BP 52: Protein structure and dynamics II

Time: Friday 9:30–11:45 Location: H 1028

Invited Talk BP 52.1 Fri 9:30 H 1028

Biophysics of light-activated ion transporters — Arend Vogt, Jonas Wietek, and •Peter Hegemann — Humboldt-Universität zu Berlin

The field of optogenetics utilizes light-activated ion transporters as channelrhodopsins (ChRs) and Arch3 for specific neuronal activation or inactivation. Recently we characterized a light-driven proton pump CsR of the arctic alga Coccomyxa subellipsoidea. Owing to the fact that the photocurrents are very large in Xenopus oocytes, we have taken this advantage to analyze the function of individual positions at the extracellular side of the retinal Schiff base chromophore respective relevance for proton transport. Modification of the highly conserved proton shuttling residue R83 or its interaction partner Y57 strongly reduced the power of the pumps and converted CsR at moderate electrochemical load into an operational proton channel with inward or outward rectification depending on the replacement. We compared these proton selective channels with various natural and artificial light-activated channels (channelrhodopsins) that are selective for protons, sodium and chloride and we derived principle for light-depending gating, ion conductance and selectivity of microbial rhodopsins.

BP 52.2 Fri 10:00 H 1028

Electrochromic shift calculations reveal the structural changes between red- and green-sensitive rhodopsins — •Florimond Collette, Marcel Schmidt am Busch, and Thomas Renger — Institut für Theoretische Physik, Johannes Kepler Universität Linz, Altenberger Strasse 69, 4040 Linz, Austria

Rhodopsins are biological pigment-proteins found in photoreceptor cells of the retina. Within the framework of a quantum chemical/electrostatic calculation scheme that has recently been successfully applied to reveal the functional states of BLUF photoreceptors [1], we estimated absorption shifts of the retinal cofactor for a series of site-directed mutants. Our calculations accurately reproduce a series of spectroscopic data and eventually the variations of the maximal absorbance in the red- and green-sensitive visual pigments.

[1] F. Collette *et al.*, *J. Phys. Chem. B* **118**, 11109 (2014).

BP 52.3 Fri 10:15 H 1028

Photo-dynamics of photo-activated adenylyl cyclase NgPAC3 from the amoeboflagellate *Naegleria gruberi* **NEG-M strain** — •Alfons Penzkofer[1], Meenakshi Tanwar[2], Sinddu Kandoth Veetil[2], Suneel Kateriya[2], Manuela Stierl[3], and Peter Hegemann[3] — [1]Fakultät für Physik, Universität Regensburg, Universitätsstraße 31, D-93053 Regensburg, Germany — [2]Department of Biochemistry, University of Delhi South Campus, Benito Juarez Road, New Delhi 110021, India — [3]Institut für Biologie/Experimentelle Biophysik, Humboldt Universität zu Berlin, Invalidenstraße 42, D-10115 Berlin, Germany

The absorption and emission spectroscopic behavior of the photo-activated adenylyl cyclase NgPAC3 from the amoeboflagellate *Naegleria gruberi* NEG-M strain was studied [1]. The flavin cofactor was found to be partly fully oxidized and partly fully reduced. The typical BLUF domain (BLUF = Blue Light sensor Using Flavin) fully oxidized flavin absorption photo-cycle dynamics with about 14 nm flavin absorption red-shift in the signaling state was observed. The quantum efficiency of signaling state formation was determined to be $\phi_s = 0.66 \pm 0.03$. A bi-exponential signaling state recovery to the dark-adapted receptor state was observed with the time constants $\tau_{rec,f} = 275s$ (fraction 0.29) and $\tau_{rec,sl} = 45min$ (fraction 0.71). The thermal irreversible protein unfolding was studied and a protein melting temperature of $\vartheta_m \approx 50°C$ was found. NgPAC3 showed light-gated adenylate cyclase activity upon illumination with blue light.

[1] A.Penzkofer et al., J.Photochem.Photobiol.A:Chem. 287(2014)19.

15 min break

BP 52.4 Fri 10:45 H 1028

Conformational Change of the Neuronal Calcium Sensor GCAP1 — •Jörg Robin, Jens Brauer, Stefan Sulmann, Christoph Lienau, and Karl-Wilhelm Koch — Carl von Ossietzky Universität, 26129 Oldenburg

The ability of photoreceptor cells to adjust to changing light conditions on a millisecond timescale relies on a well balanced interplay of two second messengers, cGMP and calcium [1]. Upon decrease of intracellular calcium due to closure of cGMP gated ion channels the guanylate cyclase is stimulated to replenish cGMP. Two sensor proteins, GCAP1 and GCAP2, regulate the activity of the guanylate cyclase in a sequential step by step order mechanism [2] by conformational change due to binding of calcium. This conformational change has recently been investigated by time-resolved fluorescence spectroscopy for GCAP2 [3]. In this study, we have site-specifically labelled each cysteine residue in GCAP1 mutants by the fluorescent dye Alexa647 and probed its local environment via time-resolved fluorescence spectroscopy. We have observed both an increase in fluorescence lifetime and in rotational correlation time for the apo compared to the calcium bound state. Our findings are supported by analysing the motional restriction of the dye in a wobbling-in-a-cone model and by molecular dynamics simulations. In conclusion, GCAP1 undergoes conformational change, but distinctly different from GCAP2. [1] Pugh, E. N. Jr. & Lamb, T. D. Handb. Biol. Phys. 3, 183 (2000) [2] Koch, K-W. & Dell'Orco, D. ACS Chem. Neurosci. 4, 909 (2013) [3] Kollmann, H. et al. ACS Chem. Biol. 7, 1006 (2012)

