Rhomboid intramembrane proteases catalyze the transmembrane domain cleavage of single-pass membrane proteins. This activity is crucial for the activation of epidermic growth factor receptor ligands Gurken, Keren and Spitz in D. melanogaster. How rhomboids recognize their substrates and select which peptide bond to cleave is not understood. We have studied the substrate specificity and peptide bond selectivity of purified rhomboids from E. coli, P. aeruginosa, D melanogaster and H. sapiens using chimeric substrates containing the transmembrane domains of Gurken, Keren and Spitz. Analysis of the proteolytic products by mass spectrometry reveals that cleavage occurs in the membrane water interface at sites that are shared by both eukaryotic and prokaryotic rhomboids. Mutagenesis of the substrates reveals a helical amino acid motif that is crucial for substrate recognition and peptide bond selection. With insight from computational data a model for the substrate-enzyme complex will be presented.

1672-Pos Board B516

Biophysical Properties of Transmembrane Segment 6 of E.coli MntH Transporter

Vera Nunukova[1], Masoud Jelokhani-Niaraki[2], Eva Urbankova[1], Roman Chaloupka[1].
[1]Charles University in Prague, Prague, Czech Republic, [2]Wilfrid Laurier University, Waterloo, ON, Canada.

The Natural Resistance-Associated Macrophage Protein (Nramp) family of secondary active divalent metal ion transporters plays an important role in a variety of biological processes, such as metal ion homeostasis, in virtually all living organisms. Due to its structural and functional homology with eukaryotic Nramps, the E. coli transporter MntH (Proton-dependent Manganese Transporter) represents a prototypic model to advance understanding of structure-function relationship in Nramp family. Synthetic peptides corresponding to the transmembrane (TM) segments of membrane proteins could serve as a suitable alternative model for studying the structure and interaction of the membrane protein TM domains with biological membranes. In this study the synthetic peptide corresponding to the sixth transmembrane segment (TMS6) of E. coli MntH and its two mutants, in which the His211 residue was substituted by arginine or alanine, were used. TMS6 was previously shown to contain two functionally important histidine residues. The H211A mutation preserves bacterial sensitivity to metal ions and facilitate H+ uptake in the presence of metal ions. In contrast, H211R does not induce metal sensitivity (1,2). The secondary structures of TMS6 and its mutants were determined in model membranes and membrane-mimicking organic environments, using CD spectroscopy. The conformation of the peptides exhibited ordered α and β conformations in these milieus. Furthermore, patch clamp measurements demonstrated that TMS6 was able to form multi-state ion channels in the presence of manganese as a physiological substrate of MntH. The mutant H211R does not show any channel-like activity and with the mutant H211A the ion channel activity was rarely observed.

1. Lam-Yuk-Tseung, S., Govoni, G., Forbes, J., and Gros, P. (2003) Blood 101, 3699-3707.
2. Chaloupka, R., Courville, P., Veyrier, F., Knudsen, B., Tompkins, T.A., and Cellier, M.F. (2005) Biochemistry 44, 726-733.

1673-Pos Board B517

Solid State NMR of Membrane Proteins: Towards Complex Structural and Functional Information for Bacterial ABC Class Importers

Dylan T. Murray[1,2], Timothy A. Cross[1,2].
[1]Institute for Molecular Biophysics, The Florida State University, Tallahassee, FL, USA, [2]The National High Magnetic Field Laboratory, Tallahassee, FL, USA.

Despite the wealth of protein structural data available today, membrane protein structural characterization continues to pose a significant challenge in structural biology. Choosing the membrane mimetic is a challenge and often detergent micelles are employed. However, detergents are prone to induce distortions in the protein structure. An emerging technique, solid state nuclear magnetic resonance (NMR), provides a path to structural characterization using an environment similar to the native one, liquid crystalline lipid bilayers.

Static solid state NMR experiments on proteins determine the orientation of the peptide planes with respect to the magnetic field. All ^{15}N-^{1}H dipolar couplings and anisotropic ^{15}N chemical shifts observed in two dimensional separated local field experiments (PISEMA) lie within a butterfly shape in the spectrum. Special patterns called PISA wheels arise for uniformly aligned protein samples which directly reflect the orientation of protein secondary structure. Using these wheels the tilt angle of each helical axis from the magnetic field and membrane normal can be determined without complete structure determination. Consequently, a single data set allows for characterization of secondary structure in the membrane mimetic as well as providing a set of high resolution peptide plane orientations that can be used directly in structural refinement.

Uniform alignment has been achieved for multiple proteins in our laboratory. Still, studies on larger membrane proteins are required to make solid state NMR a generally applicable technique for membrane protein structure characterization. One excellent example is SugAB, the transmembrane domain of an ABC importer from *Mycobacterium tuberculosis.* These transport proteins contain at least 10 transmembrane helices. This makes SugAB an excellent target for determining the utility of solid state NMR to structurally characterize large membrane proteins.

1674-Pos Board B518

Re-examination of the Role of the Amino-Terminus of SecA in Promoting Its Dimerization and Functional State

Sanchaita Das.
Wesleyan University, Middletown, CT, USA.

