Chamapsocephalus gunnari) have been investigated. A novel fluorescence assay was utilized that simultaneous monitors changes to the global protein structure, structural changes near the active site, and aggregation of the enzyme in response to increasing temperature and increasing concentration of the natural osmolyte, trimethyl amine N-oxide (TMAO), a stabilizer of protein structure. Using this assay, the reverse changes of stability and affinity for oxamate were established for both, phLDH and cgLDH. Importantly, a low-temperature (pre-denaturation) structural transition was found that precedes the high-temperature (denaturation) transition for both LDHs and coincides with increasing enzymatic activity. The structural transitions of the global protein structure and the active site are concerted for the rigid (phLDH) and not concerted for the flexible (cgLDH) LDHs. The profound contribution of entropy to G along with the higher structural flexibility increases functional plasticity of the psychrophilic cgLDH. TMAO increases stability and shifts all structural transitions to the higher temperatures for both orthologs and simultaneously reduces their catalytic activity. The multiple active and inactive along with intermediate substate conformations of the enzyme exist in equilibrium at the stage preceding irreversible thermal inactivation. This equilibrium is an essential selective factor for the adaptation of an enzyme to the environmental temperature. It seems also possible that thermal adaptation of proteins may be complemented by evolution of the cellular milieu.

285-Pos Board B50

Denatured State Loop Formation Thermodynamics of a Hybrid Polypeptide

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Previous work with the four-helix bundle protein cytochrome *c*' in *Rhodopseudomonas palustris* using histidine-heme loop formation thermodynamic methods revealed fold-specific deviations from random coil character in its denatured state ensemble. To examine the generality of this finding, we extend this work to a three-helix bundle polypeptide, the human DNA excision repair protein's second ubiquitin-associated (UBA) domain, UBA(2). We use yeast iso-1-cytochrome *c* as a scaffold, fusing the UBA(2) domain to the N-terminus of iso-1-cytochrome *c*. Using site-directed mutagenesis, we have engineered histidine into solvent accessible surface residue positions within the all-alpha fold, creating eight single histidine variants. Isothermal equilibration denaturation studies reveal that the fusion protein unfolds in a 3-state process, commencing with iso-1-cytochrome *c* followed by UBA(2). Thermodynamic stability experiments also demonstrate that the histidine residues in the UBA(2) domain strongly destabilize iso-1-cytochrome *c*. Furthermore, histidine-heme loop formation equilibria show lower apparent pK_a's compared to the pseudo-wild type variant, indicating significant interactions in the denatured state. We will compare the degree of deviation of loop stability versus loop size, relative to predictions of the Jacobson-Stockmayer relationship used in our previous work on cytochrome *c*'. This comparison will allow evaluation of sequence-based conformational bias in the denatured state of this protein.

286-Pos Board B51

Regulation of Protein Folding using Organic Solvents and Ionic Liquids

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The *Escherichia coli* expression system is frequently used to prepare various proteins in a highly efficient manner using information obtained by the Human Genome Project. However, recombinant proteins are often expressed as inclusion bodies which are biologically inactive, and a refolding reaction is absolutely required to form the correct tertiary structure. For this purpose, i*n vitro* refolding reactions of recombinant proteins are generally performed in aqueous solutions. However, it is still difficult to efficiently fold recombinant proteins into their biologically active form in aqueous solutions because aggregates are formed as the result of the hydrophobic folding intermediates. Ionic liquids have recently been employed for refolding reaction of proteins in place of aqueous solutions and successfully folded into their native conformations can be accomplished, although the folding yield was still low. In addition, organic solvents, such as trifluoroethanol, are generally used for protein folding to induce α-helix formation and may reduce the extent of hydrophobic interactions. Therefore, to regulate or suppress hydrophobic interactions during the refolding reaction, organic solvents and ionic liquids were examined in attempts to construct the native conformation or to produce a proper folding intermediate.

