we are investigating the interaction between Cx32 and ZO-1 to identify if a common mechanism of binding exists with hDlg. These data provide new insights into the regulation of MAGUK family scaffolding proteins and Cx32 interactions.

1729-Pos Board B573
Recombinant Expression Screening of P. aeruginosa Bacterial Inner Membrane Proteins
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Transmembrane proteins (TM proteins) make up 25% of all proteins and play key roles in many diseases and physiological processes. However, much less is known about their structures and molecular mechanisms than for soluble proteins. Problems in expression, solubilization, purification, and crystallization cause bottlenecks in the characterization of TM proteins. This project addressed the need for improved methods for obtaining sufficient amounts of TM proteins for determining their structures and molecular mechanisms.
We obtained plasmid clones encoding eighty-seven transmembrane proteins with varying physical characteristics, for example, the number of predicted transmembrane helices, molecular weight, and grand average hydrophobicity (GRAVY). All the target proteins were from P. aeruginosa, a gram negative bacterial opportunistic pathogen that causes serious lung infections in people with cystic fibrosis. We measured the relative expression levels of the transmembrane proteins under several culture growth conditions. The use of E. coli strains, a T7 promoter, and a 6-histidine C-terminal affinity tag resulted in the expression of 58 out of 87 test proteins (68%). In this study, factors related to overall hydrophobicity and the number of predicted transmembrane helices correlated with the relative expression levels of the target proteins.
Identifying physical characteristics that correlate with protein expression might aid in selecting the "low hanging fruit", or proteins that can be expressed to sufficient levels using this sort of expression system. The use of other expression strategies or host species might be needed for sufficient levels of expression of transmembrane proteins with other physical characteristics. Surveys like this one could aid in overcoming the technical bottlenecks in working with TM proteins and could potentially aid in increasing the rate of structure determination.

1730-Pos Board B574
Molecular Determinants of Neisserial Pathogenesis: Mapping the Interaction Between Opa I and a Human Binding Partner CEACAM1
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Neisserial Opa proteins mediate the internalization of the bacterial cell by host epithelial cells via an interaction between the extracellular loops of Opa proteins and the extracellular domains of the host binding partner present on the cell surface. The eleven Opa proteins can be subdivided into two classes on the basis of the human receptor target. The Opa_{HS} class is named for heparan sulfate proteoglycan (HSPG), while Opa_{CEA} proteins bind carcinoembryonic-antigen related cell adhesion molecules (CEACAMs), of which there are seven varieties. Significantly, each of the Opa_{CEA} proteins has a characteristic specificity for each CEACAM. Of the four extracellular loops of Opa proteins, binding specificity is attributed primarily to two, which correspond to hypervariable regions of the protein sequence. However, mutational and chimeric analyses have not revealed the sequence determinants of the hypervariable regions that are responsible for receptor target recognition. Furthermore, it has been shown that the binding requires a cooperative interaction between the two hypervariable domains, and that specificity is determined by specific pairing of the sequences. It is the goal of this study to determine at a molecular level how specificity is attained by studying the structure and dynamics of the Opa I - receptor interactions. To this end, OpaI, which binds to CEACAM1 receptors, has been cloned, expressed, purified, and refolded and the NMR backbone assignment is in progress. The progress towards structure determination will be presented. In addition, NMR data mapping the interactions between Opa I and the soluble receptor will be presented in order to characterize the functionally relevant structural interactions involved in bacterial pathogenesis.

1731-Pos Board B575
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Second-order nonlinear optical imaging of chiral crystals (SONICC) enables sensitive and selective detection of sub-diffraction limited protein microcrystals with negligible contributions from solvated proteins or amorphous protein aggregates. Under low magnificaiton (large field of view) applications, SONICC can detect individual crystals as small as 100 nm in diameter, which is conservatively 6 orders of magnitude lower that achievalbe using current methods for crystal detection in commercial screening platforms. In studies of microcrystallites of green fluorescent protein (GFP) prepared in 500 pL droplets using a crystallization micro-array, the SHG intensities rivaled those of fluorescence but with superb selectivity for crystalline regions. Furthermore, SONICC is directly compatibility with virtually all common protein crystallization platforms without modification.

