detergents induce alpha-helix formation irrespective of the intrinsic native secondary structure, a process known as "reconstructive denaturation." Although this latter phenomenon underpins the ubiquitous technique SDS-PAGE, the mechanism of SDS denaturation and the molecular nature of the SDS denatured state are not known. We use a combined biophysical and computational approach to elucidate the molecular basis of protein denaturation by ionic detergents, with a special focus on the mechanism of reconstructive denaturation by SDS.
Specifically, biophysical techniques, including CD and ITC, are used to study the interaction of a set of detergents with model peptides in parallel with molecular dynamics simulations of the same systems. Our results show that SDS and LTAC induce increased alpha-helix content in cationic and anionic peptides respectively, but not vice versa. The zwitterionic detergent lauryl-dimethylamine oxide (LDAO) has no effect on either peptide. Our MD simulations provide atomic resolution detail of the results from the biophysical experiments, and show different modes of micellar binding that correlate with the observed detergent/peptide data. These results suggest a mechanism for the reconstructive denaturation phenomenon and for SDS's universal protein denaturing action.

**2306-Pos**
**Circular Dichroism Measurements in a Microfluidic Serpentine Mixer**
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The signature spectra of circular dichroism in the far UV is a useful probe to determine the secondary structure of protein. It is now being implemented in ultra-rapid microfluidic mixers to obtain time resolved structural information of a protein during folding. We have developed a CD instrument that utilizes a serpentine mixer with a mixing time of at least 100 microseconds to explore the formation of secondary structure within the slow process of a typical two-state folder. As a first measurement we observe the change in secondary structure in the first millisecond of lysozyme folding.

**2307-Pos**
**Investigation of Collagen Glycine Substitution Mutations Leading to Disease in a Bacteria Collagen System and Collagen Like Peptides**
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Diseases such as Osteogenesis Imperfecta are caused by missense mutations in collagen which change one Gly to another residue, breaking the repeating $(Gly\text{-}X\text{-}Y)_n$ collagen sequence pattern. Two approaches were taken to investigate the effect of Gly substitutions on triple helix structure, stability and folding. In the first approach, a bacterial collagen with the normal tripeptide repeating sequence is expressed in E. coli, and compared with the homologous proteins with a mutation replacing one Gly residue by a Ser or by an Arg residue. The bacterial collagens with Gly to Ser and Gly to Arg replacements still form stable triple helical molecules but with a small decrease in stability. The effect of these mutations on folding is under investigation. In the second approach, collagen model peptides are being used to examine the effect of replacing Gly by the next smallest substituting residue Ala, introducing a mutation sequence known to cause Osteogenesis Imperfecta. Biophysical studies on these peptides are designed to understand why some Gly to Ala replacements lead to collagen diseases while others do not.

**2308-Pos**
**Understanding the Effectiveness of Synthetic Crowding Agents**
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In vitro studies on the structure and stability of macromolecules are typically performed using very dilute solutions. However, the total intracellular concentration of macromolecules is very high, resulting in an *in vivo* environment that is significantly crowded. Prior studies have proven that the nonspecific interactions that occur between individual macromolecules and their crowded surroundings have a significant effect on biochemical rates and equilibira. In other words, the mechanisms under which a protein functions in a living cell may be quite different from the conditions under which a protein is studied by biochemist in the laboratory. To gain a better understanding of the phenomenon of macromolecular crowding, researchers have begun to utilize synthetic crowding agents such as ficoll, dextran, and PEG to recreate the *in vivo* environment. Experiments are conducted to understand the properties of proteins in such conditions with the belief that these synthetic crowding agents are able to adequately mimic the intracellular environment with its multiple components of lipids, carbohydrates, nucleic acids, and proteins. These crowding agents are thought to serve as inert compounds that have no interaction with the protein in question. This study has investigated the ability of synthetic crowding agents to produce a cellular environment that is similar to that of the actual cell. The thermal denaturations and NMR spectra of lysozyme and fibroblast growth factor (hFGF) were tested in the presence of various synthetic crowding agents. This was compared with the thermal denaturation and NMR spectra of these same proteins when placed in higher concentrations of themselves. The results indicate that synthetic crowding agents are not effective in mimicking the cellular environment. With these results, the understanding of protein study in the laboratory can be furthered as techniques to create a life like laboratory environment are refined.

