model of LDL, in which the core CE molecules arranged in stacks with their sterol moieties side-by-side in the higher density regions and the fatty acyl chains extending from either side. These stacks of acyl chains in the CE core are directed outward towards the amphipathic beta-sheet domains on the top and bottom faces of the particle and are surrounded by a semicircle of flexible amphipathic alpha-helix rich domains, which is important to maintaining the structural integrity, and thus functionality, of normal LDL.

1985-Pos
Statistical Analysis and Deblurring of Class Averages in Single-Particle Electron Microscopy
Wooram Park[1], Dean R. Madden[2], Daniel N. Rockmore[3], Gregory S. Chirikjian[1].
[1]Department of Mechanical Engineering, Johns Hopkins University, Baltimore, MD, USA, [2]Department of Biochemistry, Dartmouth Medical School, Hanover, NH, USA, [3]Departments of Mathematics and Computer Science, Dartmouth College, Hanover, NH, USA.
In single-particle electron microscopy, the electron dose is limited to avoid damaging the specimen. This results in images with low signal-to-noise ratios (SNR). Class averaging techniques are used to enhance the low-SNR electron micrograph images. A class is defined as a collection of projection images taken along nominally identical projection directions. The images in each class are aligned and averaged in order to cancel or reduce the background noise. The class-averaged images with high-SNR can be used for more accurate three-dimensional reconstruction in single-particle electron microscopy. However, errors in the alignment process are inevitable due to noise in electron micrographs. This error results in blurry averaged images. Using the mean and variance of the background noise that is assumed to be Gaussian, we derive equations for the mean and variance of translational and rotational misalignments in the class averaging process. Furthermore, the blurring function representing the distribution of the misalignments is estimated using a Gaussian with the computed mean and variance of the misalignments. The blurring process in class averaging is formulated as convolution of an underlying clear image with the blurring function. We propose a deconvolution method to estimate the underlying image using the Fourier analysis in the appropriate domain. This deconvolution method is applied to artificial and experimental electron micrographs. The deburred class averages are assessed quantitatively and qualitatively.
This work was supported by NIH Grant R01GM075310 ''Group-Theoretic Methods in Protein Structure Determination.''

1986-Pos
Determining Orientation in Cryoem Single Particle Analysis
Regis A. James, Steven J. Ludtke.
Baylor College of Medicine, Houston, TX, USA.
In CryoEM single particle analysis, images are recorded of individual molecules or macromolecular assemblies in the 10-100 nm size range embedded in vitreous ice. These images approximately represent a projection of the electron density of the specimen. Due to dose limitations, these images are extremely noisy, with spectral signal-to-noise ratios generally peaking at less than 1. To perform a 3D reconstruction from such images, the orientations of all of the thousands of particle images must be accurately determined. The most common strategy for accomplishing this task is iterative projection matching, meaning that the accuracy and resolution of the structure are limited by the similarity metric used to assess the similarity of each particle image vs. a set of projection references. A wide range of metrics has been used for this purpose, such as correlation coefficient, phase residual and Fourier ring correlation, with variants in application of each. Each of these methods represent a tradeoff in sharpening the orientation vs. decreasing the probability of making a noise-based, rather than a data-based decision. We present a thorough comparison of a number of different similarity metrics when applied to particles with varying noise levels and symmetries.

1987-Pos
High-Throughput, High-Resolution Cryoem Structural Analysis of Helical Assemblies of Biological Macromolecules Toward Atomic Resolution
Takashi Fujii, Takayuki Kato, Keiichi Namba.
Graduate School of Frontier Biosciences, Osaka University, Suita, Japan.
We report structures of helical assemblies of biological macromolecules at near atomic resolution obtained by electron cryomicroscopy. These structural analyses were completed within a week. One of the factors that enabled such high-throughput, high-resolution analyses is the use of a CCD detector instead of film. Since the modulation transfer functions of CCD detectors are significantly worse than those of films, high-resolution image data are too poor to attain atomic resolution if conventional magnifications are used. The resolution of 3D image reconstructions from data collected at a magnification of 88000 was limited to ~7 Å. However, the higher magnification of 170000 solved this problem. We have reached 3.8 Å resolution for the stacked disk aggregate of TMV coat protein. The density map clearly shows the main chain and large side chains. The only disadvantage with high-magnification imaging is a small image area of CCD, making the data collection efficiency lower. However, CCD imaging is still fast enough to allow high-throughput analysis. The other factors include the thickness of vitreous ice film embedding specimen particles and the specimen temperature, both of which affect image contrast. We improved the quick-freezing method to optimize ice thickness. We also found that images recorded at ~50 K have ~1.6 times higher contrast than those at 4 K. By improving these factors in further technical development, we believe we should be able to achieve atomic resolution within a week from data collection to image analysis.

