Protein Folding & Stability II

1688-Pos Board B532
GCN4 Enhances the Stability of the Pore Domain of Potassium Channel KcsA
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KcsA, a prokaryotic potassium channel, is the first channel that has a known crystal structure of the transmembrane domain. The crystal structure of its soluble C-terminal domain, however, still remains elusive. Biophysical and electrophysiological studies have previously implicated the essential roles of the C-terminal domain in pH sensing and in vivo channel assembly. We examined this functional assignment by replacing the C-terminal domain with an artificial tetramerization domain, GCN4-LI. The expression of KcsA is completely abolishled when its C-terminal domain is deleted, but it can be rescued by fusion with GCN4-LI. The secondary and quaternary structures of the hybrid channel are very similar to those of the wild type channel according to circular dichroism and gel-filtration analyses. The thermostability of the hybrid channel at pH 8 is similar to that of the wild type but is insensitive to pH changes. This supports the notion that the pH sensor of KcsA is located in the C-terminal domain. Our result is in agreement with the proposed functions of the C-terminal domain and we show that the channel assembly role of the C-terminal domain can be substituted with a non-native tetrameric motif. Since tetramerization domains are found in different families of potassium channels and their presence often enhances the expression of channels, replacement of the elusive C-terminal domains with a known tetrameric scaffold would potentially assist the expression of other potassium channels.

1689-Pos Board B533
Multilevel Modeling To Understand The Folding Hysteresis Of GFP
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The green fluorescent protein (GFP) is known to show hysteresis during folding. Experiments indicate that this hysteresis occurs due to the cooperative behavior of the chromophore and a few prolines which occur at the edge of the GFP barrel near the chromophore containing helix. Even one-head-per-residue (C-alpha) structure-based (Go) simulations of GFP contain signatures of structural frustration near the proline regions. We explore the role of this frustration and the cooperativity of the prolines and the chromophore further by performing detailed simulations of GFP.

1690-Pos Board B534
A Proteomic-scale, Mass Spec-based Method to Probe Forced Unfolding within Cells
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Cysteine is a relatively hydrophobic amino acid and is often buried within protein folds or assemblies. We use Cys labeling of live cells under different conditions, including mechanical stress and temperature, to identify by Mass Spec those proteins and sites that are perturbed. For intracellular proteins, Cys are not tied up in disulfides, and so their reactivity to many fluorescent dyes in solution is readily determined as a function of temperature under both native and high urea conditions. When a protein is folded at low temperature, the reaction rate tends to be faster in urea, and the ratio of rates yields a thermal unfolding curve that looks very similar to results from solution methods such as Circular Dichroism. Unlike these other methods, however, the Cys labeling approach can also be applied to intact cells by using membrane-permeable, cell-viable dyes at low dose. Labeling studies of several cell types, including stem cells, lung cancer cells, and cardiomyocytes, treated under two different conditions, such as stressed vs unstressed or 37°C vs fever-like 42°C, are followed by electrophoretic separation and detailed LC/MS-MS study. What results is an identification of labeled proteins as well as differentially labeled sites in many proteins, including several structural proteins with major roles in disease and development: Myosin, Filamin, Spectrin, and Vimentin. The Cys strategy that method thus provides unique, proteomic scale measurements of protein structural changes in both solution and intact cells.

1691-Pos Board B535
Thermodynamic and Structural Origins for the Extreme Stability of Taq DNA Polymerase
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The thermal stability of Taq DNA polymerase is well known, and is the basis for its use in PCR. We have previously shown that at 25°C, Klentaq polymerase (the large fragment domain of Taq) has a folding free energy (ΔGfold) of −27 kcal/mole, making it one of the most stable monomeric proteins yet characterized. In contrast, Klentaq polymerase has a ΔGfold of only −4.7 kcal/mole at 25°C, which is quite low for a protein of its size (68kDa). Here we report full stability curves (ΔG versus temperature) for Klentaq and Klentaq, which reveal that Klentaq’s extreme free energy of folding originates from a significantly decreased energetic penalty of folding (ΔSconf). Structural analyses of the denatured-state ensembles for the two proteins indicate that the source of these energetics is a significantly smaller and less solvent accessible denatured state for Klentaq relative to Klentaq. It is also notable that although the average denatured state diameter for Klentaq is approximately 1.4X larger than for Klentaq, they both fall within the range for random-coil behavior.

1692-Pos Board B536
Thermostabilization Due to Rigidity: A Case Study of Rubredoxin
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The source of increased stability in proteins from organisms that thrive in extreme thermal environments is not well understood. Previous experimental and theoretical studies have suggested many different features responsible for such thermostability. Many of these thermostabilizing mechanisms can be accounted for in terms of structural rigidity. Thus a plausible hypothesis accounting for this remarkable stability in thermophilic enzymes states that these enzymes have enhanced conformational rigidity at temperatures below their native, functioning temperature. This study investigates the relationship between thermostability and rigidity using rubredoxin as a case study. The FIRST software is used to calculate local (residue level) and global rigidity for available rubredoxin structures and simulated mutants. Quantitative global rigidity measures indicate that an increase in structural rigidity (equivalently a decrease in flexibility) corresponds to an increase in thermostability. At the level of individual residues, hydrogen deuterium exchange experiments level indicate differential changes in flexibility between mesophilic and thermophilic rubredoxin structures that agree with computational flexibility analysis from the FIRST software.

1693-Pos Board B537
Formation Of A Core In SDS Denatured States Of Rhodopsin
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A general understanding of the folding pathway of helical membrane proteins has remained elusive due to the limited number of membrane proteins investigated to date. In order to begin to establish conditions under which the folding pathway of the mammalian membrane protein rhodopsin, a prototypic G protein coupled receptor with primary function in vision, can be studied, we have measured its secondary and tertiary structure changes under chemical denaturing conditions. A significant decrease in ellipticity at 222nm of maximally 40% is observed on adding sodium dodecyl sulfate (SDS) as a denaturant suggesting unfolding of the native helices. Tertiary structure is disrupted already at very low SDS concentrations of 0.05%, as evidenced by the loss of retinal chromophore, increase in tryptophan fluorescence and increase in cysteine accessibility. However, at intermediate SDS concentrations (0.1% to 3%), there is a decrease in tryptophan fluorescence accompanied by a simultaneous decrease in cysteine reactivity indicating formation of a compact intermediate. Light-scattering confirms that this decrease is not due to unspecific aggregation of the protein. Site-directed mutagenesis indicates that the reactive cysteines in this intermediate are located in the cytoplasmic domain of rhodopsin. At high SDS concentrations, between 7% and 15%, where a large amount of additional helix is disrupted, there is an increase in tryptophan fluorescence and cysteine reactivity. However, with further increase in SDS concentration up to 30%, there is a drop in fluorescence and cysteine accessibility suggesting formation of a core in this largely unfolded state. Existence of a folding core during early stages of folding of rhodopsin has also been postulated by the long-range interaction model and has been predicted by earlier computational experiments.