

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.

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#### 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<sup>2+</sup>. 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<sup>+</sup> 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.

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#### 1682-Pos Board B526

##### Protein-Protein Interactions And The Energy Coupling Mechanism In TonB-Dependent Transport

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