glutathione coated wells. The free cysteines are biotinylated and detected via streptavidin-HRP. We used this assay to study the amount of oxidation and palmitoylation in SNARE proteins (SNAP-25, Syntaxin 1A, and a no cysteine mutant of SNAP-25). Each protein contains 0-4 cysteine residues and results were compared to the background signal obtained from just GST. Reducing agents (Cu^{2+}, Fe^{2+}, Cystine) and oxidizing agents (cysteine and Zn^{2+}), were used to alter the extent of oxidation/reduction of cysteine residues. Alternatively, reduced cysteines were blocked by reactions with NEM or Palmitoyl-CoA. Except for palmitoylation, all reactions could be driven to near completion during a 10 minute incubation on ice. Palmitoylation (without enzymes) required incubation for 1 hour at RT and high doses of Palmitoyl-CoA to palmitoylate >50% of the cysteine residues. This assay is simple, inexpensive, and relative fast, and should allow greater elucidation of the chemistry of cysteine residues in proteins due to its high resolution.

3282-Pos

Application of Gaussian Network Model to Elucidate Functional Modes of Motion in a Glutamate Transporter

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Glutamate transporters located on the plasma membrane of glia and neurons are responsible for clearing glutamate from the extracellular space and maintaining its concentration below toxic levels. Disruption in glutamate uptake is associated with acute brain injury (ischemia, stroke, seizures) and chronic pathological conditions, including amyotrophic lateral sclerosis, Hungtington's disease and Alzheimer's disease. Moreover, glutamate transporters possess a substrate-activated chloride current which may regulate neuronal activity. Understanding the mechanism of transport is therefore, of great physiological and medical importance. High resolution structures of a glutamate transporter homolog (GltPh) from an archaeal species were determined recently (Boduker et al, 2007) allowing for establishing a basis for the structural mechanism of binding and transport at the molecular level. We used the Gaussian Network Model (GNM) for protein dynamics to elucidate the most cooperative modes of motions intrinsically accessible to the homotrimer. A concerted opening and closing of the extracellular vestibule is found to be the most dominant mode of motion near the equilibrium structure. This mode of motion is expected to aid in the substrate binding process of the transport machinery. The movement of the extracellular portions of the protein, towards the center of the aqueous cavity is supported by cross-linking experiments with mutants where residues mutated to cysteine are observed to form disulfide bridges with their counterparts in the neighboring subunit. In the X-ray structure, these residues are more than 40 Å apart. The formation of these disulfide bridges also results in reduced transport of glutamate, suggesting a functional role for the motion induced by the most cooperative GNM mode.

3283-Pos

Simulation of Membrane Sculpting by EFC F-BAR Domain Lattices

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Cells, during cellular morphogenesis, are dynamically sculpted into different compartments by membranes with the help of proteins. The BAR domain is one of the conserved protein domains that is involved in shaping cellular membranes in vivo, and is observed to induce tubule formation from liposomes in vitro. Previous simulations showed that certain lattice arrangements of N-BAR domains shape membranes into tubules (Yin, Arkhipov, and Schulten, 2009). Here we show, by means of several microsecond coarse-grained simulations of F-BAR domains in different lattice configurations on POPC/POPS membranes, that extended-FCH (EFC) F-BAR domains shape membrane in a fashion similar to what has been seen in N-BAR simulations. The membrane bending property of several F-BAR domain lattice arrangements is characterized, showing that different lattice configurations induce a range of membrane curvatures. A highly detailed view of the dynamic membrane sculpting process by F-BAR domain lattices on a microsecond timescale is obtained.

Yin, Y., Arkhipov, A., and Schulten, K. (2009). Simulations of membrane tubulation by lattices of amphiphysin N-BAR domains. *Structure* **17**(6)**,** 882-92.

