a pair of premixers and a single channel containing pillar obstacle structures. A computational fluidic dynamics (CFD) simulation, using FLUENT CFD was used to direct our mixer designs. Experimental evaluations of mixer performance at millisecond time scales were conducted by fluorescence microscopy using fluorescent dye solutions or nanoparticle suspensions. Now we have integrated a micro-mixer and several types of sprayer into silicon-based chips, and have begun to test their feasibility for TRCEM. Initial experiments have focused on the kinetics of the assembly of prokaryotic ribosomes from their constituent 30S and 50S subunits. Assembly of ribosomes has been achieved by TRCEM, and experiments to detect potential intermediates in the process are under way.

2119-Pos Board B89

Learning Mixture Networks Reveal Functional Dynamics of Molecular Assemblies

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While the last years have shown a steady improvement of the experimental techniques in molecular structural biology, it appears that the analysis of large macro-molecular machines relies more and more on an integrative modeling approach, by combining data from a variety of sources. Multi-resolution docking is an essential tool in this context as it not only permits a high-resolution reconstruction of molecular assemblies, but also provides tools to study the dynamics of those systems. Over the last decade, the field has developed a variety of software tools, primarily for the interpretation of volumetric data from electron microscopy.

The advances in new experimental techniques leads now to an integration of a larger spectrum of data sets, for example tomographic reconstructions or small-angle scattering data. This diversity of the input data results in new challenges, including the stability of the algorithms in the presence of noise and the computational cost for a multi-resolution interpretation.

A novel scoring function for hybrid modeling is introduced, relying on a feature-based description of the three-dimensional objects. The recent development of neural maps with kernel-based activation rules facilitates a reliable detection of features in arbitrary signals. These Gaussian-mixture networks are known for their accurate density estimation properties and were also used to form equi-probabilistic networks. The identical feature-extraction approach can be used for all of the different experimental techniques and the resulting point sets can be brought into registration with our efficient anchor-point matching algorithm. The performance of the new scoring measure is evaluated and the integration into a multi-resolution docking tool is discussed.

This work was supported by startup funds of the University of Texas Health Science Center at Houston, by NIH grant R01GM62968, and by a grant from the Gillson Longenbaugh Foundation.

2120-Pos Board B90

Solving Complex Puzzles: Automated Protein Complex Assembly From Cryo-Electron Microscopy Data Via Multi-Resolution Modeling

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Currently, one of the central problems in structural biology is the better understanding of large protein complexes. Cryo-electron microscopy has made a significant impact in this area due to its ability to image proteins in a near-native environment. Multi-resolution docking approaches then allow atomic models of the whole complex to be built. However, all existing tools only dock one subunit at a time, leaving the user to sift through dozens or hundreds of possible solution candidates to assemble the complete protein complex.

Essentially, choosing the correct solutions from the candidate solution list is equivalent to solving the Knapsack problem (KP). The general case of the KP has been extensively studied in the field of computer science and is extremely compute intensive to solve. In the present case, we are interested in a set of candidate solutions without any steric clashes and maximal overall agreement with the experimental volumetric map of the protein complex.

We developed a Knapsack solver targeted specifically at the assembly of protein complexes, exploiting knowledge of this problem domain to reduce the overall computational load. The solver first clusters the raw docking data to locate possible subunit neighborhoods, significantly cutting down the overall search space. Sets of candidates, consisting of one possible solution from each region, are then screened for steric clashes using a fast, octree based, clashing detector. Sets with large amounts of steric hindrance between subunits are discarded. The remaining sets are ranked by the sum of all CC scores of the contained candidates. Finally, the best complete docking solution is output.

Besides details about our novel assembly algorithm, the present report examines its performance on both simulated and experimental data sets.

2121-Pos Board B91

Structure of a Type III Restriction Endonuclease by Single-Particle Electron Microscopy

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Type III restriction endonucleases recognize DNA sequences and cleave at a specific distance from the recognition sequence. These enzymes are composed of two protein subunits: Mod, necessary for specific binding and methylation, and Res, necessary for ATP-dependent restriction. A Res_2Mod_2 subunit assembly has been determined for the best-known examples of Type III restriction endonucleases, EcoP1I and EcoP15I, however no more structural information is available yet about type III restriction endonucleases. We have used electron microscopy (EM) and single-particle image processing to start deciphering the 3D structure of EcoP15I.

Advances in single-particle reconstruction methods have made it possible to elucidate the 3D structure of many macromolecules from EM images. However, accurate 3D structural determination of non-polymeric proteins smaller than 500 kDa such as EcoP15I is still a challenge. To optimize the extraction of information from our EM dataset, we review systematically different aspects of the reconstruction algorithms such as image de-noising, alignment, and classification. De-noising is an important factor in the quality of reconstruction because images of small macromolecules have low signal-to-noise ratio. We are comparing various techniques of de-noising based on statistical and geometrical characteristics of images. We are also investigating possible improvement of alignment based on coordinate transformation, interpolation, and statistical information to reduce alignment errors. Finally, we are evaluating sophisticated classification algorithms based on statistical information of the images.

