1247-Pos Board B198
Probing Structure and Dynamics of Externalized Transmembrane Alpha Helices of S21 Pinholin Protein using Electron Paramagnetic Resonance Spectroscopy
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The last step of the bacteriophage infection cycle is cell death through lysis, the breakdown of the cell membrane. The mechanism of lysis uses two proteins, a small membrane protein called the holin and a muralytic enzyme called endolysin. The holin protein of this study is the S21 pinholin, a small hole forming membrane protein comprised of two transmembrane α-helical domains, TMD1 and TMD2. Only TMD2 is required for membrane hole formation, whereas TMD1 acts as the active inhibitory domain of TMD2. Therefore, in the mechanism of hole formation TMD1 must be externalized from the cell membrane. Although the function of TMD2 is well characterized there is a lack of information regarding TMD1. It is currently believed that TMD1 serves no functionality once externalized from the membrane. However, there is substantial literary evidence suggesting otherwise. It is hypothesized that the externalized TMD1s interact with each other using the glycine zipper α-helical packing motif to inhibit any TMD1s from looping over to close off or block the holin hole once formed. These interactions between TMD1s could also stabilize the pinhole formation and conformational changes of TMD2 inside the membrane.
This study will be the first time solid phase peptide synthesis (SPPS) will be used to make the S21 pinholin protein. The holin proteins will be site-specifically spin labeled with MTSL and the purity will be confirmed using MALDI-TOF mass spectroscopy. Next will be to determine the degree to which TMD1 is externalized through lipid system incorporation and EPR power saturation techniques. This is followed by confirmation of TMD1 α-helical structure retention post externalization. Finally, externalized TMD1 interactions will be probed using DEER and ESEEM EPR techniques to measure distances between neighboring TMD1s.

1248-Pos Board B199
Probing the Secondary Structure of Membrane Protein using Bacterial Expression System and Electron Spin Echo Envelope Modulation (ESEEM) Spectroscopy
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Membrane proteins possess a variety of functions essential to the survival of organisms. However, due to the inherent hydrophobic property, it is extremely difficult to gain the structural information. Previously, we probed the structure of an α-helical component of a small model membrane peptide, AchR M2δ, by detecting 2H modulation from a 2H-labeled Val side chain that was 3 or 4 residues away from a nitroxide spin label using ESEEM spectroscopy. In this study, for the first time, ESEEM technique was applied to determine the local secondary structure of a biological membrane protein system using bacterial expression method for incorporating the 2H-labeled Val side chain both in micelle and lipid bilayer environment. An integral membrane protein, KCNE1 with known 3D structure, was utilized as a model membrane protein to test the feasibility and validity of this ESSEM technique. The side chain of valine at position 21 (outside domain), 50 (transmembrane domain), and 95 (cytoplasmic domain) was 2H labeled (denoted as ith position). An MTSL nitroxide spin label was positioned at 2, 3, and 4 residues away (denoted i+2 to i+4, respectively) from 2H-labeled Val residue of each domain. Our results indicated that i+3 and i+4 positions have a significant 2H modulation while 2H modulation is absent at i+2 position, which is consistent with the 3.6 residue/turn periodicity of the α-helix. These results are also consistent with the micelle structure of KCNE1. This ESEEM technique was further validated using the protein incorporated into lipid bilayer of DMPC/DPC bicelle and the transmembrane domain was probed to be α-helical as well. This ESEEM technique is very powerful, efficient, and quick to examine the local secondary structure of any protein system without size limitation.

1249-Pos Board B200
Structure and Activity of the Outer Membrane Protein Ail from Yersinia Pestis
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Yersinia pestis, the causative agent of plague, is a highly pathogenic organism that spreads rapidly and causes extremely high human mortality. Y. pestis is sensitive to a restricted panel of antibiotics, however, it is classified as a Tier 1 Select Agent due to its extraordinary pathogenicity, the potential weaponization of aerolized bacteria with bio-engineered antibiotic resistance, and the lack of an effective vaccine. The outer membrane protein Ail (attachment invasion locus) is a Y. pestis virulence factor and a prime candidate for therapeutic development due to its two principal activities in mediating bacterial adhesion to host cells and promoting resistance to human complement. Here we present the backbone structure of Ail, determined by NMR spectroscopy in detergent micelles. We also describe activity assays that provide information about the interactions of Ail with its human ligands. Furthermore, we present the results of NMR and activity studies performed with Ail incorporated in phospholipid liposomes and nanodiscs. Overall the data highlight the importance of obtaining structural and functional data in the native-like heterogeneous environment of the lipid bilayer membrane, where the extracellular loops of the protein can be solvated by water and the transmembrane barrel is embedded in the hydrophobic membrane core.
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1250-Pos Board B201
Mechanisms of Membrane Protein Crystallization
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In spite of the importance of membrane proteins (MPs) in many essential biochemical pathways, there are limited high-resolution structures of this class of proteins in the Protein Data Bank. This is mostly due to the difficulties in obtaining well-diffracting crystals, as crystallization phenomena are not well-understood and crystals are obtained largely by trial-and-error. Our approach for improving in surfo MP crystallization is focused on the critical micelle concentration (CMC) of surfactant monomers and their interaction with common precipitating agents, including poly(ethylene) glycol (PEG) and 2-methyl-2,4-pentanediol (MPD). The phenomena were investigated using two model MPs - reaction center from Rhodobacter sphaeroides and outer membrane protein X expressed in Escherichia coli. Protein-detergent complexes (PDCs) of these proteins were studied at molecular and nanoscopic length scales using multiple techniques, including NMR, isothermal titration calorimetry (ITC) and small-angle scattering (i.e., SANS and SAXS) under crystallization conditions. We found that surfactant microstructure changes significantly in the presence of precipitating agents, and the CMC of surfactant monomers increases due to the favorable interaction with precipitants and additives. ITC dilution measurements showed an increase in the CMC over a broad range of crystallization conditions reported in the literature, indicating that precipitants act as co-solvents of monomeric surfactants. Additionally, NMR and scattering results suggest the formation of a complex between precipitant and surfactant molecules and shrinkage in the detergent micelle dimensions, respectively. Our crystallization trials with model MPs indicate that optimal crystallization conditions were closely correlated to the detergent concentrations at or slightly below the measured detergent CMC in a protein-free environment; excess surfactant conditions ($>$ CMC) tend to inhibit crystal formation, while lower surfactant conditions ($<$ CMC) promote aggregation. We propose a rational design of crystallization trials can be made based on a priori knowledge of surfactant CMCs under crystallization conditions.

1251-Pos Board B202
Transmembrane-Peptide Structure Formation from Coarse-Grained Simulations
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Interfacial systems are at the core of fascinating phenomena in many disciplines, such as biochemistry, soft-matter physics, and food science. However, the parametrization of accurate, reliable, and consistent coarse-grained (CG) models for systems at interfaces remains a challenging endeavor. In this work, we report recent advancements made toward the description of secondary-structure formation of peptides in a membrane environment using CG models. By combining a lipid model that can semi-quantitatively reproduce material properties of a fluid membrane bilayer [1] and a peptide model that is not biased toward one particular state (e.g., alpha-helix or beta-sheet) [2], the combined parametrization [3] allows to look at how peptide structure is affected by the membrane environment on long timescales. We illustrate the