Platform: Membrane Protein Structure & Function III

2766-Plat
Transport Dynamics in a Glutamate Transporter Homologue
Nurunisa Akyuz, Roger Altman, Scott Blanchard, Olga Boudker.
Weill Cornell Graduate School, New York, NY, USA.
Glutamate transporters are secondary active transporters, which mediate glutamate uptake from the synaptic cleft into glial cells and neurons allowing multiple rounds of neural transmission and preventing glutamate-mediated excitotoxicity. their structural dynamics is key to their function: during transport cycle, they alternate between outward facing and inward facing states, in which the substrate-binding sites are accessible from the extracellular and intracellular solutions, respectively. Crystallographic studies of a bacterial homologue Glt_{Ph} revealed that this isomerization entails trans-membrane movements of three discrete transport domains within a trimeric scaffold. Here, using single-molecule fluorescence resonance energy transfer (smFRET) imaging, we report real time observations of these movements for the first time. We labeled Glt_{Ph} with donor and acceptor fluorescent dye pairs at positions for which the inter-dye distances differ in the outward and inward states. Our observations reveal FRET states with efficiencies consistent with those predicted from the crystal structures. Remarkably, the rates of transitions between these states are modulated by substrate binding, as well as by lipid surroundings.

2767-Plat
Structural Basis of the Alternate-Access Mechanism in a Bile Acid Transporter
Xiaoming Zhou[1], Elena J. Levin[1], Matthias Quick[1,2], Yaping Pan[1], Ruchika Sharma[1], Ming Zhou[1].
[1]Columbia University, New York, NY, USA, [2]New York State Psychiatric Institute, New York, NY, USA.
The apical sodium-dependent bile acid transporter (ASBT) utilizes the Na^+ concentration gradient to transport bile acids into cells. ASBT is essential in cholesterol homeostasis and blocking of ASBT reduces cholesterol concentration in plasma. A recent structure of a bacterial homolog of ASBT in an inward-open state advanced our understanding of the transporter, but a single conformation does not resolve fundamental questions about the transport mechanism. Here we present crystal structures of an ASBT homolog from *Yersinia frederiksenii*, $ASBT_{Yf}$ in a lipid environment, in an inward-open state at 1.9 Å and an outward-open state at 2.5 Å. The structures reveal how bile acids could be transported across the membrane by a large rigid-body rotation of a six-helix domain, suggest the existence of a substrate binding site critical for translocation, and show how Na^+ could be released into the cytosol. These results build a structural framework for understanding the mechanism of bile acid recognition and transport in ASBT.

2768-Plat
Molecular Details of SERCA Regulation by Phospholamban Revealed by Paramagnetic Relaxation Enhancements and Solid-State NMR
Gianluigi Veglia, Martin Gustavsson, Vitaly V. Vostrikov, Raffaello Verardi.
University of Minnesota, Minneapolis, MN, USA.
Sarcoplasmic reticulum (SR) Ca^{2+}-ATPase (SERCA) ia a 110 kDa membrane protein that regulates cardiomyocyte relaxation by transporting Ca^{2+} into the SR. SERCA is regulated by PLN, a 52-resdiue membrane protein consisting of a helical, inhibitory transmembrane domain and a regulatory cytoplasmic domain. The cytoplasmic domain is in equilibrium between a folded T state and an unfoled R state. Phosphorylation of PLN at S16 relieves the inhibition of SERCA and, based on studies in animal models, mimicking the effects of PLN phosphorylation is a promising path for the development of therapeutics against heart failure.
Here we co-reconstituted SERCA and PLN in lipid bilayers under fully functional conditions and utilized solid state NMR to probe the molecular mechanisms of SERCA regulation. Asymmetric spin labeling and isotopic labeling of SERCA, PLN and lipids in combination with magic angle spinning experiments provided paramagnetic relaxation enhancements (PREs) that mapped the structure of the membrane protein complex. PREs were combined with oriented restraints from separated local field experiments, chemical shift perturbations and torsion angles from backbone chemical shifts to determine a structural model of the PLN/SERCA complex. The transmembrane domain of PLN remains anchored to the ATPase, while the cytoplasmic domain is membrane-associated in the T state and interacts transiently with the phosphorylation and nucleotide domains of SERCA in the R state. Phosphorylation increases the R state population and the affinity of the R state for SERCA. These results explain the non-inhibitory character of PLN mutants with increased R state population and provide crucial information for future design of therapeutics to increase SERCA function. In addition, the methodological advances described here further open the landscape to conducting structural studies of membrane protein complexes of molecular weights beyond 100 kDa.

