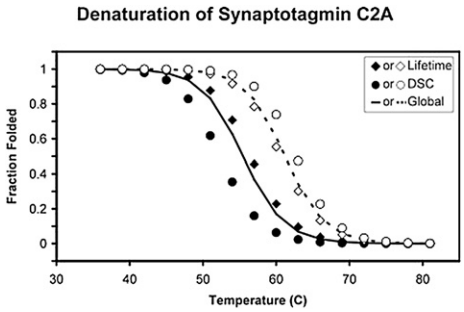

Figure 1. *Overlay of Lifetime, DSC, and Global Analyses.* Black or solid data represents protein in the absence of Ca^{2+}. White or dashed data represents protein in the presence of Ca^{2+}.

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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.

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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