and a cytoplasmic region. The extracellular region has five tandemly repeated ectodomains (EC1-EC5), with three calcium binding sites situated between each of these domains. Cell-cell adhesion is mediated by the dimerization of cadherins presented on neighboring cell surfaces. We are focusing our studies on two members of type I classical cadherins, Neural (N) - and Epithelial (E)-cadherins. In spite of high sequence similarity between E- and N-cadherin, they have distinct physiological localization implying differences in their adhesive properties. Here, we compare the spectral characteristics, stability, calcium binding and assembly properties of the first two domains of N- and E- cadherins. Spectroscopic studies of these proteins were predictable and indicated typical β-sheet conformation with only partial exposure of tryptophans. Although both proteins are stabilized by calcium, apo-ECAD12 is less stable than apo-NCAD12. Direct calcium titrations that found the proteins bind calcium with equally high affinity (−6.2 kcal/mol). There was a striking difference between these proteins in terms of the kinetics of disassembly. Analytical size exclusion chromatography experiments showed that disassembly of ECAD12 dimers is rapid and disassembly of NCAD12 dimers is slow regardless of the calcium concentration. We observe this striking difference with constructs containing only the first 220 residues of a 700 residue protein. Thus, not only is this an interesting protein folding-function question, this remarkable difference in these cadherins may explain their segregation into different physiological niches.

**2312-Pos**

**Effect of Osmolytes on Proteins**

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Cells exhibit cellular coping mechanisms when faced with osmotic stresses by importing or producing compounds called osmolytes, which aid in osmotic regulation. Proline is an example of such a compound. The primary function of these compounds is to combat the effects of dehydration in the cell. Stabilization of proteins, which are susceptible to osmotic stresses, is of key importance to the cell's health. Osmolytes have been shown to impact the stability and solubility of proteins, and certain osmolytes also exhibit the function of aiding in protein folding and refolding and in preventing protein aggregation. The mode by which osmolytes aid in protein stabilization is believed to be a solvent-oriented process by which folding is facilitated by the preferential ordering of solvent molecules, but the exact mechanism remains elusive. We characterized the supramolecular structure of proline in solution using multi-dimensional NMR spectroscopy and dynamic light scattering. The molecular mechanism underlying the stabilizing effect of proline on a protein is studied using thermal denaturation monitored by steady-state fluorescence. Results from the denaturation studies indicate that the Tm of the protein increases in the presence of increasing concentrations of proline by about 20 °C, suggesting that thermodynamic stability of the protein is enhanced upon binding to proline. Stability studies using several other osmolytes like TMAO, glycerol, 4-hydroxy proline, and betaine show that proline is the osmolyte which stabilizes the protein to the largest extent. Two-dimensional HSQC NMR experiments were used to reveal the proline binding sites on FGF-1. The results provide useful insights on the molecular mechanism of proline. The mechanism by which proline stabilizes protein is further investigated in hydrogen-deuterium exchange experiments monitored by NMR with the protein in the presence and absence of proline in viscous medium. These results provide valuable thermodynamic and binding specificity data.

**2313-Pos**

**How Stable is an Enzyme from a Thermophilic Organism? Denaturation Studies with the Esterase from *Pyrococcus furiosus* - The Role of Charge-Charge Interactions**

