Low-dose cryo-electron ptychography of proteins at sub-nanometer resolution

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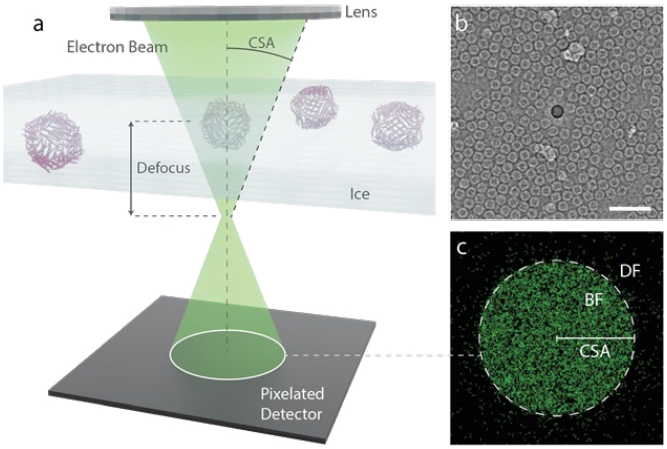
The field of cryogenic electron microscopy (cryo-EM) has seen remarkable advancements, enabling researchers to visualize biomolecular structures with ångström-level resolution. While conventional transmission electron microscopy (CTEM) combined with single-particle analysis (SPA) has played a crucial role in these developments, it faces challenges with very small proteins and large biomolecules. Specifically, small proteins often exhibit insufficient contrast, and large biomolecules can produce opaque images due to multiple scattering. Electron ptychography, a technique derived from 4D scanning transmission electron microscopy (4D-STEM), presents promising solutions to these issues. It enhances contrast through phase retrieval, aiding in imaging small proteins, and manages multiple scattering effects, which is vital for examining thicker specimens. Electron ptychography has already proven to be a powerful method for room temperature material science samples, achieving unprecedented resolutions[1]. However, the application of STEM-related techniques to biological specimens is relatively rare[2,3], and ptychographic imaging at low-dose conditions (less than 50 e/Å²) remains largely unexplored.

In our study, we applied this technique to frozen hydrated single protein samples and achieved sub-nanometer resolution with a relatively small number of particles, yielding micrographs of enhanced quality (signal-to-noise ratio, SNR). The scattering potential of single protein particles was iteratively reconstructed using the open-source software, py4DSTEM[4]. We present structures of apoferritin at 5.8 Å using 11,552 particles, a phi92 sheath structure at 8.4 Å using 1,600 particles, and a tobacco mosaic virus at 6.4 Å using 2,120 particles. The application of ptychography to biological specimens, particularly when integrated within the SPA framework for smaller frozen hydrated protein particles or combined with tomography tilt series for thicker samples (e.g., bacteria, tissue), holds great promise.

Graphic:



Keywords:

4D-STEM, ptychography, cryo-EM, low-dose

Reference:

[1] Jiang, Y., Chen, Z., Han, Y., et al. (2018). Electron ptychography of 2D materials to deep sub-ångström resolution. Nature, 559, 343-349. Available at: https://doi.org/10.1038/s41586-018-0298-5
[2] Lazić, I., Wirix, M., Leidl, M.L., et al. (2022). Single-particle cryo-EM structures from iDPC–STEM at near-atomic resolution. Nature Methods, 19, 1126-1136. Available at: https://doi.org/10.1038/s41592-022-01586-0
[3] Zhou, L., Song, J., Kim, J.S., et al. (2020). Low-dose phase retrieval of biological specimens using cryo-electron ptychography. Nature Communications, 11, 2773. Available at: https://doi.org/10.1038/s41467-020-16391-6
[4] Varnavides, G., Ribet, S., et al. (2023). Iterative Phase Retrieval Algorithms for Scanning Transmission Electron Microscopy. arXiv:2309.05250. Available at: https://arxiv.org/abs/2309.05250
[5] Küçükoğlu, B., Mohammed, I., Guerrero-Ferreira, R.C., et al. (2024). Low-dose cryo-electron ptychography of proteins at sub-nanometer resolution. bioRxiv. Available at: https://doi.org/10.1101/2024.02.12.579607