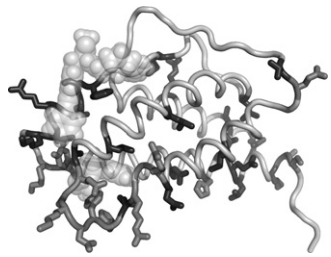
1988-Pos
SONICC: A Novel Nonlinear Optical Detection Technique for 2D Celluar Crystallography
Fei Guo, Ellen Gualtieri, Garth Simpson, Wen Jiang.
Purdue University, West Lafayette, IN, USA.
2D crystallization is one of the most powerful methods to study the structure and function of membrane proteins in their native lipid bilayer environment, yet obtaining high resolution diffraction protein crystals is the most difficult and time consuming step. Traditional techniques for protein crystal detection mostly depend on optical microscopy and the birefringence property of crystals. This situation becomes even more difficult for 2D crystallization because a negative stain check on electron microscope is required which makes it nearly impossible for high-throughput screening. Recently, UV spectroscopy has also been applied to distinguish protein crystals from salt ones. However, all these methods has detection limit which depends on the crystal size and crystallization condition. The Second Order Nonlinear Imaging of Chiral Crystals (SONICC) therefore is developed to overcome this difficulty. SONICC could selectively detect large or small non-centrosymmetric 2D and 3D protein crystals (<1um) with high signal noise ratio. Using this method, we successfully detected purple membrane (ie 2-D crystal of bacteriohrodopsin). We could even detect the crystal patch in a single living H. Salinarium R1 cell. By removing retinals from purple membrane, the signal dramatically decreased (~5 fold) due to the distortion of the crystalline order and the absence of pigment. Considering the difficulty of growing 2D membrane crystals, SONICC not only solved the current detection limitation, but also provided a new opportunity for direct detection membrane crystals in situ formed naturally or artificially by overexpression.

Molecular Dynamics I

1989-Pos
Membrane Diffusion of Tethered DPPC and Tethered PIP3-Bound Protein Systems
Michael G. Lerner, Richard W. Pastor.
NIH/NHLBI, Rockville, MD, USA.
We have used molecular dynamics simulations to investigate the diffusion of tethered proteins in lipid bilayers. Coarse-grained (CG) models of DPPC dimers were simulated in a DPPC bilayer with the MARTINI model, and single-lipid diffusion constants compared to those obtained for dimers at various tether lengths. The ratio of diffusion constants matches well with both experimental results and theoretical predictions of a simple bead model. CG models of pleckstrin homology domain (PH) bound to a lipid with a PIP_3 (phosphatidylinositol (3,4,5)-trisphosphate) head group were then constructed and compared for the monomer, tethered dimer, and tethered trimer cases.

1990-Pos
Quantifying Correlations Between Allosteric Sites in Thermodynamic Ensembles
Christopher L. McClendon[1], Gregory Friedland[2], Matthew P. Jacobson[1].
[1]University of California San Francisco, San Francisco, CA, USA, [2]Sandia National Laboratories, Joint Bioenergy Institute, Emeryville, CA, USA.
Allostery describes altered protein function at one site due to a perturbation at another site. One mechanism of allostery involves correlated motions, which can occur even in the absence of substantial conformational change. We present a novel method, ''MutInf'', to identify statistically significant correlated motions from equilibrium molecular dynamics simulations. We quantify correlated motions using a mutual information metric, which we extend to incorporate data from multiple short simulations and to filter out correlations that are not statistically significant. Applying our approach to uncover mechanisms of allostery in human interleukin-2 and other proteins, we identify clusters of correlated residues from 50 ns of molecular dynamics simulations (see figure). In

