130-Pos

Examining the Dependency of the Flexibility of Type 1 Molecular Collagen on Solvent Conditions

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Collagen is the most abundant protein in the body by mass, playing important roles in tissue architecture and mechanics. Specifically, type I collagen is responsible for the mechanical properties of tissues such as tendons, ligaments, and cornea. To fully understand the development of mechanical properties in these large-scale structures, the study of type 1 collagen on the molecular scale is vital.

Type I collagen molecules are typically on the order of 300nm in length and with reported persistence lengths ranging from 14.5nm (Sun et al.) to 170nm (Nestler et al.). Clearly there exists a large discrepancy in the literature on the flexibility of these molecules. Here we present a study of the dependency of the flexibility of type I collagen molecules on solvent conditions.

We extracted the persistence length for type I collagen molecules deposited from solution onto substrates. AFM images were used to measure the contour length and end-to-end distance of the molecules. The persistence length then was calculated from this data. We compared these results with those from molecular dynamics simulation. By examining the effects of various salt species and concentrations in the solution, our aim was to eliminate some of the confusion surrounding the conflicting measurements for the flexibility of type I collagen.

131-Pos

Crowding Effects on Protein Conformational Changes

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Conformational changes are directly linked to protein functions. Inside cells, the equilibria and transition rates between different conformations may be affected by macromolecular crowding. Recently we have developed a ''post-processing'' approach for modeling crowding effects [1]. With this approach, proteins can now be represented at the atomic level when modeling crowding effects. Here this approach is used to study how crowding affects the equilibria between open and closed conformations of 8 proteins: Trp repressor, adenylate kinase (AK), orotidine phosphate decarboxylase (ODCase), yeast protein-disulfide isomerase (PDI), hemoglobin, DNA β-glucosltransferase, cyanovirin-N, and Ap_4A hydrolase. For each protein, molecular dynamics simulations of the open and closed states are separately run. Representative open and closed conformations are then used to calculate the crowding-induced changes in chemical potential for the two states. The difference in chemical-potential change between the two states finally predicts the effects of crowding on the population ratio of the two states. Crowding is found to reduce the open population to various extents. In the presence of crowders with a 20-Å radius and occupying 35% of volume, the open-to-closed population ratios of Trp repressor, ODCase, AK, and PDI are reduced by 40%, 46%, 64%, and 80%, respectively. The reductions for the remaining four proteins are 15-30%. As expected, the four proteins experiencing the stronger crowding effects are those with larger conformational changes between open and closed states (e.g., as measured by the change in radius of gyration). Larger proteins also tend to experience stronger crowding effects than smaller ones [e.g., comparing PDI (504 residues) and Trp repressor (107 residues)]. These quantitative results and qualitative trends will serve as valuable guide for expected crowding effects on protein conformation changes inside cells.

[1] S. Qin, and H.-X. Zhou, Biophys J **97**, 12 (2009).

132-Pos

Conformational Studies of Soluble Guanylate Cyclase Through Time-Resolved Fluorometry

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We have used time-resolved fluorometry to determine the decay lifetimes of the four tryptophans in soluble guanylate cyclase (sGC). sGC is a heme sensor protein, which is able to be physiologically activated by nitric oxide (NO). Based on our lab's previous work, the binding of NO induces a conformational change, thus activating sGC. Based on this work, a model has been created using steady-state conditions. We are using the time-resolved data, a more sensitive technique to confirm the steady-state model. Time-resolved spectroscopy allows for individual tryptophan identification, as each one has a unique tau value. The unique tau values will allow for testing with the native protein since the tryptophans can be monitored individually. A temperature dependence study was performed to determine whether or not the tau values are temperature sensitive. It was determined that they are very sensitive to temperature, and in order to observe all four tau values, a temperature no higher than 20°C must be used. The four tryptophan's tau values are 0.005 ns, 0.5 ns, 3 ns, and 7 ns. Upon NO binding, although the longest tau value remains unchanged three of the four tau values increase in time. A change in lifetime can indicate multiple changes. Quenching studies are being used to help determine what is happening to the individual tryptophans upon activation. These studies have determined whether the tryptophans are moving farther from the heme or becoming more buried in the protein, providing more information about the conformational change. After the model has been confirmed using NO, the drug activator YC-1 will also be investigated.

