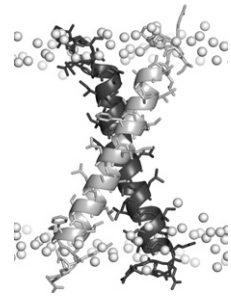
proteins, even alpha-helical ones, because the contribution of the backbone conformation is subtracted from our final measurements.
We were also able to use OMPLA to measure how the energetics of side-chain insertion vary with depth in the bilayer. Both Arginine and Leucine have their most extreme insertion energies when they are closest to the middle of the hydrophobic region of OMPLA. Further, we carried out a double mutant cycle with Arginines and discovered that the insertion of a second Arginine is aided by the insertion of the first. This result is particularly relevant for understanding the function of the voltage sensing domains of some ion channels, which may involve multiple Arginines penetrating the lipid bilayer.

1128-Pos Board B38
UV Excited Resonance Raman Analysis of Lipid Solvated Polypeptides
Christopher Halsey, Jian Xiong, Carol Roach, Renee D. Jiji,
Jason W. Cooley.
The study of membrane protein structure is hampered by a well-documented litany of technical issues associated with sample preparation, peptide solubility and sample complexity. Deep-UV excited resonance Raman (DUVRR) spectroscopy has proven to be a means by which to attain discrete structural information for some of the most dynamic or insoluble protein samples. We will present here our initial efforts to address the feasibility of using DUVRR to probe the lipid solvated polypetide backbone secondary structures.

1129-Pos Board B39
Investigation of Transmembrane Helix Hetero-Dimerization by Combinatorial Peptide Libraries and High-Throughput Measurement of FÖRSTER Resonance Energy Transfer in Liposomes
Jing He, William C. Wimley.
Receptor tyrosine kinases (RTKs) are membrane proteins containing an N-terminal extracellular ligand-binding domain, a single helical transmembrane (TM) domain, and an intracellular catalytic kinase domain. Lateral dimerization driven in part by the TM domain is proposed to be a crucial intermediate step in signal transduction across the plasma membrane. Defects in this process such as over-dimerization are closely linked to unregulated signaling and disease. Therefore, dimerization inhibitors developed using the chemical-physical basis of RTK TM domain dimerization could be promising therapeutic agents. Here we present a first step towards this goal. We designed a rational combinatorial peptide library based on the TM domain of Neu, an RTK from the Erb-B/HER epidermal growth factor receptor family (EGFR) with a pathogenic V to E mutation in the TM domain. Förster Resonance Energy Transfer (FRET) in lipid vesicles was used as a method to probe the dimerization between the library members and mutant Neu sequence. To enable high-throughput screening in liposomes, we developed a novel multi-well FRET assay. FRET donor-labeled library members and acceptor-labeled Neu peptides were incorporated into lipid vesicles in a rapid high-throughput 96-well plate format. We are using this high throughput screen to identify inhibitors of Neu dimerization. Our findings will be discussed in a context of throughput and sensitivity along with a statistical analysis that takes into account the FRET that arises from random proximity of donors and acceptors in the bilayer.

1130-Pos Board B40
Spectroscopic Studies of the Dimerization of ATP-Binding Cassette Nucleotide-Binding Domains
Maria E. Zoghbi, Guillermo A. Altenberg.
Cancer cells are frequently resistant against chemically unrelated anticancer agents or develop resistance to those agents during treatment (multidrug resistance). ATP-binding cassette (ABC) proteins such as P-glycoprotein are key mediators of multidrug resistance. They catalyze the efflux of chemotherapeutic agents out of cancer cells, preventing their intracellular accumulation and cytotoxic effect. There are two competing models to explain the mechanism of ABC exporters: 1) The alternating-access model, where large conformational changes take place during the transport cycle as a consequence of ATP-induced nucleotide-binding domain (NBD) dimerization. 2) An alternative model, which suggests that the power stroke triggered by ATP binding consists of only moderate rearrangements, with the NBDs in contact at all times during the transport cycle. Here, we performed experiments on isolated bacterial NBDs using luminescence resonance energy transfer (LRET) and tryptophan fluorescence quenching, to determine the ATP dependence on NBD dimerization. As a model, we used MJ0976, a NBD from the thermophile M. jannaschii. Under non-hydrolysis conditions (in the absence of Mg2+), tryptophan fluorescence quenching and LRET experiments showed a similar apparent affinity for ATP, in the 20-40 micromolar range. A mutation that abolishes ATPase activity increases the apparent affinity for ATP approximately 10 folds. Under conditions of ATP hydrolysis, in the presence of Mg2+-ATP, the decrease in LRET indicates that there was an increase in the proportion of NBDs in the monomeric form. The results show: 1) The feasibility of LRET studies to determine dimerization of ABC protein NBDs, and 2) That ATP-induced dimerization is a transient phenomenon. This work was supported by CPRIT grant RP101073.

