Z-contrast enhancement for small protein cryo-EM structure determination

James Z. Chen [a]

[a] Oregon Health and Science University, Portland, OR 97239, USA, chezh@ohsu.edu

Single-particle cryo-EM structure determination on small protein molecules has been challenging, mainly because of poor signal-to-noise ratio in EM data that is dictated by the low-dose imaging condition to mitigate radiation damage to the specimen. With the advent of direct electron detectors and phase plates, protein molecules as small as hemoglobin (64 kDa, [1]) and streptavidin (52 kDa, [2]) have recently been imaged and reconstructed to near-atomic resolution. Benchmarked on protein molecules of known structure in both cases, these achievements have showcased the capability of single-particle EM and, more importantly, have suggested its potential application on even smaller protein assemblies.

Electron scattering cross-sections of heavy atoms quantitatively differ from light atoms (C/N/O) abundant in protein molecules, which provides enhanced amplitude contrast in cryo-EM. It is demonstrated here that a novel 50 kDa copper storage protein (CSP, [3]) can be visualized by single-particle cryo-EM without phase-plate imaging and its density map has been reconstructed to near-atomic resolution upon multiset-CTF correction. Beyond CSP, the Z-contrast enhancement technique has broader applications in EM structural biology.

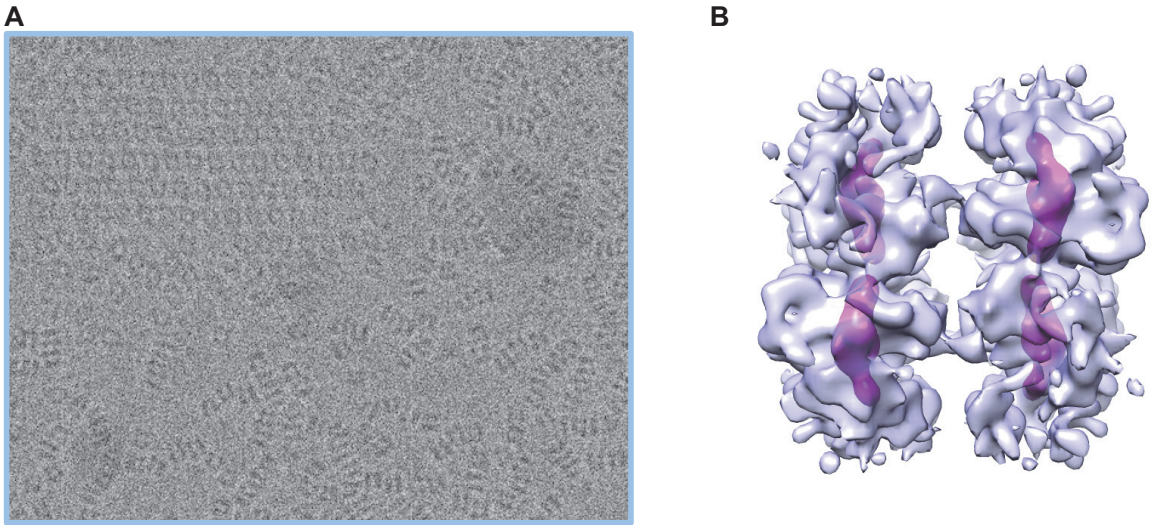

Figure 1. A) One cryo-EM micrograph of CSP particles (*1.8 μm defocus*) without phase-plate imaging. Intriguingly, a patch of molecules has self-assembled into a 2D lattice (in the upper-left area). **B)** A preliminary 3D reconstruction, in which high-density regions associated with metal ions are colored in magenta.

REFERENCES

[1] R Danev and W Baumeister, eLife **5** (2016), doi:10.7554/eLife.13046.
[2] *bioRxiv; doi: http://dx.doi.org/10.1101/457861* (2018).
[3] N Vita, *et al*, Nature **525** (2015), 140-143. doi:10.1038/nature14854.