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We have expressed the gene of the hyperthermophilic esterase (Pf2001Δ60) from *Pyrococcus furiosus.* This esterase showed to be active after boiling and has a half-life of 120 min at 75 °C. We decided to study the unfolding of this enzyme by fluorescence spectroscopy induced by urea, guanidinium hydrochloride (GndHCl) and high hydrostatic pressure (HHP). Ours results pointed out that urea is about three times more efficient than GndHCl in promoting structural perturbations in PF2001Δ60 ([D1/2]=7.1 and 2.3 M, respectively), suggesting that ion pairs are important stabilizing factors on its structure. There was almost no change in the tryptophan center of mass of PF2001Δ60 (342 nm) under HHP up to 3.1 kbar, even by staying 120 min under 2,500 bar. Interestingly, the combination of HHP with a subdenaturating concentration of urea (1 M) displaced the center of mass by ≈ 7 nm after 40 min at 2,500 bar. Since HHP enhances the electrostriction, this result reinforces the crucial contribution of salt bridges in esterase's stability. The binding of the bis(8-anilinonaphthalene-1-sulfonate) to PF2001Δ60 was increased by 2.5- and 3-fold after treatment with 2 M GndHCl or under HHP combined with urea, suggesting that these treatments convert the enzyme into a partially folded intermediate with exposed hydrophobic regions. Altogether, ours results may be an indication that the optimization of charge-charge interactions on the protein surface is a key factor for its stability. To our knowledge, this is the first time that HHP is used to access the ion pair contributions to the stability of a hyperthermophilic esterase.

**2314-Pos**

**Cysteine Reactivity as a Probe of the Proteome**

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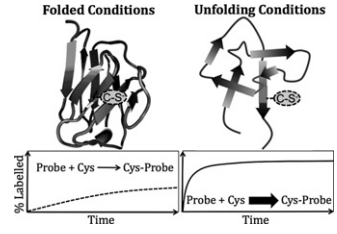
The study of protein folding and stability is typically conducted with purified proteins by methods that are exacting but lack the ability to analyze complex mixtures of proteins. To study proteins in their native environments inside a cell or isolated organelle, such as a nucleus, we have recently developed a powerful method that combines shotgun labelling with LC/MS/MS. Cysteine is a reactive but hydrophobic amino acid that can be fluorescently labelled in isolated nuclei with time resolution under stress conditions (changes in temperature). Quantitative kinetic analyses of spectra allow us to identify regions in hundreds of proteins, including nuclear lamins implicated in diseases such as Progeria, to understand the folding and interactions in situ. Select protein domains are also studied in solution, demonstrating the close correspondence to more traditional methods.




**2315-Pos**

**Nanomechanics of Ankyrin-R Repeats Probed by AFM and SMD Simulations**

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Ankyrin (ANK) repeats, identified in thousands of proteins, are composed of pairs of antiparallel alpha-helices that stack on top of each other and form super-helical spiral domains with suggestive spring-like properties, whose primary function is to mediate specific protein-protein interactions. For example, ankyrin-R links the anion exchanger in the erythrocyte membrane to the membrane skeleton and contains 24 ANK repeats that form a spiral domain. Ankyrin-R stabilizes the erythrocyte membrane and mutations in ANK repeats are documented in hereditary spherocytosis (HS), the life-threatening human anemia. Since repeats 13 to 24 (D34) of Ankyrin R are especially active in binding interactions and are subjected to the HS mutations, therefore the mechanical properties of this region of ankyrin-R are of the utmost importance. Although the conserved residues in each ankyrin repeat generate nearly identical helix-helix-loop structures, the exact positions and numbers of internal hydrogen-bonds, salt bridges and hydrophobic residue packing between repeats vary along the D34 domain and this variation is expected to modulate the elasticity, mechanical stability and mechanical unfolding/refolding properties of D34. We probed these properties of D34 directly by means of AFM-based single molecule force spectroscopy and by Steered Molecular Dynamics (SMD) simulations. By mapping the AFM force spectroscopy data onto the SMD-determined behavior of the internal network of H-bonds and salts bridges, we propose a model of the complex mechanical unfolding and refolding patterns of ANK repeats in D34.

**2316-Pos**

**Cation-Pi Interactions Contribute Significantly to the Stability of FGF and the FGFR**

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Fibroblast growth factors (FGFs) are ~ 16 kDa heparin binding proteins that regulate key cellular processes such as angiogenesis, differentiation, morphogenesis, wound healing and tumor growth. FGFRs consist of three extracellular ligand binding domains (D1, D2, D3), a single transmembrane helix, and cytoplasmic tyrosine kinase domain. Cell surface-bound HSPGs (heparan

