helices is significantly different with bulky hydrophobic residues buried deep in the membrane. Salient features of the structure will be reported in light of proton transport mechanism.

**253-Pos**

**Site-Directed Spin-Label EPR Studies Report on Drug-Induced Conformational Change of Influenza a M2 Protein**

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The M2 protein from influenza A is a pH-activated proton channel that plays an essential role in the viral life cycle and serves as a drug target. Using spin labeling EPR spectroscopy we studied a 38-residue M2 peptide spanning the transmembrane region and its C-terminal extension. We have obtained residue-specific environmental parameters in the presence of the antiviral drug amantadine to gain information about the drug bound state of M2 in POPC/POPG lipid bilayers. Power saturation studies of spin-labeled peptides reconstituted in a DOGS-NTA(Ni)-containing bilayers report on the accessibility of spin labels to nickel(II) chelated at the aqueous-lipid interface.

**254-Pos**

**HIV-1 Matrix Binding to Model Membranes Investigated By Neutron Reflectivity: Electrostatics and Binding Orientation**

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The N-terminal Matrix (MA) domain of the HIV-1 Gag protein is responsible for binding the membrane during viral assembly. A basic patch of residues localized in the MA domain confers a strong electrostatic component to this binding interaction. Through mutagenesis the putative binding interface of MA has been mapped out, but not directly determined by experimental measurements. We present neutron reflectivity measurements that resolve the one dimensional scattering length density profile of MA bound to a lipid membrane. The model membrane system used maintained the anionic surface charge density of the native viral membrane. Molecular refinement using atomic structures of MA suggests an orientation of the protein on the membrane consistent with previous mutagenesis and electrostatic modeling studies. Remarkably the MA protein maintains this orientation without the presence of the post-translational myristate group. Furthermore MA is found to only peripherally penetrate the membrane headgroups by $4.8 \pm 1.2$ Å allowing only amino acid side chains to contact the lipid headgroups. Our results find that electrostatic interactions are sufficient to preserve the correct binding motif of MA with the viral membrane.

**255-Pos**

**Oligomerization of Transmembrane Alpha-Helices Modulated By C-terminal Boundary Residues**

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In studies of the structural biology of membrane proteins, the success of strategies based on the ''divide-and-conquer'' approach, where peptides are used to model the individual transmembrane (TM) α-helices of membrane proteins, depends upon the correct identification of the membrane-embedded TM α-helix amino acid sequence within the full-length protein. In the present work, we examine the effects of excluding or including TM boundary residues on the intrinsic properties of the TM2 α-helix of myelin proteolipid protein (PLP). Using protein gel electrophoresis, circular dichroism, and fluorescence resonance energy transfer in the membrane-mimetic detergent sodium dodecylsulfate (SDS) to study parent sequence KKKK-$^{61}$AFQYVIYGTASFFFLYGALL-LAEG$^{89}$-KKKK - along with analogs containing an additional wild type Phe-90, Phe-90 and Tyr-91, and a hydrophobic mutant Leu-90 - we demonstrate that the removal of a single amino acid from the C-terminus of this TM segment is sufficient to change its intrinsic properties, with TM2 61-89 displaying only a monomeric form, but with principally dimers arising for the other three peptides. The findings suggest that deletion of critical C-terminal residue(s) tends to re-position the helix terminus toward the membrane-aqueous interface, and emphasize the potential influence of boundary residues on TM properties when utilizing peptides as models for TM α-helices. These finding may implicate a role for such residues in membrane protein folding and assembly.

