contain multiple spectral components. In this study, membrane exposed sites on model α-helical and β-barrel proteins LeuT and OmpA, respectively, were investigated using X-ray crystallography, mutational analysis, nitroxide side chain derivatives, and spectral simulations in order to obtain a motional model of the nitroxide. For each crystal structure of spin labeled LeuT, the nitroxide ring of the spin label is resolved and makes contacts with hydrophobic residues on the protein surface. In addition to the crystal structures, the spectral effect of nearest neighbor mutations and the spectral simulations suggest the spin label has a greater affinity for its local protein environment compared to water-soluble proteins. These results for spin labeled sites on LeuT and OmpA begin to explain the observed trends in membrane protein CW EPR spectra.

778-Pos Board B578

Utilizing Electron Spin Echo Envelope Modulation (ESEEM) Spectroscopy to Probe the Structure of Membrane Proteins

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New approaches are needed to more efficiently probe the structural properties of membrane proteins. A new approach has been developed to probe the structural properties of membrane peptides and proteins using the pulsed Electron Paramagnetic Resonance (EPR) technique of Electron Spin Echo Envelope Modulation (ESEEM). This technique can measure short-range distances between a nitroxide spin label and a 2H nucleus out to approximately 8Å. For this study a model membrane peptide M2δ, was constructed by solid phase peptide synthesis and inserted into a DMPC/DHPC bicelle membrane. We report for the first time, the direct detection of 2H modulation between a 2H-labeled d8 Val residue and a nitroxide spin label three and four residues away that is characteristic of an alpha-helical secondary structure. Simulations of the ESEEM data reveal a distance of approximately 6.4 +/- 0.5Å that agrees well with molecular modeling studies. ESEEM spectra in this work yielded high-quality data in less than an hour with as little as 35μg of protein sample.

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Structural Studies on the Conformation of Human KCNEL1 Membrane Protein via Electron Paramagnetic Resonance Spectroscopy

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Multi-frequency CW-EPR, Electron Spin Echo Envelope Modulation (ESEEM), and Double Electron Electron Resonance (DEER) coupled with site-directed spin labeling (SDSL), molecular dynamics modeling, and rigorous data analysis can be used to report both qualitative and quantitative information about structure and dynamics of a complex biological system. The short range distances can be measured between isotopically coupled nuclear spins and nitroxide electronic spin labels up to a distance of about 8Å using ESEEM and long range distances of 20–70Å between two nitroxide electronic spin labels using DEER. The transmembrane domain (TMD) of KCNE1 membrane protein plays a key role in the modulation of voltage gated channel activity. In order to describe the conformation of TMD of KCNE1, cysteine mutants were generated along the TMD and extracellular region of KCNE1 and further modified by MTSL nitroxide spin labels. The purified proteins were reconstituted into model membranes: Fos-Choline, LMPG micelles and POPC/POPG bilayer vesicles. CW-EPR experiments were performed on the mutants at X and Q-bands in the rigid limit and motional regime. A simultaneous multi-frequency EPR data analysis was employed to obtain the dynamic behavior of spin labels along the protein sequence. The isotropic motion of spin probe was found to decrease towards the interior region of the TMD of the protein and reaches a minimum at the G60C position indicating that the motion of the probe is hindered by the nearby overlapped hydrophobic residues and membrane environment. Additional structural information was revealed by performing ESEEM experiments on i+1 to i+5 sites, where i represents the deuterium position V502H on the TMD, and DEER was on sites V47C-I66C and V50C-S68C. The distances extracted from ESEEM and DEER are in good agreement with NAMD/ VMD and MMM modeling results.

780-Pos Board B580

Probing the Structural and Dynamic Properties of KCNE1 Using Site-Directed Spin Labeling EPR Spectroscopy

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KCNE1 (minK), a membrane protein known to modulate the function of the KCNQ1 voltage-gated potassium ion channel in the human heart, has been successfully overexpressed, purified into detergent micelles, labeled, and incorporated into POPC:POPG lipid bilayers. CW-EPR and Power Saturation EPR Spectroscopy has been used to examine the structural properties of KCNE1 in lipid bilayer vesicles, as opposed to detergent micelles. We are also currently making efforts to develop a technique which will allow quick determination of the local secondary structure of membrane proteins such as KCNE1 using ESEEM spectroscopy. It is important that these studies be conducted in lipid bilayers as opposed to micelles to better model the native membrane environment.