The SecA nanomotor promotes protein translocation in Eubacteria by binding both protein cargo and the protein-conducting channel and undergoing ATP-driven conformation cycles that drive this process. Conflicting reports exist as to whether SecA functions as a monomer or dimer during this dynamic process.Here we re-examine the role of amino and carboxyl termini of SecA in promoting its dimerization and functional state by examining three secA mutants and their respective proteins: SecAD8 lacking residues 2-8, SecAD11 lacking residues 2-11, and SecAD11/N95 lacking both residues 2-11 and its carboxyl-terminal 70 residues. We demonstrate that whether or not SecAD11 or SecAD11/N95 was functional for promoting cell growth depends solely on their vivo levels that appear to govern residual dimerization. Cell fractionation revealed that SecAD11 and SecAD11/N95 were still proficient in membrane association, although they were reduced in the formation of integral membrane SecA. The presence of a modestly higher level of SecAD11/N95 in the membrane and its ability to form dimers as detected by chemical crosslinking were consistent with the higher secA expression level and better growth property of this mutant compared to secAD11. Biochemical studies showed that SecAD11 and SecAD11/N95 displayed identical dimerization defects, while SecAD8 was intermediate between these proteins and wildtype SecA. Furthermore, both SecAD11 and SecAD11/N95 were equally defective in their translocation ATPase specific activity. Our studies show that the non-essential carboxyl-terminal 70 residues of SecA play no role in its dimerization, while increasing truncation of the amino-terminal region of SecA from 8 to 11 residues results in an increasing defect in SecA dimerization and poor in vivo function unless highly overexpressed and also clarify a number of conflicting reports in the literature and support the essential nature of the SecA dimer.

1675-Pos Board B519

Substrate Selectivity in Adic, an E. Coli Inner Membrane Arginine-agmatine Antiporter

Yiling Fang, Christopher Miller.
Brandeis University, Waltham, MA, USA.

AdiC is a membrane antiporter that transports arginine and its decarboxylation product agmatine across E. coli inner membrane. It plays a key role in the arginine-dependent extreme acid resistance. We overexpressed AdiC in E. coli and reconstituted the purified protein into liposomes. A series of arginine analogs were tested on the transporter. The permeability sequence is as follows: arg / agm > 1,5-diaminopentane, 1,6-diamino-hexane, 1,4-diaminobutane >> argininamide, lysine, ornithine, canavinine. Kinetic analysis results are K_m~ 1mM for 1,5-diaminopentane, ~3 mM for argininamide and lysine, ~15 mM for canavanine.

1676-Pos Board B520

Identification of Functionally Important Sites within the Cysteine-Free Inner Membrane Transferase Protein ArnT

Nicholas A. Impellitteri, Jacqueline A. Merten, Lynn E. Bretscher, **Candice S. Klug**.
Med Col Wisconsin, Milwaukee, WI, USA.

The bacterial inner membrane protein ArnT confers resistance to the antibiotic polymyxin in *Salmonella typhimurium* and *Escherichia coli* through the modification of lipid A, the major component of the outer surface of Gram-negative bacteria. ArnT transfers a neutral aminoarabinose moiety (L-Ara4N) onto the negative phosphate group(s) of lipid A, which significantly reduces the surface charge of these bacteria and thus prevents cationic peptides such as polymyxin from electrostatically recognizing and killing the bacteria. We have previously reported the first expression, purification and functional analysis of ArnT from *S. typhimurium*, and our studies showed that ArnT is highly α-helical and described a new *in vivo* functional assay. In this continuation of the characterization of the ArnT protein, we used the cysteine-specific maleimide-PEG_{5000} to demonstrate that all eight of the native cysteines in *S. typhimurium* ArnT are in the reduced form and therefore not involved in disulfide bonds. In addition, we created a cysteine-free protein that is structurally and functionally intact as determined by circular dichroism and the results of the new *in vivo* growth

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
Lijuan He, Nadia Shobnam, Shannon K. O'Connor, Kalina Hristova.
Johns Hopkins University, Baltimore, MD, USA.
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
Michael S. Kent[1], Jaclyn K. Murton[1], Elizabeth L. Carles[1], Darryl Y. Sasaki[2], Kenneth L. Sale[2], Jaroslaw Majewski[3], Bulent Akgun[4], Sushil Satija[5].
[1]Sandia National Labs, Albuquerque, NM, USA, [2]Sandia National Labs, Livermore, CA, USA, [3]Los Alamos National Labs, Los Alamos, NM, USA, [4]NIST, Gaithersburg, MD, USA, [5]NIST, Gairthersburg, MD, USA.
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)
Elisa J. Cabre[1], Duncan Sutherland[2], Daniel E. Otzen[2], Jesus Perez-Gil[1].
[1]UCM, Madrid, Spain, [2]iNANO Center, University of Aarhus, Aarhus, Denmark.
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
Michael J. Simon[1], Matthew C. Clifton[2], Huide Zhang[1], Mark A. Hermodson[1], Cynthia V. Stauffacher[1].
[1]Purdue University, West Lafayette, IN, USA, [2]Fred Hutchison Cancer Center, Seattle, WA, USA.
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
Natali A. Minassian[1], Meng-chin Lin[1], Karla P. Figueroa[2], Allan F. Mock[1], Giovanni Stevanin[3], Michael F. Waters[4], Stefan M. Pulst[2], Diane M. Papazian[1].
[1]UCLA, Los Angeles, CA, USA, [2]University of Utah, Salt Lake City, UT, USA, [3]Salpetriere, Paris, France, [4]University of Florida, Gainesville, FL, USA.
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
Ricardo H. Flores Jiménez, Steven M. Lukasik, David S. Cafiso.
University of Virginia, Charlottesville, VA, USA.