Electron Cryomicroscopy: From Molecules to Cells

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Electron Cryomicroscopy: From Molecules to Cells

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Abstract. Today's biomolecular electron microscopy uses essentially three different imaging modalities: (i) electron crystallography, (ii) single particle analysis and (iii) electron tomography. Ideally, these imaging modalities are applied to frozen-hydrated samples to ensure an optimum preservation of the structures under scrutiny. Electron crystallography requires the existence of two-dimensional crystals. In principle, electron crystallography is a high-resolution technique and it has indeed been demonstrated in a number of cases that near-atomic resolution can be attained. Single-particle analysis is particularly suited for structural studies of large macromolecular complexes. The amount of material needed is minute and some degree of heterogeneity is tolerable since image classification can be used for further 'purification in silico'. In principle, single particle analysis can attain high-resolution but, in practice, this often remains an elusive goal. However, since medium resolution structures can be obtained relatively easily, it often provides an excellent basis for hybrid approaches in which high-resolution structures of components are integrated into the medium resolution structures of the holocomplexes. Electron tomography can be applied to non-repetitive structures. Most supramolecuar structures inside cells fall into this category. In order to obtain three-dimensional structures of objects with unique topologies it is necessary to obtain different views by physical tilting. The challenge is to obtain large numbers of projection images covering as wide a tilt range as possible and, at the same time, to minimize the cumulative electron dose. Cryoelectron tomography provides medium resolution three-dimensional images of a wide range of biological structures from isolated supramolecular assemblies to organelles and cells. It allows the visualization of molecular machines in their functional environment (in situ) and the mapping of entire molecular landscapes.

Today, there are three categories of biomolecular electron microscopy: (i) electron crystallography, (ii) single particle analysis and (iii) electron tomography. Ideally, all three imaging modalities are applied to frozen-hydrated samples ensuring that they are studied in the most life-like state that is physically possible to achieve. Vitrified aqueous samples are very radiation sensitive and consequently, cryo EM images must be recorded at minimal electron beam exposures limiting their signal-to-noise ratio. Therefore, the high resolution information of images of unstained and vitrified samples must be retrieved by averaging-based noise reduction which requires the presence of repetitive structure. Averaging can obviously not be applied to pleomorphic structures such as organelles and cells (1,2).

Electron crystallography requires the existence of two-dimensional crystals, natural or synthetic, and averaging is straightforward given the periodic arrangement of the molecules under scrutiny. In principle, electron crystallography is a high-resolution technique as demonstrated successfully with a

number of structures, in particular of membrane proteins. Often however, the same structures can be studied by x-ray crystallography which tends to be faster and can attain atomic resolution more easily.

In contrast, EM single particle analysis, arguably a misnomer, since it involves the averaging over large numbers of identical particles, has become one of the pillars of modern structural biology. The amount of material needed is minute and some degree of heterogeneity, compositional or conformational, is tolerable since image classification can be used for further purification *in silico*. It is particularly successful in structural studies of very large macromolecular complexes where the traditional methods often fail. In principle, single particle analysis can attain near-atomic resolution but in practice, this often remains an elusive goal.

This may change, however, with the advent of new technology, in particular detectors with improved performance. But even intermediate resolution (subnanometer) structures of very large complexes can provide an excellent basis for hybrid or integrative approaches in which high resolution structures of components and/or orthogonal data, such as distance restraints, are used to generate atomic models.

Two examples will be used to demonstrate the power of EM single-particle analysis in conjunction with data from other sources. The structure of tripeptidylpeptidase II (TPPII), a giant proteolytic complex of 6 MDa has been determined by integrating the crystal structure of its basic element, a dimer of 300 kDa, into the EM density of the holocomplex (3,4). The 26S proteasome, a key player in cellular protein quality control, is smaller (2,5 MDa), but much more complex in its subunit composition; it is built of two copies each of 34 different subunits. It is a labile complex which undergoes rather large conformational changes during its functional cycle. An initial subnanometer structure (5) provided the basis for determining its subunit architecture (6) and, more recently, based upon a massive data set of about 2.5 million individual particles collected by automated image acquisition, an atomic model was obtained as well as first insights into the conformational space (6,7) (Fig. 1).

