while the entropic change can even be unfavorable. To elucidate the molecular basis of these depletion interactions, we use simulations and analytic theory. Monte-Carlo simulations follow the association of two rod "macromolecules" in binary Lennard-Jones solutions. By dissecting the association free energy change into the respective thermodynamic components, we find different cosolutes induce stabilization through different thermodynamic driving mechanisms. Even for these simple liquids, considering intermolecular interactions beyond hard-cores can result in depletion forces that are completely enthalpic. We discuss how this newly resolved mechanism originates from intermolecular interactions and solvent restructuring. Finally, a mean-field theoretical model based on regular solution theories complements the simulation analysis.

258-Pos Board B38

Protein-Protein Interactions Affect Native State Stability in Crowded Environments

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The dense, heterogeneous cellular environment is known to affect protein stability through interactions with other biomacromolecules. The effect of excluded volume due to these biomolecules, also known as crowding agents, on a protein of interest, or test protein, has long been known to increase the stability of a test protein. Recently, it has been recognized that attractive protein-crowder interactions play an important role. These interactions affect protein stability and can destabilize the test protein. However, most computational work investigating the role of attractive interactions has used spherical crowding agents and has neglected the specific roles of crowding agent hydrophobicity and hydrogen bonding. Here, we use multicanonical molecular dynamics and a coarse-grained protein model to study the folding thermodynamics of a small helical test protein in the presence of crowding agents that are themselves proteins. Our results show that the stability of the test protein depends on the hydrophobicity of the crowding agents. For low values of crowding agent hydrophobicity, the excluded volume effect is dominant and the test protein is stabilized relative to the dilute solution. For intermediate values of the crowding agent hydrophobicity, the test protein is destabilized by favorable side chain-side chain interactions stabilizing the unfolded states. For high values of the crowding agent hydrophobicity, the native state is stabilized by the strong intermolecular attractions causing the formation of a packed structure that increases the stability of the test protein through favorable side chain-side chain interactions. In addition, increasing crowding agent hydrophobicity increases the "foldability" of the test protein and alters the potential energy landscape by simultaneously deepening the basins corresponding to the folded and unfolded states and increasing the energy barrier between them.

259-Pos Board B39

Thermally Induced Structural Changes in an Armadillo Repeat Protein Suggest a Novel Thermosensor Mechanism in a Molecular Chaperone

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Molecular chaperones are commonly identified by their ability to suppress heat induced protein aggregation. The muscle specific molecular chaperone UNC-45B is known to be critical in folding myosin and is trafficked to the sarcomeres A-band during thermal stress. Here, we identify thermally dependent structural changes in the UCS chaperone domain of UNC-45B that occur in the physiologically relevant heat shock range. We show that distinct changes to the armadillo repeat protein topology result in exposure of hydrophobic patches, and increased flexibility of the molecule. These rearrangements suggest a novel thermosensor within the chaperone domain of UNC-45B. We propose that these changes may be central to the molecular mechanism of UNC-45B chaperone activity. They may function to suppress aggregation under stress by allowing binding to a wide variety of aggregation prone loops on its client.
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260-Pos Board B40

CCT5: A Model Protein Folding Machine

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The chaperonin TRiC is a 16-subunit protein complex, composed of two rings, with 8 individual subunits each (CCT1-8), that functions as a protein folding machine in eukaryotic cells. TRiC is required for the folding of approximately 10% of the human proteome, including a number of crucial cellular proteins, most notably actin and tubulin. While TRiC-mediated folding is critical for many cellular processes, its underlying mechanism is poorly understood. Mechanistic and structural elucidation of TRiC folding activity is hampered in large part by the sheer size and subunit heterogeneity of the chaperonin, and therefore most studies have focused on the functionally related but structurally less-complex chaperonins, including the thermosome and Mm-Cpn, as surrogates for TRiC architecture and function. Recently, Sergeeva et al demonstrated that human CCT4 and CCT5 subunits expressed individually in E. coli form homo-oligimeric chaperonins capable of folding native substrates. Here, we have used purified CCT5 homo-oligomer to investigate the *in vitro* chaperonin-dependent folding of human gD-crystallin and beta-actin. Modifications to the CCT5 primary sequence, including changes to the N and C termini, resulted in changes in folding efficiency, substrate recognition and suppression of aggregation. Structural, biochemical and computational studies on these modifications will provide insight into the mechanism of the TRiC folding machine.

261-Pos Board B41

How Do Group Ii Chaperonins Distinguish their Partially Folded Substrates from the Native States?

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The features in partially folded intermediates that allow the group II chaperonins to distinguish partially folded from native states remain unclear. The Archaeal group II chaperonin Mm-Cpn assists the in vitro refolding of the well-characterized β-sheet lens protein human γD-Crystallin (HγD-Crys). The buried cores of this Greek key conformation as well as the domain interface includes a variety of side chains which might be exposed in partially folded intermediates. We sought to assess whether particular features buried in the native state, and thus absent from the native protein surface, might be serving as recognition signals. The features tested were a) paired aromatic side chains; b) side chains in the interface between the duplicated domains of HγD-Crys, and c) side chains in the buried core which result in congenital cataract when substituted. We tested the Mm-Cpn suppression of aggregation of HγD-Crys mutants of these residues, during refolding after dilution out of denaturant. Mm-Cpn was capable of suppressing the off-pathway aggregation of the three classes of mutant proteins indicating that the buried residues were not recognition signals. In fact Mm-Cpn refolded most mutant HγD-Crys to levels twice that of WT HγD-Crys. This presumably represents the increased population or longer lifetimes of the partially folded intermediates of the destabilized mutant proteins. The results suggest that Mm-Cpn does not recognize paired aromatic residues, exposed domain interface, or destabilized core - but rather recognizes other features of the partially folded β-sheet, such as an exposed backbone, that are absent or inaccessible in the native state off HγD-Crys. Supported by NIH EY15834 and EY016525.

262-Pos Board B42

Real Time NMR Folding Study of the Human Gamma D Crystallin in the Presence of Metal Ions

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Cataract is a leading cause of blindness worldwide, and is due to aggregation of eye lens proteins. Age, UV radiation, oxidation, metal ions, deamidation, and truncations may be cause covalent protein damage that induces aggregation. In particular, metal ions have been implicated as a potential etiological agent for cataract. Human γD (HγD) crystallin is one of the most abundant crystallins in the lens nucleus and its non-amyloid aggregation is associated with cataracts. HγD is a highly stable protein with two homologous domains (N-terminal and C-terminal), each containing two Greek key motifs forming a β-sandwich of eight intercalated β-strands. Domain swapping from a perturbed conformation has been proposed as the mechanism of aggregation. Micromolar concentrations of Cu(II) and Zn(II) ions specifically induces the aggregation of this stable protein. In this work, NMR (H-N-HSQC) experiments were performed to study the interaction of Cu(II) and Zn(II) ions with HγD and identify possible specific metal binding sites in the protein. Moreover, we have characterized the unfolding of HγD in the presence of these ions with atomic resolution using real-time NMR spectroscopy. These studies provide further insight into the metal ion coordination properties of HγD, and the role of Cu(II) and Zn(II) in its non-amyloid aggregation. This research has been supported by: MIT-Seed Funds and NIH EY015834.