and the amplitude of the reaction was used to monitor the time course of RNA binding. The kinetics of enzyme and RNA binding followed a two-step mechanism: an initial binding was followed by a conformational change. The enzyme and RNA binding proceeded to equilibrium about six times faster at 37 oC than at 30 oC, suggesting the conformational change involved in RNA binding is temperature sensitive. Following incubation of RNA and enzyme to form an active complex, we used chemical quench-flow methods to measure the ground state binding K_d and the incorporation rate of a correct nucleotide. The fidelity of the polymerase was determined by measurement of the incorporation of incorrect nucleotides. Using these methods, we characterized the Dengue polymerase in its elongation mode by measuring the kinetics of RNA and polymerase binding and the kinetics for nucleotide binding and incorporation. Based on these data, we built a working model for studying the selectivity of Dengue polymerase.

2337-Pos

Structure-Guided Design of Novel Inhibitors of Human Uridine Phosphorylase 1

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Uridine phosphorylase (UPP) is a key enzyme of pyrimidine salvage pathways, catalyzing the reversible phosphorolysis of ribosides of uracil to nucleobases and ribose 1-phosphate. It also plays a role in the activation of pyrimidine-based chemotherapeutic compounds such a 5-fluorouracil (5-FU) and its prodrug capecitabine. In some cases, an elevated level of this enzyme in solid tumours is thought to contribute to the selective action of these drugs. Nevertheless, the therapeutic value of these fluoropyrimidine antimetabolites is often limited by their toxicity to normal tissue. To address this shortcoming, specific inhibitors of UPP, such as 5-benzylacyclouridine (BAU), have been clinically studied for their ability to moderate the cytotoxic side effects of 5-FU and its derivatives, so as to improve the therapeutic index of these agents. We have determined the high resolution structures of human uridine phosphorylase 1 (hUPP1) in complex with natural ligands and known inhibitors. The structures reveal important details underlying the architecture of hUPP1's active site and the proximate surfaces that influence binding of BAU and analogous acyclouridine compounds. This data provides opportunities for designing more potent inhibitors of this enzyme. For instance, the back wall of the substrate binding pocket is conformationally unique relative to earlier elucidated structures of microbial homologues of UPP. These features can be exploited to develop novel inhibitory compounds with improved efficacy against the human enzyme as a step toward the development of better chemotherapeutic regimens that protect normal tissues with relatively lower UPP activity.

Protein Structure II

2338-Pos

Factor Xa Dimerization and Prothrombinase Complex Formation are Competitive Process on a Membrane Surface

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Exposure of phosphatidylserine (PS) molecules on activated platelet membranes is a crucial event in blood coagulation. Binding of PS to a specific site on factor Xa (fXa) and factorVa (fVa) promotes their assembly into a complex that dramatically enhances proteolytic activity of fXa. Recent studies demonstrate that by both soluble PS and PS-containing membranespromote formation of inactive fXa dimer at 5mM Ca^{+2}. The fluorescence anisotropy of active site labeled fXa, FEGR (Fluorescein-GLU-GLY-ARG-chloromethylketone)-Xa, is decreased in the presence of PS membrane on which it forms dimer. We report now the addition of fVa to membrane-bound FEGR-Xa produced fVa- FEGR-Xa complex formation with a $K_{d,\ surface}$ approximately 60-fold lower than that characterizing FEGR-Xa surface dimerization, clearly indicating fVa strongly competed with fXa dimer formation in order to form active Xa-Va complex on the membrane surface. Analysis of FEGR-Xa fluorescence anisotropy yielded roughly constant K_d's for Xa-Va interaction with increasing Ca^{2+} concentration from 2 to 5 mM Ca^{2+} despite the fact that fXa dimer formation varied dramatically over this Ca^{2+} range. Experiments performed at varying membrane and fVa concentrations for both 23 nm and 120 nm confirmed that protein distribution between vesicles was sufficiently rapid as to overcome any possible effects of membrane discreteness. We conclude that PS-induced fXa dimerization on membrane strongly competes with fXa-fVa complex formation at high Ca^{2+} concentrations. Supported by USPHS grant HL072827 to BRL.

