microscope. Mobile fluorescent spots are observed, and their intensity and location have been tracked by fitting a 2D Gaussian function to successive frames. Analysis of the data shows that diffusion of TatA-eGFP is heterogeneous, and that the average diffusion coefficient of fluorescent TatA particles decreases when excess substrate is expressed. The latter suggests that TatA forms larger complexes upon substrate binding.

2025-Plat

Opening the SecYEG Protein Translocon

Ana Nicoleta Bondar, Douglas J. Tobias, Stephen H. White.
University of California at Irvine, Irvine, CA, USA.

The Sec61/SecYEG translocon is the central component of the major biosynthetic pathway for membrane and secreted proteins. To incorporate membrane proteins into the endoplasmic reticulum membrane of eukaryotes (Sec61), or into the plasma membrane of prokaryotes (SecYEG), the translocon relies on the opening of a lateral helical gate formed by transmembrane (TM) helices TM2 and TM7. The crystallographic structure of the closed state of the translocon (B. van der Berg et al, Nature 427, 36-44, 2003) solved in the absence of bound signal peptide provides valuable information on possible scenarios of membrane protein insertion. Nevertheless, the sequence of structural rearrangements that leads to the opening of the translocon and the geometry of the translocon in its open state remain unknown. To derive information on the structure and dynamics of possible open conformations of the SecYEG translocon, we performed prolonged molecular dynamics simulations of the SecYEG translocon in which we mutated residues determined to participate in stabilizing interactions of the closed state. We have also performed a simulation of the SecYEG translocon with a bound signal peptide. The computations provide insights into interactions essential for keeping the lateral gate closed and how perturbations of these interactions cause rearrangements of the gate.

This work was supported by research grants from the National Institute for General Medical Sciences, the NIH National Center for Research Resources, and the National Science Foundation.

2026-Plat

Real Time ^{31}P and Steady State ^{19}F/^{13}C Solid State NMR on Integral Membrane Protein *E.coli* Diacylglycerol Kinase

Sandra J. Wollschlag, Ute A. Hellmich, Clemens Glaubitz.
Center for Biomolecular Magnetic Resonance and Institute for Biophysical Chemistry, Goethe University Frankfurt, Germany.

The integral membrane protein Diacylglycerol Kinase (DGK) from *E.coli* can be used as model system for prokaryotic kinases, playing an important role in microbial physiology. In its active conformation, DGK, consisting of three transmembrane helices, forms a homotrimer with one putative active site per monomer. DGK, as the smallest known kinase, catalyses the phosphorylation of diacylglycerol to phosphatidyl acid by utilizing MgATP at the interface membrane-cytoplasm. These features make DGK an attractive model protein for research on structure of membrane proteins in lipid bilayers, as well as on its function as a lipid regulator.

Solid-state NMR is a unique tool for the investigation of membrane proteins in their native environment and for probing enzymatic reactions regardless of their compartmentalization. For such experiments, amount of protein and quality of the sample preparation are crucial. Expression, purification, reconstitution and sample preparation were optimised, so that a sample of DGK in high quality became available in amounts necessary for ssNMR experiments, while maintaining its specific activity.

A ^{31}P Real Time MAS experiment was designed and implemented, to investigate for the first time, simultaneously, ATP hydrolysis and phosphorylation of a substrate analog by a membrane protein, inside the lipid bilayer. From these data, the rate constants of enzymatic activity were determined. Furthermore, inhibition experiments with orthovanadate, $(BeF_2)_x$ and AlF_3 were carried out. The inhibiting species was identified by ^{19}F MAS NMR. This method provides the opportunity to investigate the enzymatic mechanism in real time, with atomic resolution.

A first fingerprint of structural details were obtained with a 2D ^{13}C-^{13}C MAS ssNMR PDSD type experiment on a uniformly ^{13}C/^{15}N labelled sample of a thermostable mutant- and WT-DGK. Further insight into structural details will be obtained by selective labelling.

2027-Plat

BetP - Structure and Function of an Osmosensor and Transporter

Vera Ott[1], Sascha Nicklisch[1], Igor Borovykh[2], Heinz-Juergen Steinhoff[2], Susanne Ressl[3], Christine Ziegler[3], **Reinhard Kraemer[1]**.
[1]University of Koeln, Koeln, Germany, [2]University of Osnabrück, Osnabrueck, Germany, [3]Max-Planck-Institute of Biophysics, Frankfurt, Germany.

The Na+ coupled betaine uptake system BetP of Corynebacterium glutamicum comprises three functions of stimulus (osmostress) sensing, activity regulation and solute transport. Its 2D structure was recently solved by electron and its 3D structure by X-ray crystallography. Within a trimeric structure, each BetP monomer harbours both an N- and a C-terminal domain involved in stimulus sensing and intramolecular signal transduction. Factors contributing to the sensory and regulatory function of BetP are (i) the two terminal domains, (ii) K+ ions as an osmostress related stimulus, and (iii) interaction with the surrounding membrane.

We used several techniques to analyze the contribution of the terminal sensory domains to BetP function. By scanning mutagenesis we identified the significance of single amino acids and parts of the C-terminal domain of BetP. EPR spectroscopy was applied to determine the mobility or the C-terminal domain under different functional conditions and to measure intra- and intermolecular distances in BetP. The 3D structure of BetP finally revealed a putative crosstalk between the three monomers of BetP via their C-terminal domain. On the basis of these results we suggest a novel functional model of the terminal domains of BetP during its sensory and regulatory function.

