these conditions, the predominant toxin complex that assembles is octameric. Incubation in blood causes heptameric LT complexes to lose greater than 90% cytotoxicity, whereas octameric LT complexes retain their activity. To understand the molecular mechanism of the apparent differential loss of toxin activity, we determined the pH-threshold of conversion to the channel state for each oligomeric complex. We find that the octameric toxin has a lower pH-threshold for channel formation than the heptamer. A consequence of this is that heptamers are inactivated by premature conversion to the channel state, whereas the octamers remain in the functional prechannel state. We propose that assembly of two oligomeric Atx complexes may provide a regulation mechanism for anthrax toxin cytotoxicity in both assembly at cell-surfaces and in the bloodstream. The assembly of octameric toxin complexes at the cell surface may alleviate potential assembly bottlenecks incurred by the mechanism of assembly via PA dimers, while in the bloodstream they may serve to maintain cytotoxicity during anthrax pathogenesis.

2342-Pos

An Atomic View of Fibril Structure Using Solid State NMR Approaches

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Amyloidosis is a clinical disorder caused by extracellular deposition of proteins that are normally soluble in their native conformation, but suffer conformational modifications resulting in insoluble and abnormal fibrils that impair organ function. Parkinson's disease and Alzheimer's disease are the two most common diseases associated with amyloidosis. However, many important diseases such as Prion and Huntington diseases are also amyloidogenic. To understand protein aggregation is crucial to improve the knowledge about protein-protein interactions and protein hydration and thus the thermodynamic behavior related to folding and misfolding.

The limitations of many biophysical and biochemical approaches to study fibril formation have slowed the advance in the understanding of how soluble proteins undergo conformational changes that result in aggregation. Techniques such as Atomic Force Microscopy and Transmission Electron Microscopy taken together with X-Ray Crystallography have provided some details about fibril morphology and distance correlations among monomeric units. However, samples composed of fibrils are huge, heterogeneous and extremely difficult to crystallize, which implies in a limitation to use Crystallography and Solution-State NMR. As atomic resolution models require information about the spatial coordinates of atoms, Solid-State NMR (ssNMR) has emerged as the uniquely technique able to provide these information. Improvements in distance measurements, torsion angle determination, improved decoupling sequences, and higher Magic Angle Spinning frequencies allow ssNMR to become an important tool for structural studies of fibrillar architecture. Fibrils have been shown to adopt β-sheet conformation organized in parallel or anti-parallel fashion associated with in- or out of register structures. Despite the many challenges that have been overcome, many questions remain unanswered and more improvements need to be made.

2343-Pos

Molecular Structure of Type II Collagen

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The naturally crystalline arrangement of collagen molecules within the fibrils of some tissues, allows the use of fiber diffraction methods for structural characterization. This method has the potential to give structural information about collagen type II with minimum interference from sample preparation and may give the opportunity to produce relatively detailed three-dimensional visualization of the fibrils sub-structure. Towards this end, experiments with Multiple Isomorphous Replacement (MIR) were carried out to so that a one-dimensional electron density map of native collagen structure may be determined. Several experiments were performed at the BioCAT facility at Argonne National Laboratory with variations of: sample holder designs, sample preparation procedures, heavy atoms for MIR, temperatures and setups for small and medium angle diffraction. Some more optimum combinations of these produced data of resolution 15 Å or better in the axial direction. Using these data, a subsequent study that also made use of AFM and TEM techniques, revealed that the parameters of collagen type II fibrils from lamprey notochord are very similar if not the same as collagen type II fibrils in mammalian tissues: 30 nm in diameter, axial periodicity of 67 nm, amino acid charge distribution is the same. Analysis of the X-ray diffraction derived one dimensional electron density map showed that the telopeptides, which are crucial for fibrillogenesis and organization of collagen type II tissues, have a very specific folded conformation, reminiscent of that seen in the C-telopeptide of type I collagen. The folded telopeptide conformations provide a clear picture of the intermolecular crosslink locations within the contributing collagen monomers within the 67 nm D-period. This type of structural information is essential for understanding the mechanisms of tissue development and disease pathologies.

2344-Pos

Tandem Repeats Domain in Candida Albicans Als Adhesins

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Yeast adhesins are involved in binding interactions to other cells, substrates and surfaces. Als adhesins in *Candida albicans* consists of 8 homologous proteins. The proteins are composed of an N terminal signal sequence, three Ig-like domains, a threonine rich (T) region, tandem repeats (TR), a glycosylated stalk and C-terminal GPI-anchor to the cell wall. Tandem repeats in the *C. albicans* Als adhesins consist of 2 to 36 copies of a 36-residue sequence.

Tandem repeat domain structures from six Als adhesins were modeled by Rosetta and LINUS. Both methods produced a three-stranded antiparallel β-sheet. This is consistent with circular dichroism (CD) secondary structure and atomic force microscopy domain measurements. Models of glycosylated TR domains show carbohydrates surrounding hydrophobic patches. This is the basis of protein-protein and protein-substrate interactions. In addition, the presence of tandem repeats led to enhanced non-saturable binding to polystyrene and other TR domains. Interestingly, TR domains do not affect the isosbestic point in thermal CD experiments.

This modeling structure and function of the tandem repeats in Als proteins can be applied to repeat regions in other yeast adhesins proteins.

2345-Pos

Structure-Based Models for Alpha-Helical to Beta-Helical Conformation Change in the C-Terminal of the Mammalian Prion Protein

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We employ all atom structure-based models with mixed basis contact maps to explore where there are any significant geometric or energetic constraints limiting conjectured conformational transitions between the alpha-helical (αH) and the left handed beta helical (LHβH) conformations for the C-terminal (residues 166-230) of the mammalian prion protein. The LHβH structure has been proposed to describe infection oligomers(1) and one class of in vitro grown fibrils(2,3), as well as possibly self-templating the conversion of normal cellular prion protein to the infectious form. The structure-based model uses GROMACS based molecular dynamics with a two-dimensional weighted histogram analysis method (WHAM) being applied to study projected energy surfaces. Our preliminary results confirm that the kinetics of the conformation change are not strongly limited by the large scale geometry modification, and evidence exists for a pathway linking the two conformations with a common folding intermediate, also suggested by all atom unfolding simulations(4).

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(2) Tattum M. H., et. al. Elongated oligomers assemble into mammalian PrP amyloid fibrils. J. Mol. Biol 2006; 357; 975-985
(3) Kunes K., et. al. Left handed β helix models for mammalian prion fibrils. Prion 2008; 2; 81-90
(4) See S. Dai and D.L. Cox, abstract elsewhere for this meeting

*Research supported in part by the International Institute for Complex Adaptive Matter, NSF Grant DMR-0844115

2346-Pos

Silk Fiber Mechanics from Models at Different Length Scales

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Silk is one of the most resilient fibers in nature. Consisting of an amorphous matrix cross-linked by beta-sheet rich crystalline units, silk is a hierarchically organized material the molecular details of which remain largely unknown. In order to decipher the structural determinants of its mechanical properties, we model silk at different length scales by combining molecular dynamics simulations, force distribution analysis, novel force-based coarse-grain models, and finite element methods. We predict the distinct mechanics of anti-parallel versus parallel silk crystals as force-bearing cross-links [1], and the impact of chain entanglement and crystallinity on fiber mechanics [2]. Our predictions can serve as a guide for the design of artificial silk protein analogues.

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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