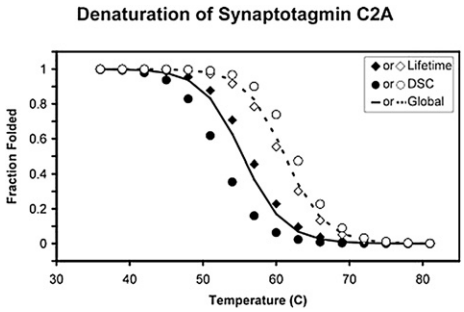

Figure 1. *Overlay of Lifetime, DSC, and Global Analyses.* Black or solid data represents protein in the absence of Ca^{2+}. White or dashed data represents protein in the presence of Ca^{2+}.

1704-Pos Board B548
Mutations Causing Early Cataract Development In Mice Destabilize Human gammaD-crystallin
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The human eye lens is composed of layers of elongated fiber cells packed with crystallin proteins at concentrations up to 400 mg/ml. Human γD-crystallin (HγD-Crys), one of the three major γ-crystallins, is a monomeric, two-domain protein found in the lens nucleus, the central region of the lens formed earliest during development. Genetic screens for mutations resulting in cataract in mice identified three mutations affecting mouse γ-crystallins. These amino acid substitutions were introduced into HγD-Crys by site-specific mutation of the cloned gene. The three mutant proteins L5S, V75D, and I90F were expressed and purified from *E. coli*. Equilibrium unfolding/refolding experiments were performed to measure the thermodynamic stability of the mutant proteins compared to wild type. Wild-type HγD-Crys was previously shown to exhibit a three-state unfolding/refolding pathway. This pathway is sequential with the N-terminal domain unfolding first, followed by the C-terminal domain. L5S and V75D also displayed three-state unfolding/refolding transitions with populated intermediates. In both cases, the first transition midpoint was shifted to lower denaturant concentrations, 0.7 M GdnHCl for L5S and 0.8 M for V75D compared to 2.2 M for the wild type. I90F exhibited a two-state unfolding/refolding transition with a single midpoint at 1.7 M. The mutant proteins all exhibited decreased thermal stability compared with wild type. Kinetic unfolding experiments confirmed that wild type unfolded through a three-state mechanism. The N-terminal domains of L5S and V75D unfolded extremely fast ($t_{1/2}$@2 s) at lower denaturant concentrations than those required for wild type. I90F was globally destabilized and unfolded through a two-state mechanism faster than wild type. These results support models of cataract formation in which generation of partially unfolded intermediates - whether due to mutation or to covalent damage - are precursors to the aggregated cataractous states responsible for light scattering.

1705-Pos Board B549
Vectorial Transport and Folding of an Autotransporter Virulence Protein During Outer Membrane Secretion
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Many virulence factors secreted from pathogenic Gram-negative bacteria are autotransporter proteins. The final step of autotransporter secretion is passage across the outer membrane (OM), mediated by a cotranslated C-terminal porin domain. Sequence analysis reveals that, despite size, sequence, and functional diversity, >97% of autotransporter passenger domains are predicted to form parallel β-helices, suggesting this structure is important for secretion. We report the folding behavior of pertactin, an autotransporter passenger domain from *Bordetella pertussis*. Despite slow but reversible folding *in vitro*, the β-helix is not prone to aggregation. Interestingly, equilibrium denaturation results in formation of a partially folded structure, with a stable core comprising the C-terminal half of pertactin. Examination of the crystal structure does not reveal any obvious reason for the enhanced stability of the C-terminus. Crystallographic data of the partially folded state shows native like structure for the C-terminus. Interestingly, the C-terminus forms a dimer with a non-native interface with a relatively low K_D ~ 0.3 μM. *In vivo*, slow folding would prevent premature folding in the periplasm, before OM secretion. Moreover, the extra stability of the C-terminal rungs might serve as a template for the β-helix formation during secretion; hence, vectorial folding of the β-helix could contribute to the energy-independent translocation. We show here that the C-terminus is the first part of the pertactin passenger domain reaching the OM, and that the C-terminus can adopt a stable structure outside the cell, prior to the completion of OM secretion. Coupled with the sequence analysis, these results suggest a general mechanism for autotransporter secretion. The combination of this data, including the lack of pertactin aggregation, could lead to new insights into the formation and prevention of protein aggregation *in vivo*.

