3D RECONSTRUCTION OF MACROMOLECULAR ASSEMBLIES AT SUBNANOMETRIC RESOLUTION

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1. INTRODUCTION

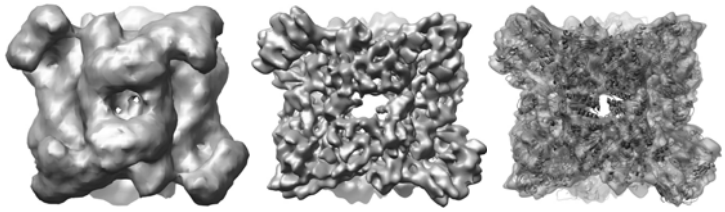
We show here an example of single-particle reconstruction of *glutamate synthase* (GltS) at resolution of 1/9.5 $Å^{-1}$. GltS is a multienzymatic complex composed of several copies of two types of subunits (alpha: 162 kDa and beta: 52.3 kDa). Present in bacteria and plants this complex is responsible for the main ammonia assimilation pathway. The reconstruction strategy is based on a recently developed method for semi-automatic image sorting aimed at removing data introducing errors in the global 3D map (drifted images) or those unable to increase global signal-to-noise ratio (images with no diffraction rings) [1].

2. METHODS AND RESULTS

We used *random conical tilt series* method [2] followed by *projection matching* refinement method [3] to compute a preliminary low resolution structure. The low-resolution volume (resolution of 1/26 $Å^{-1}$ estimated with the Fourier Shell Correlation ($FSC_{0.5}$) criterion), obtained using SPIDER software package [4], was in a good agreement with a possible alpha:6 beta:6 stoichiometry for the GltS complex (Fig. 1a).

To explore the architecture of the complex at higher resolutions, an additional set of cryoEM images was recorded on a JEOL JEM 2100F electron microscope equipped with a field emission gun. We selected 13,000 particles from micrographs of good quality using the method from the reference [1] followed by Roseman's particle picking method [5]. A high-resolution volume was obtained by projection matching, using the low resolution volume as a starting reference structure. Hence, iterative projection matching was combined with contrast transfer function correction performed using Wiener filtering of volumes from focal series [6]. Defocus was computed for each image using the program CTFTILT [7]. The angular step for projection matching was reduced gradually as the number of iterations was increasing. Also, different filters were applied on the reference volume at different stages of refinement, until resolution improved and stabilized at $FSC_{0.5}$ = 1/9.5 $Å^{-1}$ (Fig. 1b).

The atomic coordinates of the alpha subunit (PDB code: 1ea0 [8]) were then fitted in the volume, as well as a model of the beta subunit, derived from a homologous enzyme (PDB code: 1h7w [9]) (Fig. 1c). A manuscript presenting a detailed description of this project and the docking of atomic structures is in preparation [10].



(a) (b) (c)

Fig. 1 *Side view of 3D cryo-EM single-particle reconstruction of bacterial glutamate synthase. (a) 1/26 Å^{-1} resolution. (c) 1/9.5 Å^{-1} resolution. (d) Docking of atomic structures in the map shown in (c).*

3. CONCLUSION

This example shows that the reconstruction strategy must be adapted to each sample. Also, it shows how a combined use of several complementary techniques can produce a 3D model at a sub-nanometer resolution. In this case, we used tilted- and untilted-specimen images by cryo-EM, semi-automatic selection of images of good quality, single-particle analysis, 3D reconstruction, and correction of the contrast transfer function of the electron microscope.

4. REFERENCES

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