protein interior without the need for any specialized structural adaptations save for those required to stabilize the protein. Solvent-accessible surface area and depth of burial calculations indicated that all but one Lys were completely inaccessible to water. Seven Lys side chains packed into predominantly hydrophobic regions; five had Nζ atoms within 3.5 Å of one or more polar groups or crystallographic water molecules. Depth of burial or microenvironment polarity as observed in the crystal structures did not correlate with measured shifts in pK_a. ^{1}H-^{15}N HSQC NMR spectroscopy studies revealed global unfolding coupled to the ionization of two of the 25 Lys residues. Three others showed partial or local unfolding, five demonstrated localized changes in structure and dynamics, and 15 exhibited no detectable consequences. Backbone amide resonances were most similar between reference protein and the Lys-containing variant when the Lys side chains were buried shallowly or in a loop region. In the least stable variants the ionization of the internal Lys residues led to localized increase in conformational fluctuations or to conformational reorganization. These data will be useful to test the ability of structure-based pK_a calculations to account for structural rearrangements or increased dynamics in response to ionization of internal groups.

2901-Pos Board B56

Supercharging De Novo Designed Heme-Binding Proteins has Significant Effects on Solubility and Redox Characteristics

Cooper A. French.
City College of New York, New York, NY, USA.

Supercharging the external residues of natural proteins has been shown to increase solubility and thermal stability while retaining protein function. In this work we elucidate the effects of supercharging exterior residues on the solubility, folding propensities, and ligand binding properties of mutants of the de novo designed heme-binding protein HHHH.

2902-Pos Board B57

Identification and Characterization of Buried Ionizable Groups in Proteins

Joshua A. Riback, Carolyn A. Fitch, Bertrand García-Moreno E.
Johns Hopkins University, Baltimore, MD, USA.

Ionizable groups buried in the hydrophobic interior of proteins play many essential biological roles. their physical properties are not well understood. Owing to the inherent differences in the dielectric properties of proteins and of water the properties of internal and surface ionizable groups can be very different. In a previous systematic study from this laboratory Asp, Glu, Lys and Arg residues were buried at 25 internal locations in staphylococcal nuclease. Most of these internal residues titrate with highly anomalous pK_a values. Many crystal structures of these variants show that the majority of these buried ionizable residues are buried completely and in very diverse microenvironments. A survey of the properties and microenvironments of naturally occurring internal ionizable groups in proteins was undertaken with a large set of proteins from the Protein Data Bank to compare against the internal ionizable groups buried artificially in nuclease. Internal ionizable groups were identified based on accessible surface area and depth of burial using a self-consistent definition of the interface between protein and bulk water. For purposes of this survey, internal ionizable groups were characterized primarily in terms of accessible surface area, depth of burial, polarity and hydrophobicity of their microenvironments, and contact with other surface or internal ionizable groups and with crystallographic water molecules. MD simulations were also performed on a small set of proteins to examine the robustness of static structures for purposes of determination of depth of burial.

2903-Pos Board B58

Is Protein Destabilization a Widespread Factor in Genetic Disease?

Juan R. Diaz[1], Nikolay Dokholyan[2], Rachel Redler[2].
[1]University of Puerto Rico, Rio Piedras, San Juan, PR, USA, [2]University of North Carolina, Chapel Hill, NC, USA.

Protein destabilization is a known contributing factor in some genetic diseases, although the destabilizing effects of mutations as a widespread factor in genetic disease has not yet been clearly determined. In this study, a 20-protein survey was conducted in order to determine whether protein destabilization is associated with amino-acid substitutions in known disease-associated amino-acid positions. A Monte Carlo-based protein stability estimator named Eris was used to calculate the ΔΔG values for these substitutions in each protein. The ΔΔG values of every possible missense mutation for each protein were calculated and classified based on whether they occur at positions where disease-associated substitutions have been identified. For most of the proteins studied, the distributions of calculated ΔΔG for known disease-associated substitution positions displayed a trend toward more positive ΔΔG values. This indicates a tendency of substitutions in the known disease amino-acid positions to be a cause of protein destabilization. This trend in the ΔΔG shift is an indicator that protein destabilization is likely to be a general contributing factor in a wide range of genetic diseases, although the specific effect these destabilizing mutations have on each of the proteins studied here remains to be determined experimentally.

