Membrane proteins have become the target for the majority of drug related therapies in recent years; in contrast, there is limited information available on membrane proteins due to the difficulties of studying them in vitro. In order to study their structure and function, it is crucial to prepare suitable, native-like membrane mimics. Our studies involve KCNE1, a transmembrane protein located in the heart that modulates the activity of the KCNQ1 voltage-gated potassium channel. An important protein for proper cardiac function, mutations in the structure can lead to atrial fibrillation, long QT syndrome, and deafness.

In order to assess the viability of various membrane mimics for studying membrane proteins, we have utilized site-directed spin labeling (SDSL) and electron paramagnetic resonance (EPR) spectroscopic techniques, which are well established methods of studying protein structure. The CW-EPR spectral line shape analysis was conducted on an inside probe (F56C) and outside probe (R33C) in various vesicle compositions (POPC, POPG, DMPC, DOPC, DPPC, and DOPG) in order to assess the accuracy and precision of various membrane mimics. This study will provide a path for researchers working on membrane protein EPR spectroscopic studies to select a better membrane mimetic environment.

**Expression and Purification of Human A2b Receptor for Spectroscopic Characterization**

**Expression and Purification of Human A2b Receptor for Spectroscopic Characterization**

Arash Foroutan, Anne S. Robinson

Tulane University, New Orleans, LA, USA.

Adenosine A2b receptors belong to the G protein-coupled receptors family, and are implicated in asthma, regulation of cell growth, vasodilation, intestinal function, and modulation of neurosecretion. So far, there is no high-resolution structure of the protein. Thus, considering A2b receptor as a potential therapeutic target highlights the significance of conformational characterization of the protein. We used A2b receptor variant (b*a) containing A2b-R-based thermostable mutations (T98A, G119A, R123A, V208A, V240A), and A2a C-terminal residues that enable expression but retain ligand-binding function of wild type A2bR. The yeast S. cerevisiae strain BJ5464 was transformed by C-terminal residues that enable expression but retain ligand-binding function of the protein.

**Expression and Purification of Human A2b Receptor for Spectroscopic Characterization**

Megan M. Dunagan, Indra D. Sahu, Rongfu Zhang, Andrew Craig, Robert McMarrick, Gary A. Lorigan.

Miami University of Ohio, OXFORD, OH, USA.

455-Pos Board B235

Analyzing the Viability of Various Native Membrane Mimics for Membrane Proteins using Site-Directed Spin Labeling EPR

**Expression and Purification of Human A2b Receptor for Spectroscopic Characterization**

Megan M. Dunagan, Indra D. Sahu, Rongfu Zhang, Andrew Craig, Robert McMarrick, Gary A. Lorigan.

Miami University of Ohio, OXFORD, OH, USA.

Membrane proteins have become the target for the majority of drug related therapies in recent years; in contrast, there is limited information available on membrane proteins due to the difficulties of studying them in vitro. In order to study their structure and function, it is crucial to prepare suitable, native-like membrane mimics. Our studies involve KCNE1, a transmembrane protein located in the heart that modulates the activity of the KCNQ1 voltage-gated potassium channel. An important protein for proper cardiac function, mutations in the structure can lead to atrial fibrillation, long QT syndrome, and deafness.

In order to assess the viability of various membrane mimics for studying membrane proteins, we have utilized site-directed spin labeling (SDSL) and electron paramagnetic resonance (EPR) spectroscopic techniques, which are well-established methods of studying protein structure. The CW-EPR spectral line shape analysis was conducted on an inside probe (F56C) and outside probe (R33C) in various vesicle compositions (POPC, POPG, DMPC, DOPC, DPPC, and DOPG) in order to assess the accuracy and precision of various membrane mimics. This study will provide a path for researchers working on membrane protein EPR spectroscopic studies to select a better membrane mimetic environment.
Cytochrome c (cyt c) displays a striking ability to perform many seemingly disparate functions within the cell. It is responsible for shuttling electrons between complex I and II in the mitochondrial electron transport chain (ETC). Moreover, cyt c also plays a role in the regulation of apoptosis as a primary signal for apoptosis when improperly localized as a result of an interaction with the mitochondrial lipid cardiolipin (CL). We utilized reverse micelle nuclear magnetic resonance (RM-NMR) in order to investigate these different functional roles of cyt c at atomic resolution. The chemical shifts of RM encapsulated cyt c are essentially identical to the free solution protein, confirming structural fidelity. We have determined the structure of encapsulated cyt c to high resolution (0.45 Å backbone RMSD, 0.92 Å heavy atom RMSD) using standard solution NMR methods. Using pseudo-contact shifts (PCS), we find that the majority of the protein structure does not change significantly upon change in redox state. A subset of residues localized at the heme-exposed face of the protein undergo small structural changes upon change in redox state, localized to the binding site for its BC1 complex partner. The interaction of cyt c with CL was investigated by titration of the lipid into the RM encapsulated protein. The confined space effect upon protein encapsulation in the RM allowed for separation and characterization of this peripheral interaction from the subsequent lipid insertion and unfolding of cyt c. These experiments provide the first detailed interface of the initial, largely electrostatic phase of the interaction.

