effects on the NMR spectra. In particular, the resonances corresponding to the β-barrel were less affected than the loop regions indicating an interaction between some detergent micelles and the Opa extracellular loops. Once quality NMR spectra are obtained, the next task for NMR structure determination is the backbone assignment. For the Opa proteins, the amino acid content and the crowding in the random coil region of the spectrum (>50% of the protein is comprised by the extracellular loops) has created difficulties in obtaining a comprehensive assignment for structure determination. Several strategies that circumvent these difficulties will be presented.

2147-Plat
Probing the Structure of Membrane Proteins with ESEEM and DEER Pulsed EPR Techniques
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Limited structural and dynamic information on membrane proteins and peptides exist. New biophysical/structural biology methods are needed to probe these systems in a lipid bilayer. The Lorigan lab is applying unique hybrid NMR and spin-label EPR spectroscopic techniques to study membrane proteins. Magnetic resonance spectroscopic data of 15N-, 2H-labeled and/or spin-labeled membrane proteins incorporated into vesicles and bicelles will be presented. State-of-the-art pulsed EPR techniques such as 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 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. We have shown a huge improvement is sensitivity with DEER measurements at Q-band when compared to X-band.

2148-Plat
Structure of the Phospholamban/Ca^{2+}-ATPase Complex in Lipid Bilayers by Hybrid Solid-State NMR Methods
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Sarcoplasmic reticulum (SR) Ca^{2+}-ATPase (SERCA) and phospholamban (PLN) form a 116 kDa membrane protein complex that transports Ca^{2+} from the cytosol into the SR of cardiomyocytes. Owing to their importance for cardiac muscle function, PLN and SERCA have been targeted in therapeutic studies aimed at relieving heart failure. PLN consists of a transmembrane helix (domain Ib and II, residue 23-52) connected to a cytoplasmic region (domain Ia, residue 1-16) by a short loop (residue 17-22). Domain Ia is in a conformational equilibrium between an unfolded, solvent-accessible R state and a helical, membrane-associated T state. Despite the wealth of structural information about PLN (from NMR) and SERCA (from X-ray crystallography), there is still no high resolution structure of the SERCA/PLN complex.
Here, we co-reconstituted SERCA and PLN into native-like lipid bilayers under fully functional conditions and utilized hybrid solid-state NMR methods to characterize the SERCA/PLN complex. Magic angle spinning (MAS) NMR was used to determine the complete secondary structure of PLN. Paramagnetic relaxation enhancement (PRE) experiments, utilizing nitroxide spin labels covalently attached to SERCA and to the bilayer lipids, provided multiple inter-protein distances. Membrane topology was determined by oriented NMR in aligned bicelles. Finally, these restraints were implemented into XPLOR-NIH to determine the structure of R and T states of PLN in complex with SERCA. Our results show that the transmembrane domain is helical between residues 23-50 and binds to SERCA at a ~40° tilt angle with respect to the membrane bilayer. Domain Ia interacts with the cytoplasmic region of SERCA in an extended, unfolded conformation (R state) while still remaining in equilibrium with the membrane-attached helical T state. These studies show the application of hybrid solid-state NMR methods for large membrane protein complexes in lipid bilayers.

2149-Plat
NMR Structure Determination of Vpu from HIV-1
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Virus protein ‘‘u’’ (Vpu) from HIV-1 is a member of the viroporin family of small viral membrane proteins. It consists of a transmembrane (TM) domain and a cytoplasmic domain, which are associated with different biological functions. Vpu transmembrane (TM) enhances the release of newly formed virus particles from the cells as an antagonist for the human immuno restriction factor, BST-2. It is a single alpha-helix that is capable of oligomerization and exhibits ion-channel activity when incorporated into a membrane environment. The wildtype TM conducts monovalent cations, while a mutant in which a histidine is introduced mimics the properties of a proton channel. The cytoplasmic domain causes the removal of CD4 receptor from ER and its subsequent degradation. It is essential to determine the three-dimensional structure of Vpu in order to obtain an understanding of its molecular mechanisms and to develop new classes of anti-viral drugs. A combination of solution and solid-state NMR experiments are used to obtain protein structural information from different constructs of Vpu. Solution experiments gave valuable information toward obtaining an initial vpu structure. Solid-state NMR techniques are employed to study the protein in lipid bilayer, which is a more biologically relevant environment than micelles. For magnetically oriented samples (OS), proteins are incorporated into DMPC/DHPC bicelles. To complement the OS structural data and alleviate resolution problems caused from the highly dynamic regions of the protein, novel MAS experiments are being implemented to study Vpu incorporated into DMPC liposomes. 15N, 13C chemical shift as well as 1H-15N, 1H-13C dipolar couplings can be extracted based on the new experiments and converted to equivalent structural restraints. Structural features of Vpu obtained from the combination of various techniques will be presented.

2150-Plat
Structure-Function Investigation of a Transglycosylase Associated Protein from Mycobacterium Tuberculosis
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1/3 of the world’s population is infected with Mycobacterium tuberculosis 10% of whom will become sick from the bacilli. Multidrug resistant strains immune to the leading tuberculosis antibiotics, isoniazid and rifampicin, have emerged necessitating new treatments. Resuscitation from the dormant state is a key point at which infection can be fought. Cell wall reorganization is important for this activity and relies on transglycosylase enzymes to synthesize and degrade the peptidoglycan layer of the cell wall. The process is regulated by other proteins. Unfortunately, these interacting partners have not all been identified. Rv1861 is a gene product that is highly conserved across all species of Mycobacteria and may be a prime target for regulation of tranglycosylase activity in these species. Rv1861 is identified by a ’transglycosylase associated protein’ domain signature of three transmembrane alpha helices in a short (~100 amino acid) sequence. The protein also contains the Walker A, and potentially Walker B, motif which are typically involved in nucleotide binding and hydrolysis. Rv1861 is an excellent target for the investigation of activated protein-protein interactions in a membrane environment.
Here we present the initial characterization of Rv1861 reconstituted into synthetic lipid vesicles. Aligned sample solid state NMR is used to elucidate the transmembrane alpha helix orientations revealing that all helices are tilted significantly, >30°, with respect to the membrane normal. Phosphorous NMR is utilized to observe the nucleotide binding and hydrolysis activities of Rv1861. Spectroscopic detection of reaction products measures reaction rates while changes in chemical shift measure binding affinity. Magic angle spinning NMR techniques allow for the measurement of interhelical distances but first require the assignment of the highly overlapped resonances common in the correlation spectra of integral membrane proteins. Initial assignments are presented and labeling strategies are discussed.