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Stromal interaction molecule-1 (STIM1) activates store operated Ca2+ entry (SOCE) in response to diminished luminal Ca2+ levels. We have recently determined the solution structure of the Ca2+−sensing region of STIM1 consisting of the EF-hand and sterile α motif (SAM) domains (EF-SAM) (Stathopulos et al. Cell Oct 3rd issue, 2008). The canonical EF-hand is paired with a previously unidentified EF-hand. Together, the EF-hand pair mediates mutually indispensable hydrophobic interactions between the EF-hand and SAM domains. Structurally critical mutations in the canonical EF-hand, 'hidden' EF-hand or SAM domain disrupt Ca2+ sensitivity in oligomerization via destabilization of the entire EF-SAM entity. In mammalian cells, EF-SAM destabilization mutations within full-length STIM1 induce punctae formation and activate SOCE independent of luminal Ca2+. We provide atomic resolution insight into the molecular basis for STIM1-mediated SOCE initiation and show that the folded/unfolded state of the Ca2+ sensing region of STIM is crucial to SOCE regulation. (Supported by CIHR and CFI).

999-Symp
A Molecular Mechanism for CRAC Channel Activation
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The Ca^{2+} release-activated Ca^{2+} (CRAC) channel is the most intensively studied member of the class of store-operated channels, and is well known to play a critical role in lymphocyte activation during the immune response. The central mystery of store-operated Ca^{2+} entry (SOCE) has been how depletion of Ca^{2+} within the ER lumen triggers CRAC channel activation in the plasma membrane (PM). Recent breakthroughs in identifying the ER Ca^{2+} sensor, STIM1, and the pore-forming subunit of the CRAC channel, Orai1/CRACM1, have spurred rapid progress in defining a molecular mechanism. Store depletion triggers the oligomerization of STIM1 and its redistribution to ER-plasma membrane (ER-PM) junctions, where Orai1 accumulates in the plasma membrane and CRAC channels open. These dynamic structures, consisting of clusters of STIM1 and Orai1 separated by a 10-25 nm gap comprise the elementary units of SOCE. Using fusion proteins in which the Ca^{2+} sensing domains of STIM1 were replaced by FRB and FKBP12, we showed that rapamycin-induced oligomerization causes the proteins to accumulate at ER-PM junctions and activate CRAC channels without Ca^{2+} store depletion. Thus, STIM1 oligomerization itself acts as a master switch to trigger the self-organization and activation of the SOCE machinery. Oligomerization acts by enabling STIM1 to accumulate at ER-PM junctions and to bind to CRAC channels diffusing throughout the overlying PM. Orai1 is trapped and activated by binding to a cytosolic subregion of STIM1 that we call the CRAC activation domain, or CAD. *In vitro* studies with purified proteins show that CAD binds directly to purified Orai1 and crosslinks CRAC channels to form clusters. These studies support a molecular mechanism for SOCE by which the STIM1 CAD traps and activates CRAC channels at ER-PM junctions via direct binding to Orai1.

Platform L: Membrane Protein Structure

1000-Plat
High Throughput Coarse-Grained Simulations of the Insertion of Transmembrane Helices
Benjamin A. Hall, Alan Chetwynd, Richard Franzese, Mark S.P. Sansom.
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Transmembrane helices play multiple vital roles in cell function, including signalling processes, channel gating and active transport. As such, there exists a significant body of data on the biological function and structural properties of naturally occurring helices and their mutants, and on synthetic helices such as WALP and LS3 which have been used to better understand the roles of different residues in determining position and orientation of helices in a membrane. Coarse grained MD simulations are becoming an increasingly popular tool for understanding the properties of biological systems, overcoming canonical limits of atomistic simulations such as timescale or system size. Such techniques involve several manual steps, including system build, simulation set up and analysis. Here we present the Sidekick software, which enables automation of these processes, thus enabling high throughput simulations on the basis of a small set of input sequences, or of a single sequence and a scanning mutation. We demonstrate the use of this software to approach two problems; the ability of two commonly used coarse grain methods to predict insertion efficiencies of helices generated from sliding window across a larger sequence, and molecular signalling of TM2 of the methyl accepting chemoreceptor protein Tar. Our results demonstrate the value of such an HT simulation approach in the interpretation of a range of experimental data.

