of its deubiquitination activity. From these studies, we have a much better understanding of the manner in which the 26S recognizes and deubiquitinates proteins marked for proteolysis.

2001-Pos Board B771
Functionalized Ultrathin Carbon Films for Imaging Low-Abundance Biological Complexes
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By single particle electron cryo-microscopy (cryoEM), structures of low-abundance biological complexes can be obtained if the surface density of the complexes on the EM grids can be made sufficiently high for data collection. In order to prevent most of the molecules from being lost during specimen preparation, new methods are needed to retain them selectively to the surface. Such methods will permit cryoEM imaging of macromolecules that are available at subnanogram levels. Available methods along this line of thinking all add significant biomass to the background and are not so simple or robust for routine operations. We have developed a novel "bottom-up" approach to selectively anchor proteins of interest onto the surface of nanometer-thick carbon films that are routinely used for cryoEM imaging. We show that by chemically modifying the carbon films, specific ligands can be introduced onto carbon surfaces in order to enrich complexes bearing appropriate functional groups. In particular, we have been developing the chemical procedures that present on the carbon films specific ligands for His-tagged, GST-tagged and oligonucleotide-binding complexes as well as for immunoglobulin molecules. Meanwhile, we are applying this technology to the structural study of important low-abundance biological complexes by single particle EM. Our results demonstrate that the surface-engineered carbon films should have general applicability to a wide range of biophysical and biomedical problems.

2002-Pos Board B772
Efficient Automatic Detection of Filaments in Cellular Electron Tomograms based on Reduced Representation Templates
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Electron tomography is the most widely applicable method for obtaining 3D information by electron microscopy. It has become a powerful tool for elucidating 3D architectures of biological samples at resolution of about 4-6 nm and is the only method suitable for investigating polymorphic structures such as organelles, cells and tissues. However, in addition to the relatively low resolution, electron tomograms inevitably suffer from a low signal-to-noise ratio and some data-collection artifacts. These factors significantly hamper development of algorithms for reliable detection of structural features, which poses a severe barrier to progress in the field. As of today, the tasks of extracting information from these highly complex cellular tomograms are, for the most part, painstakingly carried out manually. Apart from the subjectivity of the process, the time consuming (and tiring) nature of this manual task all but precludes the prospects of the high throughput necessary to take full advantage of the method's potential.
Here, we present a novel tool for the detection of filaments in cellular tomograms that is based on reduced representation templates. Reduced representations consist of small sets of 3D points that capture the characteristics of the underlying structure. The use of these representations results in a reduction of computational complexity that allows scanning large volumes in real space in a relatively short time. This approach is specifically useful for detecting structures with higher order such as filaments and bundles. The use of reduced representations allows efficient adjustment of the scoring function for variations in signal-to-noise level, background, and surrounding environment (crowding), all factors that significantly hamper reliable detection using traditional correlation-based template matching. As a result, the approach is capable of matching or even exceeding the detection performance of a human operator.

2003-Pos Board B773
Asymmetric Small Protein Structure Determination by Individual Particle Electron Tomography
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Single-particle reconstruction of electron microscopy has been successfully used to determine high-resolution structure of proteins that are highly symmetric with a large macromolecular complex (molecular weight, MW $>$ 200 kDa). However, for asymmetric small proteins (MW $<$ 100 kDa), this approach still faces challenges to obtain intermediate resolution structure due to difficulties attaining a reliable initial model. To obtain the initial model, especially from an asymmetric small protein, we present a novel method by individual particle electron tomography (IPET). In this method, an individual protein particle was targeted and imaged from a serials of tilt-angles by electron tomography (ET) using our reported focused ET reconstruction (FETR) algorithm to reconstruct the three-dimensional (3D) structure from those tilt serial of images that were acquired from each instance of the molecule. To demonstrate the method, we imaged and reconstructed structure of small protein, cholesteryl ester transfer protein (CETP), whose molecular weight is only 53 kDa. The experimental sample was prepared by an optimized negative-staining protocol and was acquired under the near Scherzer-focus imaging condition. To investigate the reliability of the 3D density map reconstructed from single-instance particle, we statistically analyzed the map by comparing it with the crystal structure and the density map refined from this initial model, followed by the single-particle refinement procedure. Although Fourier shell cross-correlation (FSC) analysis shows the resolution of the tomographic reconstruction and single-particle reconstruction are both around 15 Å. These results suggest this approach can be used to obtain the initial model for single-particle reconstruction, moreover, the similar quality and resolution obtained from the IPET and single-particle reconstruction suggest that our reported IPET and FETR reconstruction can be used directly to determine the single-instance molecule structure at intermediate resolution (1-2 nm).

2004-Pos Board B774
High-Contrast In-Focus Imaging in Biological Cryo-EM
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A variety of devices have been proposed whose performance might be superior to that achieved when using highly underfocused images to generate contrast for cryo-EM specimens. Practical applications nevertheless continue to be limited because it has been difficult to prevent corruption of the images due to unwanted electrostatic charging that occurs during use. We have implemented two steps that - together - appear to overcome charging of one such device. This particular device consists of a microfabricated aperture that contains an opaque half circle at the center. The opaque half circle produces high-contrast "schlieren-optics" images of the low-resolution features encoded in the phase of the wave transmitted through the specimen. At the same time, however, the rest of the scattered wave passes through the open area of the aperture, resulting in conventional images at intermediate and high resolution. This hybrid double-sideband/single-sideband (Foucault knife edge) aperture is thus well suited for recording images at the optimal (Scherzer) value of defocus. Our first step in controlling unwanted charging is to coat all exposed surfaces with evaporated carbon, which ensures that the isopotential surfaces conform, as intended, to the physical surface of the aperture. The second step is to hold the device at a temperature of 300° C or more during use, in order to prevent the buildup of polymerized organic contamination during use. Although care must be taken to avoid hitting the opaque half circle directly with the unscattered electron beam, our preliminary experience is that this type of in-focus phase-contrast aperture can be used for at least a few days without showing apparent charging effects. Our proof-of-concept experiments confirm that this type of device is effective in producing high-contrast, in-focus images of cryo-EM specimens.

2005-Pos Board B775
Modeling Macromolecular Flexibility with Normal Mode Analysis in Internal Coordinates
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The prediction of molecular collective intrinsic motions is valuable for both understanding the functional conformational changes and introducing flexibility into the molecular modeling applications. A new normal mode analysis (NMA) framework in internal coordinates to approximate protein and nucleic acid flexibility is validated in diverse scenarios including Monte Carlo simulations, conformational exploration, and flexible docking and fitting. Special emphasis is placed in the flexible fitting of x-ray structures into electron microscopy 3D reconstructions for studying large macromolecular conformational changes. In this context, the implicit covalent maintenance naturally reduces the potential distortions produced when the structures are displaced along the modes in the iterative fitting procedure. The obtained results point out the sampling power of NMA in internal coordinates for capturing macromolecular conformational changes at reduced cost even at different coarse-grained levels.