2339-Pos

Sans and Osmotic Stress Approach to Study Protein Preferential Hydration and Association

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The function of biological macromolecules necessarily depends on their hydration and interactions. However, it can be challenging to appropriately and directly measure these forces. We are using a combined small-angle neutron scattering (SANS) and osmotic stress approach to directly correlate protein structure and structural transitions with the associated hydration and energetics. We performed SANS experiments on hexokinase (HK) to investigate protein preferential hydration by solute molecules, called osmolytes, and the interactions responsible for HK dimer formation. The HK monomer-dimer equilibrium plays a regulatory role but its importance to function is not entirely clear. With SANS, three regions of scattering contrast are created upon osmolyte addition: protein, protein-associated water, and bulk water/osmolyte solution. Changes in the zero-angle scattering intensity, $I(0)$, and the apparent radius of gyration, R_g, with increasing osmolyte concentration are used to quantify the number of osmolyte-excluding water molecules associated with protein. We observe the preferential hydration of HK monomer and dimer states to depend on the osmolyte chemistry and size but find the calculated hydration change accompanying the monomer-dimer transition to be independent of the osmolyte used. By experimentally exploring the forces that are important for guiding protein association, we hope to address critical questions concerning protein structure, hydration, and interactions.

2340-Pos

Understanding the Effects of Molecular Crowding on the Structure and Stability of Proteins Using NMR Spectroscopy

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Much of the research on biological proteins is performed *in vitro* (under artificial conditions) involving the isolation of the protein from the cell. The living cell, however, constitutes a very complex system, and a protein's structure and stability may be influenced by its native environment. An understanding of the effects of molecular crowding will provide important information regarding a protein's structure, dynamics, and stability *in vivo*.

This study involves the titration of ^{15}N FGF-1 with various intracellular components (to simulate an in-cell environment) followed by NMR spectroscopy to determine any chemical shift perturbation corresponding to shifting amino acid residues. Fibroblast Growth Factor 1 (FGF-1) is a protein involved in cellular proliferation, wound healing, and cancer development and metastasis. Little information is known regarding FGF-1's interactions inside the cell, as it follows a non-classical secretion pathway. To better understand the role of intracellular proteins on the structure of FGF-1, several experiments were carried out using multi-dimensional NMR spectroscopy; to the FGF-1 sample were added (1) intracellular proteins (from the purification of unlabeled FGF), (2) intracellular proteins and lysozyme, and (3) lysozyme alone (added as a control). HSQC data was obtained at regular intervals and processed using XWIN-NMR and Sparky software. A chemical shift perturbation plot was constructed from the data to show the (individual and combined) effects of the addition of the intracellular proteins and/or lysozyme on FGF-1. The preliminary results of this study indicate that a moderate number of amino acid residues were perturbed with the addition of intracellular proteins. This implies that molecular crowding plays a role in the structural conformation of FGF-1 and possibly other proteins *in vivo*.

2341-Pos

Anthrax Protective Antigen Oligomerization Regulates Toxin Activity

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Anthrax toxin (Atx) is a key virulence factor secreted by Bacillus anthracis. This three-protein toxin includes protective antigen (PA) and two enzymatic components, lethal factor (LF) and edema factor (EF), which must assemble into oligomeric complexes to disrupt cell physiology. Atx complexes are endocytosed, where they convert to a transmembrane channel that transports LF and EF into the cytosol. Assembly from monomeric components may occur in two physiological contexts: 1) in the bloodstream and 2) on cell surfaces. We have previously shown that the assembly of toxin complexes on cell surfaces produces a mixture of ring-shaped homooctameric or homoheptameric PA oligomers in a 1:2 ratio, which assemble via dimeric PA intermediates. Here we investigate how Atx complexes assemble in bovine blood. We find that, under

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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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.