assay. The investigation of over 60 cysteine mutations within ArnT identify for the first time functionally important sites within the ArnT transference. This work sets the stage for additional studies of the structure and function of this protein using biophysical approaches. This project is supported by the NIH (AI058024).

1677-Pos Board B521
Effect of Short Transmembrane Peptides on the Activation and Dimerization of an FGFR3 Pathogenic Mutant
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Mutations in the transmembrane domains of receptor tyrosine kinases (RTKs) are implicated in many human diseases. For example, fibroblast growth factor receptor 3 (FGFR3) carrying an A391E mutation in the transmembrane (TM) region is associated with cancer and Smith-Lemli-Opitz syndrome in humans. A391E mutation results in increased dimerization propensity of the receptors due to the co-expression. Currently, we are investigating the specificity and the efficiency of the inhibition by using TM peptides from other RTKs. The TM peptides which can specifically and efficiently inhibit the activation and dimerization of FGFR3 mutants are possible candidates for future therapies for diseases linked to FGFR3 domain mutations.

1678-Pos Board B522
Mechanism Of Signal Transduction Through The TLR4 Receptor Complex
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The innate immune system represents our first line of defense against microbial pathogens. TLR4 is the cell-surface receptor primarily responsible for initiating the innate immune response to lipopolysaccharide (LPS), a major component of the bacterial cell envelope. However, relatively little is known about the molecular mechanisms underlying TLR activation. Design of small molecule therapeutics to modulate immune activation will benefit greatly from a better understanding of TLR4 activation and membrane proximal events. Resolution of the molecular mechanisms requires direct structural information for the TLR4 receptor complex, including the ability to detect ligand-induced conformational changes in the components and TLR4 dimerization. To acquire such structural information at the required level of detail, we have reconstituted the system in model membranes and analyzed the TLR4 receptor complex and its structural dynamics during the transition from quiescence to activation using neutron reflectometry, cryoelectron microscopy, lifetime- and spectrally-resolved confocal microspectroscopy and total internal reflectance fluorescence (TIRF) microscopy. The results to date suggest that a conformational change in the accessory protein MD2 upon binding attenuates activation of the receptors.

1679-Pos Board B523
Study of the Effect Of Pulmonary Surfactant Protein B (SP-B) on Phospholipid Membrane Reorganization Using Quartz Crystal Microbalances with Dissipation (QCM-D)
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Pulmonary surfactant protein B (SP-B) is a hydrophobic 79-residue protein, essential for the respiratory function. SP-B is supposed to be involved in the transfer of phospholipid molecules from specific lipid/protein assemblies produced by pneumocytes into the alveolar air-liquid interface to form surface active films. The structure of surfactant and the dynamics of the respiratory surface against collapse and during inspiration is known to be critical for stabilization of the respiratory surface against collapse and during inspiration. Lack of SP-B is lethal, being its absence associated with an irreversible respiratory failure at birth. Quartz crystal microbalance with dissipation technique has been used to analyze SP-B biotic and fusogenic properties in an environment that could be closer to the surfactant multilayer stores that is formed beneath pulmonary air-liquid interface. Processes related with unpacking surfactant lipids as they are transferred into the surface and converted into multilayered forms have been attributed to SP-B function although the molecular mechanism by which the protein could perform these actions are entirely unknown.

QCM-D technique has been used to characterize how SP-B modulates the adsorption properties to surfaces of DOPC and DPPC membranes containing different physiologically-relevant protein proportions, leading to the formation of membrane stacks on the surface rather than a single supported bilayer. We have also investigated how the collapse of lipid/protein vesicles is affected or modulated by physiologically relevant factors such as lipid composition (i.e. presence of anionic lipids, which increase the effect of SP-B), presence of the other surfactant hydrophobic protein, SP-C, or the addition of Ca++. These studies have been conducted using both full-length purified porcine SP-B and selected SP-B peptide fragments.

1680-Pos Board B524
Exploration Of Conformational Changes in the RbsABC Ribose Importer Using EPR Spin Labeling
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ATP Binding Cassette (ABC) transporters are transmembrane transporters that use the energy released by ATP hydrolysis to transport a wide array of substrates. They are found in all kingdoms of life, and are implicated in various genetic conditions, such as cystic fibrosis, macular degeneration, and multi-drug resistance. The E. coli ribose transporter (RbsABC) is a multisubunit ABC transporter complex with a periplasmic ribose binding domain, a transmembrane domain dimer, and a cytoplasmic nucleotide binding domain. The ribose transporter complex has been shown to assemble and disassemble into distinct combinations of the subunits based on the presence of cofactors (ATP and an anlogue, ADP, orthovanadate, and magnesium), suggesting a series of steps for how the subunits associate and subsequently transport ribose.

