SFB 1078

Protonation Dynamics in Protein Function

Colloquium

Mon, **April 24** 2023

15:15 – 17:30

Freie Universität Berlin
Physics Department
Lecture Hall B

(Arnimallee 14, 14195 Berlin-Dahlem)

Dr. Kirill Kovalev – European Molecular Biology Laboratory, Hamburg, DE

4D crystallography of microbial rhodopsins

Microbial rhodopsins constitute a large superfamily of light-sensitive membrane proteins. They allow small organisms to utilize solar energy for their survival. Upon light illumination microbial rhodopsins undergo a photocycle – a series of transformations through several metastable intermediates – finally returning to the initial dark state. The transitions within the photocycle last from femtoseconds up to seconds and dictate the function of the rhodopsin. Determination of the high-resolution structures of not only the ground, but also of intermediate states of microbial rhodopsin photocycle is vital for understanding of its molecular mechanism. In the talk I will present our latest results obtained with 4D crystallography approach, including time-resolved serial millisecond crystallography, for the studies of various microbial rhodopsins, such as light-driven sodium and inward proton pumps. I will also describe our recent experience in use of single-particle cryo-electron microscopy as a complementary technique for the structural investigations of rhodopsins.

Prof. Frauke Gräter – Heidelberg Institute for Theoretical Studies, University of Heidelberg, DE

A protein material or a mega-enzyme? How collagen tames proton-coupled electron transfer

Proteins like collagen are perpetually subjected to mechanical forces. We have known for nearly a century that a high mechanical load on polymer materials – be it a shoe sole or rubber band – causes the rupture of chemical bonds and generates mechanoradicals. We uncovered the very same mechanism in biology: radicals form by stretching collagen, the major protein material of our body [1]. Using experiments in conjunction with simulations and machine learning we show how collagen tames its radicals and converts them to oxidative stress. Mechanically produced radicals rapidly migrate, through controlled protein-coupled electron transfer reactions, to specific amino acids in collagen that stabilize the radicals and thereby act as radical sinks [2]. We propose mechanoradicals and their transfer reactions in collagen as a theme potentially involved in tissue ageing and disease.

[1] Rennekamp et al, Nat Comm, in press, 2023, https://www.biorxiv.org/content/10.1101/2022.10.17.512491v2
[2] Kurth et al, Angewandte, in press, 2023, https://onlinelibrary.wiley.com/doi/10.1002/anie.202216610

Coffee, tea, and snacks are available during the break from 16:15 – 16:30.

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SFB 1078

Protonation Dynamics in Protein Function

Mon, **May 22 2023**

15:15 – 17:30

Freie Universität Berlin
SupraFAB

(Altensteinstr. 23a, 14195 Berlin)

Colloquium

Dr. David Buhrke – Institute for Biology, Humboldt Universität, Berlin, DE

Understanding the Cyanobacteriochrome Slr-g3 with advanced IR spectroscopy

Red/green cyanobacteriochromes like Slr-g3 bind open-chain tetrapyrroles as light-sensitive chromophores and are closely related to phytochromes. They can be photo-switched between a red-absorbing (Pr) and green-absorbing (Pg) form in a bistable and reversible manner. In my talk I will discuss how the molecular details of this photoswitching process can be understood by applying advanced IR spectroscopic methods. We applied time resolved linear and 2D IR spectroscopy to track the sequence of photocycle intermediates over many orders in magnitude in time, from the excited state decay in picoseconds to the formation of the final photoproduct in milliseconds. In the conversion from Pg to Pr form we have revealed new intermediates which precedes the Pr formation. In addition, stationary and transient 2D IR experiments measured the vibrational couplings between different groups of the chromophore among each other and with the protein. Finally, anharmonic QM/MM calculations are shown to be in good agreement with experimental 2D IR spectra of the initial and the final state of the photocycle and explain the coupling pattern.

Prof. Marilyn Gunner – City College of New York, NY, USA

How the multiplicity of protonation states in proteins supports proton transfers

Proteins are known to exist in a distribution of conformations, but the distribution of protonation states in the equilibrium ensemble is under appreciated. Recent developments in the MCCE program have enabled the analysis of protonation and conformation microstates in Monte Carlo sampling, providing insights into the diverse protonation states that exist in proteins.

Proton pumps contain proton loading sites, which undergo significant changes in proton affinity (>5 pH unit shifts) to cycle through proton-bound and released states in their reaction cycle. In this discussion, I will explore how clusters of amino acids, with multiple possible protonation states, facilitate efficient proton pumping. I will also describe how Proton Loading Sites that are made up of clusters of amino acids are often embedded in complex proton transfer networks.

Coffee and tea are available during the break from 16:15 – 16:30.