3284-Pos

A Dynamical Model for Heat Shock Protein (HSP) Transcription and its Correlation with HSP70 in vitro Experiments

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Heat-shock proteins (HSPs) are well know for their protective mechanism when cells undergo stress conditions such as heat by enhancing protein folding and mitigating apoptosis Heat-shock causes the denaturing of proteins to increase, which leads to the aggregation of denatured and functional proteins. On the other hand, cells develop thermotolerance to protect themselves from later exposure to heat shock or other stresses. Once a cell has been exposed to the heat-shock, the fate of the cell depends on the amount of protein denaturation and aggregation. The cell's ability to become tolerant of heat after the heat-shock is one of the most widely studied activating responses experimentally. In the paper, we develop three mechanistic models in a hierarchical fashion that follow a biological pathway from the initial heat-shock to the HSP70:HSF complex that dissociates to HSP70 and HSF, and aim to predict the HSP concentration in a cell. These models allow investigators to simulate the major/important mechanisms in the upregulation of HSP70. This study was performed to create a depiction of the cellular and nuclear events of the HSP70 response to the heat-shock. The data generated from the model were correlated to favorably experimental data under the assumption of time delay.

3285-Pos

Tuning Protein Dynamics by Modulating Solvent Motions through Glassy Matrices, Sol-Gels and Reverse Micelles

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Many proteins can be viewed as nanodevices whose functions are controlled by a complex array of molecular motions. Functionally important protein dynamics pose a difficult challenge for study in that they span orders of magnitude with respect to amplitudes and time scales. One approach to organizing and systematically studying protein dynamics is based on the degree to which the different protein motions are slaved to different categories of solvent motion. This approach is the basis for the solvent slaving model of Frauenfelder and coworkers and our protein dynamic state model. In the present work, we describe how sol-gels, trehalose-derived glasses and reverse micelles can be used as matrices to systematically tune these different categories of protein dynamics through modulation of solvent dynamics and confinement volume. Through this approach we can dissect out the factors that control the activation energies and onset of activation for dynamics in hemeproteins such as hemoglobin and myoglobin that control substrate/ligand diffusion within the protein as well as escape from or entry into the protein from the solvent.

3286-Pos

Structural and Dynamical Behavior of the HIV-1 Nucleocapsid Protein by Molecular Dynamics and Quantum Mechanical Simulations

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The human immunodeficiency virus-type 1 (HIV-1) nucleocapsid (NC) protein is critical for the recognition and packaging of the viral genome and has been a potential target for the development of antiviral drugs. All retroviral NC proteins contain one or two copies of an invariant array of CCHC ($Cys\text{-}X_2\text{-}Cys\text{-}X_4\text{-}His\text{-}X_5\text{-}Cys$) that are crucial for RNA genome recognition, packaging, and infectivity and have been proposed to function as zinc-binding domains. Although the solution structures of the NC protein with Zinc binding have been determined by experimental studies, the physiological relevance of zinc coordination has not been revealed. Here, we present the structural characteristics of the NCp7 protein with Zn and without Zn ions to investigate the coordination effect in the structural transitions upon inhibitory process by potential drugs by using molecular dynamics simulations with explicit water. For the elucidation of the structural and dynamic properties of the Zn-ligand binding domain in NCp7 protein, quantum mechanical calculations were executed to properly develop the force field for Zn. Upon ligand binding to NCp7 protein, correlation between experimental and computed dissociation constants will be discussed with the mechanistic variations for the ligand-binding and inhibition of NCp7 protein.

3287-Pos

Bio-Mechanical and Binding Effects in Cleavage of Von Willebrand Factor A2 Domain by Adamts13: Molecular Dynamics, Quantum Mechanics and Force Distribution

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ADAMTS13 is a plasma zinc metalloproteinase crucial for primary haemostasis, the enzymatic activity of which is biomechanically controlled. Shear stress-induced partial unfolding of its substrate, the von Willebrand factor (vWF) A2 domain, is essential for efficient cleavage of the target 1605Y-1606M peptide bond. However, the mechanisms of stress-activated vWF A2 recognition and cleavage by ADAMTS13 have yet to be fully resolved.