sulfate proteoglycans) supported dimerization or polymerization of the FGFRs are thought to be required to activate the signaling pathway. The D2 domain is suggested to bind with both HSPGs and FGFs to form a ternary complex. Xray and NMR solution structures of the D2 domain have been analyzed using the CAPTURE cation-pi program. The CAPTURE program indicates cation-pi interactions between residues Y155:R152(Xray), W191:R203 (NMR) and possibly F237:K151 (Xray). Biophysical characterization of the mutants at each cation and pi pair, identified by CAPTURE, shows a significant destabilization resulting from the Y155A, W191A and R203E mutations. Results from differential scanning calorimetry show a reduction in melting temperature by 10-14 °C for Y155A, W191 and R203 mutants of D2. The reduction in the stability of the D2 domain is corroborated by results of ANS binding, thermal denaturation and a limited trypsin digestion experiments. The HSQC of D2 Y155A shows limited chemical shift perturbation of residues in the vicinity of the mutation site. The W191A and R203E mutations show significant 1H-15N chemical shift perturbations in their HSQC spectra. The results obtained in this study show that cation-pi interactions contribute significantly to the thermodynamic stability of proteins. In addition, our results indicate that cation-pi predictions made on the solution NMR structures are more reliable than those predicted based on crystal structures.

**2317-Pos**

**Equilibrium Population of the Folding Intermediate of RNase H and its Importance in the Folding Trajectory**

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Proteins can sample a variety of partially folded conformations during the transition between the unfolded and native states. Some proteins never significantly populate these high-energy states and fold by an apparently two-state process. What factors govern which conformations are accessible to a protein as it folds? We have attempted to re-route the folding of ribonuclease H from E. coli by manipulating its regional stability. Using phi-value analysis, we compare the structures of the transition states for folding of RNases H that fold with and without a detectable partially folded intermediate and find that both versions of RNase H fold through a similar trajectory with similar high energy conformations. In light of the general importance of this species on the folding pathway, we attempted to populate the intermediate at equilibrium by destabilizing the region of the protein that is unfolded in this form. Surprisingly, a single change at Ile 25 (I25A) resulted in the equilibrium population of the intermediate under near-native conditions. The intermediate was undetectable in a series of HSQC's, revealing the dynamic nature of this partially unfolded form on the timescale of NMR detection. The dynamic nature of the RNase H intermediate may be important for its role as an on-pathway, productive species that promotes efficient folding.

**2318-Pos**

**Solvation of Hydrophobic Amino Acid Side Chains and Peptide Backbone in Aqueous Glycine Betaine and Trimethylamine N-oxide Solutions**

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There is a large class of small, water-soluble molecules broadly referred to as osmolytes that may stabilize or destabilize biomacromolecular structures. For example, trimethylamine N-oxide (TMAO) and glycine betaine are prominent stabilizers of proteins, while urea is a strong denaturant. Although these osmolytes have been employed in protein studies for more than 70 years, the mechanisms of their action are still largely unknown. One reason for this deficiency is the lack of direct thermodynamic data that can be used to quantify solute-solvent interactions versus solute-osmolyte interactions. In this work, we use high precision densimetry and ultrasonic velocimetry to examine the solvation properties of amino acid side chains and the peptide group in binary mixtures of water and TMAO or glycine betaine. Specifically, we report the partial molar volume, V°, and adiabatic compressibility, Ks°, of N-acetyl amino acid amides (alanine, valine, leucine, isoleucine, phenylalanine) and oligoglycines (Gly)1-5 in binary mixtures containing 0 to 4 M TMAO or glycine betaine. We use our volumetric results to evaluate the osmolyte-dependent group contributions of amino acid side chains and the peptide group. We analyze these osmolyte-dependent group numbers to evaluate the binding constants and elementary changes in volume and compressibility accompanying the replacement of water of hydration in the vicinity of the solutes with a TMAO or glycine betaine molecule. We compare these data with similar results previously obtained in our laboratory for the interactions of urea with protein groups. In general, we discuss the implications of our results for elucidating the mechanism of stabilization/destabilization of protein structures by osmolytes.