781-Pos Board B581

The Structural, Dynamic, and Functional Changes in the KCNE1 Membrane Protein Between Detergent Micelles and Lipid Bilayers

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Structural studies of membrane proteins in natural lipid bilayer environments are often difficult to perform because the nature of the samples produced is not conducive for the collection of reliable data. In this study KCNE1 (minK), an integral membrane protein found in the human heart, was over expressed and purified into SDS detergent micelles.. After obtaining the pure protein in micelles, we were able to successfully insert it into lipid bilayer vesicles composed of POPC and POPG using various dialysis techniques. Proper insertion of the protein into vesicles was confirmed through CD spectroscopy, CW EPR (X-Band), and Power Saturation EPR. Functional studies performed in Xenopus oocytes expressing KCNQ1 showed KCNE1 maintained its activity when removed from a micelle environment and placed in a lipid bilayer. We discuss the importance and merits of studying membrane proteins in a more native lipid bilayer environment over detergent micelles.

782-Pos Board B582

Structure and Function of Synaptotagmin 1 C2 Domains as Determined by Site-Directed Spin Labeling

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Synaptotagmin 1 (syt1) is a synaptic vesicle protein believed to act as the Ca2+ sensor for neuronal exocytosis. It consists of one N-terminal transmembrane helical segment and two C2 domains (C2A and C2B) that are connected by a short, flexible linker. The calcium binding loops of each C2 domain coordinate Ca2+ ions and bind anionic phospholipids. Syt1 also interacts with the neuronal SNARE proteins, which may play a role in the fusion process. We are characterizing the structure of syt1 both in its aqueous and membrane bound states and bound to the soluble core SNARE complex. Double cysteine mutations were engineered into a water soluble fragment of syt1 C2A-C2B and derivatized with the methanethiosulfonate spin label. Four-pulse DEER was used to obtain distance measurements between C2A and C2B in solution, with membranes, and bound to the soluble SNARE complex. The DEER-derived distances were used as restraints in a simulated annealing routine. The predominant structure is one where the C2 domains are separated by about 40 Angstroms and are oriented anti-parallel so that their Ca2+-binding loops are positioned in opposite directions. Broad distance distributions are obtained by DEER, and indicate structural heterogeneity which may be the result of the flexible linker segment joining the two domains. This structural arrangement does not change when the protein is bound to membranes or the soluble SNARE complex. Furthermore, C2A-C2B is shown to bridge bilayers, which is mediated by multiple contacts of the positive charged regions of the C2B domain and the anti-parallel orientation of C2A and C2B. The result suggests that one role for syt1 in fusion is to bridge across the vesicle and plasma membrane surfaces in a Ca2+-dependent manner. The work was supported by NIGMS grant GM 72694.

783-Pos Board B583

In the Arms of EcoRI - probing the Binding Specificity of the Restriction Endonuclease Using Electron Spin Resonance

Jessica Sarver, Katherine Stone, Jacque Townsend, Paul Sapienza, Linda Jen-Jacobson, Sunil Saxena.

Pulsed electron spin resonance (ESR) was used to probe the binding specificity of EcoRI, a restriction endonuclease that binds to and cleaves a six base pair sequence of DNA. EcoRI binds to the specific sequence GAATTC with an affinity that is 50,000-90,000-fold greater than that of a miscognate site that differs by only one base pair. Low binding affinity is also exhibited at non-specific binding sites which differ from the specific sequence by two or more base pairs. Distance measurements were performed on several spin labeled EcoRI mutants when bound to specific, miscognate, and non-specific sequences of DNA using Double Electron-Electron Resonance. These distances demonstrated that on average the arms of EcoRI, thought to play a major role in binding specificity, are similarly positioned. Additionally, noncognate (miscognate and non-specific) complexes demonstrated broader distance distributions indicating that the flexibility of the arms is greater in these complexes. Room temperature continuous wave (CW) experiments were also performed on the EcoRI mutant complexes