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3.9Å phase plate cryo-EM reconstruction of the nucleosome core particle

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Single particle cryo-EM has recently developed into a powerful technique for determining the structures of large macromolecular complexes at near-atomic resolution. This resolution revolution has been driven by the development of direct electron detectors and improved algorithms for particle classification and 3D reconstruction. However, the imaging and processing of small biological molecules (<200 kDa) by bright field phase contrast cryo-EM remains challenging. This is because small particles have low contrast, which makes them difficult to detect, align, and analyze. Here, we combine cryo-EM imaging with the use of a Volta phase plate [1] to image nucleosome core particles (NCPs) in amorphous ice [2]. We reconstituted NCP with Xenopus laevis histone octamer and the Widom 601 strong positioning DNA sequence, for which the crystal structure is available. Imaging with the Volta phase plate was done on an FEI Titan Krios operating at 300 kV, with a Gatan K2 Summit direct electron detector in counting mode. Micrographs were collected in focus with a Volta phase plate. We show that using the phase plate dramatically increases the contrast of the 200 kDa NCP molecules, improving especially detection of disk views, which are otherwise difficult to see by bright field cryo-EM. Use of the phase plate also allowed free DNA strands in the ice to be clearly identified. From 26,060 NCP particles, we reconstructed an electron density map with a global resolution of 3.9 Å, showing clear density for amino acid side chains and the phosphate backbone of the DNA (Figure 1). Our data, along with other recent near-atomic reconstructions [3], show that phase plate cryo-EM is a promising technique for imaging and determining the structures of small biological molecules at near-atomic resolution.

[1] Danev, R., Buijsse, B., Khoshouei, M., Plitzko, J. M. & Baumeister, W. (2014) Proc. Natl. Acad. Sci. 111, 15635–15640.

[2] Chua, E. Y. D., Vogirala, V. K., Inian, O., Wong, A. S. W., Nordenskiold, L., Plitzko, J. M., Danev, R., Sandin, S. (2016) Nucleic Acids Res. 44, 8013-8019.

[3] Khoshouei, M., Radjainia, M., Baumeister, W. & Danev, R. (2016) bioRxiv doi:https://doi.org/10.1101/087841

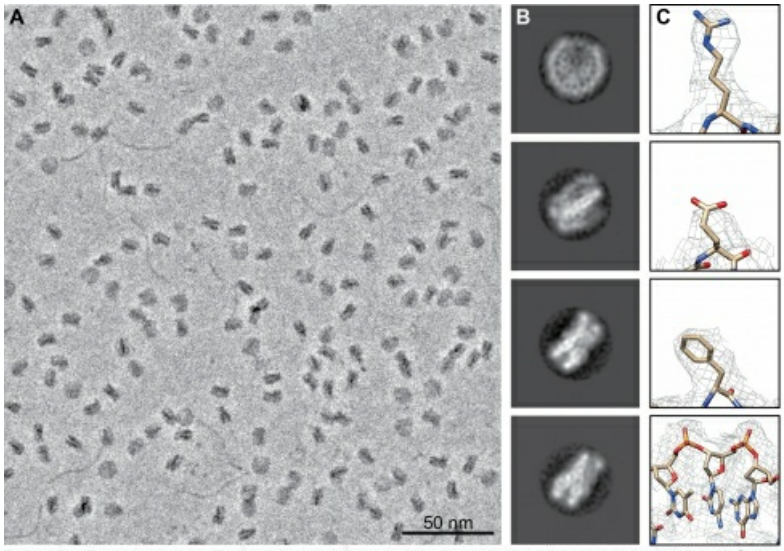

Figure 1. Phase plate cryo-EM imaging and single particle analysis of nucleosome core particles (NCPs) in vitreous ice. **(A)** Representative field view of NCPs and DNA in ice, recorded in focus with a Volta phase plate. **(B)** Representative 2D classes of NCPs in different orientations. Mask diameter is 12 nm. **(C)** Examples of amino acid side chain and DNA phosphate backbone densities from the 3.9 Å reconstruction of the NCP electron density map.

Keywords: Phase plate cryo-EM, Nucleosome core particle, Single particle analysis