Sub-3 Å Apoferritin Structure Determined with Single Position of Volta Phase Plate and Full Range of Phase Shift

Article *in* Microscopy and Microanalysis · July 2020

DOI: 10.1017/S1431927620021121

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898 – Sub-3 Å Apoferritin Structure Determined with Single Position of Volta Phase Plate and Full Range of Phase Shift

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Structure determination of small particles (less than 100kDa) is still a challenging task in the cryo-EM field. Volta phase plate (VPP) has been shown as a valuable tool by presenting several high-resolution structures of various sizes of protein1,2. However, despite the fact that VPP has been garnering attention as a compelling solution to enhance image contrast, it remains a largely artful tool whose performance couldn't be precisely controlled. In this report, we studied if a Volta phase plate position is intrinsically limited to a small number of images (a few tens) and a narrow range of phase shifts (around 90 degrees) as advocated in the field. By acquiring four datasets with different strategies of changing VPP positions, varying number of images for a single position, and across more than six months' time period, we have shown that it is feasible to image a large number of images (>400) with full range of phase shifts (0-360 degrees) using a single VPP position to obtain 3 Å and better resolution 3D reconstructions, and such results are reproducible despite noticeable variations among different VPP positions and different time periods. Datasets I and II were collected with the same sample grid and imaging condition except that the VPP position was set to change every 30 movies in dataset I as the current VPP imaging strategy suggested, while the VPP position remained the same in dataset II. Imaging on a single spot of VPP allowed us to collect enough data (dataset II) for high resolution structure determination. The apoferritin structure at 2.85 Å resolution obtained with dataset II is nearly identical to the structure reconstructed from the control (dataset I). This success with a single VPP position was initially surprising as it is at odds with the current understanding of VPP and the recommended data collection strategy involving VPP. Datasets III and IV, which were collected more than six months later, further demonstrated that the "surprising" results of dataset II obtaining high-resolution 3D reconstruction from a large number of images with a wide range of phase shifts acquired using a single VPP position were indeed a reproducible outcome of VPP. Furthermore, the comparable resolutions achieved from equal number of particles in the three different phase shift ranges indicated that the increase of phase shift does not necessarily lead to the decrease of image quality. The results in this study may provide new insights into further improvement of both efficiency and robustness of VPP, and to help turn VPP into a plug-and-play device for high resolution cryo-EM.