BP 52.5 Fri 11:00 H 1028

Phase behavior of dense lysozyme solutions — •Julian Schulze[1], Johannes Möller[1], Michael Paulus[1], Julia Nase[1], Metin Tolan[1], and Roland Winter[2] — [1]Fakultät Physik/Delta, Technische Universität Dortmund, 44221 Dortmund , Germany — [2]Fakultät für Chemie und Chemische Biologie, Technische Universität Dortmund, 44221 Dortmund, Germany

In previous studies, small angle X-ray scattering (SAXS) in combination with liquid-state theoretical approaches and DLVO theory was used to study the intermolecular interaction potential, V(r), of lysozyme solutions under the influence of varying environmental conditions as protein concentration c, temperature T, pressure p or salt concentration I. While the repulsive Coulomb term of the DLVO potential remains almost constant as a function of p, the depth of the attractive part, J(p), exhibits a non-monotonic p-dependence with a minimum at 1.5 kbar at selected T. Adding 0.5 M NaCl leads to more prominent short range interactions, especially at high c and low T. Here, the homogeneous protein solution becomes turbid due to formation of a metastable liquid-liquid phase separation (LLPS) region, where lysozyme forms small droplets of high concentration within the more dilute liquid phase. At elevated pressures, this l-l phase separation is suppressed, but due to the non-monotonic behavior of J(p), a further pressure increase leads to a re-entrant LLPS. The analysis of the SAXS data allows the construction of the c-p-T phase diagram of lysozyme solutions. As crystallization occurs in this c-p-T region as well, the diagram will help optimize crystallization conditions.

BP 52.6 Fri 11:15 H 1028

Exploring the multiscale signaling behavior of phototropin1 from Chlamydomonas reinhardtii using a full-residue space kinetic Monte Carlo molecular dynamics technique — Emanuel Peter, Bernhard Dick, Ivan Stambolic, and •Stephan Baeurle — Institut für Physikalische und Theoretische Chemie, Universität Regensburg, 93040 Regensburg

Devising analysis tools for elucidating the regulatory mechanism of complex enzymes has been a challenging task for many decades. It generally requires the determination of the structural-dynamic information of protein solvent systems far from equilibrium over multiple length and time scales, which is still difficult both theoretically and experimentally. To cope with the problem, we introduce a full-residue space multiscale simulation method [1] based on a combination of the kinetic Monte Carlo and molecular dynamics techniques, in which the rates of the rate-determining processes are evaluated from a biomolecular forcefield on the fly during the simulation run by taking into account the full space of residues. To demonstrate its reliability and efficiency, we explore the light-induced functional behavior of the full-length phototropin1 from Chlamydomonas reinhardtii (Cr-phot1). Our results demonstrate that in the signaling state the kinase is activated through the disruption of the Jalpha-helix from the light-oxygen-voltage-2-sensitive (LOV2) domain, which is followed by a stretching of the activation loop and broadening of the catalytic cleft of the ki-

nase. Literature: [1] E. Peter, B. Dick, I. Stambolic, S.A. Baeurle, Prot. Struct. Funct. Bioinf. 82, 2018 (2014).

BP 52.7 Fri 11:30 H 1028

Dynamics of the Orange Carotenoid Protein (OCP) under Photoactivation — Evgeny Maksimov[2], •Franz-Josef Schmitt[1], Thomas Friedrich[1], and Vladimir Paschenko[2] — [1]Institute of Chemistry, Bioenergetics, TU Berlin, Straße des 17. Juni 135, D-10623 Berlin, Germany — [2]Department of Biophysics, Biology Faculty, Lomonosov Moscow State University, 119991 Moscow, Russia

The cyanobacterium Synechocystis sp. PCC6803 contains a photoswitchable protein, the orange carotenoid protein (OCP) which undergoes conformational changes under illumination in the blue spectral regime. After activation by light the OCP binds to the membrane extrinsic phycobilisome (PBS) complexes and leads to non-photochemical quenching (NPQ) of the PBS fluorescence reducing the flow of energy into the photosystems under high light conditions as protection mechanism. Time- and wavelength-resolved fluorescence spectroscopy was used to image the dynamics during the photoinduced conformation change and subsequent change in the NPQ efficiency. We suppose that there is a β-ring rotation of the echinenone during photoactivation of OCP that leads to a significant red shift of the absorption spectrum. A distance change between Tyr-201, Trp-288 and the keto terminus of the pigment might break H-bonds between the protein and the chromophore which tilt the β-ring out of plane in the inactive form of OCP.