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 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.