communication include analysis of energy landscapes and network models. These models are not independent and molecular dynamics (MD) can provide knowledge of both. In this study, thermally populated substates on the free energy surface of MutS proteins are defined using all-atom MD simulations and principle component analysis (PCA). Our investigation reveals that DNA binding facilitates both, adjustment of thermal populations and major reshaping of the surface. Analysis of the collective atomic fluctuations within the protein framework of MutS establishes possible allosteric networks, which are highly dependent on the substate.

2936-Pos

Ovine Prion Polymorphisms Investigated by Threading to a Model Left Handed Beta Helical Structure using Molecular Dynamics Simlation

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We use AMBER all atom molecular dynamics (MD) to assess the stability of a model of the prion protein in its disease-causing conformation, PrPsc. The model is based upon threading the ovine prion sequence onto a template left-handed beta-helical (LHBH) structure with 18 residues per turn. Five polymorphisms in the sheep prion protein, VRQ, ARQ, ARH, AHQ, and ARR, have been identified at residues 136, 154, and 171 respectively, which are roughly 18 amino acids apart which thus align approximately on the LHBH. Threading of the sequence was thus done with an emphasis on the locations of these special sites as a means to investigate their possible role in disease susceptibility as well as investigating the overall viability of the LHBH as a structural candidate for PrPsc. In comparison to known left handed beta-helical proteins, the resulting model for VRQ is shown in all atom MD to 10 ns to exhibit similar stability as indicated by a low root mean square deviation, the presence of substantial side-chain to side-chain hydrogen bonding, and volume packing fraction. Interestingly, and in corroboration with experimental data that it is a disease resistant variant, the same model for ARR exhibits much less stability. Each polymorphic site was also investigated individually by comparing results from models with only one site different and showed a good correlation to experimental data regarding the relation of the variants to disease susceptibility.

2937-Pos

The Functional Role of Membrane Bound Proteinase 3

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Proteinase3 (Pr3) is a serine protease of the neutrophils involved in inflammation processes. Its membrane expression is a risk factor for chronic inflammatory diseases such as vasculitis or emphysema. A recent study demonstrated that Pr3 is co-localized with scramblase at the membrane and might thus be related to the externalization of phosphoserine-lipids in apoptotic cells.

Biophysical data shows a significant hydrophobic contribution to the binding of Pr3 to lipid bilayers, which is not observed for its close homolog the Human Neutrophil Elastase (HNE).

Here, we applied all-atom MD simulations to study the interactions of Pr3 and HNE with equimolar mixtures of DMPC with DMPG, DMPA and DMPS. Pr3 and HNE were introduced into previously calibrated lipid bilayers. These all-atom models enabled us to identify hydrophobic interactions of Pr3 with the lipid tails, which we did not observe for HNE. Further, we identified charge pairing of specific basic residues of Pr3 with DMPS lipids, not found for bilayers containing DMPG or DMPA. Although the substrate specificity of soluble Pr3 has been extensively studied, the influence of the membrane on its enzymatic activity is still a matter of debate. We docked peptides onto our models of membrane-bound Pr3 (mPr3). A thorough comparison of the peptide-protein interactions of mPr3 and soluble Pr3 revealed the changes in Pr3 substrate specificity induced by the membrane.

In conclusion, our MD simulations revealed the atomic details of the membrane binding of Pr3, and especially its strong affinity to phosphoserine-lipids. This allows us to propose a hypothesis on the role of Pr3 in apoptosis of neutrophils. In addition, this study contributes to the understanding of the enzymatic activity and substrate specificity of mPr3.

2938-Pos

Characterization of Electron Density Profiles and Area Per Lipid from MD Simulation of Large Undulating Bilayers

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Determination of the electron density profile (EDP) and area per lipid (A_L) from MD simulations of large lipid bilayers requires the characterization and isolation of the mesoscopic undulation dynamics. Typically, for small, flat bilayer patches, EDPs are calculated assuming a uniform bilayer normal across the trajectory. When common EDP algorithms are applied to larger systems, the undulations convolve an averaging function with the underlying "flat patch" EDP, introducing artifacts into the profile. It is necessary to decouple these undulation modes from both the protrusion and peristaltic dynamics in order to characterize an accurate EDP. We apply a 2-dimensional low-pass spatial filter, with frequency response optimized to a characteristic wave-number, q0 (as determined by Lindahl and Edholm), to define a mid-plane reference position for every atom. We present two approximations for the local curvature that are necessary when referencing this new surface. Results of both methods are in good agreement with the "flat patch" EDP. Common approaches for determining a simulated A_L underestimate the true A_L by not accounting for the out of plane components. As an alternative, we have developed two separate A_L schemes, both of which produce an increase in A_L of 1-2 $Å^2$ over the projected *xy-area*.