interleukin-2, two of the clusters with the strongest correlations highlight known cooperative small-molecule binding sites and show substantial correlations between these sites. We also present a newer approach based on the Kullback-Leibler divergence, an information-theory metric that quantifies population shifts and perturbations to the free energy landscape. Since these approaches identify pairs of residues with correlated conformations in an unbiased, statistically robust manner, they should be useful tools for finding novel or ''orphan'' allosteric sites in proteins of biological and therapeutic importance.



1991-Pos

Influence of Organic Solvents on the Structure and Enzymatic Activity of Haloalkane Dehalogenase DhaA

Morteza Khabiri.
Institute of systems bioliogy and ecology, Nove Hrady, Czech Republic.

Organisms with the ability to degrade anthropogenic chemicals are using for this purpose haloalkane dehalogenases. Hereby, a active site nucleophile attacks a carbon atom of the halogenated substrate, leading to cleavage of the carbon-halogen bond, displacement of a halide and formation of a covalent alkyl-enzyme intermediate that is subsequently hydrolyzed. The haloalkane dehalogenase DhaA from Rhodococcus rhodochrous NCIMB13064 is enzymatically active for a broad range of halogenated substrates including 1,2,3-trichloropropane (TCP) (2) and sulphur mustard (3), some of them with high hydrophobicity and low solubility in water. Improving the solubility by addition of water miscible co-solvents like dimethyl sulfoxide (DMSO) to the reaction mixture could be a way to enhance the range of potential applications for these enzymes. Therefore, in the present study we investigate effects of DMSO on structure and dynamics of DhaA using molecular dynamics (MD) simulations demonstrating that the DMSO molecules penetrate to the active site of protein and compete with the halogentaed substrate for the catalytic site histidine272. On the other hand, histidine 272 traps DMSO and thus prevents further progress of DMSO deeper into the active site. With respect to protein solubility, interactions of DMSO with the protein surface decrease the solvation energy of the protein compared to protein in pure water. However, the enzyme is clearly stable in up to 42% DMSO and we can conclude that the enzyme activity is expected to be less than in pure water, however retained.

1992-Pos

Characterizing Structure and Activity of Subtilisin Enzyme in Nonaqueous Media with Molecular Dynamics Simulations

Eugene Auh, Sihyun Ham.
Sookmyung Women's University, Seoul, Republic of Korea.

Structural and dynamical behaviors of enzyme are critical for determining its catalytic activity. Knowing that the enzyme activity may vary with different solvent polarity due to the changes in enzyme structure and flexibility, Subtilisin Carlsberg enzyme was subjected to investigate the structural variation upon different solvent systems by using molecular dynamics simulation with explicit solvents. Characterization of the structure and activity focusing on the hydration of the active site will be discussed with the effects of crystallographic water bound to the active site of this enzyme and with the solvent effects.

1993-Pos

Influence of dsDNA Architecture on Diffusion Properties in Networks

Renat N. Khaliullin[1], **Jay D. Schieber**[1], Jorge A. Iniguez-Lluhi[2].
[1]Illinois Institute of Technology, Chicago, IL, USA, [2]University of Michigan Medical School, Ann Arbor, MI, USA.

DNA is an essential element for genetic disease treatments. Its application depends on the diffusive properties of DNA through tissues. Although, there are works on linear DNA diffusion in a network environment, the dependence of the diffusion coefficient on chain architecture is not completely understood.
In this work we study dsDNA molecule behavior in a network with the discrete slip-link model. We show dependence of the diffusion coefficient on the chain molecular weight and network mesh size. We compare theoretical predictions with experimental measurements of linear dsDNA thermal diffusion in agarose gels. To analyze the influence of the architecture of the DNA molecule on its diffusion properties, the theory can be applied to star-shaped molecules. However, to obtain experimental data it requires to synthesize a well characterized star-shaped dsDNA molecule.

