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2347-Pos

Functional Roles of a Novel Structural Element Involving the $Na^+-\pi$ Interaction Present in the Catalytic Site of T1 Lipase Revealed by Molecular Dynamics Simulations

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The interaction between a cation and an aromatic ring, i.e., the cation-π interaction, is one of the strongest noncovalent forces. Metal cations such as Na^+ and K^+ can also participate in the cation-π interactions, and are known to yield significant stabilization energy. However, in biological systems, few structures containing metal-π coordination have been determined, preventing understanding of its biological roles. Recently, we have determined the crystal structure of a thermoalkalophilic lipase where a Na^+ is coordinated to a phenylalanine (Phe) in its catalytic site. To elucidate the functional roles of the Na^+-Phe complex, we performed molecular dynamics (MD) simulations of the system. Note that the current force fields cannot correctly estimate the metal-π interaction energy, requiring quantum mechanical calculations. However, their huge computational costs prohibit long-time MD simulations. Accordingly, we developed a novel scheme to calculate the interaction energy with an accuracy comparable to that of advanced *ab initio* calculations at the CCSD(T) levels, and with computational costs comparable to those of force field calculations.A comparison of the MD simulations in the presence/absence of the accurate description revealed that a significantly large enthalpy gain in Na^+-Phe substantially stabilizes the catalytic site. Thereby, the cation$-\pi$ interaction in the lipase establishes a remarkably stable core structure by combining a hydrophobic aromatic ring and hydrophilic residues, of which the latter form the catalytic triad, thus contributing to large structural changes from the complex with ligands to the free form of the lipase. Thus, we have elucidated the detailed functional roles of $Na^+-\pi$ complex with use of our presented scheme, which is currently the only way to perform long-time MD simulations with reasonable computational costs.

2348-Pos

Length Dependent Force Characteristics of Coiled-Coils

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Coiled-coil domains within and between proteins play important structural roles in biology. They consist of two or more helices that form a superhelical structure due to packing of the hydrophobic residues that pattern each helix. A recent continuum model [1] showed that the correspondence between the chirality of the pack to that of the underlying hydrophobic pattern comes about because of the internal deformation energy associated with each helix in forming the superhelix. We have developed a coarse-grained atomistic model for coiled coils that includes the competition between the hydrophobic energy that drives folding and the cost due to deforming each helix. The model exhibits a structural transition from a non coiled-coil to coiled-coil state as the contribution from the deformation energy changes.We compare simulated structures with naturally occurring structures and calculate root mean square between them. Also we studied the mechanical behavior of coiled-coils by applying force perpendicular and along the axis of coils. Our model is able to reproduce naturally occurring coiled-coils and essential features seen in unzipping experiments[2]. We explore the force-extension properties of these model coiled-coils as a function helix length and find that shorter coils unfold at lower force than longer ones, with the required unfolding force eventually becoming length independent.

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2349-Pos

Construction of a Basis Set of Signature Pockets of an Enzyme Functional Class by Structural Alignment of Multiple Binding Surfaces: Metalloendopeptidase and NAD Binding Proteins

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To understand the structural basis of protein function and to infer the biological role of a protein, we developed an algorithm for the construction of a basis set of signature pockets that are characteristic of a protein function. The signature pockets are constructed by identifying structurally conserved surface elements across binding surfaces of the same enzyme functional class. Signature pockets are then selected to form a minimalistic basis set representing the full ensemble of surfaces that an enzyme functional class can sample. By accurately locating elements on the binding surfaces that are invariant to conformational fluctuation, the signature pockets provide information on key players in enzyme function. A collection of signature pockets form a minimalistic basis set, which can be used for protein function prediction through database search. Our approach avoids the problems when an entire active site is used as a template due to conformational changes because of the dynamical nature of protein binding events. Our approach also avoids the problems when only a few key residues are used as a structural template, which often results in numerous false positives when predicting enzyme function. In addition, our method does not assume a priori a single structural template for representation of a functional class of proteins. Instead, a minimal set of distinct signature pockets are constructed to form a basis set that is able to characterize the full ensemble of binding surfaces that are capable of the specific enzyme function. We describe in detail how this approach is applied to accurately infer functional roles of the metalloendopeptidase family, which descend from a common ancestor, and of the NAD binding proteins, which have diverse evolutionary origins.

