1. Bennett MJ, Choe S, Eisenberg D. Refined structure of dimeric diphtheria toxin at 2.0 Å resolution. *Protein Sci.* (1994) 3: 1444-1463.

2150-Pos Board B136
Temperature Dependence of the Mechanical Unfolding of Single Ubiquitin Proteins
Ionel Popa, Sergi Garcia-Manyes, Julio M. Fernandez.
Single molecule atomic force microscopy (AFM) in force-clamp mode was used to study the effect of temperature on the mechanical unfolding of polyubiquitin. Single protein chains were extended at a constant force and the unfolding of individual domains was measured from the staircase increase of the contour length. The unfolding rate constant at each pulling force and medium temperature was obtained from the exponential fit to the ensemble averages of the contour length traces. The unfolding rate at zero force and the distance to transition state were then calculated from the force-dependent rate constants. By varying the temperature in the 5-45 °C range and the pulling force in the 70-210 pN interval, we find a significant thermal effect on unfolding. Fitting the temperature dependant unfolding rate at zero force k to the Arrhenius equation $k = A \exp(-Ea/kT)$ yields an activation energy Ea of 87 ± 5 kJ/mol and an exponential pre-factor $A \sim 2.3 \cdot 10^{12}$ s^{-1}. The exponential pre-factor yields a similar value to predictions of statistical mechanic calculations derived by the transition state theory (TST), of $(5.8\text{-}6.6) \cdot 10^{12}$ s^{-1} for the considered temperatures. This correlation suggests that the unfolding of proteins is not subject to frictional effects and that all interactions with the solvent molecules favor the unfolding, independent of the collision angle. This finding will prove important in assessing the energy landscape of unfolding proteins as a transition state process.

2151-Pos Board B137
Role of Internal Cavities as Determinants of Pressure Unfolding of Proteins
Jose A. Caro, Julien Roche, Jean-Baptiste Rouget, Jamie Schlessman, Angel E. García, Catherine A. Royer, Bertrand García-Moreno.
Pressure unfolds many proteins. In contrast to acid, heat and chemical denaturation, which have been studied with many proteins and which are relatively well understood, the molecular determinants of pressure unfolding of proteins are not known. Volume is the conjugate variable of pressure; therefore, the pressure unfolding of proteins is governed by differences in volume between the native and the unfolded ensembles. What is less obvious is where the differences in volume originate. Among the possibilities that have been considered are volume changes related to conformational fluctuations, volume changes related to hydration, and volume changes related to the loss of cavities present in the folded state. We have examined the role of cavities as determinants of pressure unfolding systematically, using staphylococcal nuclease as a model system. Ten different variants of a highly stable form of nuclease were engineered with single site substitutions in the hydrophobic core, designed to create cavities in the interior of the protein. High resolution crystal structures of all proteins were obtained to examine the state of the cavities and as starting structures for calculations of internal void volume with MD and MC methods. Pressure unfolding monitored with Trp fluorescence was used to measure volume changes upon unfolding. Our ultimate goal was to determine if volume changes measured in the equilibrium thermodynamic experiments correlated with the size of the cavities observed in the proteins. The mapping of experimentally determined cavity persistence ratios, combined with simulation data, provides new insight into the subtle interplay between local dynamics, water penetration, and structural properties of cavities in determining volume changes upon unfolding. Overall, the data suggest that internal cavities are one of the main determinants of the pressure-induced unfolding of proteins.

2152-Pos Board B138
Differences in Structural Rigidity between Thermophilic Proteins and their Mesophilic Homologues
Andrew Harter, Andrew J. Rader.
The source of increased stability in proteins from organisms that thrive in extreme thermal environments is not well understood. These proteins from thermophilic organisms can maintain biological functions at elevated temperatures which would render ordinary (mesophilic) proteins denatured and dysfunctional. Understanding the mechanisms responsible for thermostability has the potential to impact how effectively we can engineer thermostable analogues of proteins with specific functionalities.
If enzymes require some flexibility to function properly at their optimal temperatures, it follows that investigation of these structures at other non-native temperatures should reveal a different degree of conformational flexibility. Under such a corresponding states model, homologous mesophilic and thermophilic enzymes should have comparable catalytic efficiencies at their respective optimal temperatures because optimal activity requires a fixed degree of conformational flexibility in the active site. For thermostable enzymes, this catalytically required increase in flexibility only occurs at elevated temperatures upon the loss of local interactions. By logical extension, the remarkable stability of thermophilic enzymes is then a result of these enzymes having an increased conformational rigidity. If enzyme thermostability is dependent upon the underlying structure and its rigidity, then one ought to observe a correspondence between these traits. We hypothesize that the increased stability observed in thermophilic proteins is the result of an increased structural stability. This study investigates the various relationships between thermostability and structural stability. Our results indicate that a large degree of thermostability can be accounted for in terms of rigidity.

