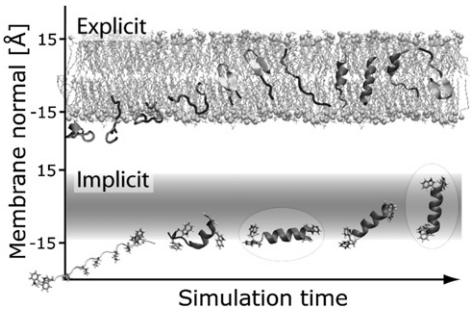
a two-stage pathway, where helical segments fold at the phase boundary before inserting, due to the large energetic penalty associated with de-solvating exposed peptide bonds.
The adsorption, folding and membrane insertion of a model peptide (WALP) was studied via microsecond-timescale molecular simulations at atomic resolution. Both an implicit model and an explicit lipid bilayer setup were used. The implicit simulations generally follow the theoretically predicted two-stage pathway. The vastly increased sampling yields fully converged thermodynamic properties such as the free-energy of folding and membrane insertion. In contrast, the explicit bilayer simulations show that after spontaneous adsorption the peptide immediately crosses the polar interface to locate at the hydrophobic membrane core. Remarkably, there is no interfacial state and the dominant configurations are deeply inserted unfolded and beta-hairpin conformers. The native trans-membrane helix formed for several hundred nanoseconds is not stable. At present the reasons for this unexpected behavior remain unclear.



1709-Pos Board B553
Forces that stabilize membrane proteins
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Understanding the energetics of molecular interactions is essential to addressing many of the central quests of biochemistry including protein structure prediction and design, relating structure to function, mapping evolutionary pathways, learning how mutations cause disease and drug design. Hydrogen bonding and Van der Waals packing are two of the fundamental molecular forces that govern the protein structure and function. Because of technical challenges, however, there have been no quantitative tests of these forces in the context of large membrane proteins. While hydrogen-bonding has been widely regarded as an important force in a membrane environment because of the low dielectric constant of membranes and a lack of competition from water, our recent double-mutant cycle analysis shows that the average contribution of eight interhelical side-chain hydrogen-bonding interactions in bacteriorhodopsin is unexpectedly modest, providing only 0.6 kcal of energy per a mol of interaction on an average, which is quite similar to the strengths measured in soluble proteins. Van der Waals packing, on the other hand, is also thought to be important, as highlighted in number of transmembrane helix dimerization motifs that drive strong helix-helix association in the absence of polar residues by providing tight knob-into-hole interactions. Van der Waals strength, reported by the slope of the correlation found between the thermodynamic stability changes that we measured in six individual cavity-creating large-to-small hydrophobic side-chain mutants of bacteriorhodopsin and the increase in cavity size observed in the refined crystal structures of the corresponding mutant proteins, is indeed quite significant and also very similar to the contributions observed in a soluble protein. Weak hydrogen-bonding and significant Van der Waals packing should be reflected in considerations of membrane protein folding, dynamics, design, evolution and function.

1710-Pos Board B554
Dna-protein Coupling Perspective In Studies Of Unfolding/folding Transitions Of A Protein
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DNA-Protein perspective in studies of unfolding/folding transitions (UFT) accounts for three arguments, both practical and theoretical. Firstly, both experimental and computational research show that properties of protein in unfolded state depend on the molecule length and not its amino acid composition - amino acid profile is not important. Meanwhile, the longer is the protein, the closer is its amino acid composition to the average genome-wide abundance of amino acids (agw aaa). Hence, it might be that the lessening of the importance of amino acid diversity in unfolded protein is consonant to degeneracy of the Universal genetic code (ugc). We show that agw aaa-profile follows closely the ugc-degeneracy profile, while other natural profiles fail.
Secondly, behind the central dogma of biology, that protein is an end product of the DNA-mRNA-Protein line, there is an assumption that common, universal features might exist in mechanisms of untwisting/unwinding of DNA material and UFT of Protein. We focus therefore on the transverse, residue-component of protein in a search of fast and high precision returns to the folded conformation.
Thirdly, the idea of intrinsic mechanical fitness factor, that natural variation and selection give rise to functional design, acknowledges that despite protein does not reproduce itself it possesses some fitness form-factor. Free energy landscape perspective produces such intrinsic score function. Along with serving as a fitness factor for natural variation and selection of DNA material, protein does possess a fitness factor of its own. In a spirit of the Protein-DNA coupling perspective, we project in a self-similar or hierarchical fashion this proposition to the residue level by introducing a residue fitness factor. Contributing to this approach is an experimental knowledge, that for each residue, there is a protein for which it is a key-residue during UFT.

1711-Pos Board B555
Stability of the Spinach Aquaporin (SoPIP2;1) in Detergent Solution and Lipid Membranes
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SoPIP2;1 is one of the major integral proteins in spinach leaf plasma membranes. It is member of the aquaporin family and a highly selective water transporter. Due to this specific characteristic SoPIP2;1 is a candidate for use in industrial membranes water-filtration applications. For this purpose it is very important to characterize whether the protein is well incorporated and stable in the different membranes to be used for the industrial water-filtration supports. Circular Dichroism (CD) and Fluorescence spectroscopy techniques have been used for the characterization of the protein and the protein-membrane complex. The secondary structure of SoPIP2;1 has been analyzed in buffer containing detergent (OG) and in membranes formed by *E.coli* lipids, DPhPC or different phospholipid mixtures. We have also tested how alkane solvents (C_{10}, C_{14} and C_{16}) affect the SoPIP2;1 structure since these solvents are used during the industrial water-filtration membrane preparation. SoPIP2;1 secondary structure is predominantly α-helical in the different environment analyzed as it is expected for the members of the aquaporin protein family. The protein shows high structural stability in detergent solutions. Thermal unfolding experiments show that SoPIP2;1 is irreversible unfolded at temperatures around 58°C. The incorporation of the protein into the different membranes has been performed using different methods and the resulting complex was tested with the techniques mentioned above. It was observed that after shaking the samples in presence of alkane solvent small changes are induced in the SoPIP2;1 structure which still have to be studied whether they could affect the protein functionality. This is the first time that different physicochemical properties of SoPIP2;1 are characterized which are prerequisites for devising an optimal protein-membrane complex for the water-filtration system with technological applications.

1712-Pos Board B556
Structural and membrane binding properties of the Prickle PET domain
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The planar cell polarity (PCP) pathway is required for fetal tissue morphogenesis as well as for maintenance of adult tissues in animals as diverse as fruit flies and mice. One of the key members of this pathway is Prickle (Pk), a protein that regulates cell movement through its association with the Dishevelled (Dsh) protein. Pk presents three LIM domains and a PET domain of unknown structure and function. Both the PET and LIM domains control membrane targeting of Dsh, which is necessary for Dsh function in the PCP pathway. Here, we show that the PET domain is monomeric and presents a nonglobular conformation with some properties of intrinsically disordered proteins. The PET domain adopts a helical conformation in the presence of 2,2,2-trifluorethanol (TFE), a solvent known to stabilize hydrogen bonds within the polypeptide backbone, as analyzed by circular dichroism (CD) and NMR spectroscopy. Furthermore, we found that the conserved and single tryptophan residue in PET, Trp 536, moves to a more hydrophobic environment when accompanied with membrane penetration and that the protein becomes more helical in the presence of lipid micelles. The presence of LIM domains, downstream of PET, increases protein