assay. The investigation of over 60 cysteine mutations within ArnT identify for the first time functionally important sites within the ArnT transferase. 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 bladder cancer and Crouzon syndrome with acanthosis nigricans. Previous work has shown that the isolated FGFR3 TM domains dimerize in detergent micelles and in lipid bilayers. Therefore, we are exploring whether the TM domain of FGFR3 can inhibit the pathogenic effects of the A391E mutation. Our preliminary data show that the activation level of a chimeric Neu_FGFR3 receptor carrying the A391E mutation could be inhibited by co-expressing short mutant FGFR3 TM peptides. We also observe a decrease in 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 TM 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 structural information at the required level of detail, we have reconstituted the system in model membranes and analyzed the TLR4 receptor complex and its dynamics during the transition from quiescence to activation using neutron reflection, cryoelectron microscopy, lifetime- and spectrally-resolved confocal microscopy, and total internal reflectance fluorescence (TIRF) microscopy. The results to date suggest that a conformational change in the accessory protein MD2 upon binding antigen causes association of the receptors.

1679-Pos Board B523
Study of the Effect Of Pulmonary Surfactant Protein B (SP-B) on Phospholipid Membrane Reorganizations 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 supposedly 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 competent to stabilize the respiratory surface against collapse along breathing dynamics. 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 lytic and fusogenic properties in an environment that could be closer to the surfactant multilayer stores thought to be 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^{2+}. 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 complicit 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 transport complex has been shown to assemble and disassemble into distinct combinations of the subunits based on the presence of cofactors (ATP and analogues, 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 protein. 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 dependence of activation in the hyperpolarized potential and dramatically slows deactivation. 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 mathematical 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-NS058500 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.
This work was supported by GM035215.

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 overexpress EGFR, were starved and stimulated with different concentrations of either transforming growth factor (TGF) or epidermal growth factor (EGF), 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, with one activation constant (Ka) describing the formation of active dimers from inactive monomers in the presence of ligands. The model is consistent with previous studies showing that TGF- and EGF-induced phosphorylations of EGFR are similar.

1684-Pos Board B528
In vitro characterization of vinculin's lipid membrane-interacting domain, helix 3
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The focal adhesion (FA) protein vinculin with its 95 kDa head domain (residues 1-858) and 30 kDa tail domain (residues 858-1066) plays an important role in cell migration and adhesion. Binding of vinculin to lipid membranes ensures these processes. Three potential membrane interaction sites (residues 940-970, 1020-1040 and 1052-1066) have been reported on the tail domain. In pull-down assays using artificial lipid membranes it was demonstrated that, when helix 3 (residues 944-972) was mutated on position K952, K956, R963, R966 to Q, its interaction with acidic phospholipid vesicles was impaired. To date, no data exist on the nature of the interaction.
Using differential scanning calorimetry on helix 3 we could show that it inserts into lipid vesicles consisting of dimyristoyl-L-α-phosphatidylcholine (DMPC) and negatively-charged dimyristoyl-L-α-phosphatidylserine (DMPS). However, when mutating the four basic residues (K952, K956, R963, R966 to Q) on helix 3, the insertion into lipid vesicles was reduced. Examining the secondary structure of the non-mutated helix 3 in the presence and absence of DMPC/DMPS lipid vesicles by CD-spectroscopy showed a conformational shift. These observations indicate that the electrostatic interactions of the basic residues on helix 3 induce the insertion into the hydrophobic core and promote the localization of the vinculin molecule to lipid membranes.

1685-Pos Board B529
Integration of Plasma Membrane in Supported Lipid Bilayers
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Supported lipid bilayers are a common biomimetic platform for biophysical studies of membrane proteins and the plasma membrane itself. They also hold potential for practical applications in biosensors. However, a limitation of the typical method of studying membrane proteins by reconstitution in pre-assembled bilayers 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 translocase heptameric channel, $(PA_{63})_7$, 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 $(PA_{63})_7$ incorporated into planar phospholipid bilayer membranes 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 LF_N (the N-terminal 263 residues of LF), on which most of our experiment were 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 $(PA_{63})_7$ channel strongly disfavors the entry of negatively charged residues on proteins to be translocated, and hence the aspartates and glutamates on LF_N 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 SO_3^-, which is essentially not titrateable, is introduced at most positions in LF_N, through the reaction of a cysteine-modified residue at those positions with (2-Sulfonatoethyl) Methanethiosulfonate (MTSES), voltage-driven LF_N 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 spectral 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 (CYS116). The photocycle was initiated by a 50 ms 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 Electrokinetic 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.