Electron cryotomography can be used to study the three-dimensional organisation of non-repetitive objects (8,9). Most cellular structures fall into this category. In order to obtain three-dimensional reconstructions of objects with unique topologies it is necessary to acquire data sets with different angular orientations of the sample by physical tilting. The challenge is to obtain large numbers of projections covering as wide a tilt range as possible and, at the same time, to minimize the cumulative electron dose. This is achieved by means of elaborate automated acquisition procedures. Electron cryotomography can provide medium resolution three-dimensional images of a wide range of biological structures from isolated supramolecular assemblies to organelles and whole cells. It allows the visualization of molecular machines in their unperturbed functional environments (*in situ* structural biology) and ultimately the mapping of entire molecular landscapes (visual proteomics) (10).

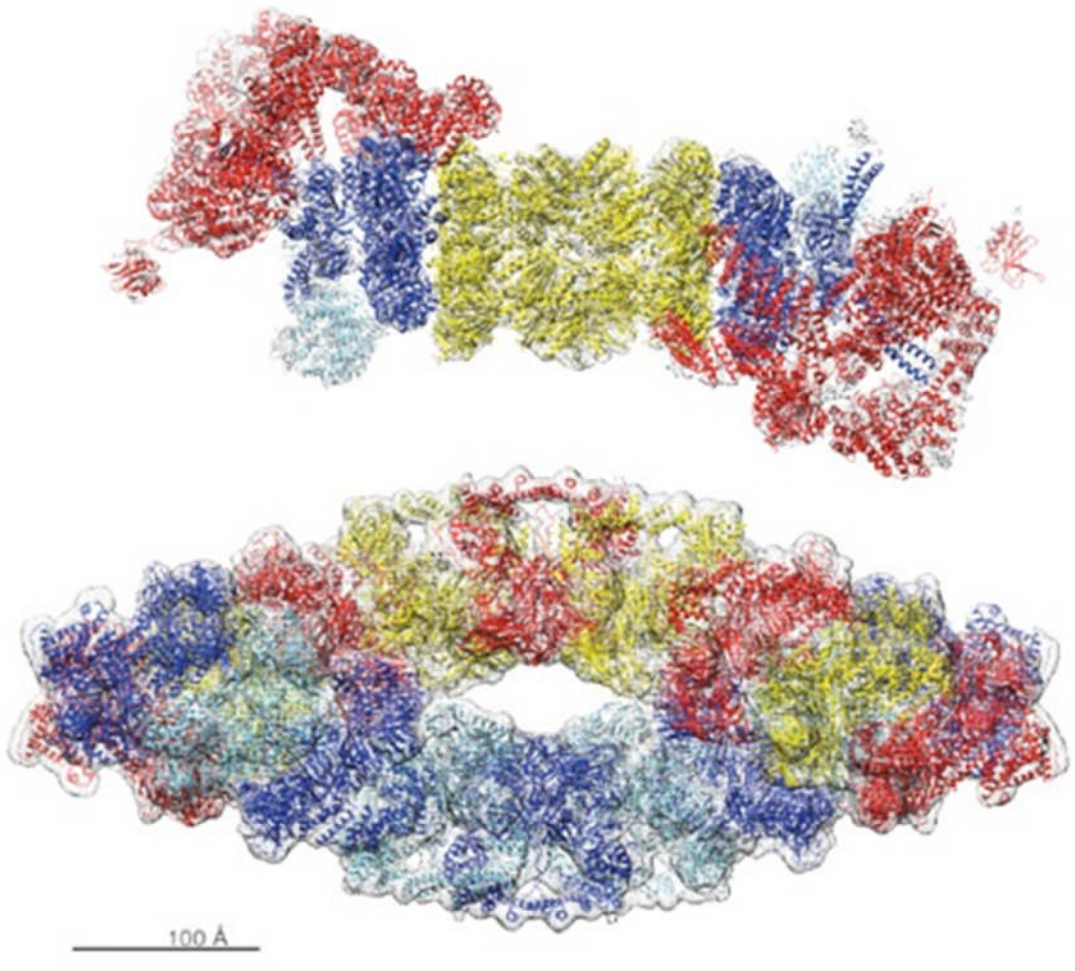

Fig.1: Structure of two large proteolytic complexes, the 26S proteasome (above) and tripeptidylprotease (below) determined by integrative approaches

Until recently the use of electron cryotomography was restricted to relatively thin samples such as prokaryotic cells or the margins of eukaryotic cells. This has changed with the advent of focused-ion beam (FIB) micromachining and developments allowing the application of this technology to samples embedded in vitreous ice. This allows the cutting of 'windows' providing views also of the interior of thicker samples such as eukaryotic cells. By combining the FIB with correlative fluorescence microscopy, it is possible now to navigate large cellular landscapes and to select and target specific areas of interest (11,12).