From our newly developed Molecular Dynamics (MD) based force distribution analysis, we find force to propagate in the cleavage-ready vWF A2 unfolding intermediate such that the target peptide bond is selectively pre-stressed and

thus putatively weakened for cleavage by ADAMTS13. MD simulations of the ADAMTS13-vWF A2 complex allowed us to identify residues adjacent to the cleaved peptide bond that are relevant for efficient A2 binding. Subsequent QM calculations using semi-empirical PM6-DH method provided relative binding energies in good agreement with experiments. The force-dependent hydrolysis of the Y-M peptide bond was explored by QM and hybrid QM/MM calculations. Mechanical stretching, induced by a shear stress, facilitates the cleavage reaction. Finally, the enzyme reduces the entropic cost of substrate binding by increasing fast-scale dynamics in the regions distant from the catalytic site (e.g. disintegrin domain).
Overall, our study reveals the subtle details of ADAMTS13 biomechanical function.

3288-Pos
Mapping the Proton Conduction Pathways in the Inner Membrane Multi-Drug Translocase AcrB
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With increasing frequency the emergence of new drug resistances constitutes a serious problem in cancer therapy or the treatment of bacterial infections. A major mechanism bacteria employ against antibiotics is based on multidrug efflux pumps extruding the drugs before they can reach their targets. In Escherichia coli the major efflux system comprises the inner-membrane translocase AcrB, the membrane-fusion protein AcrA and the outer-membrane channel TolC. AcrB functions as the engine of this complex, using proton motive force to expel a wide variety of unrelated toxic compounds such as antibiotics, disinfectants or detergents. The molecular details of how proton conduction through AcrB is coupled to drug expulsion are not fully understood yet. To gain insight into the AcrB proton conduction pathway we performed a series of 5 independent molecular dynamics simulations of AcrB in a phospholipid/water environment at a 150mMol NaCl concentration. Each monomer was considered in a different protonation state as suggested in (1), and in each run the system was simulated for at least 50 ns, using GROMACS 4.0.3 and the GROMOS96 53a6 forcefield. We report three possible proton conduction pathways through the trans-membrane domain. The pathways were identified based on the dynamics of protein-internal water molecules and monitoring their frequency of hydrogen bond formation to adjacent residues. That way we also determined residues likely involved in AcrB's hydrogen bonded network. Each residue was further characterized by its specific hydrogen bond frequency to protein-internal water. Additionally a new method was applied to analyze AcrB-internal cavities and transport tunnels in each monomer's porter domain.

3289-Pos
Application of an Inter-Protein Coarse-Grained Force Field to Binding Process of Actomyosin
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Protein-protein interaction is important for many biological processes. Especially, when molecular motors fulfill their functions, they sometimes form multi-subunit complex or move through alternate binding and unbinding processes. Therefore, it is important to model inter-protein interaction appropriately in order to reveal working mechanism of molecular motors. Coarse-grained model is often used to analyze working mechanism of molecular motors for computational efficiency, but modeling of inter-protein interaction sometimes becomes difficult problem. In this study, we apply a sequence dependent inter-protein coarse-grained force field (each amino acid is coarse-grained to one bead) that considers electrostatic interaction between charged residues and sequence dependent contact interaction [Kim and Hummer, JMB (2008)] to a molecular motor, actomyosin. Myosin is known to take detached, weakly bound and strongly bound state to its rail protein, actin, during ATP hydrolysis cycle, and the force is thought to be generated during the weakly-to-strongly transition. These binding states should be coupled to nucleotide dependent conformational changes of myosin such as open-close motion of the actin-binding cleft and rearrangement of surface loops like loop II. We will discuss the effect of the myosin conformational changes on the binding process.

