1980-Pos
Simultaneous Multicomponent Registration of High-Resolution X-Ray Structures into Electron Microscopy Maps
Mirabela Rusu, Stefan Birmanns.
University of Texas Health Science Center at Houston, Houston, TX, USA.
A structural characterization of multicomponent cellular assemblies is essential to explain the mechanisms governing biological function. Macromolecular architectures may be revealed by integrating spatial information collected from various biophysical sources. For instance, low-resolution electron cryomicroscopy (cryo-EM) reconstructions of entire assemblies can be interpreted in relation with the crystal structures of the constituent fragments. A simultaneous registration of these multiple components is beneficial when building atomic models as it introduces additional spatial constraints to facilitate the native placement inside the map. The high-dimensional nature of such a search problem prevents the exhaustive exploration of all possible solutions. Here we introduce MOSAEC (Multi-Object Simultaneous Alignment by Evolutionary Computing), a method based on genetic algorithms, for the efficient exploration of the multi-body registration search space. MOSAEC employs principles inspired by biological evolution to iteratively optimize a population of candidate solutions. The classic scheme of a genetic algorithm was enhanced with new genetic operations, tabu search and parallel computing strategies and validated on a benchmark of synthetic and experimental cryo-EM datasets. Even at very low level of detail, MOSAEC successfully registered multiple component biomolecules, measuring accuracies within one order of magnitude of the nominal resolutions of the maps, for example 35-40 A.
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1981-Pos
Integrative Structural Bioinformatics: The Sculptor Modeling Software
Stefan Birmanns.
University of Texas Health Science Center, Houston, TX, USA.
Integrative modeling techniques promise to deliver new insight by fusing data from multiple sources. Especially the docking of atomic models into low- to intermediate resolution volumetric data from cryo-electron microscopy has grown into an established technique over the last decade. In recent years the field has expanded and targets now also macromolecular systems that undergo large-scale conformational changes and models those also using data from various biophysical sources.
Although the results reported so far indicate a wide-spread applicability, the development of integrative modeling techniques on the other hand also leads to new challenges. The size and complexity of the multi-scale data sets is extremely diverse and demands novel strategies not only for the modeling approaches but also regarding pre- and post-processing and visualization. We propose a series of new modeling and analysis techniques, tailored towards handling of heteroneous data sets - heterogeneous in size, level of detail, resolution and conformation. To overcome the challenges, an interactive peak search approach is presented, coarse graining is employed to efficiently model conformational differences, and new programmable graphics cards are used to efficiently render the resulting, time-varying atomic models. The new methods are embedded in an interactive visualization tool termed Sculptor, forming together a flexible, robust and versatile interactive modeling tool. The present report highlights also the overall concept and implementation of Sculptor. Sculptor is freely available from http://sculptor.biomachina.org and can be downloaded as package for Linux, Windows and MacOSX.
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1982-Pos
Calcium Gating by the DHPR-RyR1 Pair
Montserrat Samso.
Brigham & Women's Hospital/Harvard Medical School, Boston, MA, USA.
In skeletal muscle the L-type voltage-gated calcium channel (DHPR) in the t-tubule coexists in a tight functional interaction with the sarcoplasmic reticulum calcium release channel (RyR1). By means of this interaction a depolarization-induced conformational change in the DHPR is translated into RyR1's opening, and a massive calcium release from intracellular stores. We are performing structural studies on purified DHPRs and RyRs to understand structural details involved in the gating mechanism.
The DHPR is a heteropentamer with total molecular weight of ~450 kDa. Up to now he best structural knowledge has been gained by electron microscopy, although its relative small size has limited the resolution obtained to date. Our new 25Å resolution 3D reconstruction shows two distinct parts: a main body shaped like an irregular pentagon with distinct corners, and a hook-shaped feature. Consistent with the considerable conservation of membrane topology among voltage-gated channels, a good part of the main body can be closely fitted with an atomic structure of a full-length potassium channel, and this in turn is helping to locate the RyR1-interacting domains identified using biochemistry and molecular biology techniques.
The RyR is a large homotetramer of 2.2 MDa, which has facilitated its structural study by 3D cryo-electron microscopy. Its reproducible 3D structure consists of a large cytoplasmic domain and a smaller transmembrane domain. Our 3D reconstructions of RyR1 in the open and closed states at 10 Å resolution show that the ion pathway consists of two right-handed bundles converging into a constriction (putative ion gate) that changes its diameter by ~4Å upon gating. Although the molecular distance between the putative ion gate and the closest site of proximity to the DHPR is very large (>130 Å), the conformational changes associated with gating are generalized, suggesting long-range allosteric pathways connecting these distant domains.