2028-Plat

Atomic Force Spectroscopy Measures Light Activation And Transducer Binding Induced Structural Changes In The Sensory Rhodopsin II

Leoni Oberbarnscheidt[1], Swetlana Martell[2], Martin Engelhard[2], Filipp Oesterhelt[1].
[1]Heinrich-Heine Universitaet, Düsseldorf, Germany, [2]MPI, Dortmund, Germany.

Rhodopsins are a family of seven-helical transmembrane proteins responding to light. Sensory rhodopsin II (SRII) triggers two very different responses depending on the presence or absence of its transducer: Whereas light activation of the SRII-transducer-complex triggers a signalling cascade initiating the photophobic response of the bacterium, SRII alone acts as a proton-pump.

Using single molecule force spectroscopy we analysed the stability of SRII in dark and after light activation as well as in presence and absence of the transducer, which revealed a distinct pattern of changes in the protein stability. By improving the force spectroscopic data analysis we were able to predict the localisation of occuring forces within the protein chain with a resolution of about six amino acids.

Different regions showed up, where secondary structure elements of SRII are selectively stabilised or weakened by either light activation or transducer binding or both. Independent of the presence of the transducer light activation has a destabilizing effect in the middle of α-helix G. This suggests a loss in interactions between helices G and F, which would allow an outward tilt of α-helix F as previously observed. Additionally, the unfolding curves show an increased number of rupture events in the region of helix F upon transducer binding, which is most likely due to the formation of several interactions between α-helix F and TM2 of the transducer. Most interestingly, we found a loss of some of these interactions upon light activation, which might explain transducer activation and help to answer remaining questions concerning the precise molecular mechanism. Finally, in the absence of the transducer, destabilizing effects are observed at the cytoplasmic half of helix G, which might indicate its importance for the proton-pumping properties of SRII.

2029-Plat

Conformational Dynamics of PorB, a Helical Outer Membrane Protein from C. glutamicum: a Multi-scale MD Simulation Study

Syma Khalid.
University of Southampton, Southampton, United Kingdom.

PorB is one of the major porins found in the cell wall of C. glutamicum. Somewhat surprisingly, for an outer membrane protein, the recently determined X-ray structures of PorB monomers revealed a helical topology. A model for the pentameric protein was proposed base upon (1) the required polar interior and nonpolar exterior of the porin, (2) a recurring crystal packing contact around a 2-fold axis, (3) the assumption of a simple Cn symmetry, (4) the experimentally established electric conductivity and anion selectivity and (5) the generally observed shape of porin channels. The presence of divalent cations was thought to be required to balance the charged protein residues.

We have employed MD simulations to study the conformational dynamics of the putative PorB model. We have performed multiple, atomistic simulations of the protein in the presence and absence of divalent cations. Our simulations have allowed us to study specific protein-ion interactions and thus to speculate on the role of these ions. The structural role of the interdigitation of the N-terminal and C-terminal extensions has also been studied in detail. In addition, we have studied the conformational dynamics of the PorB pore and compared its flexibility to similar (in size) pores of barrel-shaped outer membrane proteins. We have employed coarse-grained MD simulations to determine the preferred location and orientation of PorB in phospholipid bilayers.

Furthermore, these simulations have also enabled us to study the deformation of the bilayer induced by the protein- we have compared this to the bilayer deformation induced by barrel-shaped outer membrane proteins of a similar size. Thus we present a multi-scale study of the putative model of PorB- one of only two bacterial outer membrane proteins known to have a helical topology.

2030-Plat

Gating-related Structural Dynamics in the Outer Vestibule of KcsA: A Functional and Spectroscopic Analysis

H. Raghuraman, Julio F. Cordero-Morales, Vishwanath Jogini, Eduardo Perozo.
Institute for Biophysical Dynamics, The University of Chicago, Chicago, IL, USA.

KcsA is activated by intracellular protons through a large conformational change in the inner helical-bundle gate. Further, the selectivity filter and surrounding structures play a crucial role as an inactivation gate in ion conduction. Here, we monitored the conformational changes in the outer-vestibule of KcsA during gating using electrophysiological and EPR measurements to gain insight on dynamic properties of these conformational fluctuations. pH-jump measurements show that Cd^{2+} facilitates the rate of inactivation of outer-vestibule mutant Y82C-KcsA, however, there is no effect of Cd^{2+} on the non-inactivating E71A/Y82C-KcsA. This suggests that the outer-vestibule has different conformations in the inactivated and non-inactivated states of KcsA. EPR mobility results show that upon opening the lower gate, Y82C undergoes a significant conformational change only in the E71A (non-inactivating) background and not in inactivated state (wild-type). This conformational change is also evident in tandem dimer Y82C constructs, even when the hydrogen-bond network at the selectivity filter is partially perturbed. Distance measurements using cw-EPR at low temperatures show that the diagonal distance between spin labels bound to tandem-dimer Y82C and E71A/Y82C-KcsA is ~12 Å when the lower gate is closed at pH7. Interestingly, the distance between the spin labels bound to Y82C is found to be 8 and 19 Å upon gating in inactivating and non-inactivating forms of KcsA. The change in the Y82C position could be related to the effects of Cd^{2+} on the rate of inactivation in different functional forms of KcsA. The diagonal inter-spin in the closed state is in excellent agreement with the distance seen in the crystal structure of the spin label linked to Y82C-KcsA in the closed state. These results are interpreted in terms of the conformational transitions in the outer-vestibule during activation and inactivation gating.