**2309-Pos**
**Analysis of Thermal Stability of Protein 4.1R FERM Domain**
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**[Motivation and Aim]**
The crystal structure of N-terminal 30kDa domain of protein 4.1R (R30, "FERM" domain), that is a membrane skeletal protein, is three-lobe-clover. The transmembrane proteins, Glycophorin C (GPC) and band 3, and p55 bind to each different lobe of R30. Calmodulin (CaM) also binds to R30 in $Ca^{2+}$-independent manner. Binding with these proteins may stabilize R30. In the present study, we analyzed temperature dependent changes of R30 structure and its binding affinity to apo-CaM.
**[Materials and Methods]**
1) The recombinant proteins (cytoplasmic domains of GPC and band 3, p55, and R30) were purified as GST fusion protein from bacteria lysate. CaM was purified from bovine brain.
2) FT-IR (attenuated total reflection (ATR) analysis), with Tensor27 and BIO-ATRII accessory (Bruker Optics K.K.) was used for detecting of secondary structure of R30.
3) Dynamic light scattering (DLS) analysis was carried out with Zetasizer Nano ZS® (Sysmex Corp.).
4) The binding kinetics of R30 to proteins was analyzed using IAsys (Affinity Sensors). R30 dissolved in 10mM HEPES, pH7.4 containing 0.1M NaCl and 1mM EDTA was incubated at 4°C~60°C for 30 min and the binding activity was measured.
**[Results]**
1) ATR analysis of R30 showed dramatic increase in intensity of β-sheet ($1628cm^{-1}$ and $1672cm^{-1}$) with increase in temperature from 40°C to 45°C. The corresponding change was small in the presence of apo-CaM.
2) In DLS measurement, R30 became to be aggregated around 45°C.
3) R30 denatured at 50°C lost binding ability to apo-CaM, cytoplasmic domains of GPC and band 3. The binding ability of R30 to p55 did at 40°C.
**[Discussion]**
Aggregation of R30 at 45°C may be caused through its β-sheet. FT-IR results suggested increasing of intramolecular β-sheet. Actually, p55 binding site is located at the β-sheet structure rich domain.

**2310-Pos**
**Studying Protein Folding on the Ribosome One Molecule at a Time**
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In contrast to traditional in-vitro protein refolding experiments, protein folding in the cell occurs in a vectorial fashion. To what degree do the trajectories and states populated during in-vitro refolding report on in-vivo folding pathways? This question is one which requires the development of novel methodologies which enable the study of the conformational distributions and dynamics of unfolded proteins both in the context of the ribosome exit tunnel and under conditions which mimic those of the crowded interiors of living cells. Here we will describe the development of a novel approach which can address this and other related questions by using single molecule Fluorescence Resonance Energy Transfer (smFRET) to probe the conformations of ribosome-bound nascent chains.

**2311-Pos**
**Slow Disassembly of Neural-Cadherin Dimers**
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Cadherins are calcium dependent homophilic cell adhesive protein molecules that are critical for morphogenesis, synaptogenesis and synapse maintenance. Cadherins comprise an extracellular region, a single transmembrane region

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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Rodrigo V. Almeida, Orlando B. Martins, Debora Foguel.
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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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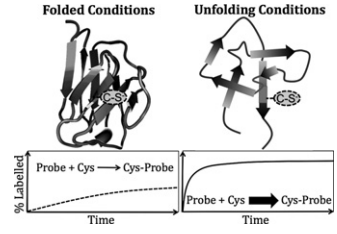
The study of protein folding and stability is typically conducted with purified proteins by methods that are exacting but lack the ability to analyze complex mixtures of proteins. To study proteins in their native environments inside a cell or isolated organelle, such as a nucleus, we have recently developed 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