1983-Pos
Ultrastructural Organization of Budding Yeast Septin Filaments Both *in vitro* and *in situ*, Analyzed by Electron Microscopy
Aurelie Bertin, Michael McMurray, Luong Thai, Galo Garcia,
Jeremy Thorner, Eva Nogales.
UC Berkeley, Berkeley, CA, USA.
Septins have been discovered more than 30 years ago as temperature sensitive mutants in budding yeast *Saccharomyces Cerevisiae*. Septins make an hourglass shaped structure of filaments bound to the inner cell membrane. Mitotic budding yeasts express five septins: Cdc3, Cdc10, Cdc11, Cdc12, and Shs1/Sep7. All, but Shs1, are essential for cell division.
Using electron microscopy of negatively-stained samples, *in vitro*, we have observed that the Cdc3-Cdc10-Cdc11-Cdc12 septin complex self-assemble into octameric rods in high salt. At lower ionic strength, septins polymerize into paired filaments. The position and identity of each subunit in the rod has been determined. This analysis revealed a symmetric organization where the different subunits are arranged in the following order: Cdc11-Cdc12-Cdc3-Cdc10-Cdc10-Cdc3-Cdc12-Cdc11. We have also shown that the subunit-subunit interfaces alternate between so called N-C and G interfaces.
To get more insight into septin organization *in situ* we have studied septin-lipid interaction using a lipid monolayer model assay. We have seen that budding yeast septins interact specifically with ($PI(4,5)P_2$). This interaction promotes filament formation and organization, even for mutants or under conditions where septins do not polymerize in solution. This interaction appears to be specifically mediated through Cdc10 and Cdc3.
We have been also analyzed the organization of septin filaments *in situ*, using electron tomography to visualized dividing budding yeast cells. 3D reconstructions of yeast sections were obtained by electron tomography using either freeze substituted samples or cryo-sections (Cemovis). Surprisingly, an array of two sets of perpendicular filaments is present at the bud neck. Cells displaying different types of septin mutations are now being analyzed.

1984-Pos
The Molecular Architecture of Human Low Density Lipoprotein and Bound Receptor Revealed by Electron Cryo-Microscopy
Gang Ren[1], Gabby Rudenko[2], Steven J. Ludtke[3], Johann Deisenhofer[4], Henry J. Pownall[3], Wah Chiu[3].
[1]University of California, San Francisco, San Francisco, CA, USA, [2]University of Michigan, Ann Arbor, MI, USA, [3]Baylor College of Medicine, Houston, TX, USA, [4]The University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA.
An elevated plasma level of low density lipoprotein (LDL)-cholesterol is a well-documented risk factor for cardiovascular disease. LDL transfers cholesterol from plasma to liver cells via the LDL receptor (LDLr). LDL is heterogeneous in composition, shape, size, density and charge and is difficult for structural study by X-ray crystallization and NMR. Here, we used electron cryo-microscopy (Cryo-EM) and image analysis to study the structures of LDL and LDL●LDLr complex. We found 1) the reconstructed LDL embedded in vitreous ice is approximated a flattened ellipsoid with planar opposing faces. 2) The reconstructed map of the LDL●LDLr complex was similar to that of LDL in shape and size, but with a ~35-45 Å protrusion attached on the surface. The protrusion matched in size to the LDL receptor beta-propeller domain. 3) The internal density distribution of LDL showed a liquid crystalline core containing three similarly sized internal high density ''isthmi''. 4) The LDL high-density regions that correspond to the apo B-100 appear as a pair of paddles connected at one end of the particle by a linker region with three separate long semicircular ''fingers'' extending from each edge of the linker region to wrap around the particle. These results allowed us to propose an architecture

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