1732-Pos Board B576
Expression, Reconstitution and Biophysical Studies of Neuronal Uncoupling Proteins: UCP4 and UCP5
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Uncoupling proteins (UCPs), located in the inner membrane of the mitochondria, uncouple ATP-synthesis from the respiratory chain by transporting protons across the inner membrane into the matrix, hence dissipating the proton-motive force and releasing heat. The neuronal UCPs (nUCPs), UCP4 and UCP5, were discovered recently (in 1998) and little is known about their structure and function. To gain further insight into the potential importance of these two proteins in the neuroprotection and neuromodulation of neurodegenerative diseases, this study will focus on the structure, function and interaction of the nUCPs with nucleotides (inhibitors) and fatty acids (activators). A recombinant version of the proteins, utilizing a hexa-histidine tag and a TEV protease site (for subsequent His-tag cleavage) has been designed, expressed as insoluble inclusion bodies, and isolated and purified using immobilized metal affinity chromatography. Subsequent reconstitution of the proteins in mild detergent (DDM and digitonin) allowed for biophysical studies by circular dichroism and fluorescence spectroscopy. Circular dichroism spectroscopy has shown that, similar to the recombinant UCP1, nUCPs possess dominantly helical structures in digitonin and DDM [1]. Furthermore, detergent-mediated reconstitution of the proteins into preformed liposomes can give more physiologically relevant structural and functional information. Comparison of the structure and function of human UCP1 (thermogenin) to nUCPs, in lipid membranes and membrane-like environments, will eventually show whether these proteins have any similarity in conformation and functional behaviour.
[1] Jelokhani-Niaraki, M., Ivanova, M.V., McIntyre, B.L., Newman, C.L., McSorley, F.R., Young, E.K. and Smith, M.D. (2008) *Biochem. J.*, **411**, 593-603.

1733-Pos Board B577
Helix-Helix Interactions in Membrane Proteins: Structural Analysis and Free Energy Calculations of Polyleucine-based Dimers
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According to Popot and Engelman's 'two stage' model, lateral interaction of helices in the membrane is a key step towards the folding of helical membrane proteins. In order to understand, how protein self-association regulates biological functions and how mutations may be involved in diseases, it is important to understand the constituent free energies. In this study, we investigated the effect of a wide range of mutations, at the 'a' or 'd' position of the heptad in the amino acid sequence, on the helix-helix interactions in a polyleucine dimer in lipid (DOPC) environment. We used molecular dynamics simulations and the thermodynamic integration method to calculate the free energies for various mutations. Our free energy calculations show that highly polar residues like Asn, Asp, Gln, Glu, Cys, and His enhance the stability of the polyleucine dimers much more than that by smaller polar residues like Ser and Thr, which agrees with previous experimental studies on similar peptides [1,2]. A past study showed that Ser, Thr, and less polar amino acids occur at a higher frequency, as compared to highly polar residues (Asn, Asp, Gln, Glu, Cys, His), in membrane proteins [2]. Recent experiments suggest that there is a high occurrence of weak H-bonds in membrane proteins [3]. Our findings and other studies suggest that membrane proteins prefer a wide range of moderately stabilizing interactions instead of strong ones, which lends them a greater degree of flexibility in terms of conformation and stability [3,4].
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3. Joh, N.H. et al. *Nature* **453**, 1266-1270 (2008).
4. Grigoryan, G. et al. *Nat. Chem. Bio*. **4**, 393-394 (2008).

1734-Pos Board B578
Assembling Within The Lipid Membrane: Viral Membrane Proteins
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Viruses encode a number of membrane proteins which are interacting with lipid membranes. One class of these proteins are known to form homo-oligomeric

assemblies which alter the permeability of lipid membrane for ions and small molecules. These so called channel or pore forming proteins can either have one, two or three TM spanning domains. Albeit structural information is available for some of them, neither the number of monomers forming the functional pore or the proper orientation of most of the monomeric units within the assembly is available.
Computational methods are used to model the functional pore architecture of some of the proteins encoded by HIV-1, SARS-Co and Polio virus. Based on these findings novel pore motives need to be discussed and verified by experiments.

