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catalytic zinc, which may mediate recognition of the positively charged NFκB subunits that are substrates of NleC. In order to begin characterizing the specificity of NleC, we verified the cleavage site of RelA as within the DNA-binding loop, which was contested in the literature. As with other Zincin family members, there are several subsites visible on NleC's surface, which likely bind residues near the scissile bond of the target and provide specificity. To tease apart the contributions to specificity between global binding and local recognition of the cleavage site, we are pursuing systematic mutagenesis of the RelA DNA-binding loop. This information will be used to define additional potential targets mediating NleC's role in O157:H7 *E. coli* virulence. Additionally, because T3SS effectors are necessarily partially unfolded during secretion into the host, we are investigating whether the stability of NleC differs from other Zincins by utilizing thermal degradation and atomic force microscopy pulling experiments.

2813-Plat

Membrane Interactions, Intrinsic Disorder, and Unknown Functions of Myelin Proteins

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The myelin sheath is a unique membrane, tightly wrapped around selected axons in the vertebrate nervous system. The multilayered myelin proteolipid membrane contains a specific set of proteins, many of which share no homology with other known proteins. We are interested in the structure, function, interactions, and dynamics of myelin proteins and use multidisciplinary methods to study these relationships. We have solved crystal structures of myelin-specific proteins, characterized their membrane-binding properties, and initiated experiments to pinpoint details of protein dynamics. Among our most recent results are a comprehensive X-ray crystal structure-based characterization of the reaction cycle of the myelin enzyme CNPase, the identification of several myelin-specific proteins as intrinsically disordered molecules, and the analysis of molecular dynamics in myelin proteins and the lipid bilayers they interact with by neutron scattering. All the results will be important in understanding the formation of the myelin membrane multilayer, as well as the roles the myelin proteins play in myelination and myelin-related diseases.

Platform: Membrane Structure II

2814-Plat

The Complexities of the E.Coli Cell Envelope: Details Revealed by MD Simulations

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Gram-negative bacteria such as E.coli are protected by a surprisingly complex cell envelope. The cell envelope is composed of membranes that form a protective barrier around the cells, and control the influx and efflux of solutes via various routes and mechanisms. To study the influence of the bacterial membranes on the dynamics of embedded outer membrane proteins (OMPs), we have created virtual E.coli inner and outer membranes. Our atomistic-level models incorporate the heterogeneity of the various lipid types, including the lipopolysaccharide molecules of the outer membrane, the mixture of phospholipids in the inner membrane and some of the peptidoglycan of the periplasmic space. We have performed a series of simulations exploring how these various membrane components influence the structure and dynamics, and therefore function of the proteins that reside within them. Furthermore, we have used coarse-grain models to enable simulation of much larger, multi-protein systems on longer timescales, thus we are able to simulate systems of over 1 million particles on microsecond timescales. In summary, here we provide an update on our efforts to model an entire organelle; the cell envelope of E.coli.

2815-Plat

Insertion of Functional Proteins into Bilayer Lipid Membrane using a Cell-Free Expression System

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The incorporation of well-conformed membrane proteins within lipid bilayer is an important challenge because these proteins play a major role in every living cell and are key factors in cell-cell interaction, signal transduction and transport of ions and nutrients. To insert an integral membrane protein in a lipid bilayer it is important to separate the lipid bilayer from the supporting solid substrate in order to minimize interactions of the protein with the substrate and to provide adequate space for the protein incorporation.
We chose to produce two kind of lipid bilayer membrane, a suspended membrane in which alpha hemolysin nanopores were produced and incorporated and a tethered Bilayer Lipid Membrane (tBLM), spaced from the surface by a tethering molecule as a polyethylene glycol (PEG), for the incorporation of a transmembrane protein like Aquaporin Z.
The two membrane proteins were produced directly on the top of the lipid bilayers using a cell-free expression system, without any purification. This alternative technique is not affected by cell physiology and allows producing membrane proteins in a correct conformation without toxicity limitation, protein aggregation or misfolding. To demonstrate that these proteins produced with this cell free expression system are inserted and functional in a lipid bilayer, we used Quartz Crystal Microbalance with Dissipation monitoring (QCM-D), Atomic Force Microscopy (AFM), Surface Plasmon Resonance (SPR) and ion current recording experimentations.

2816-Plat

Highly Resolved Structure of a Floating Lipid Bilayer: Effects of Calcium Ions and Temperatue

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Biological membranes are heterogeneous and highly dynamical organizations of lipids and proteins that define the outer boundary of a cell. Solid supported lipid bilayers have often been studied as model systems to understand the structures and properties of such cellular membranes. Such systems are constrained to a planar geometry and the strong support-membrane interaction could disrupt the inherent structural and functional properties of the membranes. To accurately mimic the biological membranes with their natural thermal fluctuations, curvature deformity and in-plane mobility of lipid molecules, a soft support is required. Hence, we have studied a double bilayer where the upper floating bilayer has the structural freedom to reproduce the morphology of a cellular membrane. Electron density profiles obtained from 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) bilayers by reflectivity studies using synchrotron x-rays provide unprecedented structural details of this system. Further, the effects of Ca^{2+} ions on the structure of these systems and the distribution of these ions near the bilayer have been investigated. These ions are preferentially bound to the head group region of the bilayer, which leads to a tight packing of lipids in the film. They cause the bilayer to thicken by increasing the hydrophobic core of the bilayer. Again, these ions are observed to intensify the flexibility of the bilayers, which is exhibited by the increased interfacial roughness. With the added Ca^{2+} ions, the inter bilayer separation is found to increase as a function of temperature and finally the floating bilayer unbinds from the adsorbed one. Such an effect was not observed in the absence of these ions.
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2817-Plat

Synthetic Lipid Nanotubes as Cell-Cell Junctions for Inter-Cellular Ca^{+} Propagation and for Cell Contraction Monitoring

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We previously discovered that the main component of bacterial cell membranes, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), self-assembles into single-wall tubes with an outer diameter of 19.1 $\pm$ 4.5 nm and a length of close to 1 mm (synthetic lipid nanotubes, LNTs). [1]
By exploiting the interaction of living cells (REF52) with LNTs, we demonstrated cell contractility recording. The newly generated LNTs pattern upon cell spreading exhibited structural fingerprints characteristic for cell types and conditions. Monitoring the fluorescent-labeled LNTs and paxillin accumulated at the focal adhesions of fibroblasts simultaneously, we proved that the nucleation of new LNTs is linked to the cell's focal adhesions and contractile activity.