1001-Plat
Structural models of Alzheimer's Abeta channels
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Amyloid beta (Aβ) peptides involved in Alzheimer's disease form Ca^{2+} permeant ion channels. We have been developing models of these channels to be consistent with experimental findings. These models are refined and evaluated by molecular dynamics (MD) simulations. In our segment nomenclature, residues 1-14 are called S1, residues 15-28 S2, and residues 29-42 S3. The models we find most consistent with functional properties have a pore lining formed by 6-12 S1 segments in a β-barrel structure. The cation selectivity of the pore is due to negatively charged residues at positions 1,3,7, and 11 that extend into the pore. This category of models may differ by the number of strands, whether these strands are parallel or antiparallel, and the structures of the protein that surround the S1 β-barrels. Heavy metal ions and histidine-containing peptides block Aβ channels and inhibit Aβ-induced apoptosis. Zn^{2+} and Cu^{2+} binding sites are formed in our models by E11, H13, and H14 residues. Clustering of these residues at the entrance to the pore is more pronounced in the parallel models, and they are nearer the axis of the pore in the 6-stranded models. The 6-stranded β-barrel models are also more consistent with blockade of the channels by Tris cations. We have developed hexameric and dodecameric models in which S2 and S3 segments are helical, and dodecameric models in which S2 and S3 segments form a 24-stranded β-barrel that surrounds the pore-forming S3 β-barrel. However, the models most consistent with microscopy studies of the channels are composed of 36 subunits with only a fraction of the S1 segments forming the pore. These models are hexamers of hexamer in which S3 segments of each hexamer forms a 6-stranded β-barrel that is exceptionally stable during MD simulations.

1002-Plat
Pore Formation and Structure of the Twin Arginine Translocase Subunit TatA from B. subtilis
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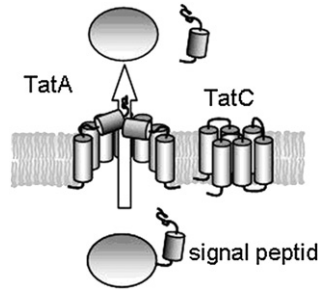
Cells have developed sophisticated transport machineries to allow proteins to cross membrane barriers. In bacteria, the twin arginine translocase (Tat) can translocate proteins even in the folded state. In *B. subtilis*, a membrane protein complex consisting of two subunits, TatA and TatC, is responsible for the Tat translocation process. TatA is believed to form a nanometer size pore trough which the protein is transported, whereas TatC is involved in recognition of the target protein signal peptide.
To get insight into the mechanism of the Tat translocation in *B. subtilis*, we studied the structure of the pore-forming subunit TatA and the pore assembly pursuing two complementary experimental approaches. The structure of individual TatA monomers in membranes or membrane-mimetic environments was characterized using solid state and solution NMR. The formation of oligomeric assemblies of TatA in the membrane, on the other hand, was investigated using in-plane neutron scattering of TatA reconstituted in aligned membranes. This way, we were able to both derive a detailed structural model of TatA, and to characterize pores formed by TatA.



1003-Plat
Lysophospholipid Micelles Sustain Diacylglycerol Kinase in Active and Stable Form for Biochemical and Structural Studies
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One of the challenges in the study of membrane proteins is that many methods require the proteins be soluble and stable in aqueous solution, which can be solved by the use of detergent micelles to provide an environment that mimics the hydrophobic environment of biological membranes. Yet, it remains to be determined which detergents are the best in sustaining the structural conformation and biological function of the proteins of interest. In this study, we used *Escherichia coli* DAGK, which is an integral membrane protein, to screen detergent conditions that can maintain the catalytic activity of the protein and are suitable for structural studies using NMR spectroscopy. We learned that

preparation of *E. coli* DAGK in lysophospholipids, LMPC and LMPG, allows the protein to be catalytically active as observed using a spectrophotometric assay. We also found that the ^{15}N-TROSY-HSQC spectrum quality of these samples is comparable to that of a sample prepared in dodecylphosphocholine (DPC), which is the detergent used to determine the structure of *E. coli* DAGK in the absence of the substrates. In addition, we noticed that in the two lysophospholipid conditions, even though addition of substrates does not alter peak dispersion on the ^{15}N-TROSY-HSQC spectrum of DAGK significantly, we can map the catalytic site by monitoring the peaks that shifts as the substrates are titrated. Altogether, our data indicate that the use of lysophospholipids in sample preparation allows us to acquire structural information of DAGK in its active conformation with the substrates bound at the catalytic site. This work is supported by NIH grant R01 GM47485.

1004-Plat
Mixing and Matching Detergents for Membrane Protein NMR Structure Determination
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One major obstacle to membrane protein structure determination is the selection of a detergent micelle that mimics the native lipid bilayer. Currently, detergents are selected by exhaustive screening because the effects of protein-detergent interactions on protein structure are poorly understood. In this study, the structure and dynamics of an integral membrane protein in different detergents is investigated by nuclear magnetic resonance (NMR) and electron paramagnetic resonance (EPR) spectroscopy, and small angle X-ray scattering (SAXS). The results suggest that matching of the micelle dimensions to the protein's hydrophobic surface avoids exchange processes that reduce the completeness of the NMR observations. Based on these dimensions, several mixed micelles were designed that improved the completeness of NMR observations. These findings provide a basis for the rational design of mixed micelles that may advance membrane protein structure determination by NMR.