**256-Pos**

**Structure, Dynamics and Topology of the N-terminus and First Transmembrane Segment of APJ**

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APJ is a G-protein coupled receptor expressed in the cardiovascular system, central nervous system and several other tissues. Activation of APJ by the peptide ligand apelin has defined roles in cardiovascular regulation, in glucose metabolism and in tumour growth. Transmembrane proteins such as APJ are difficult to study due to expression, solubility and refolding difficulties. For this reason we have produced a fragment of APJ containing the functionally essential N-terminal region and first transmembrane helix of the receptor (APJ55). Through a combination of circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopy we have characterized APJ55. CD spectroscopy indicates that APJ55 only properly refolds in specific detergents, with the anionic detergents sodium dodecylsulphate and 1-palmitoyl-2-hydroxy-sn-glycero-3-[phospho-rac-(1-glycerol)] (LPPG) being the best. NMR spectroscopy has provided an initial structure of APJ55. As a complement to this structure, relaxation studies and paramagnetic spin label titration demonstrated the dynamics and topology of APJ55 in the LPPG micelle. Finally the structure of APJ55 has been placed into the context of full length APJ using a homology model. APJ55 provides a new system to probe apelin-APJ interactions and is a basis for study of additional regions of APJ.

**257-Pos**

**CD and EPR Structural Studies on the KCNE1 Protein in a Lipid Bilayer**

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KCNE1, also known as minK, is a membrane protein responsible for modulating the KCNQ1 voltage-gated potassium ion channel in the human heart. Previous *in vivo* electrophysiological studies have shown that KCNQ1 loses its functionality in the absence of KCNE1, showing that KCNE1 is an essential protein for proper heart function (Sanders et al., Biochemistry 2007 46:11459-11472). Though KCNE1 has been extensively studied in micelle detergent systems, little work has been done to study the protein in an actual lipid bilayer-membrane system. Our current research uses biophysical techniques such as circular dichrosim (CD) spectroscopy and electron paramagnetic resonance (EPR) spectroscopy to characterize and compare KCNE1 proteins in various micelle and lipid bilayer environments using both qualitative and quantitative methods. Our CD spectroscopy experiments have shown that KCNE1 undergoes a change in secondary structure when removed from a micelle environment and placed in a lipid bilayer. We have used EPR spectroscopy to show that the dynamic properties of KCNE1 also change when taken out of micelles and inserted into lipid bilayers. Calculations have been done to quantify these differences in the structural and dynamic properties observed for KCNE1 in micelles and lipid bilayers.

**258-Pos**

**Accessory Alpha-Helix of Complexin I Can Displace VAMP2 Locally in the Complexin-Snare Quaternary Complex**

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The calcium-triggered neurotransmitter release requires three SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins: synaptobrevin 2 (or VAMP2) on the synaptic vesicle and syntaxin 1 and SNAP-25 at the presynaptic plasmas membrane. This minimal fusion machinery is believed to drive fusion of the vesicle to the presynaptic membrane. Complexin, also known as synaphin, is a neuronal cytosolic protein that acts as a major regulator of synaptic vesicle exocytosis. Stimulatory and inhibitory effects of complexin have both been reported, suggesting the duality of its function. To shed light on the molecular basis of the complexin's dual function, we have performed an EPR investigation of the complexin-SNARE quaternary complex. We found that the accessory α-helix (amino acids 27-48) by itself has the capacity to replace the C-terminus of the SNARE motif of VAMP2 in the four-helix bundle and makes the SNARE complex weaker when the N-terminal region of complexin I (amino acids 1-26) is removed. However, the accessory α-helix remains detached from the SNARE core when the N-terminal region of complexin I is present. Thus, our data show the possibility that the balance between the activities of the accessory α-helix and the N-terminal domain might determine the final outcome of the complexin function, either stimulatory or inhibitory.