1994-Pos

Electric Field Effects on Water and Water-Vacuum Interfaces in Molecular Dynamics Simulations

Jane HyoJin Lee[1], Zachary A. Levine[2,3], P. Thomas Vernier[4,3], Mayya Tokman[1], Michael E. Colvin[1].
[1]School of Natural Sciences, University of California, Merced, Merced, CA, USA, [2]Department of Physics and Astronomy, University of Southern California, Los Angeles, CA, USA, [3]MOSIS, Information Sciences Institute, Viterbi School of Engineering, University of Southern California, Marina del Rey, CA, USA, [4]Ming Hsieh Department of Electrical Engineering, Viterbi School of Engineering, University of Southern California, Los Angeles, CA, USA.

Understanding the behavior of water in atomic-scale detail is essential to explaining the microscopic dynamics of such phenomena as electroporation, electrospinning, electrospraying, and electric-field-driven evaporation. In this study we employ molecular dynamics simulations to investigate water-vacuum systems under the influence of an externally imposed electric field. SPC and SPC/E water models are used to describe nanodroplets and interlaced water-vacuum slab configurations. The dynamics of these systems is studied in detail with respect to the strength of the applied electric field, and we note the importance of a proper representation of long-range electrostatics in the simulations. Water behavior is analyzed from both structural and energetic points of view. We discuss how such characteristics as the system geometry, local water density, and water molecular dipole orientation change as the electric field is increased. These changes are described in the context of interplay between pressure, surface tension, and electrodynamic dipole-field and dipole-dipole interactions. For example, we find that for nanodroplets containing ~900 water molecules there is a critical electric field strength above which there is a jump in the alignment between the water dipole and the field. Concomitant with the dipole alignment is a distortion of the droplet shape from a sphere to a prolate ellipsoid oriented along the electric field. We also study formation of small-scale structures at the water-vacuum interface in both droplet and slabs configurations and investigate the relationship between these structures and a subsequent creation of pore-like bridges between the water slabs in the water-vacuum-water systems.

1995-Pos

A Molecular Dynamics Simulation Study of the 9_25-11 Dnazyme

Walter R. Scott, Jason Thomas, David M. Perrin.
University of British Columbia, Vancoiver, BC, Canada.

DNAzymes (catalytic DNA) have recently attracted increased research interest with an eye towards applications as therapeutic agents and biosensors, among others. Advantages over proteins include an increased resistance to hydrolysis and cost-effective production.
Most DNAzymes recruit divalent metal ions as cofactors, however D.M. Perrin and coworkers have recently synthesised a M2+-independent, multiple turnover DNAzyme (Dz9(25)-11 by the inclusion of two kinds of modified nucleotides (8-histaminyl-dA and aminoallyl-dU in place of dA and dT, respectively) which afford enhanced catalytic rates that are attributed to the roles of electrostatic (cationic amine) catalysis as well as both general base and general acid catalysis (imidazoles). In this regard, Dz9(25)-11 functions as a sequence specific RNaseA mimic.
The use of DNAzymes in a number of applications notwithstanding, structural and dynamic information about DNAzymes in general is scarce compared to proteins. Moreover there is no X-ray structure of Dz9(25)-11, however, D.M. Perrin and coworkers have conducted a site-directed chemical study from which proximity information between specific nucleotides can be inferred.
Here, this information is incorporated into an atomistic, fully solvated model of Dz9(25)-11using the GROMOS96 biomolecular simulation package. The structure, dynamics and putative function of the DNAzyme is discussed in light of the simulation results.

1996-Pos

Oxidative Damage in Lipid Bilayers: A Reactive Molecular Dynamics Study

Joseph Fogarty, Sagar Pandit.
Univeristy of South Florida, Tampa, FL, USA.

Current simulations of lipid bilayers focus on their structural and compositional properties. Chemical reactivity between cell membranes and extra cellular species is an unexplored field for molecular dynamics. To explore this new area of research, we have simulated a lipid bilayer composed of 200 POPC lipids (along with 50 waters per lipid) and its reaction with a simple peroxide for 8 ns using Reactive Molecular Dynamics (Purdue Reax). The specific chemical pathways of oxidative damage can be determined from these simulations and a greater insight into the process can be achieved.