three initial conformational states from X-ray crystal structures is performed. The modes with greatest variance qualitatively agree with the slowest ENM modes, indicating a PCA analysis on the FIRST/FRODA ensemble of conformations produce consistent predictions. However, in order to determine a statistically significant interpretation of the PCA modes, a negative control was performed that replaces a biologically relevant displacement vector with a random displacement vector (decoy). Surprisingly, the decoys produce similar high correlation to the same few greatest variance PCA modes. Our results put serious question on the soundness of the common interpretation of high overlap between a conformational displacement vector and a mode. Further analysis shows that biologically relevant motions found in myosin V require many modes. As such, inferring specific mechanisms based on a single mode is generally difficult to impossible. To facilitate a quantitative justification in identifying a biologically relevant pathway, the protein motion is decomposed further by projecting PCA modes into structural domains, and correlated motions are identified via mode-coupling. We present a critical comparison to recent predictions for the conformational pathways in myosin V, where statistical significance is monitored. This work is supported by NIH grant 1R21HL093531.

148-Pos

Modeling Drkn Sh3 Domain Using Sequence Specific Wormlike Chain Model

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Though the wormlike chain (WLC) model has successfully described the statistical properties of fully denatured polypeptides, the lack of sequence details and attractive forces made it less successful in describing unfolded states in folding conditions. To cover the limitation while keeping the model's efficient feature, we have developed a sequence specific wormlike chain model. Computationally, secondary structure constraints from the secondary chemical shift measurements of drkN are integrated into the construction of each wormlike chain. Then the probability distribution is reweighted to bias compact conformations in which residues of similar hydrophobicity are located near each other. This model has been tested on two mutants (C2 and C60) of the Drosophila drk N-terminal (drkN) SH3 domain. drkN exists in approximately 1:1 equilibrium between folded and unfolded state in water, which gives us the opportunity to monitor the contact quenching of tryptophan 36 by either cysteine under all denaturing conditions. The experimental results exhibit a relatively slow kinetics, which implies slow intramolecular diffusion. The reweighted pairwise distance distributions are also compared to the Paramagnetic Relaxation Enhancement (PRE) data for drkN.

149-Pos

Comparison of λ Cro Solution Ensemble To Its Open and Closed Crystal Forms

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λ Cro is a small dimeric bacteriophage transcription factor consisting of multiple crystal structures. Its X-ray images range from a more closed DNA-free conformation relative to an open form when bound to DNA. Considered a flexible ''dumbbell'' with a pliable β-sheet region connecting two more rigid α-helical domains, the subunits of λ Cro may undergo a relative rotation. This suggests a possible induced fit DNA-binding model (1). In solution the protein likely accesses a range of conformations encompassing its DNA-bound open and DNA-free closed forms. However, the snap-shots from X-ray crystallography capture just one of these structures, making it difficult to recompose the λ Cro solution ensemble. Moreover, the crystal may have randomly selected a conformer un-representative of its dominant physiological form. Here we show the transition between the open and closed forms of λ Cro to occur with no large energy barrier in solution. We have performed 50 ns all-atom Molecular Dynamics (MD) simulations starting from both structures in explicit solvent using the Amber10 force field. The trajectory started from the closed form undergoes transitions to a structure intermediate of the open and closed conformations. The simulation begun from the open form closes spontaneously. We have extended our work in a Generalized Born continuum model as a basis for enhanced sampling techniques to obtain the free energy landscape of λ Cro conformational change. This will provide insight into the protein's DNA recognition mechanism. We anticipate our solution simulations to be the starting point to study the effects of crystal environments on λ Cro conformation (2). [1] B. M. Hall, et al. (2008) J. Mol. Biol. 375, 802-811. [2] I. I. Vorontsov and O. Miyashita (2009) Biophys. J. doi:10.1016/j.bpj.2009.08.011

Protein Folding & Stability I

150-Pos

Rheology of Small Volume Antibody Formulations and Kinetics of Surface Induced Protein Fibrillation

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This study shows how the QCM-D technology can shed light onto complex protein-protein interactions by rheological characterization of high concentration antibody solutions and measuring amyloid growth kinetics. Quartz Crystal Microbalance with Dissipation (QCM-D) is a surface sensitive technique which simultaneously measures the mass and viscoelastic properties of adsorbed layers and bulk fluids. Bulk fluid characterization was demonstrated by varying the pH of a humanized monoclonal antibody in 10mM sodium acetate or MES buffer and flowing ~ 350 ul of this solution at a rate of 50 ul/min across a QCM-D sensor. The storage (G') and loss moduli (G'') of the IgG2 antibodies were measured between a pH range of 4 - 6.2 at a variety of different frequencies. The ratio of G''/G' was greater than 1 for all solutions investigated with a maximum found at pH 5.5 indicating the greatest liquid-like behavior relevant both to administration and also physical characterization of the formulation itself. Surface fibrillation was studied by anchoring an insulin seed fibril onto an inert QCM-D surface and measuring the uptake of additional insulin as a function of time that was later correlated to AFM images of the surface. Growth kinetics of insulin were measured by QCM-D onto seed surfaces and found to be 83 +/− 0.2 pg/s or 8.1×10^9 +/- 1.4×10^6 individual insulin molecules per second. In the absence of seed fibrils insulin uptake was negligible. The rates of fibril growth accurately follow Arrhenius type behavior with an enthalpic activation barrier of $\Delta H^{\ddagger} = 24.4$ +/- 1 kcal/mol and a Gibbs free energy of 6.1 +/− 2 kcal/mol.