**259-Pos**

**Osmolytes Modulate Conformational Transitions in Solvent-Exposed Regions of Two Outer Membrane Proteins**

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Electron paramagnetic resonance (EPR) spectroscopic studies using site-directed spin labeling (SDSL) have been used to investigate local structure and conformational exchange in different regions of two *E. coli* outer-membrane TonB-dependent transporters: BtuB and FecA. It is known that the

TonB-binding region of these transporters, called the Ton box, undergoes dramatic structural changes from an immobile to a mobile configuration upon substrate binding. However, this change is not observed in the crystal structures of BtuB. When SDSL is performed with protecting osmolytes such as polyethylene glycols (PEGs), which are contained in the buffers used in crystallization, this order-disorder transition of the Ton box is abolished in BtuB. Here we find that the substrate-dependent Ton box transition in FecA is also modulated by PEGs, and we show that the five residues N-terminal to the Ton box of BtuB are in conformational exchange between folded and unfolded states. Upon addition of PEGs, this equilibrium is shifted towards the folded state. Larger molecular weight PEGs produce a larger apparent shift in the conformational free energy, consistent with the finding that PEGs are excluded from protein surfaces as a result of steric interactions. The observation of conformational exchange in the N-terminus provides an explanation for differences seen between the *in surfo* and the *in meso* crystal structures of BtuB and suggests that each of these structures represents one conformational substate among a family of substates that are normally sampled by the protein. This work illustrates how SDSL and osmolytes may be used to characterize and quantitate conformational equilibria in membrane proteins.

**260-Pos**

**Proximity of the EGF Receptor Kinase Domain To the Plasma Membrane**

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Understanding the activation mechanism of the epidermal growth factor receptor (EGFR) is of fundamental importance in anticancer drug development. Crystal structures establish the two kinase domains in the receptor dimer bind to and activate each other by an allosteric mechanism. However, information about the conformation of the juxtamembrane (JM) region and the proximity of kinase domains to the membrane in live cells is lacking. Electrostatic potential calculations suggest that both the positively charged N-terminal JM region and a positive face of the kinase domain could bind to the negatively charged inner leaflet of the plasma membrane. The objective of this report is to investigate the proximity of the kinase domain of EGFR to the plasma membrane. We truncated the EGFR after the kinase domain and genetically tagged it with the monomeric yellow fluorescent protein (mYFP). We measured FRET between a plasma membrane target cyan fluorescent protein (PMT-CFP) and the truncated EGFR- mYFP (tEGFR-mYFP). The observed FRET is consistent with the hypothesis that the kinase domain is located close to the membrane in the absence of ligand. Addition of epidermal growth factor (EGF) produced a decrease in FRET, which indicates the kinase domain may dissociate from the membrane during activation.

**261-Pos**

**Distinct Topologies For the HIV-1 Transmembrane Glycoprotein gp41 C-Terminal Tail on Cellular and Viral Lipid Membranes**

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The HIV-1 envelope (Env) transmembrane protein, gp41, is typically considered a type I membrane protein with an extracellular N-terminus, a single membrane-spanning domain, and a C-terminus forming a ~150 residue intracytoplasmic tail. However, published studies indicate an alternative or dynamic topology for portions of the C-terminal tail (CTT) that results in exposure of CTT segments on the membrane surface. To distinguish between these alternative models, we evaluated the accessibility of a reference CTT sequence, the ''Kennedy epitope'' (KE), in viral and cellular membranes to map CTT topology relative to the lipid bilayer. KE accessibility in cell-associated Env was defined by reactivity of native or VSV-G epitope-tagged KE to specific monoclonal antibodies (MAbs), measured by FACS of intact cells. In parallel, KE exposure in virion-associated Env was characterized by MAb binding to intact virions as measured in immunoprecipitation and surface plasmon resonance (SPR) spectroscopy assays. FACS analyses of live cell-associated Env demonstrated significant reactivity of the KE with MAbs to native or VSV-G epitope-tagged KE. No reactivity was observed in cells expressing Env with the VSV-G epitope substituted into a cytoplasmic domain. In contrast to cellular Env, KE in the context of virions failed to react with MAbs directed to the native KE sequence, as measured both by immunoprecipitation and SPR assays. However, MAbs specific for a membrane-proximal sequence bound virions in both assays. Together, the results of these accessibility assays indicate that the KE sequences of gp41 are accessible to antibody binding in cell surface-expressed, but not virion-associated, Env. These observations suggest that the CTT may assume distinct topologies (reflected in KE exposure) that depend on the membrane environment (viral/cellular) and that parts of the CTT may be (transiently) exposed on the membrane surface.