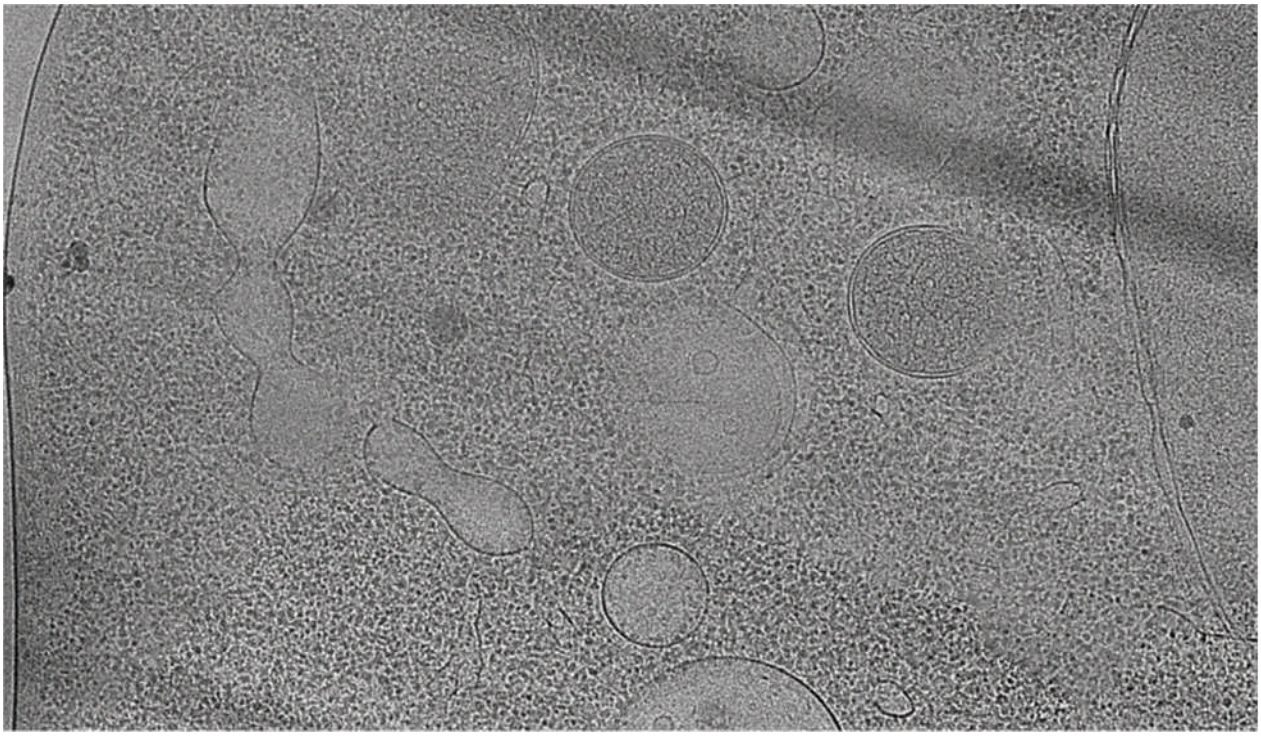

Fig. 2: TEM image of a ‚window' cut into an ice-embedded Dictyostelium cell by focused ion-beam micromachining

Given the poor signal-to-noise ratio of the tomograms, it can be challenging to interpret them and take advantage of their rich information content. Image denoising can improve the signal-to-noise ratio by reducing the noise while preserving the features of interest. Segmentation separates the structures of interest from the background and allows their three-dimensional visualization and quantitative analysis. Larger molecular structures can be identified in tomograms by pattern recognition methods using a template structure and once their location and orientation is determined, identical structures can be extracted computationally and averaged. Therefore electron tomography has unique potential to bridge the divide between molecular and cellular structural studies, perhaps the most exciting frontier in structural biology (13).

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