3290-Pos
Fluctuation Theorem Applied to F1-ATPase
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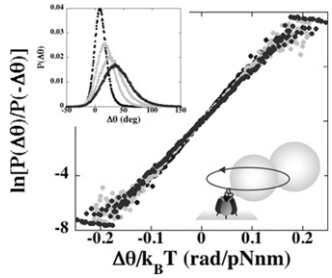
The fluctuation theorem (FT), which is one of fluctuation theories based on non-equilibrium statistical mechanics and represents the property of an entropy production in a small system, was experimentally verified in a motor protein F_1-ATPase (F_1). The theorem has been applied to several experimental systems such as colloidal particle systems and RNA hairpins. Those systems were in non-equilibrium when the operations were added to the systems and the entropy productions were measured for those non-equilibrium processes. Unlike those systems, F_1 is an autonomously non-equilibrium system in which the rotor γ subunit rotates in the stator $\alpha_3\beta_3$ ring upon ATP hydrolysis. Can FT be applied to such an autonomous system? Noting that the entropy production of the probe visualizing the rotation of F_1 is a product of the rotary torque and the angular velocity, we introduced the representation of FT appropriate for the torque measurement. The torque measured through our method was compared with that measured conventionally. In addition to the verification, we applied the theorem to a mutant F_1 and another motor protein V_1. The applicability of FT should be expanded to the wide range of biological systems *in vitro* and *in vivo*.



Protein Folding & Stability: Computational Approaches

3291-Pos
Computational Search of Evidence for the Division of Amino Acids into Transitional Groups in a Virtual State Protein. Phase I: Equilibrium Fluctuations
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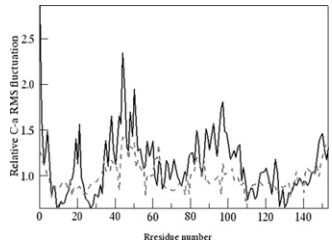
Core-Shell model postulates that beyond certain resolution, non-equilibrium states are virtual, non-testable as proteinous, because they are not ''domesticated'' by evolution. We propose that during (un)folding, the {Cα, Cβ, Cγ, Cδ, Cε}-backbone of residue does an elementary move, transverse to peptide backbone. Sliding mechanism assigns amino acids into three transition groups based on residue stereochemistry. According to model, this division is masked in folded conformation. Search of evidence for such division begins with study of fluctuation of folded molecule, using united-residue protein model of sperm whale myoglobin to simulate Monte Carlo conformational trajectories. For alpha-carbon fluctuations along polypeptide chain, reasonable qualitative agreement between simulated and crystallographic B-factor (PDB ID: 108M) profiles is reached. Nearly equal amount of average rms-fluctuation contribution is found for T-groups: T1 (1.13 ± 0.12Å); T2 (1.17 ± 0.14Å); T3 (1.18 ± 0.09Å); with 1.13 ± 0.11Å being for the whole molecule. Model predicts constant-rate built-up of rms-fluctuation amounts as protein unfolds. Myoglobin has high symmetry of architecture and unusual sextet of amino acid pairs. For comparison, their rms-fluctuation amounts are:
[Trp(2),Asn(2)] (1.38 ± 0.57Å);
[Met(3),Tyr(3)] (0.85 ± 0.00Å);
[Pro(4),Arg(4)] (1.25 ± 0.14Å);
[Gln(5),Thr(5)] (1.14 ± 0.09Å);
[Ser(6),Asp(6)] (1.25 ± 0.18Å);
[Phe(7),Val(7)] (1.08 ± 0.12Å).



3292-Pos
Multiscale Modeling and Design of Molecular Conformational States
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Some of the emerging goals in modern medicine are to uncover the molecular origins of human diseases, and ultimately contribute to the development of new therapeutic strategies to rationally abate disease. Of immediate interests are the roles of molecular conformational ensembles and dynamics in certain cellular processes leading to human diseases and the ability to rationally manipulate these processes. We developed a multiscale approach, which utilizes rapid Discrete Molecular Dynamics (DMD) simulations. We demonstrate that by using this approach we can predict protein structure, conformational ensembles of the unfolded protein states, and uncover the folding kinetics of biological molecules. Furthermore, using computation and experiment, we demonstrate that by using computational molecular design we can manipulate these states. We will describe several recent studies that demonstrate our multiscale modeling and design approach.