conformation. Results reveal that a toroid can indeed form under the biologically relevant forces for the viral packing motor (up to 100 pN). To understand the effects of the highly stressed toroid on DNA structure, we then employ molecular dynamics (MD) simulations starting with the rod model-computed equilibrium as the initial condition. Following equilibration in MD, we construct an approximate density map using the final predicted toroid to compare with the recently published cryo-EM data. The computed density map correctly predicts the major dimensions of the toroid as well as regions of high and low density. Finally, we simulate the start of the ejection process by integrating the rod model forward in time upon sudden removal of the protein ''tail knob.'' The resulting fast dynamic collapse of the toroid suggests that its function could be to prime the ejection of the remaining viral genome.

2142-Plat

Structure and Dynamics of the Bacterial Chromosome in E. coli

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We address the question of whether the E. coli chromosome is folded into a self-adherent nucleoprotein complex, or alternately whether it is an essentially unfolded DNA molecule. We do this by in vivo visualization of the whole chromosome, via fluorescence of a controllably-expressed fusion of gfp and the nucleoid-associated protein Fis. Space-time studies of the nucleoid in live E. coli cells shows a relation between chromosome segregation and cell division under different growth conditions, and it also shows how domain structure and overall conformation of chromosomes vary during rapid and slow growth. These observations support a self-adherent filament model of the chromosome, as opposed to a non-self-adherent ''self-avoiding polymer'' model. We have also developed methods for isolation of single bacterial chromosomes, in order to study the bacterial chromosome outside of the cell, to establish their spatial organization and mechanical properties, and to study how those properties are changed by varied external conditions. Our further objective is to directly examine nucleoid mechanical properties using micromanipulation methods which could provide further insight into bacterial chromosome folding.

Platform: Membrane Protein Structure II

2143-Plat

The Structure of the Chemokine Receptor CXCR1 in Phospholipid Bilayers and Interactions with IL-8

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CXCR1 is a receptor for the chemokine interleukin-8 (IL-8), a mediator of immune and inflammatory responses. Strategically located in the cell membrane, CXCR1 binds to IL-8 with high affinity, and subsequently transduces a signal across the membrane bilayer to a G-protein activated second messenger system. Here, we describe the three-dimensional structure of human CXCR1 determined in phospholipid bilayers under physiological conditions by solid-state NMR spectroscopy. We use a five-step approach: (1) prepare a sample of a uniformly 13C/15N labeled CXCR1 in proteoliposomes by bacterial expression, purification, and refolding. (2) Resolve individual signals with MAS solid-state NMR experiments. (3) Assign each signal to a specific residue. (4) Measure two or more orientation-dependent frequencies for each residue and some distances. (5) Calculate de novo three-dimensional structure. The availability of a method for determining the structures of unmodified GPCRs in their native environment of phospholipid bilayers under physiological conditions has the potential to transform the field of structural determination of membrane proteins, and to accelerate the discovery of drugs that interact with membrane-associated receptors. Progress towards determining the structure and function of IL-8 bound to CXCR1 in membrane environments will be described.

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Heterooligomerization of Membrane Peptides Involved in Tuberculosis: MgtC and MgtR

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Recent results have converged to highlight the role of hydrophobic peptides that form a novel class of active molecules in *Escherichia coli* and *Salmonella enterica serovar Typhimurium*. These peptides are apparently able to interact with membrane proteins leading to their inactivation through an unfolding process. MgtR, a highly hydrophobic 30 amino acid peptide, expressed in *Salmonella typhimurium* is one of these inactivating molecules. It has been shown that within the macrophage, the interaction between MgtR and the membrane protein MgtC leads to a decrease of Salmonella replication rate. This phenomenon can be explained by the importance of MgtC for the survival of the bacterium at low magnesium concentrations.

To our knowledge, an interaction between two different peptides (heterooligomer) has never been studied in a membrane environment by NMR. Relying on the sequence homology between *Salmonella typhimurium* MgtC and *Mycobacterium tuberculosis* MgtC, the interaction between MgtR from *Salmonella typhimurium* and the transmembrane helix #4 of *Mycobacterium tuberculosis* MgtC is studied.

Beyond the challenge consisting of solving a membrane protein structure, Solid state NMR and EPR are two powerful techniques to decipher protein dynamics and structure within the membrane hydrophobic core. Understanding the molecular mechanism of these peptide-protein interactions would offer great opportunities in pharmacology and drug design.

Structural studies using solid state NMR spectroscopy of liposomal preparations for magic angle spinning experiments and oriented bilayer samples for additional structural restraints have been performed. EPR distance measurements were also performed on theses peptides in the membrane environment. The use of the NMR and EPR restrains combined, allowed us to propose a hypothetical interaction between these peptides in the membrane core.

2145-Plat

Solution Structure and Peptidoglycan Interaction of Rv0899, a Virulence Factor from Mycobacterium Tuberculosis

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Rv0899 is a membrane protein encoded by an operon that is required for fast ammonia secretion, rapid pH neutralization and growth of M. tuberculosis in acidic environments. The gene is restricted to pathogenic mycobacteria and, thus, is an attractive candidate for the development of anti-tuberculosis chemotherapy. Rv0899 contains three independently structured domains. The N-terminus (Rv0899-M; residues 1-80) includes a membrane-anchoring sequence of 20 hydrophobic amino acids (residues 28-50) that is required for membrane translocation. The central region (Rv0899-B; residues 81-194) contains two consecutive repeats of the BON (Bacterial OsmY and Nodulation) domain (pfam04972), a conserved, putative lipid binding sequence that is found in some bacterial osmotic shock protection proteins. Finally, the C-terminus (Rv0899-C; residues 195-326) contains an OmpA-like domain (pfam00691), a periplasmic peptidoglycan-binding structure found in several types of bacterial membrane proteins. Using NMR spectroscopy, we determined the high-resolution structures of Rv0899-B and Rv0899-C. Rv0899-B does not form a transmembrane beta barrel and has an undocumented mixed alpha/beta fold. Furthermore, we found Rv0899-B has a specific zinc binding site and associates with lipid vesicles in a pH dependent manner. The structure of Rv0899-C adopts the characteristic OmpA-like family fold. It exhibits pH-dependent conformational dynamics, with significant disorder at neutral pH and a more ordered structure at acidic pH. Rv0899-C associates tightly with polymeric peptidoglycan isolated from M. tuberculosis and also associates with a soluble peptide intermediate of peptidoglycan biosynthesis. This enabled us to characterize the peptidoglycan binding site where five highly conserved Rv0899-C residues, including two key arginines, establish the specificity for DAP- but not Lys-type peptidoglycan. The three-dimensional structure of Rv0899 suggests alternative possible modes of membrane association and provides a molecular basis for the screening of small molecules with anti-TB potential.

2146-Plat

Strategies for the Solution NMR Structure Determination of Beta-Barrel Membrane Proteins

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To date, a handful of β-barrel membrane proteins have been determined with solution nuclear magnetic spectroscopy (NMR) with the first reported in 2001. The bottlenecks seem to be protein sample preparation and obtaining near-complete assignment. In the process of determining the structures of two β-barrel membrane proteins, Opa_{50} and Opa_{60} from *Neisseria gonorrhoeae*, strategies for obtaining quality NMR spectra and backbone assignments were established. The effects of detergents on the global fold and NMR spectral quality were determined. Although all detergents stabilized the overall fold (after refolding in dodecyphosphocholine), there were variable