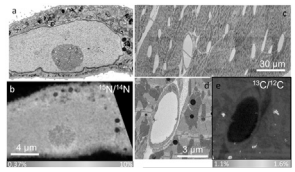
up to 50 nm. This capability has enabled several important studies on biological samples. Backscattered electron imaging has the ability to create morphological map from single cell to tissue scale. Combining the two techniques, we are able to obtain structural and isotopic information from the same sample, which can be useful to study complex biological problems at both single cell and tissue scale. (1) Single Cells: glutamine metabolism has been studied by correlative analysis, which makes it possible to measure the uptake of isotopically labelled molecules in specific organelles in single cells (Fig 1a-1b). (2) Tissues: various mouse tissue samples have been imaged, which enables to trace 13C-labelled lipids in any structures throughout the tissues (Fig 1c-1e).



3020-Pos Board B712

A Time-Resolved CRYO-EM Study of Ribosome Subunit Association by Mixing-Spraying

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The emergence of a high-throughput data processing pipeline and efficient classification algorithms has revived the idea of time-resolved cryogenic electron microscopy (cryo-EM), i.e., capturing time-dependent structures in a biological specimen using cryo-EM. The blotting method now routinely used to prepare cryo-EM specimen is sufficient only to study a reaction in the second to minute range. Capturing faster reactions, in the sub-second range, has been a practical challenge, due to the required step of depositing the specimen on the grid. To address this challenge, Lu et al. (2009) have developed a novel method to prepare time-resolved cryo-EM specimens, by using a nano-fabricated mixing-spraying chip. The mixing-spraying chip allows a two-component reaction to proceed inside the chip for tens to hundreds of milliseconds (ms), which is then stopped by fast freezing. Here we used the improved mixing-spraying method to study ribosome subunit association, and particularly to capture the conformational changes of the ribosome in this reaction. Ribosome subunit association is a pivotal step in translation initiation. Previous ensemble kinetic studies suggested that the ribosome subunit association is a multi-step process, with the ribosomal inter-subunit bridges sequentially formed. Using time-resolved cryo-EM, we were able to capture the association reaction in a pre-equilibrium state by mixing the two ribosomal subunits and reacting for 140ms. However, the fraction population of the associated ribosomes in each conformation was stable from 140ms to 75min, indicating that the ribosome undergoes conformational changes faster than the 140ms time frame upon association of the subunits. Thus, we have demonstrated that the mixing-spraying method of time-resolved cryo-EM is able to visualize the states of macromolecules in a reaction within a sub-second time frame.

3021-Pos Board B713

Visualizing Biological Samples in Liquid Solution by Electron Microscopy

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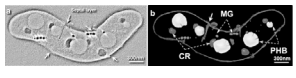
Liquid water is essential to life on earth. The cell, the basic component of all living organisms, functions through the action of proteins in a liquid environment. Consequently, the ability to directly visualize protein structure at nanometer-resolution in a liquid environment would dramatically advance mechanistic understanding of protein function in cellular activity. Current techniques in imaging biological samples in liquid solution are limited by micrometer scale resolution, Fluorescence-labeled, dehydrated, fixed, stained or frozen in ice.

Here, we report an electron microscopic approach for imaging label-free, near-native biological samples in liquid environment. By passing electrons through a thin layer of liquid-buffer sealed within a micro-chamber, we directly visualized the structure of proteins, viruses, bacteria and human cells at nanometer resolution, even achieved their 3D structures. For example, the electron tomographic 3D reconstruction (Figure) of a label-free living bacteria, Magnetospirillum magneticum in growth medium showed a smooth outer shell and internal components, such as poly(3-hydroxybutyrate) (PHB), chromosomal mass (CR), magnetosomes (MG) and internal septal layer. These results suggest the method offers both a general-purpose and high-throughput tool for imaging the structure of biological samples in near-native physiological liquid conditions.



3022-Pos Board B714

Cryo-Em Studies of DRP1 Self-Assembly Provide Insights into the Mechanism of Mitochondrial Fission

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Dynamin-related protein 1 (Drp1) belongs to a family of large GTPase proteins that regulate membrane dynamics and morphology. Self-assembly of cytosolic Drp1 into larger oligomers on the surface of mitochondria is essential for ensuing membrane fission. Preliminary studies show that in vitro reconstitutions of Drp1 emulate key features of the mitochondrial division machinery and provide a model system for evaluating conformational changes that drive membrane remodeling. To understand Drp1 self-assembly, cryo-electron microscopy (cryo-EM) will be used to determine the 3D structures of both the pre-assembly state and helical oligomers. More specifically, recombinant Drp1 forms stable tetramers in solution that are amenable to EM analysis. In the presence of non-hydrolyzable GTP analogs and/or synthetic liposomes, Drp1 further assembles into helical oligomers with varying diameters, suggesting ligand-induced conformational changes. Cryo-EM studies will determine the 3D structures of reconstituted Drp1 oligomers to reveal key intermolecular interactions and conformational changes that drive Drp1 self-assembly and mediate mitochondrial membrane fission.

3023-Pos Board B715

Cryo-Tomography of Vitrified Bacterial and Human Cells by Scanning Transmission Electron Microscopy

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Cryo-transmission electron tomography (CET) has emerged as a vital tool for structural biology studies of cells and viruses. Direct imaging of fully hydrated, vitrified material represents the state of the art for preservation of biological samples. Lacking heavy metal stains, CET relies on phase contrast typically obtained by defocusing the sample. The dependence on phase coherence, as well as cumulative radiation damage on frozen hydrated specimens, impose an inherent upper limit on sample thickness and usable tilt range. Even with energy filtration to remove the contribution of inelastic scattering, CET suffers from "missing wedge" effects and low signal-to-noise ratio. Scanning transmission electron microscopy (STEM) circumvents the need for phase contrast with incoherent detection, and has recently been extended to biological tomography. However the weak electron scattering by light elements was thought to preclude its application to unstained cryogenic specimens. We show this not to be the case. To the contrary, we find that raster scanning permits a higher total dose than conventional wide-field imaging, and the independent STEM detection in bright and dark field detectors provides sufficient contrast to show detailed cellular architecture similar to that provided by wide-field tomography. An important difference is that the specimen remains dynamically in focus even at high very tilts up to 70°. This significantly improves the depth resolution in reconstructions. Sample thickness limitations are also relaxed. We demonstrate the cryo-STEM tomography (CSTET) method using unstained, vitrified bacteria and human epithelial cells.

3024-Pos Board B716

Compressed Sensing Methods for Electron Tomography of Cellular Structure

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Optimization-based image recovery algorithms exploiting sparse representations, such as compressed sensing (CS), are emerging as powerful tools in image denoising and dosage reduction for biomedical imaging. The efficacy of CS recovery for any imaging application hinges on the structure of the images to be reconstructed, the structure of the measurement process, and the structure of image noise. In particular, the utility of CS-ET for undersampled tomogram reconstruction relies on the image satisfying constraints of an a priori image structural model, a dependency, which hints at the theoretical connections between CS and regularization techniques for image recovery. We examine these issues in the context of electron tomography (ET) for membranous cellular organelles, analyzing the ultrastructural sparsity in the datasets, and addressing the structure of the shot noise. Using numerical simulations, we compare reconstructions from the standard tilt series acquisition technique and a randomized tilt-angle variant, against a benchmark CS measurement scheme. To assess