151-Pos

Direct Observation of the Tug-Of-War During the Folding of a Mutually Exclusive Protein

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Although most protein folding studies are carried out on single-domain proteins, over two thirds of proteins in proteomes are multi-domain proteins. A significant fraction of these multi-domain proteins are domain-insertion proteins, in which one guest domain is inserted into a surface loop of a host protein. Intricate thermodynamic and kinetic coupling between the two domains can have profound impacts on their folding dynamics, which has not been probed yet. Here we engineered a mutually exclusive protein as a model system to directly illustrate one such complex dynamic process: the 'tug-of-war' process during protein folding. By inserting a guest protein I27w34f into a host protein GB1-L5 (GL5), we engineered a novel mutually-exclusive protein GL5/I27w34f, in which only one domain can remain folded at any given time due to topological constraints imposed by the folded structures. Using stopped-flow techniques, we obtained the first kinetic evidence that the guest and host domains engage in a folding tug-of-war as they attempt to fold, in which the host domain folds rapidly into its three-dimensional structure and is then automatically unfolded driven by the folding of the guest domain. Our results provided direct evidence that protein folding can generate sufficient mechanical strain to unravel a host protein. Using single molecule atomic force microscopy, we provide direct evidence for the existence of a conformational equilibrium between the two mutually exclusive conformations. Our results highlight important roles played by the intricate coupling between folding kinetics, thermodynamic stability and mechanical strain in the folding of complex multi-domain proteins, which cannot be addressed in traditional single-domain protein folding studies.

152-Pos

Protein Oligomerization: Thermodynamic and Structural Analysis of the Dimerization of Beta-lactoglobulin

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Protein oligomers are widely found in living organisms and they are also crucial in some neurodegenerative diseases. The aim of this work is to analyze the thermodynamics and stability of protein oligomers employing β-lactoglobulin as the experimental model.

β -lactoglobulin is a small, globular and compact protein which, in solution, is in equilibrium with its dimeric form. The association equilibrium can be affected through changes in total protein concentration, temperature and pH. The dimer is the prevalent species at neutral pH. We studied the dissociation process of β-lactoglobulin induced by temperature employing different techniques at pH = 6.7. We performed calorimetric experiments at different values of total protein concentration and we observed that the endotherms of

β-lactoglobulin at pH = 6.7 are biphasic and they show a clear dependence with protein concentration. While the transition that appears at higher temperature is insensible to changes in protein concentration the first transition is stabilized when protein concentration is increased. This result allowed proposing a mechanism that involves dissociation of the dimer in the first instance and subsequent denaturing of the monomer. According to the thermodynamic model of a dimer dissociation presented in a previous work (1), it was determined through calorimetric profiles simulations, that only a dimer with negative dimerization enthalpy shows the dependence on protein concentration observed in these experiments. We also performed isothermal titration calorimetric studies and the association parameters of β-lactoglobulin were obtained at pH = 6.7. The dimerization process is exothermic in accordance with the model. Furthermore, temperature induced β-lactoglobulin dissociation at pH = 6.7 was monitored with infrared absorbance spectroscopy and we can state that there are structural changes related to the dissociation of the dimer.

(1) J. Phys. Chem. B. 112 (45): 14325-14333, 2008.

153-Pos

Structure and Folding Thermodynamics of MfpA, a Pentapeptide Repeat Protein From *mycobacterium Tuberculosis*

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The *Mycobacterium tuberculosis* protein MfpA confers bacterial resistance to the antibiotic fluoroquinolone. MfpA is a dimer in solution and in the crystal. The C-terminal α helices of two monomers form the dimer interface. The shape and distribution of the negative charge on the surface of MfpA mimics those of DNA (1). We quantitatively explored the unfolding, refolding and aggregation of MfpA as a function of temperature, urea concentration and the anionic surfactant SDS by circular dichroism (CD) and intrinsic fluorescence. These analyses reveal a structural transition followed by aggregation of intermediate conformers; the intramolecular and intermolecular interactions occur almost simultaneously. Stacking of phenylalanine side chains stabilize the N-terminal portion of MfpA's pentapeptide thus expanding on the motif of DNA mimicry. The high Arrhenius activation energy of aggregate formation rationalizes the nature of the kinetic trap shown earlier (2) that facilitates aggregate formation. Although secondary structure contents can not be calculated accurately for α/β proteins from their CD spectra (3), the increased α-helical content and a long-wavelength shift of the fluorescence emission maximum show intramolecular secondary and tertiary structure changes along the structural transition of MfpA. Overall, the unfolding and refolding of MfpA in solution is described by the 'double funnel' energy landscape where the 'native' and 'aggregation' funnels are separated by the high kinetic energy barrier that is not overcome during *in vitro* refolding.