1005-Plat
Structure and Dynamics of TM Domains of Human Glycine Receptor in LPPG Micelles
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The structure and backbone dynamics of the entire four transmembrane domains of human glycine receptor α1 subunit (GlyRTM1234) were studied in LPPG micelle. Using triple-resonance multi-dimensional NMR methods, over 80% residues were assigned. The chemical shift index clearly showed that most residues in the TM region were in helical conformation, consistent with a four-helix-bundle TM protein. We have collected sufficient amount of distance, H-bond, and dihedral angle restraints from NOE and chemical shift data, as well as long-range distance restraints from paramagnetic relaxation enhancement measurements. The structure model, determined using the available restraints, showed a kink between Trp72 and Cys76 in the beginning of TM3 domain. Moreover, the end of TM2 domain, S53 to S56, is more flexible compared to the rest of TM2. In contrast, part of TM23 loop, V63 to V66, showed a helical secondary structure. Backbone dynamics measurements indicated the existence of significant internal motions of TM1234 in LPPG micelles. The relaxation data also resulted in an overall rotational correlation time of ~33.4 ns, estimated based on the non-flexible helical residues. This overall tumbling time corresponds to a 66.8 kDa protein-LPPG complex with ~98 LPPG molecules per GlyRTM1234. The size is confirmed by the dynamic light scattering measurement. We suggest that the flexible end of TM2 and the beginning of TM3 can better coordinate the coupling between the TM2-TM3 loop and the extracellular domain. This coupling is thought to be important for mediating channel gating. In addition, it is confirmed that the early termination of TM2 helix in GlyR is an intrinsic property and is independent of the presence or absence of other TM domains (Funded by NIH R37GM049202 & R01GM069766).

1006-Plat
Conformational Cycle Of A Bacterial Homolog Of Human Neurotransmitter Sodium Symporters
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Neurotransmitter:sodium symporters (NSS) control the magnitude and duration of synaptic signaling through active reuptake of specific neurotransmitters. While it is known that the transmembrane sodium gradient supplies the energy for transport, the coupling mechanism to substrate translocation from its primary binding site (S1) is poorly understood. Conformational rearrangements of extracellular and cytoplasmic regions of the protein are thought to regulate alternating access to S1, and substrate binding at a second site (S2) located near the extracellular region of the transporter, to act as a symport coupler (Shi et al 2008 Mol Cell 30, 667). We used spin labeling and EPR spectroscopy to investigate the conformational dynamics of a highly homologous bacterial member of the NSS family, the leucine transporter LeuT, for which the crystal structure is known (Yamashita et al 2005 *Nature* 437, 215). Changes in global and local structural constraints derived from the EPR analysis and induced by sodium and leucine binding were then correlated to conformational changes in the LeuT structure in proteoliposomes. Sodium binding was found to increase the distance between the probes as assessed from global rearrangements measured in the extracellular region of LeuT. In contrast, subsequent leucine binding in the presence of sodium was found to decrease the distance between the probes. Consistent with these observations, sodium binding increases spin label mobility and water accessibility at positions within the S2 site. Furthermore, leucine binding increases spin label order and decreases water accessibility. These results suggest a model in which sodium binding to LeuT primes the transporter for substrate binding in S1 by increasing the population of an ''outward-facing'' conformation, exposing the substrate permeation pathway. With a filled S1, the extracellular pathway constricts, consistent with the occluded state observed in the LeuT crystal structure.

1007-Plat
X-ray Footprinting Studies on Photoactivation of Bovine Rhodopsin
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Rhodopsin, the visual G-protein coupled receptor (GPCR) in the rod cells of the vertebrate retina, is fundamental to vision. The light-activated intermediate of rhodopsin, Meta-II, initiates a signalling cascade that culminates in an electrical impulse in the visual cortex of the brain. The molecular details of agonist-induced structural change that are likely to be conserved among the members of the GPCR super family are not fully understood. We used X-ray Footprinting to study the conformational change in rhodopsin in solution upon photoactivation. Purified rhodopsin is exposed in tens of milliseconds with high flux focused X-rays. The hydroxyl radicals that are generated by photolysis of water react with the solvent accessible side chains and form stable modification products. The peptic fragments are analyzed by mass spectrometry to quantify the extent and identify the sites of oxidation. Monitoring the changes in the radiolytic modification as function of the exposure time provides information that is directly correlated with the solvent accessibilities of individual peptide or side chain residues within the protein. The difference in solvent accessibilities between dark state and light activated Meta-II state shows conformational changes near the retinal binding site, but not a large structural change as predicted by some models of GPCR activation. Labeling was also observed in the trans-membrane helical regions, this was also unexpected. We introduce a novel O^{18} labeling method to determine if transmembrane labeling arises from exchange with bulk water or is mediated by activation of bound, conserved water molecules in the GPCR structure. This is a novel approach that can probe the details of bound water structure and dynamics that function in a number of ion channels and receptors.

Platform M: Cell & Bacterial Mechanics Motility

1008-Plat
Physical Description of Mitotic Spindle Orientation During Cell Division
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During cell division, the duplicated chromosomes are physically separated by the action of the mitotic spindle. The mitotic spindle is a dynamic structure of the cytoskeleton, which consists of two microtubule asters. Its orientation defines the axis along which the cell divides. Recent experiments on dividing cells, which adhere to patterned substrates, show that the spindle orientation depends on the spatial distribution of cell adhesion sites. In the present work we show that