Our preliminary 2D averages of EcoP15I show an oval structure of ~160x100 Å major and minor axes, and at least four distinguishable domains. The different classes correspond to different various projection angles, readily providing 3D structural information. Our preliminary 3D reconstruction confirms the above finding. The enhancement prospects of the image processing developments will be discussed in the context of the determination of the 3D structure of EcoP15I.

2122-Pos Board B92

Intact Flagellar Motor Architecture Revealed by Cryo-Electron Tomography

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Motility is an important component of the pathogenesis of bacteria and the bacterial flagellar motor is considered to be the most proficient biological machine for this purpose. The influx of protons through the motor produces either counterclockwise or clockwise flagellar rotation, resulting in translational motion or 'tumbling', respectively. Although the rotor portion of the bacterial flagellar motor has been purified and studied for decades, very little is known about the stator portion of the motor and its relationship to the rotor. We used high-throughput Cryo-Electron Tomography (Cryo-ET) and cutting edge image analysis to obtain 3-D structures of the intact flagellar motor assembly associated with native snap frozen bacteria at a level of detail that has not been previously observed. By averaging the 3-D volumes of ~1280 flagellar motors, we obtained a detailed model of the intact flagellar motor showing both stator and rotor assembly in its native cellular environment at about 3 nm resolution. We have also been able to identify distinctive structural changes resulting from the mutation of a flagellar gene. This is direct mapping of a single genetic code change into the 3-D structure of a functioning molecular machine in situ. Our results provide new insights into the motor structure and the molecular basis for bacterial motility.

2123-Pos Board B93

New Insight Into Desmosome Structure By Whole Cell Cryo-electron Tomography

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Previous electron tomograpic study of conventional plastic sections offered the first 3D insight into desmosome structures and suggested flexibility within the extracellular domains of desmosomal cadherins. And cryo-electron microscopy and tomography of vitreous sections indicated the extracellular inter-desmosmal interface was characterized by highly ordered straight rod-like structures with 5 nm periodicity and a cluster architecture model was generated by subvolume alignment and average. Meanwhile, some other studies suggest that

the desmosome is both a complex and dynamic structure. To better define and understand the precise entire structures of desmosome in both extracellular space and in intracellular spaces, new sample preparation techniques and imaging approaches will be important. Instead of conventional plastic section and cryo-section, we studied the desmosome structures in whole cells which were grown on electron microscopic grids coated with supporting film and which were plunge frozen to liquid nitrogen temperature. In this sample preparation, there was no or minimal manipulation of the structures compared to any other sample preparation techniques. Another major advantage of this study is we were able to image the entire desmosome rather than thin sections. We collected data on a 300 kV accelerating voltage microscope equipped with in-column energy-filter. Three-dimensional reconstructions and post-reconstruction analysis have offered a number of new insights into desmosome structure which may also shed light on the mechanism of desmosome assembling.

2124-Pos Board B94

Mitochondrial Fission is Mediated by Conformational Changes in the Dynamin-related Protein, Dnm1

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DNM1, a dynamin-related protein, plays an essential role in mitochondrial fission in yeast. Dnm1 contains a GTPase, middle and GTPase-effector domains, similar to dynamin, but lacks the Pleckstrin homology (PH) and proline-rich domains that are essential for dynamin function during endocytosis. Previous studies show that Dnm1 forms spiral structures larger than dynamin (~110 nm for Dnm1 vs. 50 nm for dynamin), and this increased diameter coincides with the size of mitochondrial constriction sites observed in vivo (1). Additionally, Dnm1 drives membrane tubulation and readily organizes into a helical array in the presence of liposomes. We have solved the three-dimensional structure of Dnm1-lipid tubes using single-particle helical reconstruction methods. These tubes have an overall diameter of ~125 nm with close to 30 repeating subunits per turn. We observe no interaction between the Dnm1 protein and the lipid bilayer, consistent with the lack of a PH domain in Dnm1. The organization of the Dnm1 oligomer is similar to dynamin-lipid tubes, but significant differences exist that mediate an altered conformational change. When GTP is added to dynamin tubes, the helical array constricts from 50 nm to 40 nm (2). Dnm1 tubes, which have an initial diameter of ~110 nm, rapidly constrict to less than 60 nm when GTP is added, and the protein quickly dissociates from the lipid bilayer. This work demonstrates that Dnm1 plays an active role in constricting the mitochondrial membrane during fission in a GTP-dependant manner. When compared with dynamin structures, Dnm1 provides a better understanding of the basic structural features essential for membrane fission.