1706-Pos Board B550
Can We Measure the Thermodynamic Stability of Membrane Proteins in a Lipid Bilayer Environment?
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Experimental determination of the free energy (ΔG) stabilizing the structure of membrane proteins (MPs) in their native environment has been hampered by MP's aggregation and precipitation outside the lipid bilayer. Recently we have demonstrated that the latter process can be prevented by the use of fluorinated surfactants, FTACs, which act as chaperones for MP insertion without partitioning in the membrane themselves [Biophys. J. 2008 94:4348-4357]. Here we combine the advantages of the chaperone-like ability of FTACs with the sensitivity of fluorescence correlation spectroscopy measurements to determine ΔG of bilayer insertion of model MPs. First, we calibrate our approach by examining the effects of chaperoned insertion on ΔG of transmembrane insertion of Annexin B12. We find that a shorter-chained surfactant, FTAC-C6, for which the working concentration range of 0.05-0.2 mM falls below CMC=0.33 mM, has a mild effect on an apparent ΔG. In contrast, additions of a longer-chained FTAC-C8 (CMC=0.03 mM) result in a steep and non-linear concentration-dependence of ΔG. Then, we applied the same methodology to the pH-triggered insertion of diphtheria toxin T-domain, which is known to be affected by non-productive aggregation in solution. We find that the correction of the ΔG value needed to compensate for un-chaperoned insertion of the T-domain exceeds 3 kcal/mole. A relatively shallow and linear dependence of the ΔG for Annexin B12 and T-domain insertion on FTAC-C6 concentration is encouraging for future applications of this surfactant in thermodynamic studies of the stability of other MPs. We will test our approach using model transmembrane WALP and KALP peptides. Supported by NIH GM069783(-04S1).

1707-Pos Board B551
Which Potential Role Can Cellular Membranes Play in the Misfolding of SOD Protein Involved in ALS Syndrome
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The neurodegenerative disease amyotrophic sclerosis (ALS) is closely connected to single site mutations of the Cu/Zn superoxide dismutase (SOD) protein, whose pathological conversion into misfolded aggregates is a hallmark of ALS. However, *in vitro* folding studies of the most aggressive SOD mutants do not correlate at all with their *in vivo* behavior of early onset of ALS and very short patient survival times. Therefore, potential interactions of SOD proteins with neuronal membranes were suggested as a possible molecular driving mechanism, similar as seen for membrane mediated accelerated aggregation of Aβ-peptide in Alzheimer disease or synuclein in Parkinson. Combined CD, solid state NMR and calorimetry experiments indicate clearly, that membrane association of the SOD protein modifies its secondary structure depending on the presence of negatively charged lipid compounds and the SOD mutation. As a potential mechanism for the toxic effect of miss-aggregated SOD the ability to disrupt lipid membranes was investigated with a dye leakage assay. The essay proves the ability of SOD to disrupt lipid vesicles. We could identify an important role of the surface charge and a complex dependency on the oxidation state for this process.

1708-Pos Board B552
Folding Peptides Into Lipid Bilayer Membranes
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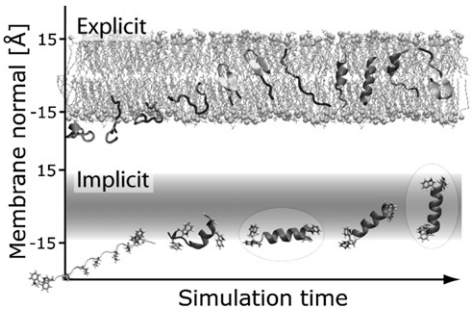
The folding and integration of peptides into lipid bilayers remains one of the most intriguing processes in biophysics, as it cannot be directly observed at sufficient temporal and spatial resolutions. From a physical chemistry perspective transfer of solvated peptides into a hydrocarbon phase should follow

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