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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
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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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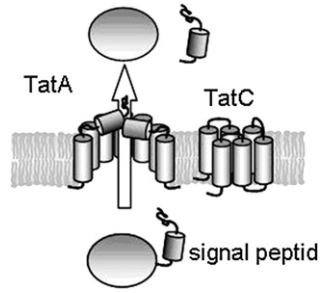
Cells have developed sophisticated transport machineries to allow proteins to cross membrane barriers. In bacteria, the twin arginine translocase (Tat) can translocate proteins even in the folded state. In *B. subtilis*, a membrane protein complex consisting of two subunits, TatA and TatC, is responsible 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