2350-Pos

Using Structure Recurrence to Define Protein Domains

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Domains are basic units of protein structure and essential for exploring protein fold space and structure evolution. With the NIH Protein Structure Initiative and other structural genomics initiatives worldwide, the number of protein structures in PDB is increasing dramatically and domain parsing needs to be done automatically. Most of the existing structural domain parsing programs consider the compactness of the domains and/or the number and strength of internal (intra-domain) versus external (inter-domain) contacts.
Here we present a completely different approach. Taking advantage of the growing number of known structures in the PDB, the chains are parsed solely by using recurrence of similar structures that appear in the structural database. A non-redundant set of 6373 protein chains was selected as the target data set and 128 benchmark chains from pDomains were used as query chains. For each query chain, one against all target structure comparisons were performed using VAST. Then the VAST cliques were collected and the protein residues were clustered using mathematical procedures akin to those used for analyzing the microarray data. These clusters define domains. NDO scores were used to compare the results with SCOP and CATH domain boundaries as well as with those from other parsing programs.
Our algorithm gave results that were comparable to those of several existing programs. It handles segmented domains equally well as non-segmented domains. The structures that contribute the cliques that define a domain may contain distant evolutionary information of the domain.

2351-Pos

2°Struc - the Protein Secondary Structure Analysis Server

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Protein secondary structure can be defined by the pattern of hydrogen bonding between backbone amide and carboxyl groups, whereby the protein is constrained to adopt repetitive dihedral angle conformations. "Define Secondary Structure of Proteins" (DSSP)(Kabsch and Sander, 1983) is the *de facto* standard for annotation using rules similar to those described by Pauling and Corey, (1951) to assign eight secondary structure states. However, other methods have been developed to address problems including poor edge residue definition, low-resolution structure elucidation and Cα only structures.
These methods define secondary structure in different ways resulting in a wide variation in assignment at the amino acid and segment levels. To enable investigation of this variation we present 2°Struc; a web server that analyses protein secondary structure content derived from a number of available methods. The output is in five sections. **Protein structure summary** details the 'whole protein' percentage structure content and provides a numerical comparison of each method relative to DSSP using several commonly applied metrics including percentage similarity and Matthews correlation coefficient. **Structure summary by chain** displays percentage content and provides an option to compare each structure assignment method using the Jmol molecular viewer. **Multiple structure alignment** uses a three-state representation colored to display secondary structure assignments relative to PDB and UniProt sequence records for each method. A majority vote consensus is also provided. **Original multiple structure alignment** provides a second colored alignment displaying unmodified structure assignments. **Sequence structure alignments** shows comparative unmodified and modified three-state output relative to UniProt and ATOM record sequences, with an option to download a PDF file containing

information about the method run, percentage structure content, chain length and diagram of the three-state structure assignment, also provided.
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2352-Pos
An Automated Approach to Segregate and Identify Functional or Disordered Loop Regions in Protein Structures using their Ramachandran Maps
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The loops which connect or flank helices/sheets in protein structures are known to be functionally important. However, ironically they also belong to the part of protein whose structure is least accurately predicted. Here, a new method to isolate and analyze loop regions in protein structure is proposed using the spatial coordinates of the solved 3D structure. The extent of dispersion among points of successive amino acid residues in the Ramachandran map of protein region is utilized to calculate the Mean Separation between these points in the Ramachandran Plot (MSRP). Based on analysis of 2935 protein secondary structure regions obtained using DSSP software, spanning a range from 2 to 64 residues, taken from a set of 170 proteins, it is shown that helices (MSRP < 17) and strands (MSRP < 64) stand effectively demarcated from the loop regions (MSRP > 130). Analysis of 43 DNA binding and 98 ligand binding proteins revealed several loop regions with clear change in MSRP subsequent to binding. The population of such loops correlated with the magnitude of backbone displacement in the protein subsequent to binding. Can changes in MSRP quantify the temporal oscillations in dihedral angles among structured/unstructured regions in proteins? Molecular dynamics simulations (10 ns) revealed that deviations in MSRP among different snapshots in the trajectory were at least two-fold higher for unstructured proteins (PDB codes: 2SOB, 1LXL, 2HDL & 1VZS) in comparison with folded proteins (PDB codes: 1BGF & 1MUN). Additionally it was observed that deviations in MSRP were highest amongst loop regions, while it was lowest amongst alpha-helical regions. The above results validate use of MSRP parameter as a tool to identify & investigate functionally active loops and unstructured regions in protein structures.

2353-Pos
Origins of Thermophilicity in Endoglucanases
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Endoglucanases are involved in the initial stages of cellulose breakdown–an essential step in the bioprocessing of lignocellulosic plant materials into bioethanol. Although these enzymes are economically important, we currently lack a basic understanding of how some endoglucanases can sustain their ability to function at elevated temperatures needed for bioprocessing, while others with the same fold cannot. In this study, we present a detailed comparative analysis of both thermophilic and mesophilic endoglucanases in order to gain insights into origins of thermophilicity. We used the CAZy (Carbohydrate-Active enZymes) database to build our endoglucanase protein data sets and analyzed their sequences and structures. Our results demonstrate that thermophilic endoglucanases and their mesophilic counterparts differ significantly in their amino acid compositions. Strikingly, these compositional differences are specific to protein folds and enzyme families and lead to modification in hydrophobic, aromatic, and ionic interactions in a fold-dependent fashion. However, when it comes to thermophilicity, there is a caveat of applying general heuristic rules to specific proteins: although thermophilicity in endoglucanases is usually conferred through altering amino acid composition, in some cases even a single-point mutation is sufficient to convert a mesophilic protein into a thermophilic protein. Here, we provide fold-specific guidelines to control thermophilicity in endoglucanases that will make production of biofuels from plant biomass more efficient.