To further explore the conformation of the complex in the presence of various cofactors, EPR spin labels were introduced to the periplasmic ribose binding domain. The EPR spectra confirms previously observed data suggesting that the ribose binding domain is strongly bound to the transmembrane domain in the resting state. Additionally, data suggest that the ribose binding domain binds in two steps, forming an initial weak interaction, then a strong interaction that results in a reduction of affinity for ribose.

1681-Pos Board B525
Distinct Functional Effects of Kv3.3 Mutations Associated with Spinocerebellar Ataxia Type 13
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Spinocerebellar Ataxia Type 13 is an autosomal dominant genetic disease characterized by ataxia, oculomotor abnormalities, and the death of cerebellar neurons. SCA13 is caused by mutations in the Kv3.3 voltage-gated K+ channel gene, including R366H in S2; R420H and R423H in S4; and F448L in S5. SCA13 exists in two forms with infant or adult onset. There is a strong genotype-phenotype correlation between the disease-causing mutation and the age of onset of symptoms. The functional effects of the mutations fall into two categories. R366H, R420H, and R423H are non-functional when expressed alone and exert strong dominant negative effects when co-expressed with wild type Kv3.3 or other members of the Kv3 subfamily. The stoichiometry of suppression differs among the three R-→H mutations, with R366H subunits less disruptive to function than R420H or R423H. In contrast, F448L is a dominant gain of function mutation that affects channel gating. F448L shifts the voltage depen- dence of activation in the hyperpolarized potential and dramatically slows de-activation. These changes in Kv3.3 gating are not significantly different whether F448L is expressed alone or co-expressed with wild type subunits. Kv3 channels, including Kv3.3, facilitate high frequency firing in neurons. The SCA13 mutations are expected to alter the excitability of cerebellar neurons, which express high levels of Kv3.3. Since the functional effects of the mutations are distinct, it is likely that they will have distinct effects on the excitability of cerebellar neurons. This hypothesis is supported by mathe- matical modeling of firing behavior in cerebellar neurons. Differential effects on neuronal excitability are likely to underlie the symptoms of SCA13 and may help to explain the differences in the age of disease onset. This work was supported by NIH grant R01-N058500 to DMP.

1682-Pos Board B526
Protein-Protein Interactions And The Energy Coupling Mechanism In TonB-Dependent Transport
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BtuB is a TonB-dependent outer membrane transporter of vitamin B12 in E. coli. In this work, we investigated the interaction between BtuB and the inner membrane protein TonB using site-directed spin labeling (SDSL). In CHAPS/POPC mixed micelles, the Ton box of BtuB undergoes an order-to-disorder transition upon addition of vitamin B12 which appears to be identical to that seen in POPC bilayers. Under these conditions, addition of a C-terminal fragment of TonB broadens the EPR lineshapes, indicating that there is an ordering of the Ton box and an interaction between the transporter Ton box and this C-terminal fragment. Residues N-terminal to the Ton box do not appear to interact with TonB. These changes appear to be independent of the addition of the substrate, vitamin B12. The EPR data obtained are generally consistent with the crystal structure that has been obtained for this complex (Shultis et al. Science 312, (2006)); however, preliminary distance measurements using DEER indicate that there may be multiple states of TonB when it is bound to BtuB. Spin labels incorporated into TonB also become ordered upon interaction with BtuB, and the EPR lineshapes indicate that there is a decrease in backbone dynamics of TonB upon association with BtuB. An EPR based equilibrium binding assay was carried out to determine the affinity between this C-terminal TonB fragment and BtuB, and was performed using either labels on BtuB or labels on TonB. Both labels indicate that there is an affinity of approximately 50 μM between BtuB and TonB, which is, unexpectedly, independent of substrate addition.

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1683-Pos Board B527
A Phosphorylation-Based Model for EGFR Activation as a Function of Ligand Concentration
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On the basis of receptor phosphorylation assays, a simple mechanistic model has been proposed to describe the activation of the epidermal growth factor receptor (EGFR) as a function of ligand concentration. A431 cells, which over-express EGFR, were starved and stimulated with different concentrations of EGFR, ranging from 0 - 5000 ng/ml. Phosphorylation of Tyr1068 in EGFR appeared to plateau when cells were stimulated with 5000 ng/ml, suggesting that the maximal activation was reached. Quantitative analysis of Western blots revealed that the activation of EGFR can be described with a simple model, in which only a single fluorescent label is required. The label provides a new tool for studies of photosensitive proteins.