2939-Pos

A Coarse Grained Molecular Dynamics Study of the Formation and Structure of Bicelles

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Our goal is to study interactions between bicelles and membrane associated proteins by computer simulations. In this preliminary study we have tested different representations of the bicelle lipids within the MARTINI coarse grain (CG) model. Bicelles are lipid disk-shaped systems with properties that are believed to resemble those of a native lipid bilayer. This has made them useful for studies of membrane associated proteins by a range of biophysical techniques, such as circular dichroism, liquid and solid state NMR and in diffraction studies. Bicelles normally consist of two different kinds of lipids, one with short hydrophobic tails and one with longer tails. The structure and composition of a bicelle depends on both the temperature and the ratio between the long and short tailed lipids, also known as the q-factor. The results from the CG molecular dynamics (MD) simulations are compared to NMR data, and by that revealing that this method can account for the experimental observations made of bicelle structures. Furthermore, reversed CG united atom MD simulations using the GROMOS96 force field indicate that the bicelles formed are stable on the time scale simulated. The results from the CG MD of bicelles also underline the importance of choosing a proper mapping of atoms to the CG beads.

2940-Pos

Coarse-Grained Molecular Dynamics Simulations of Pegylated Lipids

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Polyethylene oxide (PEO) and polyethylene glycol (PEG) are polymers with the subunit C-O-C. Due to their low toxicity and high solubility in water, they have been conjugated (or PEGylated) to the drug transporters such as vesicles and micelles. Experimental results have shown the phase behavior of lipid/PEG-lipid mixtures and characterized their sizes using a disk model with apparent hydrodynamic radius. In this work, we perform coarse-grained molecular dynamics simulations of self-assemblies of a mixture of lipids and PEGylated lipids. Simulations with various concentrations of PEGylated lipids lead to formation of liposomes, bicelles, and micelles, and their sizes were characterized. Phase diagrams show dependence of PEG concentration, length, and temperature.

2941-Pos

Improving Internal Peptide Dynamics in the Coarse-Grained Martini Model: Application to Amyloid Peptides

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The assembly of misfolded protein aggregates into amyloid fibrils is at the heart of the development of neurodegenerative disorders such as Alzheimer's disease and prion diseases [1, 2]. Despite its significance, the driving forces behind the aggregation of peptides and protein misfolding are not well understood. To gain molecular insight into the aggregation of amyloid peptides, we carry out computer simulations using the recently developed MARTINI coarse-grained (CG) model [3]. Compared to the more traditional atomistic simulations, CG models offer the possibility of following protein folding events, which typically occur on the millisecond timescale. The current MARTINI model, in particular, is able to reproduce a wide range of lipid properties as well as lipid-protein interactions for rigid proteins. Protein folding and aggregation however often involves significant transitions between secondary structures and hence requires that the proteins be flexible during the simulations. We will present recent

advances on our extension of the MARTINI model to more accurately describe the internal flexibility of peptides and small proteins. The model is applied to simulations of amyloid peptides of different lengths in water. Its performance is assessed by comparing the distributions of various structural properties with their counterparts from atomistic simulations.

References:
1. Lührs, T. et al., Proc. Natl. Acad. Sci. USA, 102, 17342-17347 (2005)
2. Nelson, R. et al., Nature, 435, 773-778 (2005)
3. Monticelli, L. et al., J. Chem. Theory and Comput., 4, 819-834 (2008)

2942-Pos

Biophysics of Transmembrane Pores - Interactions by Coarse-Grained Molecular Dynamics Simulation

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The hydrophobic thickness of lipid bilayers has been shown to influence the biological activity of transmembrane (TM) pores which are of central importance to the biology of cells and to a number of nanotechnological applications. Here we report on a systematic exploration of protein pores and their interactions with lipid bilayers via coarse-grained molecular dynamics (CG-MD) simulation. Until recently computational studies on these interactions have focused on simplified models. To extend to a wider range of more biologically representative models of TM pores and their interactions with lipid bilayers, CG-MD simulations were employed to initially study a set of 72 pore-lipid bilayer systems. Both main structural classes of membrane proteins (alpha-helical and beta-barrel) were represented by the eight pores investigated and the nine bilayer systems (phosphate-phosphate distances: 2.8 - 5.3 nm) sample a wide range of local hydrophobic mismatch conditions. Lipid bilayer perturbation due to pore insertion, the dependence between hydrophobic mismatch and the observed pore tilt angle, and the local de-mixing of lipid types around a pore in mixed-lipid bilayers were all analysed. The local lipid bilayer perturbation caused by the inserted pores suggests possible mechanisms for both lipid bilayer-induced protein clustering and protein-induced lipid de-mixing - both driven by the hydrophobic mismatch. This has been further investigated by a series of CG-MD simulations of multiple TM pores in large planar lipid bilayer patches. To study the impact of membrane curvature on protein-lipid interactions, analogous simulations with vesicles (diameter: 31 nm) are currently being conducted.