2904-Pos Board B59

Copper Ion Induces N-Terminal β Structure in Amyloid β Protein in Reverse Micelles

Hiroaki Komatsu, Paul H. Axelsen.
University of Pennsylvania, Philadelphia, PA, USA.

A hallmark of Alzheimer's disease is the accumulation of insoluble fibrils in the brain composed of amyloid beta (Aβ) proteins with parallel in-register cross-β-sheet structure. It has been suggested that the aggregation of monomeric Aβs into fibrils is promoted by "seeds" that form within compartments of the brain that have limited solvent due to macromolecular crowding. In our previous report, a crowded macromolecular environment was mimicked by encapsulating monomers of Aβs into reverse micelles (RMs), formed from AOT, sodium bis(2-ethylhexyl) sulfosuccinate. We demonstrated that the crowded environment of the RMs induced the formation of β-strand seed structures for nucleating amyloid fibril formation.[1] Copper is a redox-active metal with many important biological roles, and the dysregulation of copper is intimately involved in the pathogenesis of Alzheimer's disease. In the present study, we have encapsulated a short 1-16 residue form of the Aβs as well as the 40- and 42-residues forms into RMs and examined them in the presence of copper ion with transmission Fourier-transform infrared spectroscopy. We propose a mechanism for the effect of copper ion on the formation of amyloid fibril seeds, involving the formation of a coordination complex with the His residues at position 13 and 14 on a pair of Aβ molecules and inducing in-register alignment of the N-terminal residues.

Ref. (1) Yeung, P. S.-W., and Axelsen, P. H. (2012) *J. Am. Chem. Soc.*, *134*, 6061-6063.

2905-Pos Board B60

Overcoming the Cellular Crowding Effect: VlsE-FRET is Destabilized Inside Cells

Irisbel Guzman Sanchez, Martin Gruebele.
University of Illinois at Urbana-Champaign, Urbana, IL, USA.

The understanding of protein folding dynamics inside cells is crucial for the development of new therapies against protein folding and aggregation diseases. In addition, many processes inside cells such as gene expression, association and dissociation processes, as well as protein degradation are controlled by protein stability and kinetics. Fluorescence Relaxation Imaging (FReI) instrument combines temperature jump, FRET and cell imaging to study dynamic processes inside cells from the microseconds to the seconds time scales. using FReI, we have carried out experiment to calculate the melting temperature (Tm) and relaxation time (τ) of a constructed FRET pair protein of the Variable Lyme Disease Surface Antigen E (VlsE). VlsE-FRET has an in vitro Tm= 38 ± 1 °C and τ = 0.5 ± 0.1 s. In contrast, VlsE-FRET has an in cell Tm = 35 ± 1 °C and τ = 2 ± 2 s. Also, the in vitro data support a single exponential unfolding process with β = 1.0 ± 0.1, but the in cell data does not with β = 0.8 ± 0.2. Interestingly, VlsE-FRET has two distinct populations in cell with melting temperatures of 33 ± 1 °C and 36 ± 1 °C. These data support that some proteins can overcome the crowding effect inside cells due to the contribution of chemical/electrostatic forces. VlsE has different regions that undergo antigenic variation in order to evade the immune system of the host. Probably, the VlsE-FRET destabilization in cell is part of the trigger mechanism for the extensive genetic and antigenic variation that allows VlsE to evolve.

2906-Pos Board B61

Quantitative Characterization of the Interaction between Sucrose and Native Proteins via Static Light Scattering

Di Wu, Allen P. Minton.
NIDDK,NIH, Bethesda, MD, USA.

Compatible osmolytes, such as sucrose, are small organic compounds that are synthesized in cells to protect proteins and other macromolecules from the effect of osmotic stress. Our present work is to quantitatively characterize the interactions between native globular proteins and sucrose, which has long been known to stabilize proteins against denaturation by heat or chaotropes, and has been shown to attenuate structural fluctuations in native proteins. The composition-dependent static light scattering of binary mixtures of each of four dilute globular proteins–bovine serum albumin (BSA), ovalbumin, ovomucoid, soybean trypsin inhibitor (STI), and sucrose were measured over a broad range of sucrose concentrations. A conventional analysis of the dependence of excess light scattering of a single macrosolute in a continuum solvent yields unphysical results. Then the analysis is based upon multicomponent scattering theory, treating sucrose as well as protein as a scattering species, and is shown to yield reliable information about the nature and magnitude of