2611-Pos Board B41
Interactions between Pairs of Charges Buried in the Hydrophobic Interior of a Protein are Unexpectedly Weak
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Internal ion pairs buried in the hydrophobic protein interior are essential for many important biochemical processes, including H+ transport, e- transfer, ion homeostasis, and catalysis. Despite the importance of these pairs, their properties remain poorly understood. It has been suggested that, for some systems, medium or long-range Coulomb interactions between buried groups could play a role in determining biological function. In principle, this should only be possible if the protein interior behaves like a medium of low dielectric constant, as assumed in most electrostatics models. These motifs are then of special interest not only for their functional roles, but also as probes to examine the balance between Coulomb and hydration energies experienced by buried charges. As the distance between the internal charges increases, the balance of these energies will disfavor burying the groups in the charged state, eliminating any Coulomb interaction between them. To examine the balance between Coulomb and dehydration energies in the protein interior we engineered a series of double variants in a highly stable variant of staphylococcal nuclease. Each variant included an internal histidine paired with either an aspartate or glutamate buried at various internal positions throughout the protein interior. This set of proteins used to probe the distance dependence of potential Coulomb interactions. No significant electrostatic coupling was observed for pairs that interacted through medium- or long-range Coulomb interactions. When the distance between the groups was short, favorable coupling energies were only observed when the side chains of the pair could achieve a geometry favorable for a H-bond; spatial proximity alone was insufficient to create favorable Coulomb interactions. Simple electrostatics models that describe ionized states as point charges interacting through space are unlikely to be able to reproduce these data.

2612-Pos Board B42
A Reason for Long Tales
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Transcription Activator-Like Effectors (TALEs) are bacterial virulence factors containing a domain of repeats that recognize specific DNA sequences and reprogram transcription activation of invaded plant cells. TALE genes encode 5 to 30 repeats with average pairwise repeat identities greater than 91%. Most variability arises from only two positions termed Repeat Variable Diresidues (RVDs), which confer DNA binding specificity. Crystal structures of free and DNA-bound TALEs (Deng et al. *Science* 2012) show a large conformational change upon DNA binding. Thus, DNA binding is likely coupled to the free energy of tertiary structural change between the TALE repeats. We are interested in quantifying this relationship and relating it to folding cooperativity using nearest-neighbor (''Ising'') models.
To investigate the length dependence of folding, we created a set of consensus TALE constructs of varying length. Solubilizing N- and C-terminal caps are needed to favor monomeric protein in solution as detected by sedimentation velocity analytical ultracentrifugation. Capped consensus TALE repeat constructs have alpha-helical secondary structure as measured by farUV CD. Urea-induced unfolding transitions of TALE repeat arrays were measured and show cooperative unfolding transitions as well as increases in stability with length. These data are well-fitted by an Ising model, which separates the contributions of intrinsic and interfacial free energies. TALE repeats have an unfavorable intrinsic folding free energy of 5.3 kcal/mol and a favorable interfacial free energy of −6.8 kcal/mol. Using these values, the length dependence of TALE stability can be modeled, and shows TALEs under 5 repeats to be unfolded. Using the Ising parameters, we find that partially folded states with a single repeat unfolded are energetically accessible. Population of these partially folded states may be important for DNA binding.

2613-Pos Board B43
Effect of Gamma Radiation on the Structural and Functional Integrity of IgG
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Plasma-originated commercial intravenous immunoglobulin, which is used for a variety of clinical purposes, has been studied to determine the effect of virus-inactivating doses of gamma irradiation on the structural and functional characteristics of the protein. A detailed analysis has been performed in response to a concern that the use of conventional gamma irradiation may damage biologically active proteins. The results demonstrate that although gamma irradiation of the IgG may have some impact on protein structure, the damage can be reduced or even prevented by appropriate irradiation conditions. At the virucidal dose of gamma irradiation (50 kGy) and a temperature of −80oC, the integrity of the polypeptide chain of immunoglobulin and the secondary structure of IgG can be completely protected, while conformational changes in tertiary structure are significantly minimized to a level that preserves functional activity. The irradiated IgG retains specific antigen-binding properties and Fc-binding activity, indicating that the conformational integrity of the most important structural regions is not affected by gamma-irradiation. These results present strong evidence that gamma irradiation treatment can be effectively implemented for inactivation of pathogens in IgG solutions that are used for intravenous injection.

2614-Pos Board B44
Photoacoustic Calorimetry Studies of Ferric Cytochrome-C Folding using an No Photo-Trigger
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In this study, NO is utilized as a photo-trigger together with photoacoustic calorimetry to probe the kinetics, enthalpy and molar volume changes associated with the earliest folding events in ferric Cytochrome-c (Cc3+). The ferric heme protein was examined under different denaturing conditions including guanidine hydrochloride (2.8M GdnHCl) and Sodium dodecyl sulfate (SDS 0.4mM) (both in 50 mM Hepes buffer, pH ~7.5) along with NO resulting in the disruption of the axial heme Methionine-80 heme bond, triggering the partial unfolding of the complex. Under these conditions photo-dissociation of NO leaves the protein in a conformational state that favors refolding of the protein. The PAC data reveals three kinetic phases taking place subsequent to photolysis regardless of the denaturant environment. Specifically, in the presence of 2.8 M GdnHCl photolysis gave rise to kinetic events with lifetimes of <20 ns, ~860 ns, and ~6 μs that were associated with $\Delta H/\Delta V = -25$ kcal mol-1/-9 mL mol-1, 26 kcal mol-1/15mL mol-1, and 9 kcal mol-1/-26 mL mol-1, respectively. In the presence of 0.4 mM SDS, kinetic events were observed with lifetimes of <20 ns, ~640 ns, and ~8 μs with corresponding $\Delta H/\Delta V$ of −11 kcal mol-1/-1 mL mol-1, 5 kcal mol-1/3 mL mol-1, and 30 kcal mol-1/-9 mL mol-1, respectively. The data suggests a uniform mechanism for the early folding events occurring in the folding of Cc3+ complex subsequent to NO photo-dissociation which are attributed to NO dissociation from the heme, followed by reorganization of the distal pocket (i.e hydrogen bond formation/breakage, NO solvation, etc.) and potentially a intermolecular binding of Methionine (80 or 65) or Histidine (23 or 33) to the heme iron upon folding.

2615-Pos Board B45
Simulation of Pressure-Induced and Temperature-Induced Denaturation of Phosphoglycerate Kinase
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Phosphoglycerate kinase (PGK) is a 415-residue protein composed of two domains of almost equal size. Under the heat-induced denaturation, folding and unfolding of PGK is a two-state process. However, folding and unfolding of PGK under pressure-induced denaturation,is a three-state process. How the temperature and pressure affect the denaturation of PGK is still unknown. We aim to provide a molecular explanation for thermal and pressure denaturation by using coarse-grained molecular simulations where the interactions of residues experience expulsion of water molecules.

2616-Pos Board B46
Intermolecular Interactions in Highly Concentrated Protein Solutions Upon Compression and the Role of the Solvent
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The influence of high hydrostatic pressure on the structure and protein-protein interaction potential of highly concentrated lysozyme solutions up to about 370 mg mL^{-1} was studied and analyzed using small-angle X-ray scattering (SAXS) in combination with a liquid-state theoretical approach. In the concentration region below 200 mg mL^{-1}, the interaction parameters of lysozyme solutions are affected by pressure in a nonlinear way, which is probably due to significant changes in the structural properties of bulk water, i.e., due to a solvent-mediated effect. Conversely, for higher concentrated protein solutions, where