ubiquitination active sites, raising questions of how NEDD8 and ubiquitin are transfered, and how neddylation stimulates ubiquitination. Here we performed molecular dynamics simulations to address these questions. CRLs have cullin as scaffold holding two arms. One arm, substrate binding protein, binds to substrates; the other arm, Rbx protein, binds to E2. In our simulations, we observed big conformational changes on both arms. The flexible linker on the arm of Rbx1, serving as a hinge to rotate the RING domain, thus brings E2 toward substrate to shorten the 50-60 Å distance gap to a minimum of 13 Å, while the flexible linkers on the other arm of the substrate binding protein could also serve as hinges to rotate the substrate binding domain1,2 and bring substrate toward E2 thus further shorten the distance by 7~12 Å to bridge the ubiquitin transfer distance gap. The distance gap for neddylation could also be shortened due to the conformational change during the simulations. We therefore propose that a large ensemble of conformations could provide the possible conformation to bridge the distance gap for ubiquitin transfer and NEDD8 transfer, and that neddylation stimulates the ubiquitination by stimulating conformational change of CRLs and generating a larger conformational ensemble. This project is funded by NCI contract HHSN261200800001E.

1. Liu J, Nussinov R. Proc Natl Acad Sci USA 105:901-6, 2008
2. Liu J, Nussinov R. PLoS Comput Biol 5:e1000527, 2009

143-Pos

Evolutionary Analysis of Conformational Changes in Allosteric Proteins

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Allostery is fundamental to control numerous biological processes and coupled to the conformational rearrangements of protein structure. The changes of residue interaction networks upon ligand binding or protein-protein interactions impact protein dynamics and function. General principles on how conformational rearrangements of residues are encoded in protein sequences remain unknown. Here, we show that the residues that are evolutionary coupled with many partners mediate the conformational rearrangement of protein allostery. Highly evolutionarily coupled residues are involved in a dynamic network which participates in the smooth transition of two allosteric states; protein allostery is built up from the interaction rearrangements of these residues. We show that the evolutionary principles of protein conformational change provide the insight into the mechanisms controlling allosteric regulation and propose a new method to identify the key residues involved in the structural transition.

144-Pos

The Adenylate Kinase Transition Requires Many Easy Motions, Not a Few Hard Ones

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Conformational transitions are functionally important in many proteins. In adenylate kinase (AK), two small domains (LID and NMP) close over the larger CORE domain; the reverse (opening) motion limits catalytic turnover. Previous experiments and computations have also shown that local motions are important. Here, we hypothesize that the open/closed (O/C) transition rate depends on many low-barrier motions rather than a few high-barrier ones. To test this hypothesis, we simultaneously characterize the contributions of rigid-body (Cartesian), backbone torsional, and contact motions to the transition state (TS). O, C, and TS sub-ensembles are derived from a double-well Go simulation based on the native contacts of the O and C crystal structures. In Cartesian space, LID closes approximately two-thirds toward CORE in the TS, and NMP closes about halfway, substantially reducing rigid-body entropy. In backbone dihedral space, the TS dynamics of LID are more consistent with the higher-entropy O ensemble, while the TS dynamics of NMP, CORE, and most interdomain hinges are more consistent with the lower-entropy C ensemble. In contact space, contacts unique to C (C-contacts) with lower interresidue distances in the O state are more likely to form in the TS than those with higher distances; the CORE-LID/NMP interfaces remain sufficiently open to bind the ligand. Thus, the TS ensemble derives substantial enthalpy from C-contacts but at a relatively low entropic cost. Together with the observation that Cartesian, dihedral, and contact motions important to the transition are weakly correlated, these results strongly support our hypothesis that many degrees of freedom are important to the TS of AK. Finally, this work may complement structural analysis and protein dynamics experiments toward identifying structural features for allosteric design in proteins.

145-Pos

Functional Pathways in Proteins Are Uncovered By Strong Disorder

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We propose a simple and computationally inexpensive method to determine the few residues that control the communication between functionally linked regions. A protein structure is represented as a network of residues whereby edges are determined by intra-molecular contacts[1], weighted by amino-acid pair potentials[2]. The optimal path lengths that are operative under diverse perturbation scenarios are investigated for robust residue communication[3]. Pathways along which the maximum weights are minimized (strong paths) are found to be descriptive of communication during extreme events such as allosteric control and binding. This is a kinetic viewpoint whereby the rate of signal propagation is determined along paths with the lowest barrier to be surpassed.

Here, we examine 90 interacting proteins with structurally non-redundant interfaces. We study every strong path that connects the interacting proteins by recording residue pairs forming bridges between the protein-protein complexes. We then focus on those pairs that appear along the predominant fraction of these paths. Although nearly half the surface area of a protein is involved in protein-protein interactions, this approach delineates the few key contacts that control the communication between protein complexes. We compare the results from the current approach to those from computational hot spots[4]. We find that over 60% of the most used pairs correspond to a pair of hotspots and 92% of the mostly used pairs correspond to at least one hotspot on either partner protein of the complex. The results are further corroborated by experimental findings[5,6].

1. Atilgan AR, Akan P, Baysal C. Biophys J. 86,85(2004).
2. Thomas PD, Dill KA. PNAS 93,11628(1996).
3. Atilgan AR, Turgut D, Atilgan C. Biophys J. 92,3052(2007).
4. Tuncbag N., Gursoy A., Keskin O. Bioinformatics 25,1513(2009).
5. Fischer et al. Bioinformatics 19,1453(2003).
6. Lee et al. Science 322,438(2008).

146-Pos

Long Time Scale Dynamics of Molecules With Internal Rigid Fragments

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Due to the large number of degrees of freedom which are present in protein systems it is still a major challenge to monitor the dynamics of molecules out to the long time scales on which functionally important conformational changes occur. We have developed a rigid body Newtonian dynamics method, in which local high frequency motion of the molecule are naturally frozen out by decomposing the molecule into linked rigid bodies, thus decreasing the number of degrees of freedom monitored in the system. The factor of ca. 10 increase in the time step which comes from the elimination of high frequency motion in rigid body Newtonian dynamics can be further extended by elimination of explicit water molecules using a Langevin dynamics prescription in which friction and random force terms are added to the potential function to mimic the effect of the solvent. As a first step in this direction we have implemented a Langevin prescription for normal modes vibrational analysis of molecules with internal rigid fragments which is developed by Durand et al. [Biopolymers, 34, 759 (1994)]. Two simple illustrative examples representing signal propagation in the membrane-bound gramicidin-A dimer with two different initial conditions are presented as numerical applications for i) rigid body Newtonian dynamics (anharmonic PES) and ii) Langevin dynamics with internal rigid fragments in the harmonic field, respectively.

147-Pos

Critical Assessment of the Statistical Significance of Simulated Motions in Myosin V

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Elastic Network Models (ENM) and Principal Component Analysis (PCA) on Molecular Dynamics (MD) simulations are well-established computational methods that identify protein motions. Both methods have limitations in sampling conformational diversity. We employ an alternative method that more efficiently samples conformations by performing a rigidity analysis of myosin V using Floppy Inclusions and Rigid Substructure Topography (FIRST). We then generate trajectories of conformations using the Framework Rigidity Optimized Dynamics Algorithm (FRODA) that is approximately four orders of magnitude faster than MD. PCA analysis of the alpha carbon positions on 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