Isopenicillin N synthase, a key enzyme in β-lactam biosynthesis alongside other 2OG-dependent oxygenases. By expanding my scientific repertoire into time-resolved crystallography and spectroscopy, I gained profound insights into the dynamic interplay governing enzyme catalysis.

Recently I was awarded a Wellcome Career Development Award, with which I have established an independent research group at the University of Oxford; we will apply the so-called "molecular movie" approaches to diverse enzymes. In my group, our research focuses on oxygenases including those involved in bioluminescence, such as luciferases. Leveraging cutting-edge technologies at Diamond Light Source and X-ray free electron laser facilities, we scrutinize enzyme reactions *in crystallo* and complement those findings with comprehensive solution studies employing NMR, mass spectrometry, UV/VIS, EPR, and luminescence-based methods—following our guiding principle, ‘‘Understanding catalysis is our inspiration.’’

As a visiting researcher at Diamond Light Source, my group is involved in innovative method development focusing on rapid O_2-evolving technologies for time-resolved crystallography. Our efforts span biological and chemical strategies to rapidly generate O_2 alongside the synthesis of metal photocages, with the goal of promoting rapid and homogeneous reaction initiation *in crystallo*.

Given the interdisciplinary nature of our pursuits, collaboration lies at the heart of our research ethos. Our work fosters fruitful partnerships within the Oxford community and beyond, engaging with leading experts worldwide. These collaborations are pivotal in addressing the fundamental scientific queries surrounding catalytic control and dynamics during macromolecular catalysis, underscoring the collective endeavor that is driving scientific advancement.

Alice R. Walker
Wayne State University, Detroit

Simulations from photons to proteins for rational design

I studied chemistry at the University of Michigan-Dearborn as an undergraduate student and worked in industry for two years as an analytical chemist before my PhD studies at University of North Texas. There, I studied combined quantum mechanics/molecular mechanics method development and classical force field protein simulations. My work focused on applying computational chemistry and dynamics to explore structure-function relationships, particularly for mechanisms of DNA repair in the AlkB family and in DNA polymerases. Much of my work involved collaborations with experimental groups that used FRET to probe the dynamics of proteins, and I became fascinated by the idea of interpreting such experiments by simulating fluorescence directly. I then moved to Stanford University for my postdoctoral research, where I expanded into excited state dynamics simulations of both proteins and small molecules of biological interest. Here, I also expanded into GPU-accelerated large-scale QM/MM simulations that probed new biochemical phenomena and enhanced studies of structure-function relationships: for example, corroborating X-FEL data on isocyanate hydratase.

In 2021, I began my independent career as an assistant professor at Wayne State University in Detroit, Michigan, USA. Here, my lab has taken a multiscale approach to investigating complex biological systems, doing method development for force field parametrization, automated workflows, classical dynamics, QM/MM, and excited state dynamics. We combine these techniques to understand complex systems: for example, simulating the diffusion of sulfate ions around a fluorescent protein, and its overall motion, to improve its kinetic function and lower its pH sensitivity as a new *in vitro* sulfate sensor. Continuing in this vein, we have similarly applied various electronic structure methods to understanding the behavior of fluorescent proteins as they evolve on the excited state surface, comparing both crystallographic and spectroscopic data to interpret their mechanisms. This work has been applied to the study of fluorescent sensors for various ions, in which we determine key residues and mutations of interest.

With large-scale QM/MM simulations (>600 atoms described with QM), we corroborate high resolution X-FEL crystallographic data from structure to excited state dynamics. While the techniques of our work are consistent, we apply them to an array of systems to investigate their structure-function relationships. For example, we have recently probed the excited state dynamics of cam-P450 and demonstrated good agreement with key protein residue motions as the heme group evolves from an excited singlet to triplet to quintet state. Similarly, we have applied our techniques to understand unusual curvatures of residues at the dimer interface of isocyanide dehydrogenase, where a QM description is required for agreement with high-resolution crystal structures. Our work bridges unusual experimental phenomena and measured signals with high-level quantum mechanical simulations in order to probe new and fascinating biological systems.

Kartik Ayyer
Max Planck Institute for the Structure and Dynamics of Matter, Hamburg

Ultrafast structural dynamics without precise triggering

My main scientific interest is in imaging the dynamics of ultrafast processes in order to understand and eventually control them. The standard paradigm to achieve this has been the pump-probe experiment, where one starts the process at a known time using a pump pulse and then probes the time evolution of the system at different time delays. But many processes are initiated not by a strong external trigger pulse but rather by effects like temperature or diffusion, which are difficult to precisely control. There are efforts to make such dynamics more ‘‘triggerable’’ in order to make the stroboscopic movies, but I am interested in another approach, enabled by rapid advancements in two areas.

The first is the development of high repetition-rate X-ray free-electron lasers (XFELs). These sources, like the European XFEL in Germany and the LCLS-II in the USA, can produce 1,000s of images per second with atomic resolution and femtosecond time resolution, which is the timescale of fluctuations around room temperature. The pulses are also bright enough that one can obtain sufficient information from single-nanoscale objects to enable classification and sorting. Thus, these 2nd generation X-ray lasers enable one to study these dynamics without precise trigger pulses by just probing

a sufficiently large number of systems one at a time and reconstructing the so-called "molecular movie" *post facto* computationally.

This brings me to the other advancement, which is in machine learning, especially in generative networks. Using these techniques—like variational autoencoders, generative adversarial networks and vision transformers—one can learn the structural dynamics purely from the dataset, without training data. This requires two factors: sufficient sampling of the latent space to be studied and sufficient information from single patterns, both of which are now becoming possible.

My research work focuses on bringing these two things together to study problems in structural biology, condensed matter physics, and nanoscience. In my research group at the Max Planck Institute for the Structure and Dynamics of Matter (MPSD) in Hamburg, Germany, we design and lead experiments at these facilities but also do theoretical work testing new algorithms and measurement approaches. We study systems ranging from lead halide perovskites and plasmonic nanoparticles to viruses and protein cages.

The experiments themselves are inherently collaborative, involving domain experts in the systems under study but also experts in aspects like sample delivery and instrumentation, which are non-trivial challenges with these sources. Our role is in analyzing the terabytes of data to reconstruct the target dynamics and in the aspects of experimental design that are relevant to extracting the relevant information.

After obtaining my B.Tech. in Engineering Physics from IIT Delhi, I did my PhD at Cornell University in Physics in 2014, where I studied reconstruction algorithms toward biomolecular single particle imaging with XFELs. This then led to a postdoctoral position at Deutsches Elektronen Synchrotron (DESY), where I learned more of the experimental craft going into XFEL imaging experiments. In the process, I think I have become what Benjamin Nachman calls a *data physicist*: someone who sits at the interface between experimental and computational research in order to explore opportunities arising from better data analysis methods.

DECLARATION OF INTERESTS

C.F.M.: There are 2 patents related to the Electrochemical Leaf; one has been granted and another has been filed. US Patent 11,694,852 B2 was granted July 2023. This patent has also been filed in Europe under European patent application no. 17713411.1, and the European Patent office has sent us a Notice of Intention to Grant Second Patent, WO2024009074A1, filed in July 2023.

R.I.E.: Three patent applications related to time-resolved sample preparation for cryo-EM have been filed by the Francis Crick Institute.