1735-Pos Board B579
Predicting Strained Regions for Folding and Protein-Protein Interaction in Bacterial Outer Membranes
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Beta-barrel membrane proteins are found in the outer membrane of Gram-negative bacteria, mitochondria, and chloroplasts. Exposed on the surface of bacterial cells, many beta-barrel proteins are initial sites of attachment by bacteriophages and bacterial toxins. Little information is known on the stability of local regions of these membrane proteins, and how they interact with each other. We have developed a model to quantify energy level of transmembrane strands in beta-barrel membrane proteins. We showed that our predictions on PagP are consistent with recent experimental NMR studies of protein dynamics [1] and mutant studies of folding stability [2]. We further found that the strands located in the interfaces of protein-protein interactions are considerably less stable by our model We have further developed a method to predict interface of protein-protein interaction of beta-barrel membrane proteins. In a data set of 25 beta-barrel membrane protein structures with less than 32% pairwise sequence identity, we can predict whether the protein will form a monomeric or multimeric structure with 91% accuracy and identify the protein-protein interaction interface with an accuracy of 86% in leave one out tests using sequence information only. We also report results of predicting genome-wide protein-protein interaction in bacterial outer membranes.
[1] Evanics F., Hwang P.M., Cheng Y., Kay L.E. and Prosser R.S. (2006). Topology of Outer-Membrane Enzyme: Measuring Oxygen and Water Contacts in Solution NMR Studies of PagP. *J. Am. Chem. Soc.,* **128,** 8256-8264.
[2] Huysmans G.H.M., Radford S.E., Brockwell D.J. and Baldwin S.A. (2007). The N-terminal Helix is a Post-assembly Clamp in the Bacterial Outer Membrane Protein PagP. *J. Mol. Biol.,* **373**, 529-540.

1736-Pos Board B580
Molecular Dynamics Simulation of Transmembrane Helix Dissociation
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In a typical single-molecule force spectroscopy (SMFS) experiment with a transmembrane protein (TM), a protein surface loop or tail is grabbed by the atomic force microscope cantilever and helices unwind as the TM is pulled in a direction perpendicular to the plane of the membrane. This process is unrelated to the insertion of helical segments from the translocon into the bilayer in a direction parallel to the plane of the membrane. We now use molecular dynamics simulations (MD) to consider a SMFS experiment in which TMs are pulled apart in the plane of the lipid bilayer, so that the helix-helix interactions can be studied. Parallel-versus perpendicular-pulling energy. We calculated the work of separating the helices of the glycophorin A dimer in a sodium dodecyl sulfate (SDS) micelle. Pulling perpendicular to the membrane plane requires about four times the work as parallel pulling to separate the helices. Point pulling. Using MD, we pulled Ala 82 of glycophorin A in a direction parallel to the membrane plane. For 150 ps, the two helices separated smoothly, essentially as rigid rods. However, both Ile 76 side chains remain in van der Waals contact, locking the dimer together. Further pulling unfolds the backbone around Ala 82, until the Ile 76 groups separate after 600 ps. Similar results were observed for a two-helix fragment of bacteriorhodopsin in an SDS micelle. Pulling on Ile 52, the helices separate smoothly for 150 ps. However, during this motion, Leu 13 and Leu 61 remain locked in van der Waals contact. We conclude that SMFS of helical membrane proteins pulled in the direction of the membrane plane would provide valuable information about the interactions that stabilize integral membrane proteins. Experimental implementation will require development of tethering groups compatible with bicelles.

