

Electron Cryomicroscopy: From Molecules to Systems

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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. It has been particularly successful with membrane proteins; their properties favor the formation of 'membrane crystals'. In principle, electron crystallography is a high-resolution technique and it has indeed been demonstrated in a number of cases – some of them high-profile structures – that near-atomic resolution can be attained. The difficulties encountered in obtaining well-ordered two-dimensional crystals and the low yield of high-resolution images limit throughput in electron crystallography and often put it at a disadvantage in competition with other methods, in particular x-ray crystallography.

Single-particle analysis is particularly suited for structural studies of large macromolecular complexes. The amounts of material needed for the analysis is minute and some degree of heterogeneity (impurities, conformational variability) 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 (~1 nm) structures can be obtained relatively easily, it often provides an excellent basis for hybrid approaches in which high-resolution structures of components (subunits, domains) are integrated into the medium resolution structures of the holocomplexes. Where other methods fail, hybrid approaches can provide most valuable structures with pseudoatomic resolution.

Unlike electron crystallography and single particle analysis, both requiring deterministic and, hence, repetitive structures, electron tomography can be applied to structures which are subject to stochastic variations and therefore non-repetitive. Most supramolecular structures inside organelles and 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 (2-4 nm) three-dimensional images of a wide range of biological structures from isolated macromolecular assemblies to organelles and cells. It allows the visualization of molecular machines in their functional environment and the mapping of entire molecular landscapes, i.e. the generation of molecular atlases from which interaction networks can be deduced.

Two large proteolytic complexes will be discussed in some detail to exemplify the potential of single particle analysis and the usefulness of hybrid approaches: Tripeptidylpeptidase (TPP) II and the 26S proteasome. To illustrate the opportunities provided by electron tomography, recent work on the nuclear pore complex and on higher order ribosome structures (polysomes, 100S ribosomes) will be discussed.

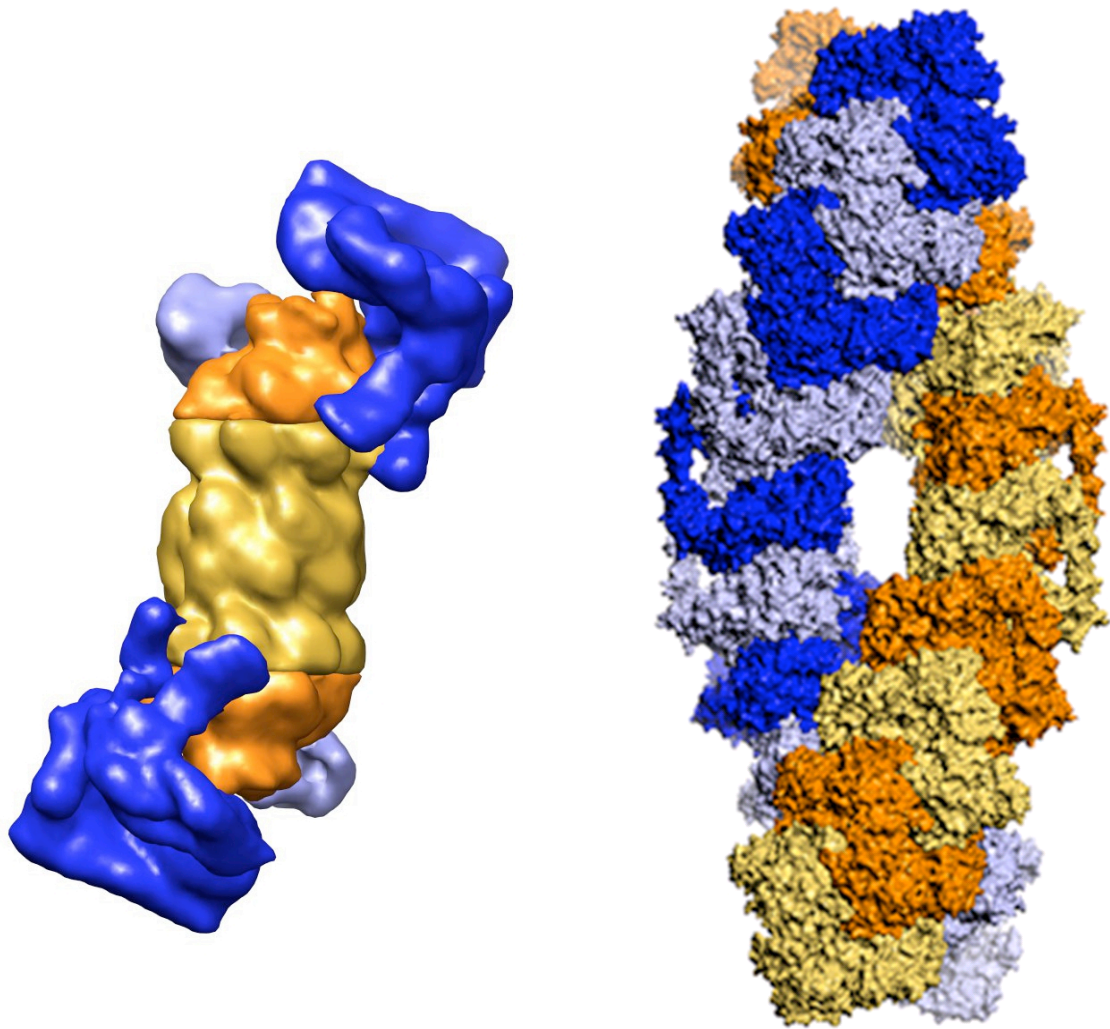


FIG. 1. Structures of the 26S proteasome (left) and tripeptidylpeptidase (TPP) II (right) as determined by single-particle analysis (26S) and a hybrid single-particle EM – x-ray crystallography approach (TPPII).

References

- [1] A. Sali et al., *Nature* 422 (2003) p216.
- [2] O. Medalia et al., *Science* 298 (2002) p1209.
- [3] C. Robinson et al., *Nature* 450 (2007) p973.
- [4] M. Beck et al., *Science* 306 (2004) p1387.
- [5] M. Beck et al., *Nature* 449 (2007) p611.
- [6] F. Brandt et al., *Cell* 136 (2009) p261.
- [7] A. Leis et al., *Trends Biochem. Sci.* 34 (2009) p60.