2769-Plat
Charge Asymmetry in the Proteins of the Outer Membrane
Joanna S.G. Slusky, Roland L. Dunbrack.
Fox Chase Cancer Center, Philadelphia, PA, USA.
Outer membrane β-barrels (OMBBs) are the proteins found in the outer membranes of bacteria, mitochondria and chloroplasts. There are thousands of β-barrels reported in genomic databases with approximately 2-3% of the genes in gram-negative bacteria encoding these proteins. These proteins have a wide variety of biological functions including active and passive transport, cell adhesion, catalysis, and structural anchoring. Of the non-redundant OMBB structures in the Protein Data Bank, half have been solved over the past five years. This influx of information provides new opportunities for understanding the chemistry of these proteins.
Assessing this class of proteins bioinformatically, we have determined a dramatic asymmetry in the charge distribution of these proteins. For the outward-facing amino acids of the beta barrel within regions of similar amino acid density for both sides of the membrane, the extracellular side of the membrane contains more than three times the number of charged amino acids as the periplasmic side of the membrane. Moreover, the overall preference for amino acid types to be in the outward leaflet of the membrane corresponds roughly with hydrophobicity. This preference is irrespective the protein's oligomeric state and is demonstrably related to the structure of the outer membrane itself. Finally, the charge asymmetry of proteins in the outer membrane has important implications for how we understand the mechanism of outer membrane protein insertion.

2770-Plat
Site-Directed Spin Labeling Reveals Multiple Modes for Regulating Protein-Protein Interactions in Bacterial Outer Membrane Transport
Jessica Sarver, Audrey Mokdad, **David S. Cafiso**.
University Virginia, Charlottesville, VA, USA.
Structural transitions in three outer-membrane bacterial transport proteins have been investigated using site-directed spin labeling and EPR spectroscopy. In the vitamin B12 transporter BtuB and the ferric citrate transporter FecA, EPR spectra reveal the existence of a substrate-dependent order-to-disorder transition in the energy coupling motif (Ton box), which is localized at the periplasmic surface of the transporter. In BtuB, the Ton box unfolds into the periplasm, and in FecA, an N-terminal transcriptional domain disengages from the Ton box. Both these events expose the Ton box and initiate interactions with the inner membrane protein TonB, which drives transport. However, this disorder transition is not observed in all transporters, and in the ferrichrome transporter FhuA the EPR spectra indicate that the Ton box is constitutively disordered. Distance measurements using double electron-electron resonance (DEER) indicate how the FhuA Ton box is regulated, and each transporter regulates interactions with the inner membrane protein TonB using a distinct molecular mechanism. This work indicates that a variety of mechanisms may used to regulate protein-protein interactions in a single transporter family.

2771-Plat
VDAC1 Topology in the Outer Mitochondrial Membrane: The Final Answer
Francesca Guarino[1], Marianna Flora Tomasello[2], Andrea Magrì[1], Agata Impellizzeri[1], Simona Reina[1], Angela Messina[1], **Vito De Pinto**[3].
[1]University of Catania and INBB, Catania, Italy, [2]University of Catania, Catania, Italy, [3]university of Catania, Catania, Italy.
The first two authors equally contributed.
Voltage Dependent Anion Channel (VDAC) is a pore forming protein located in outer mitochondrial membrane (1). Its lack is lethal in human (2) and it is involved in cellular cross-talk, important in the apoptotic cascade (3). Its structure is a transmembrane β-barrel (4) organized in 19 β-strands and a N-terminal α-helix probably important for gating (5). However the sidedness of VDAC inside the membrane still remains unresolved (4). This issue is essential in order to define the pore structural determinants interacting with soluble proteins. We expressed, in HeLa cells, a recombinant hVDAC1 carrying C-terminal tag including the hemagglutinin-tag (HA), the specific caspase 3/7 cleavage site (DEVD) and 7 histidine residues (7xHis). DEVD amino acidic sequence can be cleaved upon apoptosis induction by staurosporine. A complementary Fluorescence Protease Protection assay was performed where cell protein are exposed to protease digestion after plasma membrane permeabilization. If