The conundrum of depositing half-maps for experimental cryoEM data

Zbyszek Otwinowski[1]

[1]UT Southwestern

zbyszek@work.swmed.edu

In electron microscopy, the resolution of results has a softer character compared to X-ray crystallography. The core procedure involved in estimating resolution is comparing two reconstructions derived from two halves of the data.

The 3D reconstruction process uses image data and the end results are real-space maps. However, intermediate calculations are performed mainly in reciprocal space, and due to the low signal-to-noise ratio of the starting images, elaborate filtering and weighting are necessary to produce the best possible results. Phase contrast imaging, which is the core of cryoEM methodology, necessitates complex CTF filtering, supplemented by additional weights resulting from correcting for motion, particle quality, etc. 3D reconstruction from multiple back projections is performed in reciprocal space, where data from multiple images are merged according to their statistical estimators (weights). The merging is enabled by the particles having finite size, and so data from local environments in reciprocal space could be statistically averaged. The particle finite size description is converted into using a particular filter, for example a Kaiser-Bessel, truncated Gaussian, or a sinc function. This is equivalent to applying a particle mask, but of a rather simple shape.

Reconstructions based on two disjoint halves of the data are part of the normal workflow in cryoEM. However, what is compared is full, accumulated data in reciprocal space that involves both signals and weights. Sometimes accompanying maps are also produced (as output), but they are not part of the iterative calculations and they lose critical information about the distribution of weights. For cryoEM SPR data deposited to the PDB, there is a recent requirement to deposit half-maps which are unfiltered and unmasked. These maps are not suitable for validating results because they are missing critical information in the half-reconstructions; this is of particular significance in the case of preferred orientation, which is a frequent occurrence in cryoEM SPR.

The requirement for submitting these half-maps has created a conundrum due to relying on the idea that half-maps fully represent information in reciprocal-space half-reconstructions. The underlying problem of validation of cryoEM reconstructions has many layers and is without an obvious solution. I will discuss possible ways out of this conundrum.