Recent advances in high-resolution cryo-EM structure determination

Sjors HW Scheres*

*MRC Laboratory of Molecular Biology, Cambridge Biomedical Campus, CB2 0QH, Cambridge, UK

EMAIL scheres@mrc-lmb.cam.ac.uk

Radiation damage is the main limiting factor in imaging biologically relevant macro-molecular complexes with electrons. Still, it has been estimated that 3D reconstructions should be possible to near-atomic resolution, i.e. ~3 Å, by combining projection images of a few thousand particles with a molecular mass of ~100 kDa [1]. In practice, resolutions obtained have been much lower, even when combining more and/or larger particles. However, this field is undergoing rapid changes, and near-atomic resolution reconstructions have now been reported for data sets comprising only tens of thousands of particles [2,3]. I will discuss two advances that underlie this sudden progress: direct-electron detectors; and statistical refinement algorithms.

Newly developed direct-electron detectors have a significantly improved signal-to-noise performance compared to conventional detection media like photographic film and charged-coupled device (CCD) detectors. The improved signal-to-noise performance allows for better reconstructions to be calculated from smaller amounts of particles. Moreover, these detectors can be read at high speeds, with frame rates in the range of 17-400 s-1. As frozen macro-molecular complexes typically move upon irradiation with electrons, recording movies while the sample is being imaged allows correcting for beam-induced movements, which further improves resolution [2,3].

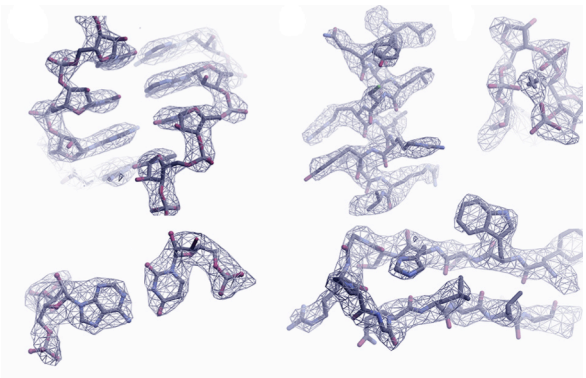

Figure 1. *Reconstructed cryo-EM density for parts of the large subunit of the yeast mitoribosome.*

The development of a regularised likelihood optimisation algorithm for single-particle reconstruction [4], in combination with a strict prevention of overfitting [5], has further improved the quality of cryo-EM density maps. Optimisation of a regularised likelihood target provides an elegant derivation of the optimal (i.e. Wiener) filter for the reconstruction task. The resulting algorithm infers many parameters from the data, so that expert user decisions may largely be avoided. Moreover, for macro-molecular complexes that adopt multiple three-dimensional structural states, in silico classification may be used to identify structurally homogeneous subsets of the data.

As an illustration of the combined power of the new detectors and the statistical image processing approach, I will discuss the structure determination of the large subunit from the yeast mitochondrial ribosome, or mitoribosome. Using less than fifty thousand individual particles, we obtained a 3.2 Å resolution density map (Fig. 1). This map allowed us to propose a nearly complete atomic model, for which approximately 600 kDa of protein and RNA molecules was built de novo. This work was funded by the Medical Research Council under grant MC_UP_A025_1013.

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[3] Liao et al, Nature 504 (2013), p. 107.

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[5] SHW Scheres and S Chen, Nature Methods 9 (2012), p. 853.

[6] A Amunts et al, Science (2014) in press.