**2319-Pos**

**Site-Specific Determination of Conformational Flexibility from a Side Chain Perspective: Native State Thiol Exchange of E. coli Ribonuclease H**

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Tools such as crystallography and hydrogen exchange (HX) have revealed a wealth of knowledge about protein function, folding, and dynamics. Here we explore the use of thiol exchange (SX) to gain further insight into the energy landscape of E. coli ribonuclease H (RNase H). Similar to HX, SX investigates the solvent accessibility and conformational fluctuations of specific positions in a protein, but while HX measures exchange of the backbone amide proton, SX takes advantage of cysteine's unique reactivity to measure solvent accessibility of the side chain. Native state SX results for a hyperstabilized mutant of E. coli RNase H reveal a partially unfolded form (PUF) at equilibrium with the native state, as is seen by HX. The structured regions of the PUFs measured by the two techniques agree overall, with some slight differences due to probing the side chain rather than the backbone. Moreover, while for some positions the SX experiments revealed this equilibrium information, the same experiments yielded direct kinetic information about protein opening events. Thus, in one set of experiments we have measured both kinetic and equilibrium parameters describing the folding of E. coli RNase H.

## Enzymes

**2320-Pos**

**Functional Effects of N-Metal Binding Domain Deletion and Specific Mutations on the ATP7B (Wilson Disease) Copper ATPase**

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We obtain high yield heterologous expression of ATP7B (Wilson disease) protein in COS1 cells infected with adenovirus vector. The recombinant protein recovered with the microsomal fraction of the infected cells undergoes high levels of phosphorylation with ATP through the minute time scale, most of which involves serine residues ($Ser^{478}$, $Ser^{481}$, $Ser^{1121}$ and $Ser^{1453}$), as demonstrated by proteolysis and mass spectrometry (J Biol Chem. 2009; 284:21307-16). We now find that incubation within the second time scale yields mostly alkali labile phosphorylation which we attribute to formation of phosphoenzyme catalytic intermediate (EP). In fact, this rapid phosphorylation does not occur following $D^{1027}N$ (conserved catalytic aspartate in P-ATPases) or C983A/C985A (transmembrane copper binding domain site) mutations. The WT phosphoenzyme intermediate reaches steady state levels within 2 seconds, and undergoes 3 $sec^{-1}$ turnover at 30 °C. We also find that the H1069Q mutant (nucleotide binding domain mutation found in Wilson disease) does not form the catalytic phosphoenzyme intermediate within the second time scale, and reduces phosphorylation of serine residues as well. Finally, we find that an extensive deletion eliminating the first five out of six copper sites of the N-metal binding domain (NMBD, a unique feature of ATP7A and B which is not present in other P-type ATPase) does not interfere with formation and rapid turnover of phosphorylated enzyme intermediate. It is noteworthy that, as in previous work with the bacterial copper ATPase CopA (J Biol Chem. 2008; 283: 22541-9), mutation of the NMBD copper site close to the A domain sequence slows substrate utilization kinetics, indicating interference with A and N domains movements. (Supported by 5 R01 HL069830-08).

**2321-Pos**

**Characterization of Membrane Bound Phospholipase-Lipid Complex**

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Phospholipases are interfacial enzymes that catalyze hydrolysis of lipids in membranes. Their activity is significantly higher at the surface of lipid aggregates than on monomeric substrates. Enzymatic activity occurs in three sequential steps of 1. enzyme-interface binding; 2. bound enzyme-lipid binding at the active site; 3. lipid hydrolysis. The interface binding in step 1 puts the enzyme in an "open" conformation. In recent work we formulated a specific role for the complex formed in step 2 between the membrane-bound enzyme and phospholipid in the rate of hydrolytic cleavage of the lipid, which involves the thermodynamic properties of the complex. In this work we present results of thermodynamic characterization of the enzyme-lipid complex in vesicles. Existence of an energy barrier for the complex formation is postulated. The heat capacity of the formation of the complex in vesicles was measured by Differential Scanning Calorimetry. The DSC thermograms indicate the existence of a peak in