residues important for peptide specificity and affinity to the PDZ domain. PST's of all eight solved crystal structures of T-cell lymphoma invasion and metastasis 1 (Tiam1) PDZ domains are mapped using knob-socket analyses. The comparison among the PST maps of all Tiam1 PDZ-ligand structures reveal determinants of peptide affinity and specificity. The PST maps of PDZ complexes allow categorization of the important interactions between ligand and the PDZ domain binding pockets. Interestingly, all the ligands bind as a 6th sheet to the PDZ's 5-strand β-sheet. The extension of the β-sheet provides additional knobs and sockets to pack with PDZ's second α-helix H2. In agreement with previous experimental analyses, the peptide P_0 and P_{-2} positions directly interact with the PDZ domain, but the binding is more complex. Comparison between different PDZ domains and their respective peptides show that the P_0 position packs primarily against the first coil, and the P_{-2} position packs into H2. Therefore, the peptide interaction with a PDZ domain can be separated into 3 distinct interactions: 1) extend the β-sheet, 2) interactions of this extended β-sheet with the α-helix H2 (specifically P_{-2}), and 3) P_0 packing into the first coil. This set of rules provides a simple yet discrete guide to designing better binding peptides to a PDZ domain. Moreover, this result demonstrates the utility of the Knob-Socket construct in the analysis and design of peptide ligand binding with a protein domain.

Posters: Protein Stability, Folding, and Chaperones II

2033-Pos Board B49

Quantitative Prediction of Bacterial Fitness from a Protein's Energy Landscape

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The biophysical properties of proteins provide a link to how molecular mutations might exert their effects on cellular fitness. A good candidate for examining this relationship between genotype and phenotype is TEM beta-lactamase, a bacterial enzyme that confers antibiotic resistance by hydrolyzing penicillin-like antibiotics. It is known that four missense mutations in TEM result in increased activity against third-generation cephalosporins, such as cefotaxime. These mutations affect both the catalytic efficiency and the stability of TEM, but neither of these properties alone are enough to predict bacterial fitness. Circular dichroism (CD) data suggest that TEM folding includes at least one intermediate, and western blots provide evidence that changes in the stability of this intermediate ensemble relative to the native ensemble correlates with changes in cellular protein abundance. This is further supported by pulse proteolysis experiments indicating that the intermediate ensemble is susceptible to degradation. We hypothesize that intermediate stability can be quantitatively related to protein abundance, thus allowing us to create a mathematical model to predict bacterial fitness, as measured by minimal inhibitory concentration (MIC), from the activity and stability of a TEM mutant.

2034-Pos Board B50

Prediction of New Stabilizing Mutations Based on Mechanistic Insights from Markov State Models

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Protein stabilization is fundamental to enzyme function and evolution, yet understanding the determinants of a protein's stability remains a challenge. This is largely due to a shortage of atomically-detailed models for the ensemble of relevant protein conformations and their relative populations. For example, the M182T substitution in TEM β-lactamase, an enzyme that confers antibiotic resistance to bacteria, is stabilizing but the precise mechanism remains unclear. Here, we employ Markov state models (MSMs) to uncover how M182T shifts the distribution of different structures that TEM adopts. We find that M182T stabilizes a helix that is a key component of a domain interface. We then predict the effects of other mutations, including a novel stabilizing mutation, and experimentally test our predictions using a combination of stability measurements, crystallography, NMR, and *in vivo* measurements of bacterial fitness. We expect our insights and methodology to provide a valuable foundation for protein design.

2035-Pos Board B51

Probing Protein Folding Landscape by Using Combined Force Spectroscopy and Molecular Dynamics Simulations

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Most proteins, even those with complex folding trajectories and rough energy landscapes, successfully navigate the funneled folding landscape to achieve the correct native conformations. However, some proteins misfold, sometimes resulting in some neurodegenerative diseases and cancers. Such misfolding is thought to occur via partially folded high-energy structures accessible from the native state or during the folding process. Structural and energetic characterization of such high-energy species is important but experimentally challenging due to their rare and transient nature under native conditions. Single-molecule force spectroscopy allows investigation of the force dependent movement of the transition state ensemble and detection of high-energy intermediates, which may be populated at high force. Molecular dynamics simulations allow atomistic characterization of structures and multiple folding pathways on the energy landscape. Here, we probe important protein sub-states and folding pathways of the second PDZ domain of PTP-BL by using a combination of single molecule optical tweezers experiments and molecular dynamics simulations.

2036-Pos Board B52

The Structural Basis of Thermostability in an Engineered Variant of the Engrailed Homeodomain

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The Engrailed Homeodomain (EnHD) is a three-helix bundle transcription factor with an ultra-fast folding rate, making it an attractive choice for computational and experimental thermostability studies. The Mayo group designed an engineered variant of EnHD called UVF, which both folded successfully and had a melting temperature >99°C, much higher than the melting temperature of 52°C for EnHD. We constructed hybrid protein models to test several hypotheses. First, we hypothesize that the buried residues of UVF contribute more to its thermostability than its surface residues. To test this hypothesis, we constructed two hybrid models combining the sets of buried and surface residues of EnHD and UVF. We also hypothesize that the lack of hydrogen bonds between buried and surface residues of UVF enable these regions of the protein to move independently and potentially increase thermostability. To test this, we created models of EnHD without these hydrogen bonds and compared its stability with UVF. Finally, engineering proteins for stability often comes at the expense of function, and the stability-focused design of UVF removed EnHD's DNA-binding residues. We constructed various models of UVF reintroducing the DNA-binding residues of EnHD to find a suitable model. Here, we report all-atom, explicit-solvent molecular dynamics simulations of EnHD, UVF, and our hybrid models at 25°C and 100°C and assess the proteins' stabilities.

2037-Pos Board B53

Chemical Chaperone Activity of NAD^+ in Protein Folding

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Folding of most proteins occurs in the complex and crowded cellular environment, and the efficient folding in this environment is critical for the fitness of the organism. Still, our understanding of how the complex chemical environment affects protein folding in cells is largely limited. Recently, we discovered that NAD^+ has a significant effect on the folding of E. coli glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Originally, we found from our proteomics screen that GAPDH is apparently destabilized in the presence of ATP. A follow-up biophysical characterization demonstrated that the apparent destabilization results from selective stabilization of a dimeric intermediate of GAPDH, not the native tetramer, by ATP. Moreover, GAPDH folds significantly faster in the presence of a physiological concentration of not only ATP but also NAD^+, which is a cofactor for GAPDH. Investigation of the effects of the structural fragments of NAD^+ on the folding of GAPDH revealed that NAD^+ facilitates the folding of GAPDH through the interaction between its adenosine moiety and a partially folded intermediate of GAPDH. A mutagenesis study of the binding pocket elucidated that the adenine-binding subsite of the NAD^+-binding pocket is responsible for the effect of NAD^+ on GAPDH folding. Based on the results, we propose a novel mechanism by which NAD^+ functions as a chemical chaperone in protein folding.