2153-Pos Board B139
Domain Swapping Promoted by Charges in the Hydrophobic Core of a Protein
Jamie L. Schlessman, Victor S. Khangulov, Daniel A. Karp, Michael J. Harms, Michael S. Chimenti, Bertrand Garcia-Moreno E.
Charges are more stable in water than in the dehydrated and relatively hydrophobic interior of proteins; therefore, internal ionizable groups usually destabilize the native state. They can also stabilize alternative conformational states, as illustrated here by two different variants of staphylococcal nuclease with Arg substitutions at the internal positions Thr-62 or Val-66. In contrast to internal Lys, Glu and Asp residues in nuclease studied previously, which titrate with highly perturbed pK_a values, Arg-62 and Arg-66 titrate with normal pK_a values. Crystal structures of the T62R and V66R variants are indistinguishable from that of the reference protein except at the N-terminal β strand (approximately residues 1 - 19), which in the structures of T62R and V66R variants is exchanged between neighboring molecules. In this domain-swapped state the charged moieties of Arg-62 and Arg-66 are fully exposed to water, which is consistent with their unperturbed pK_a values. These domain-swapped proteins illustrate potential consequences of the high affinity of charges for water: a single mutation that introduces an ionizable amino acid into the hydrophobic core of a protein can result in extensive structural reorganization. These novel examples of domain swapping as the result of a point mutation suggest an efficient mechanism for the evolution of multimeric proteins but with potentially deleterious consequences. They suggest that ionizable groups are eliminated from internal hydrophobic environments in proteins when they are not essential for structural or functional reasons, not only because they destabilize the native state, but also because they can compromise the solubility of proteins, by stabilizing aggregation-prone states.

2154-Pos Board B140
Malondialdehyde as a Fluorescent Probe for Misfolded Protein in Cells
Rongqiao He, Ya Jie Xu, Min Qiang, Chan Shuai Han.
Malondialdehyde that is produced under the oxidative stress and other pathological conditions results in changes in the structure and function of protein. We incubated BSA, fibrinogen and other proteins with malondialdehyde and then measured the changes in structure and function by using fluorescence, Thioflavin-T fluorescence, CD, electron microscopy, as well as atomic force microscopy. A new fluorescence (λ_{ex} 410 nm; λ_{em} 470 nm) was formed with quantum yield 0.49 in the reaction of protein with malondialdehyde. Fibrinogen was inactivated in the coagulation up to 5-hour incubation with malondialdehyde. We also observed amorphous protein aggregations in the presence of alondialdehyde. Moreover, the malondialdehyde-modified BSA could be transferred into neuroglia cells and a light blue fluorescence in cytoplasm could be detected under fluorescence microscope. This suggests that modification by malondialdehyde induces misfolding of protein and the formation of a new fluorescent protein derivative, which can be employed as a probe for misfolded protein in living cells.

2155-Pos Board B141
Volumetric Characterization of Interactions of Protein Groups with Glycine Betaine
Yuen Lai Shek, Tigran V. Chalikian.
We report the volumetric properties of N-acetyl amino acid amides with unionizable side chains and oligoglycines in binary solutions of water and glycine betaine, a protective cosolvent. We analyze these data within the framework of a statistical thermodynamic formalism to determine the association constants for the reaction in which glycine betaine binds to the peptide backbone and amino acid side chains replacing four water molecules. The association constants are linked to the free energy of transfer of functional groups from water to a glycine betaine solution, G_{tr}. Transfer free energy, G_{tr} is the sum of a change in the free energy of cavity formation, G_C, and the differential free energy of solute-solvent interactions, G_I, in a concentrated glycine betaine solution and water. We compare our results with