Understanding the dynamics of macromolecular machines by Three-Dimensional Electron Microscopy: The methods and some of their applications

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Abstract:

The study of large and potentially flexible macromolecular complexes is not an easy task for any experimental technique. Still, three-dimensional electron microscopy (3DEM) offers the potential to analyze in three-dimensions the structure of large complexes without imposing restrictive size limits or requiring the growth of any type of crystals.

The principles of 3DEM are rooted in the field of medical tomography, where a set of 2D projection images of a 3D object obtained from different projection directions are combined into a quantitative estimation of the 3D structure of the object. In the case of 3DEM the images are obtained with the help of an electron microscope, achieving a typical spatial resolution between 1 and 2 nm. It should be noted that the interaction between electrons and matter is relatively strong, forcing the use of very low electron doses in the images of the complexes, which results is very poor electron statistics in the images and, consequently, a very strong noise, typically many times more than the signal coming from the macromolecule itself.

The unique characteristic of 3DEM as compared to other experimental techniques in structural biology is that the experimental information, the 2D images, are obtained from each of the complexes individually rather than representing an "ensemble" information. However, the experimental 2D images are very noisy, requiring some level of averaging to obtain a reliable 3D structure. It is the trade-off between the unique capacity to visualize individual complexes and the need to average them that determines the 3DEM capacity to classify the images being obtained into structural classes, and, in this way, to better understand the flexibility of te complex.

In this presentation we will review a number of examples in which 3DEM has successfully provided new structural information on the structure of large and flexible macromolecular complexes, presenting in each case the methods that have been used for their determination.

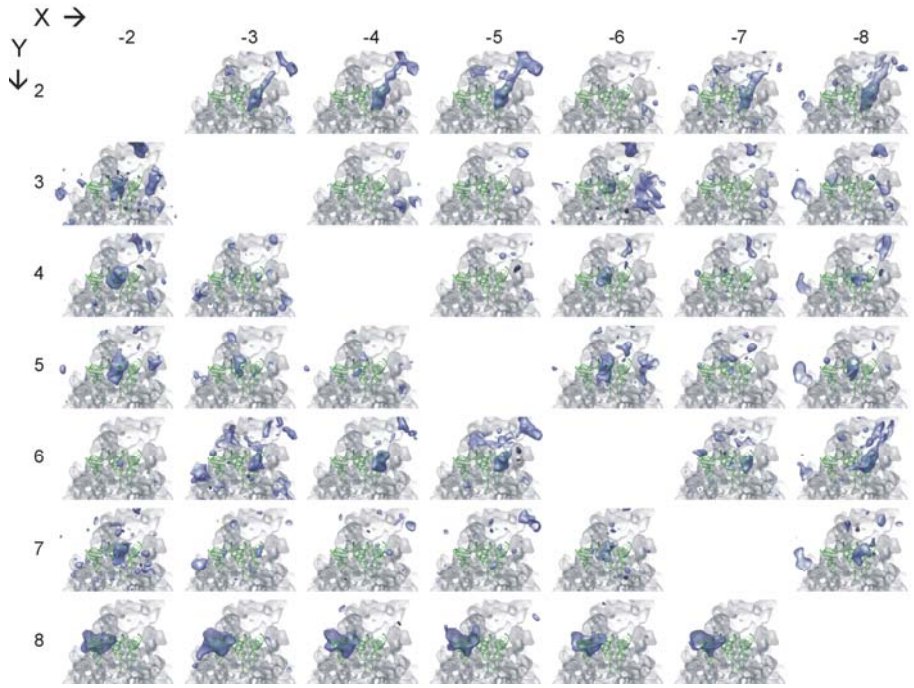

Figure 1: Maximum likelihood classification (ML3D, Scheres et al., 2007) was performed to divide a dataset of 74,400 70S ribosome·tmRNA·SmpB complexes into eight classes based on the maxima of their probability distributions over all orientation and class assignments. Shown are the difference maps calculated between classes 2 to 8 of the 70S·tmRNA·SmpB complexes. The view is towards the 50S subunit (surface rendered in grey) with most of the 30S subunit removed to highlight the subunit interface. The A, P and E sites are defined by the positions of tRNA molecules in the corresponding positions from the pdb-entry 1GIX. Difference maps (blue) have been surface rendered at 0.5nm. Inspection of these difference maps played an important role in the interpretation of these classes as follows: class 1: damaged ribosomes, mainly 50S particles. Classes 2 and 6: Ribosomes binding tmRNA•SmpB, with SmpB protruding into the A site. Classes 3, 4, 5, 7 and 8: Ribosomes with low occupancy/variable conformation of tmRNA, with density in P (classes 3, 4, 5 and 7) or E site (class 8) (Cheng et al., 2010).