1131-Pos Board B41
Structural Aspects of Transmembrane Domain Interactions of Receptor Tyrosine Kinases
Eduard Bocharov, Konstantin Mineev, Dmitry Lesovoy, Marina Goncharuk, Sergey Goncharuk, Olga Bocharova, Pavel Volynsky, Roman Efremov, Alexander Arseniev.
Specific helix-helix interactions of single-span transmembrane domains of receptor tyrosine kinases are critical for their lateral dimerization and biological function. Establishing structure-function relationship as well as rational drug design requires precise structural information about this class of biologically significant bitopic membrane proteins. A combination of NMR, optical spectroscopy, protein engineering and molecular modelling made it possible studies of the nature and the mechanisms of important transmembrane helix-helix interactions inside the supramolecular complexes mimicking membrane environment. Using a robust strategy we investigated recombinant transmembrane fragments from different families of receptor tyrosine kinases: EphA, ErbB and FGFR, which play important roles in normal and in pathological conditions of human organism by providing cell signalling, maintaining cellular homeostasis and controlling cell fate. We established spatial structure and internal dynamics of the homo- and heterodimeric transmembrane domains, characterized diverse transmembrane helix-helix packing interfaces and obtained detailed picture of intra- and intermolecular interactions in membrane. The already available information about structural-dynamic properties of the dimeric transmembrane domains of studied receptor tyrosine kinases along with the available biophysical and biochemical data provides useful insights into their functioning in the human organism at the atomic level.



1132-Pos Board B42
Insights into the Membrane-Interaction Properties of the *PSEUDOMONAS Aeruginosa* Type III Secretion Translocator Popd
Fabian B. Romano Chernac, Kyle C. Rossi, Min Chen, Alejandro P. Heuck.
In order to infect human cells, pathogenic Gram-negative bacteria depend on their ability to secrete toxins into the cytoplasm of an adjacent eukaryotic host cell. A conserved and efficient delivery mechanism, called the type III secretion (T3S) system, acts as a syringe to inject these toxic proteins called ''effectors'' which need to translocate through the eukaryotic plasma membrane. In *Pseudomonas aeruginosa* PopB and PopD are essential for effector translocation. Substantial genetic and biochemical data support a model with PopB and PopD inserting into the target membrane and forming an oligomeric translocon pore, however virtually nothing is known about this critical transmembrane complex.
Our primary goal is to determine the arrangement of PopD in membrane assembled translocons. What segments of PopD interact with the membrane? Does PopB influence the PopD/membrane interaction? Using membrane binding assays, conductivity measures in planar lipid bilayers, site-directed fluorescence labeling, and multiple fluorescence approaches we have analyzed the interaction of PopD with model membranes and the pore formation activity of the translocator in the presence and absence of PopB.
Despite both proteins can individually form pores in membranes, we found that PopD oligomerizes with PopB and forms pores with an inner diameter of approximately 30Å. Our analysis also showed that the PopD predicted transmembrane segment (residues L119-V137), does not adopt a transmembrane orientation. Our data support a model that consists in the binding of the unfolded translocators to the membrane, oligomerization, and formation of discrete transmembrane pores.