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SFB 1078

Protonation Dynamics in Protein Function

Thurs, **June 8 2023**

15:15 – 16:30

Freie Universität Berlin
SupraFAB

(Altensteinstr. 23a, 14195 Berlin-Dahlem)

Colloquium

Prof. Ronald Clarke – Department of Chemistry, University of Sydney, Australia

Hidden Secrets of Ion Pump Function

The Na^+,K^+-pump (or Na^+,K^+-ATPase) is one of the most important energy-transforming systems of animal physiology. It utilises the free energy of adenosine triphosphate (ATP) hydrolysis to do the electrical work of pumping Na^+ and K^+ ions against their electrochemical potential gradients across the plasma membrane of all living animal cells. In this way the chemical energy of ATP is transformed into a non-equilibrium Na^+ concentration gradient across the membrane as a store of energy [1]. For the discovery of the Na^+,K^+-pump [2], Jens Christian Skou of the University of Aarhus, Denmark, received the 1997 Nobel Prize in Chemistry. The energy stored in the Na^+ gradient is utilised as an energy source to drive numerous vital physiological processes. These include nerve impulse propagation, muscle contraction, and nutrient reabsorption in the kidney.

The Na^+,K^+-pump is an integral membrane protein. Since 2007 several crystal structures of the protein have been determined by X-ray crystallography and cryo-electron-microscopy [3]. However, none of the published structural studies have been able to resolve the protein's intrinsically disordered N-terminus, and hence the structures have provided no information on the role that this region of the enzyme plays in the ion pumping mechanism. Information from biophysical and bioinformatic analyses is mounting [4], however, that the N-terminus is involved in a crucial lipid-protein interaction, whereby positively charged lysine residues of the N-terminus interact electrostatically with the lipid head-groups of negatively charged phosphatidylserine (PS) molecules on the cytoplasmic face of the plasma membrane. PS is concentrated in the cytoplasmic leaflet of the plasma membrane of all animal cells via a phospholipid flippase embedded in the membrane, which also uses the energy derived from ATP hydrolysis to maintain transverse lipid asymmetry across the plasma membrane. The electrostatic interaction between the N-terminus and the membrane provides an ideal regulatory mechanism for the Na^+,K^+-pump. A decrease in charge of the N-terminus by the phosphorylation of conserved serine and tyrosine residues allows for an "electrostatic switch" mechanism, whereby the N-terminus is released from the membrane, altering the overall conformation of the protein, and modulating its ion pumping activity.

[1] R. J. Clarke and X. Fan, Clin. Exp. Pharmacol. Physiol. 2011, 38, 726.
[2] J. C. Skou, Biochim. Biophys. Acta 1957, 23, 394.
[3] J. P. Morth et al, Nature 2007, 450, 1043.
[4] E.-L. Blayney et al, Int. J. Mol. Sci. 2023, 24, 67.

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SFB 1078

Protonation Dynamics in Protein Function

Mon, **June 12, 2023**

15:15 – 16:30

Freie Universität Berlin
Physics Department
Hörsaal B

(Arnimallee 14, 14195 Berlin-Dahlem)

Colloquium

Prof. Renee Frontiera – Department of Chemistry, University of Minnesota, Minneapolis, MN, USA

Super-resolution and chemically-specific Raman imaging

Super-resolution microscopy techniques have revolutionized imaging and found numerous applications in probing and understanding biological processes on nanometer length scales, due to their unprecedented ability to break the optical diffraction limit by several orders of magnitude. However, most current super-resolution microscopies require fluorophore-labeled samples, which are highly subject to degradation, limiting the scope and time resolution of super-resolution measurements. Our lab has developed an imaging technique capable of achieving similar spatial resolution without the need for external fluorescent labels, by combining aspects of stimulated Raman microscopy with stimulated emission depletion microscopy. This microscope provides far-field Raman images with resolution well below the diffraction limit. In addition to providing nanoscale information on chemical composition, the technique also enables interrogation of local environmental impacts on chemical reaction dynamics. We have used this chemical specificity of Raman spectroscopy to uncover the role of intracellular proteins in mediating the unpackaging of polymeric gene therapy delivery vehicles. This trackable delivery system should be broadly applicable to study nucleic acid delivery mechanisms. Overall, Raman chemical imaging is a powerful approach for sub-diffraction resolution as well as for providing mechanistic insights in complex environments.

About Prof. Renee Frontiera

Renee R. Frontiera is the Northrop Professor of Chemistry at the University of Minnesota. Her research group uses Raman spectroscopic techniques to examine chemical composition and chemical reaction dynamics on nanometer length scales and ultrafast time scales. She received her Ph.D. in 2009 from the University of California – Berkeley in Richard Mathies' group, and did her postdoctoral research with Richard Van Duyne. Her research group at the University of Minnesota was founded in 2013, and she is the recent recipient of an NSF CAREER award, a DOE Early Career award, and an NIH Maximizing Investigators' Research Award (MIRA). She was named one of *Chemical & Engineering News*'s "Talented 12", and has won a Journal of Physical Chemistry Lectureship, the American Physical Society's "Future of Chemical Physics" lectureship, and a Camille Dreyfus Teacher-Scholar award.