Dioxiane and 1-hexyl-3-methylimidazolium chloride (HMIM) were examined for the refolding reaction of prouroguanylin, as a model protein, which contains three disulfide bonds. Prouroguanylin was able to fold into its native conformation at a low concentration of HMIM or dioxane. Surprisingly, prouroguanylin was still able to form native disulfide bonds even at 80% HMIM or dioxane although prouroguanylin was not able to fold into its native structure under denaturing condition using urea or guanidine hydrochloride. Therefore, the results indicate that those solvents are suitable candidate solvents for use in protein folding studies. The results will be discussed in this paper.

287-Pos Board B52

Glycine Betaine Reverses Osmotic Shock Induced Protein Destabilization in Living Cells

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Cells utilize several mechanisms for adapting to changes in osmotic pressure. Bacteria, including *Escherichia coli,* can grow in a wide range of osmolarities. Increasing the external osmolarity (*i.e.,* hyperosmotic shock) causes water efflux, reduction in cell volume and accumulation of osmolytes such as glycine betaine. This volume reduction increases the crowded nature of the cytoplasm, which is expected to affect protein stability. In contrast to traditional theory, which predicts that more crowded conditions can only increase stability, recent work shows that crowding can destabilize proteins through transient attractive interactions. Here, we quantify protein stability in living *E. coli* cells before and after osmotic shock in the presence and absence of glycine betaine. The 7-kDa *N*-terminal SH3 domain of *Drosophila* signal transduction protein drk (SH3) is used as the model protein because it exists in an equilibrium between a folded state and an unfolded ensemble. Labeling SH3 with a fluorine on its sole tryptophan facilitates NMR-based detection of both states simultaneously, allowing quantification of the free energy of unfolding *in vitro* and in living *E. coli* cells. We find that hyperosmotic shock decreases SH3 stability, consistent with the idea that weak interactions are important under physiologically relevant crowded conditions. Subsequent uptake of glycine betaine returns SH3 to the stability observed without osmotic shock. These results highlight the effect of transient interactions on protein stability in cells and provide a new explanation for why stressed cells accumulate osmolytes.

288-Pos Board B53

Fluorescence Evidences for Non-Homogeneity and Residual Structure of Denatured States

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About 30 % of human proteins do not fold into a stable 3D arrangement of secondary structure elements, but stay predominantly unfolded -similar to proteins under highly denaturing conditions. These proteins are involved in many cell signaling processes. Their characterization poses a great challenge for current experimental methods as they consist of an ensemble of rapidly interconverting conformations. Intense debate exists on the possibility that they show, to certain extent, residual structure, which might facilitate folding or enhance ligand binding. To study the unfolded state conformational heterogeneity using Förster resonance energy transfer (FRET), we used the lysozyme from the phage T4 (T4L) in denaturing conditions as a model system. We built an elastic network model that spans T4L's topology in order to evaluate local and global conformational changes by combining ensemble (ensemble time-resolved fluorescence lifetime and anisotropy) and single-molecule spectroscopic (multiparameter fluorescence detection, photon distribution analysis, (filtered) fluorescence correlation spectroscopy) methods. Through extensive comparison of models, we identified regions with apparent residual structure under highly denaturing conditions, which might serve as folding nuclei; and additionally we showed that chemically denatured T4L is not a random coil as previously thought. By using obtained distance restraints we determined that denatured T4L shows a native-like mean structure, albeit larger in size compared to the native state. We demonstrate here the necessity of careful data interpretation, but also the potential of a multidimensional approach to characterize an ensemble of states, which can be applied generally to unstructured or denatured proteins.

289-Pos Board B54

Selection Maintaining Protein Stability at Equilibrium

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Recently it was indicated that fitness costs due to misfolded proteins are a determinant of evolutionary rate and selection originating in protein stability is a driving force of protein evolution. Here we examine protein evolution under the selection maintaining protein stability.

