An unbiased computational structural modelling approach is presented to address two potential routes building up bundles of Vpu, a sequential and simultaneous route. In a fine grained docking approach in combination with molecular dynamics simulations [1, 2] the transmembrane domain of Vpu is assembled. A ridge-of-alanines motif [3] is likely to set the dimeric structure of the assembly. Independent of the assembly route lowest energy bundle structures adopt configurations with tryptophans (Trp-23) pointing inside the bundle. Applying short MD simulations structural stability of a series of bundles is assessed.

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2397-Pos Board B167

Bioinformatic Analysis of Aquaporin Protein Lipid Requirements

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A striking feature of cell membranes is the lipid compositional diversity with more than two hundred different lipid species. Thus otherwise structurally and functionally similar proteins must cater for the differences in their surrounding environment. It seems however, that membrane proteins do not only adapt to a given environment, in fact proteins often exhibit requirements for the presence of specific lipids, serving as cofactors for the correct function, folding and stability of the given protein. In order to study these requirements we compared the trans-membrane protein-family of aquaporins, a membrane channel-protein that facilitates the transport of water molecules across the membrane and which are found in a wide range of organisms. We based our analysis on HotPatch a neural network method developed by Pettit *et al.* (Pettit, F. K. *et al. J. Mol. Biol.* **2007** 369, 863-879). This allowed us to compare protein sites involved in specific lipid interactions and the character of the residues involved. Understanding the functionally important features of the lipid requirements of membrane proteins may assist in the understanding of the optimal design of either protein and/or biomimetic membranes for applications such as biosensors, where reconstitution of functionally intact membrane-proteins in an artificial membrane is a necessity.

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Thermodynamic Measurements of Bilayer Insertion of a Single Transmembrane Helix

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Accurate determination of the free energy of transfer of a helical segment from aqueous into a transmembrane conformation is essential for understanding and predicting of the folding and stability of membrane proteins. Until recently, direct thermodynamically sound measurements of free energy of insertion of hydrophobic transmembrane peptides were impossible due to peptides' aggregation outside the lipid bilayer. Here we overcome this problem by using fluorinated surfactants that are capable of preventing aggregation, but, unlike detergents, do not themselves interact with the bilayer. We have applied previously introduced FCS (Fluorescence Correlation Spectroscopy) methodology [Posokhov et al., Biophysical J. 2008, 95:L54-56] to study surfactant-chaperoned insertion into preformed POPC vesicles of the two well-studied dye-labeled transmembrane peptides of different lengths: WALP23 and WALP27. Interpolation of the apparent free energy values measured in the presence of surfactants to a zero surfactant concentration yielded free energy values of -9.0 and -10.0 kcal/mole for insertion of WALP23 and WALP27, respectively. Circular dichroism measurements confirmed a predominantly helical structure of peptides in lipid bilayer, in the presence of surfactants and in aqueous mixtures of organic solvents. From a combination of thermodynamic and conformational measurements we conclude that the partitioning of a 4-residue LALA segment in the context of a continuous helical conformation from aqueous environment into the hydrocarbon core of the membrane has a favorable free energy of 1 kcal per mole. Our measurements combined with the predictions of the Wimley-White hydrophobicity scale indicates that the per residue cost of the helical backbone partitioning is unfavorable and equals $+0.13$ kcal/mole. Supported by NIH GM069783.

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FRET Investigation of Membrane Protein Folding: Evolution of Tertiary Structure of Soluble and Transmembrane Domains during Folding into Synthetic Bilayers

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This paper describes an investigation of the folding mechanisms of an integral membrane protein. A key goal is to determine the role of the soluble domain during the insertion of a transmembrane domain into synthetic bilayers. Towards this end, we report Förster resonance energy transfer (FRET) efficiencies between donor (tryptophan) and acceptor (1,5-IAEDANS) pairs that are located on the transmembrane and soluble domains of outer membrane protein A (OmpA). The FRET efficiencies are correlated to the evolution of distances and tertiary structure under the assumption of orientational averaging. Analysis of the kinetics reveals that the full-length protein, which contains both soluble and transmembrane domains, displays slower folding rates compared to the truncated variant, which is comprised of the transmembrane domain only. This difference in rates may reflect an increase in the number of kinetic traps during folding, or indicate alternate pathways. These measurements of the formation of tertiary structure and the role of a soluble domain on the kinetics of folding may aid in the elucidation of the mechanisms of membrane protein folding and dynamics.

2400-Pos Board B170

Structure and Dynamics of the Human Antimicrobial Peptide Dermcidin Oligomer: It is an Ion Channel

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Dermcidin (DCD) is one kind of antimicrobial peptides (AMPs), which is secreted into human sweat and protects human body against Gram-negative and -positive bacteria. Like most of the other AMPs, it carries great potential as new antibiotic. However, the functional mechanism of DCD and most of the AMPs is still elusive. One of the hypothesis of their function mechanisms involves their oligomerization and pore formation in bacterial membranes. Indeed, we have recently obtained the first crystal structure of the DCD oligomer, which represents a well-defined channel structure composed of six DCD monomers. Molecular dynamics (MD) simulations have been carried out on this novel channel structure, using the newly established ''computational electrophysiology'' method. We found that, the DCD hexameric channel structure is stable when embedded in the model membranes composed of POPE/POPG (3:1). This channel acts as a very efficient water permeation channel, as well as an ion channel with a conductance around 60 pS. Furthermore, it takes a tilted orientation around 20-30 degrees relative to the membrane surface normal to reduce the hydrophobic mismatch, due to its ~8-nm length which is twice of the membrane thickness. Interestingly, the ions enter and exit the channel from its side windows rather than from the ends of the channel, thus forming a very unique ion permeation path. These findings bear direct significance for the functional mechanisms of DCD and the AMP family on bacterial membranes.

2401-Pos Board B171

The AcrAB-TolC Multidrug Efflux Pump: An Alternative Complex Model

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In Escherichia coli, AcrAB-TolC serves as the major multidrug efflux pump using proton motive force to extrude drugs out of the cell. Whereas X-ray structures have been solved separately for the individual components, the best structural information on the assembled efflux pump is a docking structure based on biochemical cross-linking data [1]. Detailed considerations of this structure reveal discrepancies to the available experimental data. Furthermore, 100 ns Molecular Dynamics (MD) simulations show a tendency for complex disassembly between AcrB & TolC. These findings and the X-ray structure of the AcrAB homologue CusBA [2] showing six periplasmic adaptor proteins arranged in a shifted orientation compared to the three AcrA molecules in the docking structure, suggest that the docking structure might be incorrect. Based on the CusBA structure, recent publications as well as MD simulation of AcrA anchored to the membrane we propose an alternative model of the assembled AcrAB-TolC complex.

References:

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