and weak affinity for wild-type MarA. Early results suggest that even minimal reorganization in the core can generate novel protein binding specificity.

136-Pos

Global Conformational Change Induced By Single Amino Acid Residue of Photoactive Yellow Protein in Time Domain

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We studied the conformational changes of protein from view point of the diffusion coefficient change. We successfully monitored that the photocycle kinetics of site directed mutants and compared the results with wild-type PYP. The role of isomerization and effect of surrounding amino acid residues during photocyle were investigated. The replacement of Lys by a small Ala residue remarkably altered the conformation of protein. The details of the experiments will be discussed later.

137-Pos

Exploration of Free-Energy Profiles With Conformational Changes of Proteins

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Under physiological conditions, proteins fluctuate around their native state and often undergo conformational changes on interacting with ligands. Thermal fluctuations are critical to the dynamics of nanoscale structures like proteins, and conformational changes can stabilize the binding energy of these molecules; therefore, both these factors are crucial for the retention of protein function. Recently, X-ray crystallographic studies have revealed conformational differences between the liganded and unliganded states of proteins. The conformational transition induced in proteins upon ligand binding can be explained by 2 representative models, the induced-fit model and the preexisting equilibrium dynamics. However, it remains unclear as to whether these models appropriately describe the actual dynamics of proteins.

Here, we performed molecular dynamics (MD) simulations for the lysine/arginine/ornithine (LAO)-binding protein and the maltose-binding protein (MBP). We used the umbrella sampling approach to examine the free-energy profiles governing the conformational changes induced in these proteins upon ligand binding. The conformational transition mechanisms of LAO-binding protein and MBP are believed to differ, being characterized by the preexisting equilibrium dynamics and the induced-fit model, respectively. However, our results revealed that the conformational transition mechanism of LAO-binding protein is based on a combination of the preexisting equilibrium dynamics and the induced-fit model, rather than solely on the former, while the mechanism of MBP is based mainly on the induced-fit model. And it was also suggested that the fluctuations in the apo state are important for the conformational changes and the protein function in both of these proteins.

138-Pos

Large-Scale Conformational Sampling of Proteins Using Temperature-Accelerated Molecular Dynamics

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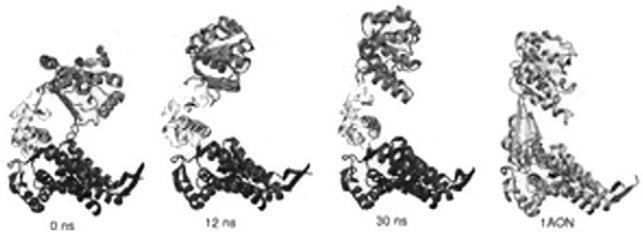
An adaptation of temperature-accelerated molecular dynamics (TAMD) is presented which permits conformational sampling of multidomain proteins in all-atom, explicitly-solvent molecular dynamics simulations. The method is simple to implement, requires no target bias, and is designed to allow the system to hyperthermally explore the free-energy surface in a given set of collective variables computed at the physical temperature. Our collective variables are Cartesian coordinates of centers of subdomains identified using a structure-based clustering algorithm. The method is applied to the GroEL subunit in its t-state, and the HIV-1 envelope gp120 in its sCD4/17b-bound state. For GroEL, the method induces in about 40 ns conformational changes that substantially recapitulate the t-to-r″ transition: the apical domain is displaced by more than 30 Angstroms, with a twist of almost 90 degrees, and RMSD relative to the r″ conformer is reduced from 13 to below 5 Angstroms, representing a fairly high degree of predictive capability. For gp120, the method predicts a conformational transition involving realignment of inner and outer domains to expose residues distal to the bridging sheet. The method gives an estimate of 10 kcal/mol for the free energy barrier between conformers in both cases.



139-Pos

Molecular Dynamics Simulation Study of Isolated Hamp Domain

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The HAMP domain is a linker region in prokaryotic sensor proteins which functions in two-component signal transduction pathways. HAMP exhibits a parallel coiled coil motif comprising four helices and transfers the signal from the sensor domain to the transmitter domain, usually a kinase. We present MD simulations of isolated HAMP (from A. fulgidus) in both the activated state and the inactive state, using structural data from wild type and mutant HAMP domains. Our simulations show that subtle changes in the hydrophobic core of HAMP lead to larger rearrangements in the coiled coil complex. The implications of these results for other signal transduction proteins containing HAMP are discussed.

140-Pos

Application of Linear Response Theory on Protein Networks For Identifying Allosteric Transitions

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We developed a fast and accurate method to predict residues that play an important role in allosteric transitions of single protein domains called perturbation response scanning (PRS). This method treats the protein as an elastic network and uses linear response theory (LRT) to obtain the residue fluctuations upon external perturbation. By sequentially exerting directed random forces on single-residues along the chain of the unbound form (i.e. by perturbing each residue one by one along the chain) and recording the resulting relative changes in the residue coordinates using LRT, we can successfully reproduce the residue displacements from the experimental structures of bound and unbound forms. Rigorous analysis of the response fluctuation profiles upon random perturbation of each residue, we identify the highest response residues that mediate long-range communication in proteins. Based on a structural network without reference to the dynamics of the bound forms, a dominant intermolecular signaling pathway of PDZ domain proteins (PSD-95 and hPTP1E) and cAMP-dependent protein kinase (PKA) can be identified.

This method can determine not only residues that play an important role in allostery but can be utilized to determine multiple receptor conformation for flexible docking scheme.

141-Pos

Orientation Dependent Residue Energies For Proteins Coarse-Grained From Atomic Force Fields

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Coarse-grained models for protein simulations can potentially access longer time-scales in larger protein systems than atomic level models. Here, a coarse-grained residue-pair potential, with distance and orientation dependency, is derived from equilibrium ensembles of residue pairs generated by molecular dynamics (MD). In particular, the Boltzmann inversion method is used to determine the energies. The residue-pair potential is used in the folding simulations of six small proteins, (28-67 residues) containing a variety of secondary structures. For the proteins tested, folding simulations by Monte Carlo methods generates structures similar to the native ones. However, these native like structures were among the lowest in energy for alpha helical proteins but not for proteins containing extended beta structures. It is also found that a careful balance between local and non-local interactions is essential.

142-Pos

Conformational Control of Ubiquitination in the Cullin-Ring E3 Ligase Machinery

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Tagging proteins by polyubiquitin is a key step in protein degradation. Cullin-RING E3 ubiquitin ligases (CRLs) facilitate ubiquitination by transfer ubiquitin from ubiquitin-conjugating enzyme E2 to the target protein. Neddylation by conjugation of ubiquitin-like protein NEDD8 to cullin can stimulate ubiquitination process. However, crystallography indicates a 25-35 Å distance between neddylation activate sites and a 50-60 Å distance between

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.

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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].

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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