Protein fitness studied is a generic form of fitness costs due to misfolded proteins; $s = \kappa \exp(\Delta G / kT) (1 - \exp(\Delta\Delta G / kT))$, where s and $\Delta\Delta G$ are selective advantage and stability change of a mutant protein, ΔG is the folding free energy of the wild-type protein, and κ is a parameter representing protein abundance and indispensability. The distribution of $\Delta\Delta G$ is approximated to be a bi-Gaussian distribution, which represents structurally slightly- or highly-constrained sites. Also, the mean of the distribution is negatively proportional to ΔG.
The evolution of this gene has an equilibrium point ΔGe, the range of which is consistent with observed values in the ProTherm database. The probability distribution of Ka/Ks, the ratio of nonsynonymous to synonymous substitution rate per site, over fixed mutants in the vicinity of the equilibrium shows that nearly neutral selection is predominant only in low-abundant, non-essential proteins of $\Delta Ge > -2.5$ kcal/mol. In the other proteins, positive selection on stabilizing mutations is significant to maintain protein stability at equilibrium as well as random drift on slightly negative mutations, although the average is less than 1. Slow evolutionary rates can be caused by high protein abundance/indispensability and large effective population size, which produce positive shifts of $\Delta\Delta G$ through decreasing ΔGe, and by strong structural constraints, which directly make $\Delta\Delta G$ more positive. Protein abundance/indispensability more affect evolutionary rate for less constrained proteins, and structural constraint for less abundant, less essential proteins.
(Reference: *J. Theor. Biol.*, **391**, 21-34, 2016.)

290-Pos Board B55
Effects of Flanking Disorder on the Behaviour of Ordered Domains
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Both structured proteins and those containing intrinsic disorder have been the subject of much investigation; especially as disorder relates to protein-protein interactions. However, there has been little exploration into the influence of these elements on each other. Do IDPs regulate the stability and folding mechanisms of connected structured regions and *vice versa* do ordered domains affect the affinity of IDRs for their ligands e.g. by affecting the association kinetics? To try and answer these questions we use biophysical methods and progressive deletions to vary the length of disordered regions flanking ordered domains in two model systems of one and two globular domains, respectively.

291-Pos Board B56
The Effect of Polydisperse Crowding on Protein Stability
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The dense, heterogeneous cellular environment is known to affect protein stability through interactions with other biomacromolecules. The effect of excluded volume due to these biomolecules, also known as crowding agents, on a protein of interest, or test protein, has long been known to increase the stability of a test protein. The cellular environment is heterogeneous not only in terms of its chemical composition, but also in terms of the sizes of the biomacromolecules, or crowding agents, present. It has been shown experimentally that the effect of polydisperse or mixed crowding agents has a non-additive effect, i.e. that there is an optimal mixing ratio where the effect of the crowding agents is larger than that of monodisperse systems of each crowder. Here we investigate the role of polydisperse crowding on two small test proteins: the helical trp-cage and the beta-hairpin GB1m3. For each test protein, a series of simulations using crowding agents of two different sizes in various ratios were conducted. Crowding agents used were either spherical excluded-volume only crowders or proteins with fixed backbones. In particular, we relate the non-additivity to the excluded volume of the crowding agents. We also show that protein-crowder interactions can play a large role in either enhancing or offsetting the effect of the crowding agent excluded volume.