2354-Pos
Thermophilic Adaptation of Protein Complexes Inferred from Proteomic Homology Modeling
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What can Nature teach us about mechanisms securing unique and stable interfaces in native protein complexes and preventing aberrant assembly of their parts in misfolded conformations? As protein complexes must remain in their native conformations at physiologically relevant temperatures, thermal adaptation requires adjustment of pertinent interactions. Based on high-quality sets of structural templates and sequences of 127 complete prokaryotic proteomes with the optimal growth temperature (OGT) from 8 to 100 C°, we performed homology modeling of complexes and monomeric proteins and analyzed trends in their sequences and structures related to thermal adaptation. With model of protein stability including negative and positive components of design, we investigate compositional biases and their correlations with the habitat temperatures specific for protein complexes. Specifically, we show how positive charges work in negative design preventing aggregation and how they contribute to positive design stabilizing both the native interface and the overall structure of the complex. Aggregation propensity of interfaces is higher than the one of surfaces and increases with OGT helping to form native complexes in harsh environments. Thermophilic trends obtained in high-throughput proteomic homology modeling illuminate sequence/structure determinants of molecular mechanisms working in protein complexes. We show that these tends are generic for both obligatory and transient complexes. They can be instructive, therefore, in experimental efforts on design of protein complexes and preventing aberrant assembly.

2355-Pos
Protein-Protein Docking Using a Brownian Dynamics Simulations Approach
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A successful protein-protein docking method provides theoretical understanding of how two or more proteins combine and interact with each other at the atomic level. It involves some unsolved problems partly due to the huge sampling space. Here we present an adapted Brownian Dynamics (BD) method used to predict the structure of protein complexes. The BD protein docking approach includes two steps, 1) global BD sampling; 2) local energy minimizations. In the first step, we run thousands of independent BD simulations to explore the entire possible conformational spaces of the protein complexes. The proteins are treated as two rigid bodies, and the translational and rotational motions are simulated for one of the proteins (protein II) around the other (protein I). The intermolecular forces and torques between proteins are given by the sum of electrostatic and exclusion forces. In the second step, we conduct local energy minimizations for all protein complexes obtained from the step one, and rank them by interaction energies. To reduce the computational costs for energy evaluations, we developed a grid-based force field to represent protein I and solvation effect. The rigid-body energy minimizations of the protein complexes are based on the downhill simplex method using the newly developed force field. The prediction quality of this newly developed BD protein docking approach is evaluated on a re-docking experiment for predicting the acetylcholinesterase-fasciculin complex (PDB entry 1FSS). The result shows that 100,000 independent BD runs generated 32797 protein complexes for the subsequent local energy minimizations. The root mean square deviation (RMSD) between the predicted lowest energy and the crystal structures is 0.17 Å. In conclusion, this adapted BD protein docking approach could be used for prediction of other protein complexes, and help better understanding protein-protein interactions.

Protein Aggregates II

2356-Pos
Both the Formation and Polyphenol-Induced Dissociation of Various Amyloid Fibrils are Accompanied by ROS Formation
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Fibrillization of amyloid polypeptides is accompanied by formation of reactive oxygen species (ROS), which, in turn, is assumed to further promote amyloid-related pathologies. Different polyphenols, all of which are established antioxidants, cause dissociation of amyloid fibrils. In this study we address the latter, poorly understood process. Specifically, we have investigated the dissociation of Aβ42 fibrils by six different polyphenols, using electron microscopy and spectrofluorimetric analysis. Simultaneously, we have monitored the production of ROS using electron spin resonance (ESR) and the commercially available peroxide assay kit. Using the same methods, we found that curcumin, one of the most potent destabilizing agents of Aβ42, induces dissociation of fibrils of other amyloid polypeptides [Aβ40, Aβ42Nle35, islet amyloid polypeptide and a fragment of α-synuclein].
When the solution contained traces of transition metal, all the dissociation reactions were accompanied by ROS formation, independent of the presence of a methionine residue. Kinetic studies show that the formation of ROS lags behind dissociation, indicating that if casual relationship exists between these two processes, then ROS formation may be considered a consequence and not a cause of dissociation.
Understanding of our results and of their implications requires further research.