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Breaking to the Subnanometer Resolution Range of Cryo-EM SPA Reconstructions Obtained from 120 kV LaB_6 TEM

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Cryo-EM single particle analysis (SPA) is routinely used to determine high-resolution 3D reconstructions of biomolecules that allow building of their atomic models [1, 2]. Nevertheless, preparation and screening of samples with suitable particle concentrations and minimal heterogeneity remains the main bottleneck in the SPA workflow. Lack of high-quality specimens hinders the effective use of the mid-range and high-end TEM microscopes which collect data in continuously increasing throughput.

Lower-end cryo-TEM microscopes, typically equipped with a LaB_6 source, are often used for initial characterization of purified biomolecules before high-resolution cryo-EM SPA data collection. The use of these microscopes is mostly limited to assessment of sample concentration and distribution of particles, including their homogeneity, orientation spread and stability in a thin ice layer of the cryo-EM specimen [3]. However, many cryo-EM facilities also require a preliminary sub-nanometer 3D reconstruction as a proof of sample suitability for high resolution imaging to guarantee an efficient use of their instruments and resources. Lower brightness and coherence of the LaB_6 electron beam as well as relatively lower sensitivity of used CCD and CMOS cameras at low electron doses generally prevented achieving resolutions better than 10-15 Å on LaB_6 TEMs.

Here, we analyzed performance of the 120 kV LaB_6 Talos L120C microscope equipped with the CETA-S 16Mpix CMOS camera that has been optimized for low dose applications. Considering the typical settings used in high-resolution cryo-EM imaging, parameters of the C-TWIN objective lens and DQE of the Ceta-S camera at the 0.72 Å/pix pixel size, acquired images should contain information beyond 7-Å resolution for applied defocus <1.5 µm (Figure 1A). To validate these theoretical predictions, we imaged thin continuous carbon film of ~10 nm thickness to approximate scattering from a dense protein monolayer in ice. Experimental results are in agreement with the theoretical predictions and indicate that CTF signal can be detected and fitted to 5-7 Å resolution from -0.5 µm to -1.5 µm defocus (Figure 1B). A cryo-EM dataset of the apoferritin sample imaged at similar conditions in very thin ice provided micrographs whose CTF could be also fitted to 5-7 Å resolution (Figure 2A). A 3D map of apoferritin was reconstructed to ~6 Å resolution from ~12,000 particles. At this resolution, α-helices of the apoferritin secondary structure are resolved as rod-like densities and their conformation fits well with the known atomic model of apoferritin (Figure 2B).

The obtained results demonstrate feasibility to achieve subnanometer cryo-EM SPA reconstructions of biomolecules imaged on LaB_6 TEMs at 120 kV acceleration. These instruments can be therefore used not only for initial optimization of cryo-EM samples and initial characterization of their overall molecular architecture, but also to reveal their internal structure, protein fold and inter-subunit interfaces.

