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.
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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