133-Pos

Understanding the Role of Ankyrin Domain of the 43-Kda Subunit of the Chloroplast Signal Recognition Particle in Protein Targeting

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Light-harvesting chlorophyll-binding proteins (LHCPs) play an important role in photosynthesis. Photosynthesis occurs in the chloroplast of plant cells, while LHCPs are synthesized in the cytoplasm. One of the many paths utilized to target proteins to the thylakoid membrane of chloroplasts is the signal recognition particle (cpSRP) pathway. LHCPs form a transit complex with cpSRP43 and cpSRP54, the two subunits of the chloroplast signal recognition particle, in the cytoplasm before being targeted and integrated into the thylakoid membrane of the chloroplast. The transit complex binds to Albino 3 (Alb3), a protein in the thylakoid membrane. Of specific interest is the Ankyrin (Ank) domain of cpSRP43. Ank domains are found in a wide variety of proteins and they are found to be functionally important. In the LHCP-cpSRP complex, part of the Ank domain is responsible for binding LHCP to the complex. Recent studies have shown that the Ank domain of cpSRP43 plays a significant role in binding to Alb3 and in LHCP integration. The present study aims to understand the interactions between the c-terminal end of Alb3 (cAlb3) and the Ankyrin domain (Ank) of cpSRP43. The proposed research plans to characterize the conformation and stability of Ank and Alb3 using circular dichroism and fluorescence. The binding affinities of Ank and cAlb3 will be examined using isothermal titration calorimetry. Finally, the binding interface between Ank and cAlb3 will be mapped using ^{1}H-^{15}N chemical shift perturbation. The results of this study are expected to achieve a greater understanding of LHCP integration to the thylakoid membrane and protein transport in general.

134-Pos

Understanding the Conformational Preferences of Alanine Heptapeptide By Theoretical Approaches

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The secondary structure of the polypeptide polymer is the key determinant of the three-dimensional structure, which is prerequisite for a specific chemical or structural function. Among the many possible secondary structures, the α-sheet has been received a great attention because it was proposed as the possible intermediate conformation to form the beta-sheet fibrils in the aggregation process. Including the α-sheet conformation, the eight possible secondary was investigated by using the density functional theory to estimate quantitatively the thermodynamic properties. The relative thermodynamic properties are discussed in terms of the thermodynamic stability, gibb's free energy, enthalpy, entropy, dipole moment, and the charge separation.

135-Pos

Reengineering Protein Specificity By Repacking the Hydrophobic Core

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Engineering of protein function frequently focuses on amino acids that directly contact the ligand or substrate. However, it seems plausible that mutating buried residues away from the binding surface could also affect protein function. Previous work in protein folding has shown that, while the core of a protein is densely packed, it can be rearranged and accommodate new compositions while preserving function. The hydrophobic core is often thought as a scaffold for the active site. We wondered if the core could have a larger role in protein activity and if rearrangements in the hydrophobic core could change function. We have taken advantage of a system developed to investigate transcription factor specificity and used it to probe the role of core repacking in evolving protein function. MarA is a small transcriptional activator that binds a range of promoters to trigger expression of genes involved in antibiotic resistance. We have made mutant libraries of MarA which represent >104 unique variants of the core of the protein. We screened our library against promoters with strong

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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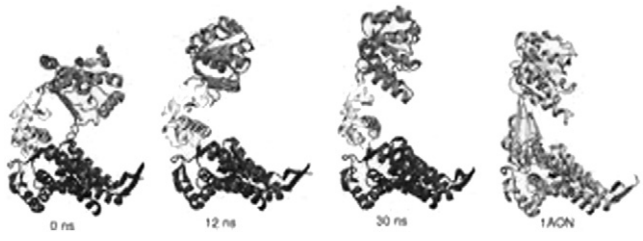
An adaptation of temperature-accelerated molecular dynamics (TAMD) is presented which permits conformational sampling of multidomain proteins in all-atom, explicitly-solvent molecular dynamics simulations. The method is simple to implement, requires no target bias, and is designed to allow the system 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