2943-Pos

Hybrid Simulations: Atomistic Peptides in Coarse Grained Solvent

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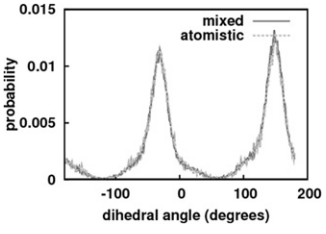
Here we present a multiscale simulation approach to study aggregation of small peptides. We develop a protocol that uses atomistic trajectories and the Iterative Boltzmann method to construct a mixed representation of the peptide/solvent system, in which the peptide is described with the united atom GROMOS forcefield and the solvent is described with effective potentials derived from atomistic radial distribution functions. For a test system composed of di-alanine peptide and octane as solvent, we show that the peptide structural properties (see figure) as well as solvation free energy obtained in the hybrid approach matches results obtained from reference atomistic simulations. Next, the model is used to calculate the potential of mean force between two peptides to evaluate how the reduced representation of the solvent influences solute aggregation properties. We show that accurate reproduction of the octane and di-alanine solvation free energy as well as careful choice of the degree of coarse-graining of the peptide-solvent interactions are important to obtain a realistic potential of mean force. Finally aggregation of many peptides is studied with this multiscale protocol. The future applications include aggregation and folding of atomistic peptides and proteins in coarse grained bilayers.



2944-Pos

Discrete Molecular Dynamics as a Tool to Test Soluble Protein Models

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Discrete molecular dynamics (DMD) has been applied in many areas of protein folding and aggregation because relatively long time scales can be simulated. Coarse-graining and implicit solvent models have been implemented to maximize its efficiency. However, there has been no rigorous evaluation with DMD on experimentally known structures. Here, we ran DMD with four-bead peptide model on X-ray structure of a human serum retinol-binding protein and NMR structure of a regulator of G-Protein signaling, and compared the results to those of simulations by NAMD (all atom molecular dynamics with explicit water). DMD showed larger root mean squared deviation (RMSD) from the starting conformation compared to NAMD though tertiary structures were mostly maintained. We developed a new implicit solvent model for DMD based on Miyazawa-Jernigan interaction pair potential that has advantage of unambiguous implementation to address dependencies in implicit solvent models. Results obtained with the Miyazawa-Jernigan implicit solvent model were comparable to those obtained with a previously used implicit solvent model based on Kyte-Doolittle hydropathy scale. We ran DMD and NAMD on incorrectly folded models. Structures of the incorrectly folded models were very poorly preserved during the DMD simulations. Both methods were able to distinguish between the correct and incorrect structures based on differences in the magnitudes of the RMSD from the starting conformation. Hence, we suggest that DMD may be useful as a tool to test soluble protein models.

2945-Pos

Determining the Molecular Basis of Disease in Single Nucleotide Polymorphism Variants using Wavelet Analysis

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Single nucleotide polymorphisms (SNPs) are the bases of a wide range of diseases and disorders, yet 3D structures and experiments often show little or no clear difference between variants, shedding little light on how a SNP leads to disease. Molecular dynamics (MD) simulations, in contrast, have shown differences between variants, but these are often very subtle and difficult to identify. Wavelet analysis is a data mining technique that has shown promise in determining the often subtle events that occur during an MD simulation. Here, we apply wavelet analysis to MD simulations of the variants of several SNPs and show how the technique can be used to isolate differences between the variants that are otherwise extremely elusive. We demonstrate that wavelet analysis can be especially useful in proteins with several mutations and show it to be a valuable technique for understanding the molecular basis of such diseases.

2946-Pos

Brownian Dynamics Simulation of FCS Measurements on Single Fluorophore-Labeled Superhelical DNA

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We investigated the dynamics of a single-fluorophore-labeled pUC18 plasmid through a Brownian dynamics algorithm, followed up by a simulation of the Fluorescence Correlation Spectroscopy (FCS) process. Recent experimental FCS measurements indicated a sensitivity of the monomer mean square displacements in DNA circles towards superhelicity. Simulations with homogeneous DNA elasticity and local straight equilibrium are not sufficient to reproduce this observed behavior. But inserting permanently bent sequences into the DNA, which favor end loop formation, caused a dependence of the calculated FCS correlation curves on superhelical density. Furthermore, our simulations allow us to take into account the orientation of the fluorophore in polarized excitation, which might explain the observed appearance of a Rouse-like regime at intermediate time scales.

2947-Pos

Investigation of Fluorescent DYE-DNA Interactions using Multidimensional Adaptive Umbrella Sampling Simulations

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Fluorescence resonance energy transfer is often used as a "molecular ruler" to measure distances between fluorescent dyes attached to a biomolecule. This technique relies on the assumption that these dyes rotate freely around the linkers that attach them to the molecule. In the case of Cy3 attached to the 5' end of DNA, it is known from NMR studies that the dye stacks on top of the first base pair, but recent fluorescence anisotropy studies show that there is still some rotation about the linker on short time scales. To explore the dynamics of this system more fully, we performed atomistic molecular dynamics simulations using GAMUS, a recently developed multidimensional adaptive umbrella sampling method. Using the method, the free energy basins of the DNA/Cy3 system were characterized in terms of five dihedral angles along the linker between Cy3 and the DNA.