Structural Investigations of an Amphipathic Region of the Twin-Arginine Translocase Tata Subunit

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suggestive of a lipid binding or structural function, and provides insights to the role of Rv0899 in *Mtb* virulence.
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3248-Plat
Structural Investigations of an Amphipathic Region of the Twin-Arginine Translocase Tata Subunit
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The twin-arginine translocase (Tat) system is used by many bacteria and plants to move folded proteins across the cytoplasmic membrane. Tat substrates contain a signature S/TRRxFLK twin-arginine motif in their N-terminal sequence. In most bacteria, the translocon consists of the TatABC subunits where TatA is the postulated pore subunit through homo-oligomerization with other TatA protomers, whereas TatBC is the substrate-receptor complex. The predicted structure of TatA includes a transmembrane helix, an amphipathic helix and a potentially unstructured C-terminal region. Biochemical and structural investigations were targeted at a peptide which represents the amphipathic region consisting of residues 22 to 44 of TatA (TatAH2). The dual topology of the region corresponding to TatAH2 in TatA was previously shown to be dependent on the membrane potential (Chan *et al*. 2007 *Biochemistry* 46: 7396-404), and thus warranted further investigations on its role for protein translocation. NMR and CD spectroscopy of TatAH2 show that it adopts helical structure in a membrane mimetic environment, in comparison to the random coil structure in aqueous solution. Microcalorimetry studies also show that it interacts with DPPG lipid vesicles to affect the phase transition temperatures. The solution NMR structure of TatAH2 shows conformation flexibility of the peptide around the acidic Asp31 at the center of the helix, a residue potentially important for the function of the TatA pore. The C-terminal half (residue 32 onwards) is α-helical, whereas the N-terminal half (23 to 30) has helical-like structure, suggesting that TatAH2 does not form a 'typical'α-helix.

3249-Plat
Investigating the Structural and Dynamic Properties of Membrane Proteins with Solid-State NMR, CW-EPR, ESEEM, and DEER Spectroscopic Techniques
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Currently, we have very limited structural and dynamic information on membrane proteins and peptides. New spectroscopic methods are needed to probe these systems in a lipid bilayer. In order to address these issues, the Lorigan lab developing unique hybrid solid-state NMR and spin-label EPR spectroscopic techniques. Magnetic resonance spectroscopic data of ^{15}N-, g^{2}H-labeled and/or spin-labeled membrane proteins incorporated into vesicles and bicelles will be presented. State-of-the-art solid-state NMR and pulsed EPR techniques such as Magic Angle Spinning (MAS) NMR, Electron Spin Echo Envelope Modulation (ESEEM) spectroscopy, and Double Electron-Electron Resonance (DEER) spectroscopy will be used. The ESEEM technique can determine short to medium range distances (out to about 8 Å) between a site-specific nitroxide spin label (MTSL) and a nearby NMR-active isotopic labeled residue for a variety of different peptides and proteins which ultimately can be used to determine the difference between an α-helical and β-sheet secondary structure. DEER can be used to measure distances between 2 spin labels out to about 70 Å. The advantages and disadvantages of applying solid-state NMR and EPR spectroscopy to probe the structural and dynamic properties of membrane proteins will be discussed.

3250-Plat
Secondary-Structure Analysis of Human Peripheral Cannabinoid Receptor CB2 Based on Solid-State ^{13}C-,^{15}N-MAS NMR and Molecular Dynamics Simulations
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The secondary structure of the human peripheral cannabinoid receptor CB2 was investigated according to chemical shift data obtained on lipid-reconstituted, functional CB2. The CB2 receptor, uniformly labeled with ^{13}C and ^{15}N, was produced by bacterial expression, purified in milligram quantities, and reconstituted in an agonist-bound form into unilamellar liposomes. One- and two-dimensional ^{13}C and ^{15}N spectra were recorded by solid-state magic-angle spinning (MAS) NMR. Homogeneity of the reconstitution was examined by measurement of the ^{31}P chemical-shift anisotropy of the membrane phospholipids and functional integrity monitored by a G-protein activation assay. The acquired one- and two-dimensional ^{13}C-, and ^{15}N-MAS NMR spectra of CB2 were compared with C_α, C_β, C=O, and N-H chemical shifts predicted from a computer model of CB2, obtained in microsecond time scale, all atom molecular dynamics simulations. Although experimental spectra suffered from heavy superposition of resonances, the comparison of theoretical and experimental spectra gave insights into secondary structure of protein domains. There is sufficient spectral resolution to partially assign the resonances of specifically labeled CB2. The prospects for spectroscopic targeting of structural changes that take place upon CB2 activation by a combination of NMR experiments and computer modeling will be discussed.

3251-Plat
Spectroscopic Investigation of Phosphorylated Pentameric Phospholamban Bound to the Sarcoplasmic Calcium ATPase
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The sarco(endo)plasmic reticulum Ca-ATPase (SERCA) is the main enzyme responsible for the reuptake of Ca2+ into the sarcoplasmic reticulum (SR) during muscle relaxation. Phospholamban (PLN), a homopentameric membrane protein expressed in the SR, binds to and decreases the rate of Ca2+ transport of SERCA. This inhibitory action is relieved when PLN is phosphorylated at residues Ser-16 or Thr-17 in response to different signal pathways. Although a great deal of knowledge has been accumulated on the functional consequences of PLN phosphorylation, very little is known about the molecular details leading to relief of inhibition. Previous work suggested a crucial role of protein topology in the complex between SERCA and PLN, with the latter showing different tilt angles in the presence and absence of SERCA. Our hypothesis is that SERCA activity is regulated by changes in topology in PLN transmembrane helices upon phosphorylation. In order to probe for the orientation of protein domains, solid-state NMR in oriented lipid bilayers has established itself as the method of choice in many studies. Here we present data on the topological changes taking place in phospholamban pentamer in the presence of SERCA upon phosphorylation at Ser-16 or Thr-17. SERCA and PLN are reconstituted in lipid bilayers and uniaxially aligned either mechanically (on glass plates) or magnetically (upon formation of bicelles). The two proteins are fully functional under these conditions as demonstrated by the ability of SERCA to transport Ca2+ in functional assays as well as PLN dependent decrease in apparent affinity for Ca2+.

3252-Plat
Protein Folding at the Membrane Interface: The Structure of Nogo-66
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Compelling evidence indicates that repair of damage to the central nervous system (CNS) is inhibited by the presence of protein factors within myelin that prevent axonal regrowth. Myelin growth inhibits and their common receptor have been identified as targets in the treatment of damage to the CNS.
We have recently determined the NMR structure of one of the myelin growth inhibitors, the neurite outgrowth inhibitor (Nogo). We studied the structure of this protein alone and in the presence of dodecylphosphocholine micelles to mimic the natural cell membrane environment. Using several paramagnetic probes, we have defined portions of the growth inhibitor that are accessible to solvent (and consequently the Nogo receptor). Mutagenesis probed through phage-display confirms that the positions predicted to be extra-cellular are sensitive to receptor binding. Using computational docking methods and the mutagenesis results, we calculated the optimal protein-protein interface between our structure of Nogo and the Nogo receptor. The structure of Nogo and the predicted Nogo/receptor inhibitory complex structure will be presented.
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