**262-Pos**

**Membrane Topology of Hepatitis C Virus Protein NS4B**

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Hepatitis C virus (HCV) protein NS4B is a poorly characterized highly hydrophobic integral membrane protein which is associated with membranes of the ER or an ER-derived modified compartment. NS4B induces the formation of intracellular membrane changes that are visible by electron microscopy, the so called membranous web. This membranous web has been postulated to be the HCV RNA replication complex. Since morphogenesis and budding has been suggested to take place in ER modified membranes, a function of NS4B might be to induce a specific ER membrane alteration that serves as a scaffold for the formation of the HCV replication complex. If that were true, NS4B might have a critical role in the HCV cycle. It has been predicted that NS4B possess four / five transmembrane (TM) domains. Two helical elements have been predicted in the C-terminal part of the protein, pointing out to a yet unknown common function of the C-terminal globular part. Additionally, RNA binding properties through a nucleotide-binding motif have recently been reported, as well as a new membrane association segment in the N-terminal portion of the protein. Many questions remain unanswered about the NS4B topology but due to the highly hydrophobic nature of the protein, a detailed structure determination using experimental techniques will not be obtained in the near future. In order to define the membrane topology of NS4B we have analyzed the spatial distribution of the spatial hydrophobicity of thirty-one sequences of protein NS4B pertaining to different strains representing the seven major genotypes of HCV. Our study shows that NS4B has at least five and possibly six TM domains.

**263-Pos**

**Evolutionary Conservation of Phospholipid-Binding Sites in Membrane Proteins**

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Membrane proteins evolved to function in a diverse phospholipid environment. Experimental evidence suggests that some phospholipid molecules are recruited by membrane proteins as co-factors or ligands that tightly bind to specific sites on the protein surface and play important functional roles. In this work we have assessed the evolutionary conservation of phospholipid-binding sites in several alpha-helical and beta-barrel membrane proteins. We first identified the membrane protein surface residues and residues that are in close contact with co-crystallized phospholipids in the x-ray structures of adrenergic receptor, photosynthetic reaction center, Kcsa potassium channel, formate dehydrogenase and ferric hydroxamate uptake receptor using methods of computational geometry such as Delaunay triangulation and alpha shape. We next collected orthologous cDNA sequences for every protein and used posterior probability analysis of evolutionary selection pressure measured as ω-ratio with the aid of PAML package to identify phospholipid-facing residues under strong purifying selection pressure. We show that protein residues interacting with co-crystallized phospholipids are collectively more conserved than the rest of the phospholipid-facing residues with statistically significant p-values in the range 10e-7 - 10e-3. Additionally, we found that every phospolipid-binding site on the membrane protein surface contains from 3 to 5 residues that experience strong purifying selection pressure similar to the functionally important buried residues.

**264-Pos**

**Transmembrane Protein Association in a Biomimetic Medium**

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A plethora of membrane proteins studies have been performed on micellar systems containing detergent solubilised proteins. A major drawback of these systems is their rather poor mimicry of biological membranes. Therefore, development of new biologically relevant membrane systems is justified.
We illustrate an original approach combining fluorescence recovery after fringe pattern photobleaching (FRAPP) with the use of an optically isotropic, extremely fluid phase of bilayers (the sponge phase). In this biologically relevant system, well suited for spectroscopic studies, the spacing between two adjacent bilayers and the thickness of the bilayers can be easily and very precisely tuned. So when the membranes are sufficiently separated, the only possible interactions occur between proteins embedded in the same bilayer, whereas when the membranes come together, interactions between proteins embedded in adjacent membranes may also occur.