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.

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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