292-Pos Board B57
PAPS-Synthase: Dissecting Folding of a Large and Naturally Fragile Protein *In Vitro* and *In Cellulo*
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PAPS synthases are bifunctional enzymes providing the cell with the sulfate donor PAPS (3'-phosphoadenosine-5′-phosphosulfate), which is further used by sulfotransferases for modification of several biomolecules (e.g. steroids). Isoform PAPS synthase 2 (PAPSS2) has been shown to be fragile within *in vitro* experiments, but is stabilized by binding of its endogenous ligands. Mutations affecting its activity lead to disease states like bone and cartilage malformation as well as metabolic diseases. Compared to *in vitro* conditions, the cellular milieu is crowded by large biopolymers resulting in intermolecular interactions and excluded-volume effects affecting each biopolymer inside the cell.
To understand the stability of this large enzyme (70 kDa), we conducted biophysical studies on the individual domains, APS kinase and ATP sulfurylase, as well as the full length protein. Using *Fast Relaxation Imaging* we studied disease-relevant mutants of PAPSS2 directly within the cell. The data provide novel thermodynamic insights into PAPSS2 stability inside the cellular milieu and its influence on the naturally destabilized protein. These results also unravel new insights into disease mechanisms of PAPSS2 mutations.

293-Pos Board B58
Effects of Salt or Cosolvent Addition on Thermal Stability of a Protein: Relevance to those on Solubility of a Hydrophobic Solute in Water
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The thermal stability of a protein is changed upon addition of a salt or cosolvent. The solubility of a hydrophobic solute (e.g., argon or methane) in water is also influenced by the addition. Interestingly, the addition which decreases the solubility usually enhances the thermal stability. This suggests that the hydrophobic effect is a principal factor governing the stability change, because the decrease and increase in the solubility, respectively, are ascribed to enhancement and reduction of the effect. However, urea decreases the solubility but lowers the stability. Bromide and iodide ions decrease the solubility but lower the stability of a protein with a large, positive net charge. In these cases, the stability change is influenced by the changes in not only the hydrophobic effect but also other physical factors. We show for hydrophobic solutes that the integral equation theory where the solute and solvent particles are modeled as hard spheres with different diameters can reproduce the experimental data for the following items: salting out by an alkali halide and salting in by tetramethylammonium bromide, increase in solubility by a monohydric alcohol, and decrease in solubility by sucrose or urea. The orders of cation or anion species in terms of the power of decreasing the solubility can also be reproduced for alkali halides. With our model, the analyses are focused on the roles of entropy originating from the translational displacement of solvent particles. As the products, we clarify the pivotal physical origin of the hydrophobic effect and present a new view on the Hofmeister series. We show how the series is expressed when the hydrophobic effect dominates and how it is modified when other physical factors are also influential.

294-Pos Board B59
Residual Structure in the Denatured State of a Three-Helix Bundle Protein
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The denatured state of a protein is canonically described as an ensemble of non-interacting random coil conformations. By studying a helical bundle protein under denaturing conditions, we have found that residual structure persists in its denatured state. Our work uses the upstream Ubiquitin-Associated domain, UBA(1), of the HHR23A protein as a model system to study residual structure in the denatured state ensemble. Multi-dimensional nuclear magnetic resonance (NMR) experiments ^{1}H-^{15}N HSQC, HNCO, and HNCA were used to find backbone atom secondary chemical shifts which correspond to protein secondary structure with amino acid resolution. By comparing chemical shifts in moderate- to highly denaturing conditions of 4M, 5M, and 6M guanidine HCl to a reference state of 7M GdnHCl, UBA(1) shows residual helical content among all three helices. In contrast, the helical propensity is predicted by the program Agadir to be higher in helix 2 and negligible elsewhere, with the assumption that helices do not interact. The presence of relatively uniform helical content among all three helices may indicate stabilizing tertiary interactions despite highly denaturing conditions. To further probe tertiary interactions, single-residue mutants of UBA(1) will be used to determine if residues in the hydrophobic core or turn regions are contributing to stability of residual structure in the denatured state.

295-Pos Board B60
Phosphorylation Induced Global Structural Destabilization of a Small Protein Domain
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Protein phosphorylation at a single or multiple sites is utilized to regulate protein functional outcomes and overall cellular activities through signaling pathways. At a molecular level, the addition of a phosphoryl group may alter the function of a protein through distinct and versatile mechanisms. These include