Supported lipid bilayers are a common biomimetic platform for biophysical studies of membrane proteins and the plasma membrane itself. They also hold promise for prion research (PrP). However, a limitation of the typical method of studying membrane proteins is that the proper structure and function of proteins may rely on the presence of other membrane components. Toward the creation of a more comprehensive platform for studies of membrane proteins, we explored two methods of creating cell-derived supported bilayers (CDSBs). Bilayers were formed either via vesicle fusion or vesicle fusion to Langmuir-Blodgett lipid monolayers, and they were analyzed using fluorescence microscopy. The lateral mobility of lipids was assessed by fluorescence recovery after photobleaching (FRAP). In addition, impedance spectroscopy was used to measure the electrochemical properties of the CDSBs.

1686-Pos Board B530
Evidence for Proton-Coupled Protein Transport through the Anthrax Toxin Channel
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The toxin produced by Bacillus anthracis, the causative agent of anthrax, is composed of a translocon heptameric channel (PA63)n, formed from protective antigen (PA), which allows its two substrate proteins, lethal and edema factors (LF and EF), to translocate across a host cell’s endosomal membrane, disrupting the cellular homeostasis. It has been shown that (PA63)n incorporates into lipid bilayers and forms a channel capable of transporting LF, EF and other small proteins. Protein translocation through the channel is driven by a proton electrochemical potential gradient, on a time scale of seconds with S-shaped kinetics. A paradoxical aspect of this is that although LFN (the N-terminal 263 residues of LF), on which most of our experiment was performed, has a net negative charge, it is driven through the channel by a cis positive voltage. We have explained this by claiming that the (PA63)n channel strongly interacts with the cell membrane lipids, resulting in a broadening of EPR lines, and hence the aspartates and glutamates on LFN enter protonated (i.e. neutralized), and therefore the translocated species is positively charged. Upon exiting the channel, these protons that were picked up from the cis solution are released into the trans solution, thereby making this a proton-protein symporter. Here, we provide further evidence of such a mechanism by showing that if only one SO3-C0, which is essentially not titratable, is introduced at most positions in LFN, through the reaction of a cysteine-modified residue at those positions with (2-Sulfonatoethyl) Methanethiosulfonate (MTS-E), voltage-driven LFN translocation is drastically inhibited.

1687-Pos Board B531
Spectral Shift FRET Assay and its Applications for Studying the Dynamics of Proteorhodopsin
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We present a highly sensitive method of studying the dynamics of photosensitive membrane protein proteorhodopsin (PR), by using spectral shift FRET. In normal FRET, the rate of energy transfer depends on the spatial separation of donor and acceptor. In spectral shift FRET, the rate of energy transfer depends on the spectral separation, which varies in response to changes in the chemical environment of one of the chromophores. Our method is particularly suited to macromolecules that contain an endogenous chromophore that undergoes chromatic shifts, in which case only a single fluorescent label is required. The label serves as a fluorescence donor, and the endogenous chromophore serves as an environmentally sensitive quencher. Proteorhodopsin found in marine bacterioplankton is a membrane protein that functions as a light-driven proton pump, converting light energy into chemical energy by creating a proton motive force across the bacterial membrane. The retinal chromophore undergoes dramatic conformational shifts during the photocycle. Bodipy-Texas Red (TR) was incorporated into a blue-absorbing variant of PR on the cytoplasmic side through a single endogenous cysteine (CYS1116). The photocycle was initiated by a 50 ns pulse at 490 nm, and the ensuing dynamics were probed by measuring the fluorescence quantum yield of Bodipy-TR (excitation at 600 nm, detection at 650-700 nm). The signal from a single PR-containing 340 nm lipid vesicle was sufficient to monitor the dynamics of the photocycle, thereby providing a highly sensitive method to monitor microbial rhodopsins. In future studies, a vesicle containing a single PR molecule will be trapped using an Anti-Brownian Electrokinesis trap, and fluctuations in the dynamics of PR will be observed using spectral shift FRET. The technique of spectral shift FRET provides an important new tool for studies of photosensitive proteins.