1737-Pos Board B581
The Interactions Of Secondary Structural Elements In The Achitecture Of Membrane Proteins
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Membrane proteins exist in an anisotropic environment which strongly influences their architecture, stability and folding. Previous studies have mainly focused on the transmembrane domains of alpha helical membrane proteins. However it is becoming increasingly apparent that their architecture is richer than a simple bundle of transmembrane alpha-helices, notably due to helix deformations and other membrane embedded structures. Here, we make use of available databases and tools to investigate the weight and role of structural heterogeneity in the supra molecular organization of membrane proteins. Using a non redundant subset of alpha helical membrane proteins, we have annotated and analysed the statistics of several types of structural element such as incomplete helices, intramembrane loop, helical extensions of helical transmembrane domains, extracellular loops and helices lying parallel to the membrane surface. The different structural elements and their composition were studied in relation to their immediate membrane environment. Calculation of hydrophobicity using different scales show that different structural elements appear to have affinities coherent with their position in the membrane. The considerable information content found in the amino-acid compositions of the different elements, suggests that the annotation scheme used might be useful for structural prediction. In a second step we have investigated the energetics of interaction between the structural elements. We show that while the folding in integral membrane polypeptides seems to be dominated by the interactions between transmembrane helices the same is not true of the assembly of complex membrane proteins. In most multisubunit membrane proteins the interactions between multiple domains are important in driving the assembly. Notably interactions at the surface of the membrane and between soluble domains are often very important.

1738-Pos Board B582
A Novel Pain Management Paradigm: Conformational Studies of the Mu-Opioid Receptor
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The mu opioid receptor (MOR) is a Class A G protein-coupled receptor. Naloxone, a MOR antagonist, has been shown experimentally to have partial agonist properties in a transmembrane helix 4 (TMH4) S196L/A MOR and full agonist activity in a triple mutant that combines the TMH4 mutation (S196L) with two mutations in TMH7, T327A and C330S (Claude-Geppert et. al, J. Pharmacol. Exp. Ther. 2005). We hypothesized that the loss of a serine in TMH4, the loss of a threonine in TMH7 and addition of a serine in TMH7 will modify the wild-type (WT) conformation of both helices and that this change may result in naloxone preferring the MOR activated state. The hydrogen bonding capacity of Ser/Thr residues in α-helices can be satisfied by an intrahelical hydrogen bond interaction, in either the *g-* or *g+* conformation, between the O-γ atom and the *i-3* or *i-4* carbonyl oxygen . Ser/Thr residues in the *g-* conformation can induce a bend in an α-helix (Ballesteros et. al, Biophysical J. 2000), as well as changes in wobble angle and face shift (Zhang et al. Mol. Pharmacol. 2005). Using the Monte Carlo/simulated annealing technique, Conformational Memories, we explored the effect of Ser/Thr in *g-* on the conformation of WT MOR and the mutant TMH4 and TMH7s (Whitnell et. al, J. Comput. Chem. 2007). For TMH4, the (wobble angle, face shift) was found to be (-36.4°, 22.1°) for WT vs. (5.7°, 4.6°) for S196L , causing the top of TMH4 to move away from the TMH bundle. For TMH7, a significant difference was found in the average wobble angles of WT(52.52° ± 21.72°) vs. mutant TMH7 (120.55° ± 23.43°), causing the top of TMH7 to move into the bundle. [Support: NIH DA023905 and DA021358]

1739-Pos Board B583
The Kv1.2 Paddle Chimera Channel in a Lipid Bilayer
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The Kv1.2 paddle chimera structure (Long et al. 2007, Nature 450: 376) is the first voltage-dependent ion channel structure that reveals all residues at high resolution. In addition, fragments of lipid acyl chains and headgroups are also revealed. We present results of an atomistic molecular dynamics simulation of the Kv1.2 paddle-chimera tetramer in a POPC bilayer in excess water. The initial configuration includes the crystallographic waters and lipid fragments (reconstructed as POPC molecules) observed in the crystal structure (PDB ID 2R9R). Similar to previous simulation studies of Kv1.2 (Treptow and Tarek 2006, Biophys. J. 90:L64; Jogini and Roux 2007, Biophys. J. 93:3070), we find the basic side chains of the S4 helix in a polar environment formed by water molecules, lipid headgroups, and acidic side chains. As suggested by the crystal structure, the H-bond network that solvates each voltage sensor domain is interrupted by F233, a side chain highly conserved among Kv channels. The lipid fragments observed in the crystal structure are fully consistent with the lipid bilayer configuration and present a distinctive dynamics that contrasts with the bulk-like behavior of the lipid molecules far from the protein.