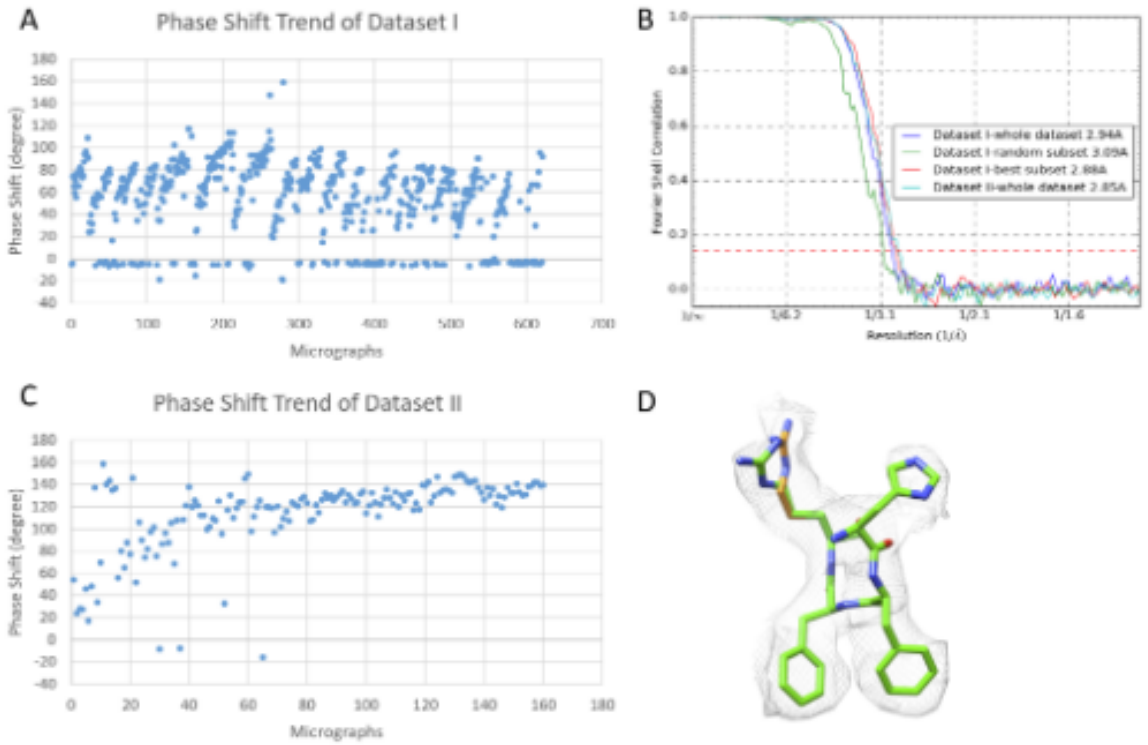

Figure 1. Figure 1. The time-course of GCTF-determined phase shifts for dataset I (A) and dataset II (C). (B) Noise-substitution corrected FSC curves of the dataset I and the subsets of dataset II. (D) The zoom-in view of the high resolution density of dataset II.

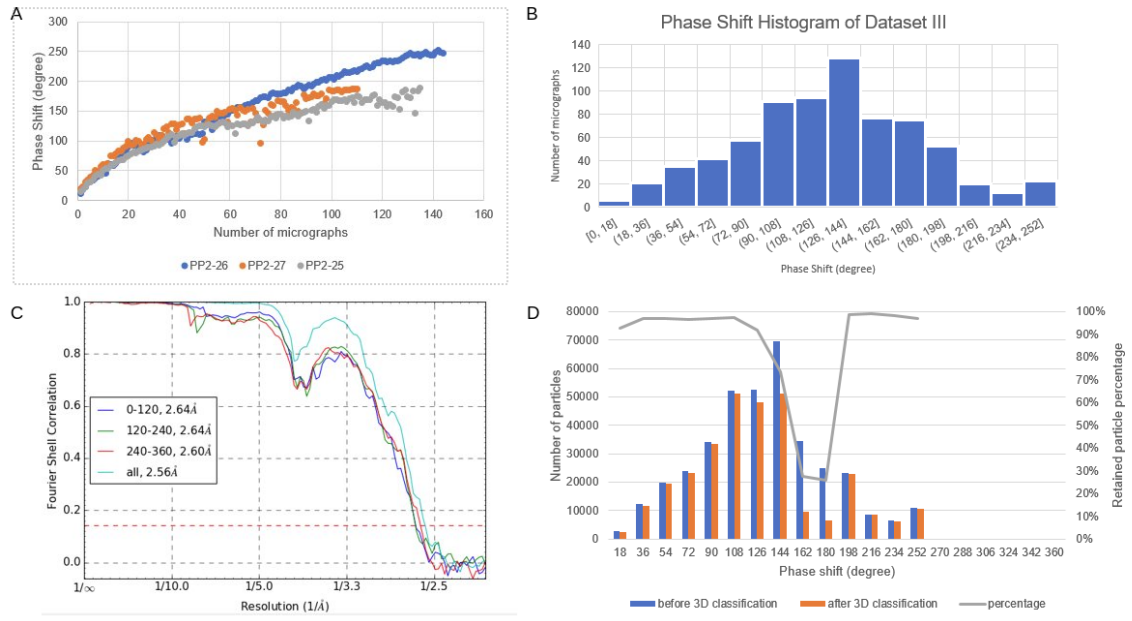

Figure 2. Figure 2. (A) The time-courses of phase shifts of three different Volta phase plate spots. (B) The phase shift histogram of dataset III determined by CTFFIND4. (C) The FSC curve of the half maps reconstructed with all particles and with particles in the phase shift range of 0-120, 120-240 and 240-360 degree. (D) The distribution of the number of particles before and after 3D classification in RELION and the percentage of retained particles after 3D classification as a function of phase shift.

https://doi.org/10.1017/S1431927620021121

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[2] R Danev, D Tegunov and W Baumeister, eLife 2017;6:e23006.

[3] The authors acknowledge funding from the NIGMS U24 GM116789 (W.J.), NIAID R01 AI111095 (W.J.), NINDS 1U01NS110437 (W.J. and R.V.), and NINDS R01 NS050227 (R.V.). We thank the Purdue Cryo-EM Facility (http:// cryoem.bio.purdue.edu) for the use of the Titan Krios microscope.