Coffee and tea are available after the talk from 16:15 – 16:30.

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SFB 1078

Protonation Dynamics in Protein Function

Mon, **June 19, 2023**

15:15 – 16:30

Freie Universität Berlin
SupraFAB

(Altensteinstr. 23a, 14195 Berlin-Dahlem)

Colloquium

Dr. Chen Song – Institut für Analytische Chemie, Universität Leipzig, DE

Solid-state NMR on trehalose-embedded phytochromes

Phytochromes represent a diverse family of photoreceptors that enable plants and microorganisms to adapt to changes in ambient light environment. Common to all phytochromes is the temperatureregulated thermal reversion. Despite methodological advances, short-lived photoproducts of phytochromes remain challenging for biophysical techniques such as solid-state NMR that require repetitive measurements due to rapid thermal reversion. To inhibit this light-independent reaction, we recently developed a simple method for photoproduct stabilisation by incorporating the protein into amorphous trehalose glasses (TGs). The resulting trehalose matrices exhibit the outstanding efficacy in this regard for long periods of time (weeks) at room temperature. More importantly, the advantages of trehalose–protein glassy matrices also allow to isolate individual transient reaction intermediates and to measure them separately under non-cryogenic conditions. In this talk, I will provide an overview of our recent solid-state NMR results of the TG-trapped photocycle states of a phytochrome-related cyanobacteriochrome which reflect changes in the local electronic structure, geometry, dynamics, and charge distribution of the chromophore during the photocycle.

Coffee and tea are available after the talk from 16:15 – 16:30.

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SFB 1078

Protonation Dynamics in Protein Function

Mon, **July 10, 2023**

15:15 – 17:30

Freie Universität Berlin
SupraFAB

(Altensteinstr. 23a, 14195 Berlin-Dahlem)

Colloquium

Prof. Ana Damjanovic - John Hopkins University, Baltimore, MD, USA

New mechanism of ion channel selectivity

Prof. Clemens Glaubitz - Goethe University Frankfurt, Frankfurt am Main, DE

Light-induced adaptation, oligomer- and lipid interactions of membrane proteins

The Glaubitz Lab works on photoreceptors (1-4), membrane-based photochemical tools (5) as well as ABC transporters and GPCRs (6,7). The methodological approach is centered around solid-state NMR spectroscopy in combination with dynamic nuclear polarization. This research lecture will focus on our work on native and artificial photoswitches in the form of light-driven ion pumps and photolipids. Microbial rhodopsins such as proteorhodopsins show a high degree of environmental adaptation by optimizing their absorption maximum. It will be demonstrated how biochemical data in combination with solid-state NMR and QM/MM approaches have been used to disentangle the factors which determine the remarkable sequence-dependent color shifts in this protein family, which is required to maintain their ion pumping activity (1). Furthermore, specific, light-induced interactions at the protomer interfaces in these pentameric complexes during the photocycle will be described and discussed (2-4). In the context of artificial photoswitches, the effect of photoswitchable lipids (AzoPC) onto lipid membranes and functional structural properties of embedded proteins will be described (5).

(1) Mao, J. et al. submitted & Mao, J. et al. Structural basis of the green-blue color switching in proteorhodopsin as determined by NMR spectroscopy. J. Am. Chem. Soc. 136, 17578-17590, doi:10.1021/ja5097946 (2014).
(2) Jakdetchai, O. et al. Probing the photointermediates of light-driven sodium ion pump KR2 by DNP-enhanced solid-state NMR. Sci Adv 7, doi:10.1126/sciadv.abf4213 (2021).
(3) Mehler, M. et al. Chromophore Distortions in Photointermediates of Proteorhodopsin Visualized by Dynamic Nuclear Polarization-Enhanced Solid-State NMR. J. Am. Chem. Soc. 139, 16143-16153, doi:10.1021/jacs.7b05061 (2017).
(4) Maciejko, J., Kaur, J., Becker-Baldus, J. & Glaubitz, C. Photocycle-dependent conformational changes in the proteorhodopsin cross-protomer Asp-His-Trp triad revealed by DNP-enhanced MAS-NMR. Proc Natl Acad Sci U S A 116, 8342-8349, doi:10.1073/pnas.1817665116 (2019).
(5) Doroudgar, M., Morstein, J., Becker-Baldus, J., Trauner, D. & Glaubitz, C. How Photoswitchable Lipids Affect the Order and Dynamics of Lipid Bilayers and Embedded Proteins. J. Am. Chem. Soc. 143, 9515-9528, doi:10.1021/jacs.1c03524 (2021).
(6) Kaur, H. et al. Coupled ATPase-adenylate kinase activity in ABC transporters. Nature communications 7, 13864, doi:10.1038/ncomms13864 (2016).
(7) Joedicke, L. et al. The molecular basis of subtype selectivity of human kinin G- protein-coupled receptors. Nat. Chem. Biol. 14, 284-290, doi:10.1038/nchembio.2551 (2018).

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