461-Pos Board B241 Prion Proteins and Mechanisms of Interaction with Model Membranes Patricia Soto 1, William Graft1, Roger Gonzalez2, Chad Nieri2, Bo Zhao1, Jason C. Bartz2, 1Physics, Creighton University, Omaha, NE, USA, 2Biology, Creighton University, Omaha, NE, USA.

Prions are infectious agents responsible for transmissible spongiform encephalopathies, a fatal neurodegenerative disease in mammals, including humans. Prions propagate biological information by conversion of the nonpathological version of the prion protein, PrPc, to the infectious conformation, PrPSc. To shed light on the biogenesis of PrPSc on the cell surface, we report on multiscale molecular modeling studies of enthalpy-driven binding modes of PrPc to model membranes and the conformational response of PrPc to such binding events. Our preliminary results suggest the existence of preferential binding spots on the PrPc surface driven by favorable protein-membrane electrostatics interactions. Upon binding, the conformational space of PrPc is reduced.

462-Pos Board B242 Functional Characterization of Human Rhodopsin Mutations by Fluorescence Imaging Caihong Jiang, physics, east china normal university, Shanghai, China.

Rhodopsin is the membrane receptor responsible for photoreception in the vertebrate retina. Over 120 point mutations in rhodopsin have been found to be related with autosomal dominant retinitis pigmentosa (ADRP) and the congenital stationary night blindness (CNBS). Despite of several mutations with intense studies, like P23H, a majority of rhodopsin mutations still need further investigations. In order to have extensive and quick functional characterization of these mutations, here we utilize fluorescence imaging to monitor rhodopsin cellular distribution, which reveals to us much useful information, like if rhodopsin has normal transportation to the cell membrane, interrupted glycosylation or protein aggregates formation. The experiments are carried out through the following process: First, a series of human rhodopsin mutations were constructed, which include mutations responsible for both ADRP and CNBS, like G89D and G90D. Second, wild-type rhodopsin was expressed in 293S GnTi- cells with homogeneous residues than periplasmic or extracellular loops. This effect can be seen in both the molecular interactions that mediate the entry of a foreign body into non-phagocytic cells.

463-Pos Board B243 Coexistence of Native-Like and Non-Native Misfolded Ferricytochrome C on the Surface of Cardiolipin Containing Liposomes Leah A. Pandiscia, Reinhard Schweitzer-Stenner. Chemistry, Drexel University, Philadelphia, PA, USA.

Cytochrome c, in spite of adopting a rather rigid structure around its prosthetic heme group, is rather diverse with regard to its function and structural variability. On the surface of the inner membrane of mitochondria it serves as an electron transfer carrier. However, at conditions, which have not yet been unambiguously identified, it can adopt a variety of non-native conformations some of which exhibit peroxidase activity. Cardiolipin-containing liposomes have served as ideal model system to investigate the various modes of interaction between cytochrome c and the inner mitochondrial membrane. We probed the binding of horse heart ferricytochrome c to liposomes formed with 20% tetraoleoyl cardiolipin and 80% dioleyoyl-sn-glycerol-3-phosphocholine as a function of lipid/protein ratio by fluorescence, fluorescence anisotropy, and visible circular dichroism spectroscopy. A global analysis of our data revealed the existence of three binding sites on the protein which causes rather different degrees of protein unfolding. We found that two of the three modes of interaction between protein and liposome led to conformational changes. A more native-like state or a higher population of the native state is obtained in the presence of NaCl, which also leads to a nearly total inhibition of the binding via the two lower affinity protein binding sites. Overall, we can rationalize the two-state equilibrium between a compact C and an extended E-state proposed by Pletneva and coworkers. We conjecture that the bound state produced by the high affinity site 1 binding might bear the closest relationship to the protein which functions as electron carrier in the mitochondria. The higher E-state population produced by site 2 and 3 binding is likely to increase the protein’s capability to function as a peroxidase.

464-Pos Board B244 Neisserial Opa Protein Dynamics and Interaction with Host CECAM Receptors Marissa K. Kieber, Tsegaa Solomon, Linda Columbus. Department of Chemistry, University of Virginia, Charlottesville, VA, USA.

Human pathogens Neisseria gonorrhoeae and N. meningitides are unique in their utilization of opacity-associated (Opa) proteins to mediate bacterial uptake into non-phagocytic cells. Opa proteins engage either heparan sulfate proteoglycan (HSPG) or carboxy-terminal antigen-related cellular adhesion molecules (CEACAMs) to hijack host cellular mechanisms, which induces bacterial engulfment. The Opa family of proteins are eight stranded β-barrels with four extracellular loops. Regions in loops two and three contain hypervariable sequences where Opa variants and dictate receptor specificity. We aim to investigate the structural determinants of Opa-receptor interactions. Overall loop dynamics of OpaAβ1, a CEACAM-binding Opa variant, were determined using CW-EPR and combined with the limited NMR relaxation data. Results indicate that the loops and hypervariable regions are highly mobile on the nanosecond timescale. Initial DEER experiments measured distances between Opab and CEACAM in the complex and preliminary models consistent with these distances will be presented. Determining the interactions between Opa and CEACAM will provide an understanding of the molecular interactions that mediate the entry of a foreign body into non-phagocytic cells.