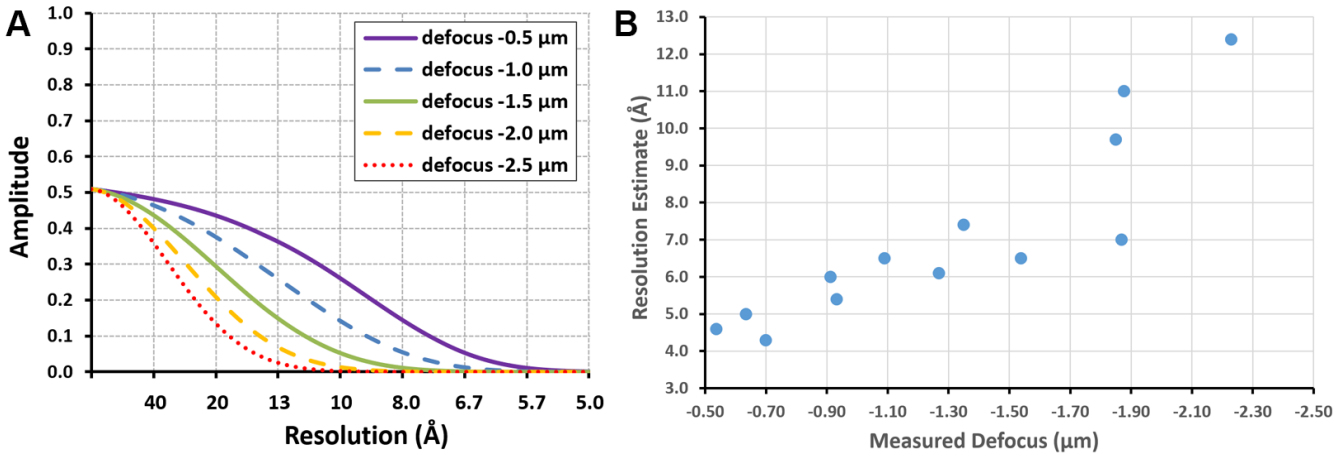

Figure 1. Resolution of CTF signal in images acquired at typical cryo-EM settings: (A) Theoretical CTF envelope functions calculated at different defoci and weighted by DQE of the Ceta-S camera at 0.72 Å/pix pixel size. (B) Estimated resolution of CTF fits in images of thin carbon (~10 nm) collected at the typical cryo-EM settings (parallel illumination, 0.72 Å/pix, ~50 e^{-}/Å^2/s dose rate, 0.8 s exposure).

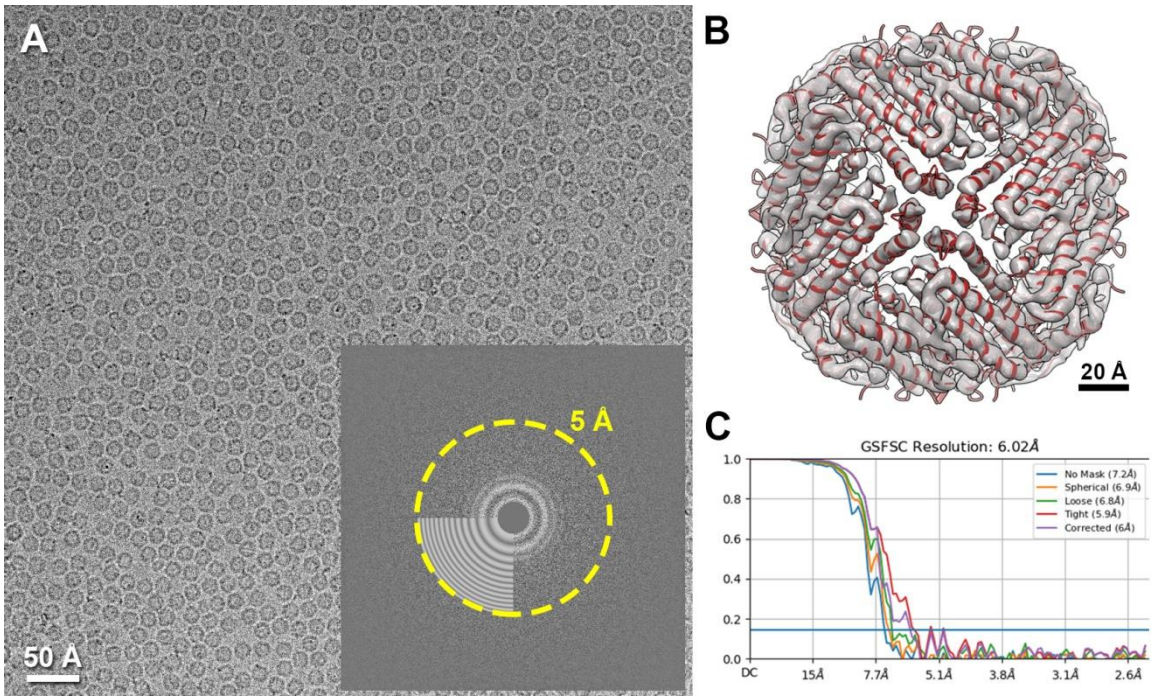

Figure 2. Cryo-EM SPA reconstruction of apoferritin using the 120 kV LaB_6 TEM: (A) Micrograph acquired at -1.0 µm defocus and 40 e^{-}/Å^2 total electron dose. Power spectrum and its CTF fit are shown in the inset at the bottom right. (B) Rendering of the reconstructed 3D map (grey density) with rigid body fitted atomic structure of apoferritin (red ribbon). (C) Gold standard FSC plot indicates the achieved resolution of ~6 Å from the dataset of ~12,000 particles.

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