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2125-Pos Board B95

The Structure Of Phosphorylase Kinase Holoenzyme At Subnanometer Resolution, Location Of The Catalytic Subunit And The Substrate Glycogen Phosphorylase

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Phosphorylase kinase (PhK) coordinates hormonal and neuronal signals to initiate the breakdown of glycogen. The enzyme catalyzes the phosphorylation of inactive glycogen phosphorylase b (GPb), resulting in the formation of active glycogen phosphorylase a (GPa) and the stimulation of glycogenolysis. PhK is one of the largest of the protein kinases (MW 1.3 x 106) and contains four copies of four subunits: α,β,γ and δ. Here we present a 9.9Å resolution structure of PhK determined by electron cryo-microscopy single-particle reconstruction. The enzyme has a butterfly-like shape comprised of two lobes with 222 symmetry. This 3D structure has allowed us to dock the catalytic γ domain, whose crystal structure is known, to the PhK holoenzyme at a location that is towards the ends of the lobes. The kinase domain is not involved in homo-dimer interactions. We have also determined the structure of PhK decorated with GPb at 18-Å resolution, which shows GPb located at the end of the lobes. Comparison of PhK/GPb complex with the volume of density for the GPb dimer derived from the X-ray model indicates that only one subunit of GPb is localized. Careful examination of the electron microscope images revealed a mixture of large and small PhK particles. In addition to the large particles described above we have determined the reduced size particles at 9.8Å resolution. This structure was consistent with a proteolysed activated form of PhK that had lost the α subunit and possibly the γ subunit.

2126-Pos Board B96

Cryo-EM Structure of Functional BK Channels in Lipid Bilayers

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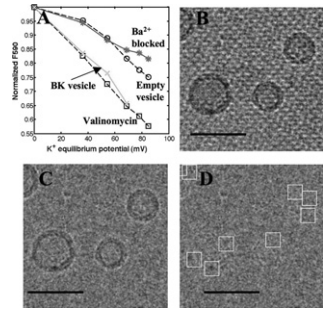
A natural way to preserve the native conformation of membrane proteins for structural study is to reconstitute them into liposomes (lipid vesicles). Here we applied this method to single-particle cryo-EM imaging of the hSlo large-conductance voltage- and Ca^{2+}-activated potassium channel (BK channel). In an assay using the JC-1 voltage-sensitive dye, proteoliposomes develop a membrane potential that is equal to that produced by valinomycin, and to that of empty liposomes when the BK channels are blocked by barium (A). For cryo-EM imaging the reconstituted BK proteoliposomes were tethered to a 2D streptavidin crystal (B). From this image the periodic crystal information was removed (C) and a liposome model of electron scattering was used to subtract the lipid bilayers (D). For 3D reconstruction, single-particle images (boxes) were oriented with constraints based on the spherical vesicle geometry. A structure based on these particle images will be presented. Scale bar in the figures is 50 nm.



2127-Pos Board B97

Functional Tests Of Purified Slack Channel Protein For Cryo-em Structure Determination

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The Slack (slo2.1) gene product is a sodium-gated potassium channel. We seek to image it using single-particle electron cryo-microscopy (cryo-EM). A first step is to verify that the expressed and purified protein is functional. Slack is expressed in HEK cell lines, solubilized, and purified by antibody-affinity. We have employed three methods to check the integrity of the solubilized and reconstituted Slack protein. Western blots verify the reconstitution of protein into floated vesicles. Fusion of these vesicles with planar bilayers yields sodium-sensitive K+ channel activity, while flux assays using the voltage-sensitive dye JC-1 show a high specific activity.

2128-Pos Board B98

Three-dimensional Reconstruction of Bovine Papillomavirus at Near-atomic Resolution by Single Particle Cryo Electron Microscopy

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Our reconstruction sets a new benchmark. We were able to assign many side chain rotamers and build a complete atomic model including previously undetermined segments, which suggests a novel mechanism of viral assembly.

2129-Pos Board B99

High-Resolution Electron Microscopy of a Rotavirus Particle

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Rotavirus is a non-enveloped, icosahedral, double-strand RNA virus that is a major cause of gastroenteritis in children. The infectious viral capsid is composed of four structural proteins arranged in three layers: VP4 and VP7 in the outer layer, VP6 in the middle layer, and VP2 in the inner layer. Using electron cryo-microscopy (cryo-EM) and single-particle reconstruction, we have determined the structure of a double-layer particle (genome encapsidated by VP2 +VP6) coated with outer-layer protein VP7. At about 4 Å resolution, the structure reveals most of the polypeptide path of VP7, and enables *de novo* modeling of its N-terminus, which closely interacts with VP6. We were able to detect conformational differences in the bound VP7 and the double-layer rotavirus particle (DLP) compared with the recently determined crystal structure of VP7 and the cryo-EM structure of the DLP, respectively. The observed differences suggest structural changes in the virus particle necessary for RNA release during viral infection.