2369-Pos Board B61
Structural and Functional Interactions between Hsp90 and the Cholera Toxin A1 Subunit
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Cholera toxin (CT) moves from the cell surface to the endoplasmic reticulum (ER) where the catalytic CTA1 subunit separates from the holotoxin and unfolds due to its intrinsic instability. Unfolded CTA1 then moves through an ER translocon pore to reach its cytosolic target. Due to the instability of CTA1, it must be actively refolded in the cytosol to achieve the proper conformation for modification of its G protein target. The cytosolic heat shock protein Hsp90 is involved with the ER-to-cytosol translocation of CTA1, yet the mechanistic role of Hsp90 in CTA1 translocation remains unknown. Potential post-translocation roles for Hsp90 in modulating the activity of cytosolic CTA1 are also unknown. Here, we show by isotope-edited Fourier transform infrared spectroscopy that Hsp90 induces a gain-of-function in disordered CTA1 at physiological temperature. Only the ATP-bound form of Hsp90 interacts with disordered CTA1, and its refolding of CTA1 is dependent upon ATP hydrolysis. Surface plasmon resonance experiments found that Hsp90 does not release CTA1, even after ATP hydrolysis and the return of CTA1 to a folded conformation. The interaction with Hsp90 allowed disordered CTA1 to attain an active state and did not prevent further stimulation of toxin activity by ADP-ribosylation factor 6, a host cofactor for CTA1. Our studies suggest CTA1 translocation involves a ratchet mechanism which couples the Hsp90-mediated refolding of CTA1 with CTA1 extraction from the ER. Continued association of Hsp90 with refolded CTA1 allows the cytosolic toxin to attain an active conformation but does not protect it from proteasomal degradation.

2370-Pos Board B62
Context-Dependent Folding: Sequence-Encoded Strategies for Stabilizing a Protein Subdomain in Isolation
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Many proteins are made up of smaller structural units called subdomains. Dissecting proteins into these small units is important for protein engineering and design. In part, the ability to create novel fusion proteins relies on a subdomain's ability to fold autonomously. Subdomains are usually identified as regions that have high contact density, in other words, they have more residue-residue contacts within a specified region than they do with other areas of the protein. Due to their high contact density, subdomains are often assumed to be able to fold in isolation. We evaluate this hypothesis, by examining the foldability of a subdomain from T4 Lysozyme (T4L). In full-length T4L, the N-terminal subdomain has high contact density yet its sequence does not fold in isolation. This suggests that high contact density is not always enough for subdomain folding. To explore this, we have extended the boundary to the complete the sequence of a helix found in the full-length protein that has no contacts with the rest of the subdomain. We find that even without increasing predicted contacts, the sequence is able to fold autonomously. To determine if the sequence of the helix is important, we created a fusion protein that appends an alanine-based helix, which is able to fold in isolation, to the original N-terminal subdomain sequence. Surprisingly, we find that this structural mimicry allows the sequence to fold. In total, our findings point to the importance of considering not just considering the contact density when choosing boundaries of subdomains. We also demonstrate strategies to stabilize the folding of otherwise unfolded subdomain sequences.

2371-Pos Board B63
Denaturant Probes Quantify the Preferential Burial of Amide Surface in Forming the Key Transition State and Early Intermediates in Protein Folding
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Protein-folding has been extensively studied, but many questions remain regarding the mechanism. Characterizing early-unstable-intermediates and the high-free-energy transition-state (TS) will help answer some of these. Recently we used effects of denaturants (urea, GuHCl) and temperature on folding and unfolding rate constants and overall-equilibrium constant, as probes of surface-area changes in protein-folding. We interpreted denaturant kinetic m-values and activation heat-capacity changes for 13 proteins to determine amounts of hydrocarbon and amide surface buried in folding to and from TS, and for complete folding (Guinn et al, PNAS '13). Predicted accessible-surface-area (ASA) changes for complete folding agree in most cases with structurally determined values. We find that TS is advanced (50-90% of overall surface-burial) and that surface buried is disproportionately amide, demonstrating extensive formation of secondary-structure in early-intermediates. Models of possible pre-TS intermediates with all elements of the native secondary-structure for several of these proteins, created and analyzed using PyMol, Coot, and Surfracer, bury less amide and hydrocarbon surface than predicted for TS. Extensions of this approach to deduce models for transition-states and early-intermediates in folding of other proteins in the data set are in progress to test the proposal that TS generally has both native secondary-structure and sufficient organization of other regions of the backbone to nucleate subsequent (post-TS) formation of tertiary interactions. To facilitate these analyses, we have developed a program (Calculate_Unfolded_ASA.py) to predict ASA of fully-extended unfolded state, shown to be a good model for denaturant-induced unfolding. The approach developed here provides proof of concept for the use of denaturants and other solutes as probes of amount and composition of the surface buried in coupled folding and other large conformational changes in TS and intermediates in protein processes.

2372-Pos Board B64
Alteration of the Cytochrome C Folding Pathway in Solution and Within Sol-Gel Glasses by Addition of Hofmeister Salts
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The ferric cytochrome *c* (un)folding mechanism in the presence of steric constraints and salts is examined. Unfolding was initiated both thermally and with chemical denaturants. Sol-gel encapsulation was used to constrain the protein to a volume close to the "molten globule state." Hofmeister salts were added singly and in pairs to alter protein stability. UV/VIS absorption spectroscopy and a basis spectra fitting analysis were used to determine the populations of each protein conformation along the folding pathway. These species can be differentiated by their axial heme ligands. Four species exist in solution: the native HM state (His18/Met80), the partially folded HW (His18/water) and HH (His18/His33) intermediates, and the 5C (water) unfolded state. An additional unfolded state found only within the sol-gel contains an unligated four-coordinate heme sequestered from aqueous solution. Solution results indicate that the thermal and chemical denaturization pathways are not the same, but that both involve significant backbone rearrangement. Further, while every species observed in solution is also observed in the sol-gel, their relative populations are different, indicating that backbone motions are hindered by encapsulation within the sol-gel matrix. The ranking of salts in the Hofmeister series (from stabilizing to destabilizing protein structure) was found to persist in the sol-gel samples, and in some cases a larger effect on protein stability was found in the sol-gels. Additionally, it was found that addition of multiple salts changed the protein's stability in an additive manner. Both the (un)folding kinetics and the accessible conformations were found to depend on the identity of ions present. These results are discussed in terms of the hydrophobic effect and an altered water structure within the confines of the gel pores.

2373-Pos Board B65
Protein-Folding Studies using Hybrid TIRF SmFRET-Magnetic Tweezers
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Optical tweezers are a useful research tool for applying forces to single proteins and measuring the resulting end-to-end extension increases that accompany unfolding and other force-induced conformational changes. They do, however, have a few limitations: they can only observe changes in extension along the axis of force application; the accompanying changes in extension must be on the order of at least a few nanometers to be resolved above the noise of the instrument; and particularly for multi-domain and polyproteins, an observed change in extension cannot always be localized to a specific region of sequence. Our experimental setup overcomes these limitations by measuring distance changes using single-molecule Förster resonance energy transfer (smFRET) produced from a total internal reflection fluorescence (TIRF) microscope incorporating magnetic tweezers. Individual protein molecules are conjugated to