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154-Pos

Concentration and Ion Induced Effects on Nucleotide Binding, Aggregation and Thermal Unfolding Transitions of Reca

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The *Escherichia coli* protein, RecA, catalyzes the DNA pairing and strand exchange reactions that are utilized in DNA recombination and repair. Buffer and salt conditions are known to influence the activity, aggregation state and thermal unfolding of RecA. We have used circular dichroism (CD), fluorescence, infrared and dynamic light scattering studies (DLS) to better understand the salt-induced effects on RecA structure, substrate binding and unfolding. CD and infrared studies were performed in order to monitor the thermally induced unfolding of RecA in the presence of a variety of salts and/or nucleotide and DNA substrates. Previous studies in our laboratory have shown that the concentration and identity of the salt ions resulted in unique influences on RecA unfolding transitions and stability. Unfolding studies performed under salt conditions known to activate RecA's ATPase activity in the absence of DNA showed thermally stable RecA structures. Additional characterization of these stable structures using DLS and fluorescence experiments shows unique aggregation states and nucleotide binding for some of the thermally stable RecA structures. A comparison of the influences of different ions on RecA unfolding, aggregation and nucleotide binding will be presented. These studies may help to elucidate how different ions influence RecA activity, structure, aggregation, and stability.

155-Pos

Influence of Matrix Metalloprotease on the Flexibility of Type I Collagen Fibrils Studied By Atomic Force Microscopy

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Collagen forms the main connective tissue in the body. Collagen turnover is intimately linked with healing of wounds, embryo development and tissue regeneration. Furthermore, the breakdown of collagen in various pathologies such as inflammatory arthritis and cancer is linked to disease progression and is accompanied by profound changes in its structure and mechanical response. Therefore, there has been increased interest in the study of mechanical properties of single collagen fibrils in the past decade. Nevertheless, the influence of the metalloproteases, which degrade collagen fibrils both in healthy tissue and a number of disease conditions, on mechanical properties of collagen has never been studied. In this work we present an investigation of the influence of MMP1 on the bending of type I collagen fibrils. Angular distribution of the segments of single collagen fibrils can be used to characterize the flexibility of collagen fibrils. High resolution images of the type I collagen fibrils were acquired by atomic force microscope (AFM) under ambient conditions in tapping mode. Angular distributions of segments of each collagen fibril were evaluated by an image recognition program from the resulting images. These distributions were compared and analyzed for native collagen type 1 fibrils and after treatment with matrix metalloprotease 1 (MMP1).

156-Pos

Population Analysis of Folding Intermediates From Time-Resolved and Spectral Fluorescence of Single-Tryptophan Apoflavodoxin

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The fluorescence of native apoflavodoxin (i.e. flavodoxin without cofactor FMN) from Azotobacter vinelandii has been extensively used to investigate thermal and denaturant-induced protein (un)folding. The protein populates an off-pathway molten globule-like intermediate during its equilibrium (un)folding. Fluorescence of apoflavodoxin arises mainly from its three tryptophans (Trp74, Trp128 and Trp167). With time-resolved fluorescence anisotropy of wild-type apoflavodoxin, tryptophan-tryptophan energy migration has been quantitatively measured to follow distance variations between two tryptophan pairs during apoflavodoxin (un)folding (N.V. Visser et al. (2008) Biophys. J. 95, 2462-2469). In this study we have followed a more general approach to analyze the time-resolved and steady-state fluorescence results of the single Trp74-mutant apoflavodoxin (Trp128 and Trp167 are replaced by phenylalanine), when it is gradually unfolded by addition of increasing amounts of guanidine hydrochloride. Singular value decomposition (SVD) of data matrices has been used to determine the number of species. In both types of experiments SVD shows the presence of three significant independent components. Therefore we can conclude that the three-state model (native, intermediate and unfolded state of the protein) should be used for further analysis. Each set of experimental data was globally analyzed using the three-state model finally yielding the relative concentrations of all species in the denaturation trajectory. The equilibrium thermodynamic properties were then determined from simultaneous and separate fits of the concentrations obtained from time-resolved and steady-state fluorescence data.

157-Pos

Photon-By-Photon Analysis of Single Molecule Fluorescence Trajectories of a Fast Folding Protein

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Förster resonance energy transfer (FRET) experiments of single protein molecules can be used to measure the structural and dynamical properties of sub-populations, as well as the kinetics of transitions between sub-populations. However, the timescale for study of both the dynamics and kinetics has been limited. To obtain folding and unfolding rate coefficients from FRET trajectories, for example, the bin size must be sufficiently long to calculate an accurate FRET efficiency, but also much shorter than the waiting times in the folded and unfolded states. In cases where the photon count rate is too low for these conditions to hold, an alternative approach is to analyze the photon-by-photon trajectories using maximum likelihood methods. Unlike ensemble methods, where