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