Membrane Protein Structure & Function II

1114-Pos Board B6
Structural Evidence for Functional Lipid Interactions in the Betaine Transporter BetP
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Bilayer lipids contribute greatly to the stability of membrane transporters and are crucially involved in their proper functioning. However, the molecular details of how these lipids affect membrane transport is limited to the few atomic resolution structures of channels and transporters revealing functional lipid interactions. As a consequence, despite their biological importance, bilayer lipid-protein interactions are rarely considered in the molecular description of a transport mechanism, which are difficult to detect in X-ray structures per se. Lipids are often depleted from the detergent-protein complex during isolation or appear too unordered to identify in the crystal. Overcoming these difficulties, we report here on a new structure of the osmotic stress-regulated betaine transporter BetP in complex with anionic lipids. Activity regulation in BetP depends strongly on the presence of negatively charged lipids. We observe eight fully resolved palmitoyl-oleoyl phosphatidyl glycerol (PG) lipids bound to one BetP trimer, mimicking parts of the membrane leaflets. Our data reveal that the PG lipids interact with key residues in transport and regulation. Lipids, while likely being involved in the correct assembly of the BetP trimer also offer important communication sites between the protomers that might be required during stress sensing and transport regulation. The lipid-protein interactions observed in BetP reiterate the influence that the surrounding membrane can have on membrane protein function and urge a more holistic approach towards understanding membrane transport, especially for LeuT-like fold transporters.

1115-Pos Board B7
The Photoreceptor Rhodopsin is a Constitutively Active Lipid Flippase
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The transbilayer transport of lipids is essential in both eukaryotes and prokaryotes, yet the spontaneous rate of flipping is too slow to support cellular life. Physiologically relevant rates of flipping are achieved through the activity of two classes of lipid transporters. The first couples ATP hydrolysis to unidirectional lipid flipping in order to maintain asymmetric membranes, such as the eukaryotic plasma membrane. The second class of transporters facilitates bidirectional, ATP-independent movement of lipids across biogenic and specialized membranes. In a recent report (Menon et al. (2011) Curr. Biol. 21, 149-153) we identified opsin as the first ATP-independent flippase. We now report that opsin's flippase activity is constitutive, and not linked to its established function as a light sensor. We expressed thermostable variants of opsin in COS-7 cells and assayed activity of the purified proteins in a reconstituted system. We tested three structurally discrete signaling states of opsin: dark-adapted rhodopsin with the endogenous inverse agonist 9-cis retinal; the meta-rhodopsin II intermediate containing the agonist all-trans retinal in the constitutively active M257Y background; and the ligand-free light-adapted opsin. All constructs demonstrated rapid ($t_{1/2} < 10$ s), ATP-independent flip-flop of zwitterionic phospholipid probes in both dark- and light-adapted conditions. These results suggest that the flippase activity of opsin, and likely other Type-A GPCRs such as the β1-adrenergic receptor which we also previously showed to have flippase activity, is localized to the relatively immobile transmembrane helices 1 - 4, or the amphipathic helix 8. These data, as well as the results of ongoing experiments on the flippase activity of dynamically constrained rhodopsin constructs, will be presented.
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1116-Pos Board B8
Production, Characterization and Refolding of G-Protein Coupled CB2 Receptor from Inclusion Bodies
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The human Cannabinoid Receptor 2 (CB2) is a Rhodopsin family G protein coupled receptor (GPCR) expressed highly in immune cells. Recent studies demonstrate the role of the CB2 receptor in attenuating bone cancer induced pain, reducing microglial activation in Alzheimer's disease and regulation of bone mass in osteoporosis. In this regard, detailed structure function knowledge of the mechanism of receptor activation will allow understanding of the ligand binding site, resolve the molecular basis of ligand functionality and also lead to the rational designing of more potent ligand. However majority of biophysical techniques which can be employed require large amounts of purified, native CB2. This poses the one of the biggest challenge for further studies. To this end we are using TrpLE, an N' terminal fusion partner with the CB2 receptor to direct its expression to inclusion bodies in the *E. coli*. High yielding GPCR inclusion bodies were solubilized and extracted to homogeneity in the presence of denaturing detergent concentrations. Inactive receptors were analyzed for its ligand binding ability and extent of secondary structure. The inactive receptor fusion protein was detergent exchanged for the removal of fusion partner by factor Xa cleavage. Purified CB2 receptor inclusion bodies are currently under refolding trials using detergents (DDM), lipids (POPC/POPS) and amphipols (A8-35) alone and in combination. These studies are being conducted in parallel and will allow determining the best refolding environment for the membrane protein receptor. Understanding of the refolding process would greatly enhance CB2 structure function studies which are currently limited by protein availability and facilitate understanding of protein lipid interactions during membrane protein refolding and/or stabilization.
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1117-Pos Board B9
Characterizing Interactions between Surfactants and Membrane Proteins with Special Emphasis on Crystallization
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In spite of the importance of membrane proteins (MPs) in many essential biochemical pathways and cell functions, there are a few structures of this class of proteins in the Protein Data Bank. One of the major bottlenecks to a greater number of solved structures is obtaining well-diffracting crystals. Crystallization remains largely a trial-and-error process, due to the complexity of crystallization solution conditions that require surfactants to solubilize MPs, and also different types of precipitants to induce crystal formation. As such, developing a more systematic crystallization strategy based on insight into the effect of interactions between solution components can be an important step for obtaining new structures of MPs.
The approach of the current research to improving MP crystallization emphasizes the importance of surfactant phase behavior, self-aggregation in the form of micelles, as well as surfactant interactions with MPs and common precipitating agents, such as poly(ethylene) glycol (PEG). Our efforts are aimed at obtaining a better picture of the kind of interactions and structures that surfactants can form in solution conditions of interest. Using a model membrane protein - reaction center from Rhodobacter sphaeroides - in different types of crystallization experiments, we have shown that variations in surfactant concentration, even a few millimolars, can have a significant effect on the outcome of crystallization. This effect is readily described by phase diagram of the surfactant used to solubilize the MP. Beyond a critical concentration threshold, most of the MP and the surfactant separate into a new phase, and it is the composition of this phase that seemingly determines the eventual outcome of the crystallization trial. Precise measurement and modulation of surfactant concentration may be a key ingredient of developing a successful crystallization strategy.

1118-Pos Board B10
Role of Lipid Interactions in Regulation of Betaine Transport across the Membrane
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For membrane transporters, lipid-protein interactions are a major aspect to be considered when it comes to a molecular mechanism. Our knowledge of how membrane transport is functionally affected by surrounding lipids is in fact surprisingly limited despite its huge biological impact. One reason is the low number of atomic resolution transporter structures that show specifically bound lipids that might play a functional role. Here we discuss the role of lipids on betaine transport regulation for two secondary osmolyte transporters: (a) the trimeric betaine symporter BetP that is osmotic stress-regulated on activity level in a lipid-dependent manner, and (b) the renal BGT-1 transporter that shows increased insertion into to the plasma membrane upon hyperosmotic stress. Based on our structural and functional data we describe how lipid-protein interactions in BetP and BGT-1 are not only involved in the correct assembly but play also important roles in transport regulation.