Z-Contrast Enhancement for Small Protein EM Structure Determination

Heavy atoms/ions have distinctively different electron scattering profiles from light atoms (namely C, N and O) abundant in protein amino acids, which can provide enhanced amplitude contrast in cryo-EM imaging. Facilitated by this physical property, my lab has been investigating the structure and function of *Bs*Csp3, a copper storage protein that can bind numerous (60+) Cu(I) ions in its tetrameric assembly (total MW <50 kDa). In the process, we have also been formulating theoretical models to characterize the effect of Z-contrast enhancement in small protein cryo-EM imaging. In a pilot study, my lab has collected a cryo-EM dataset of Cu(I)-bound *Bs*Csp3 on a local Titan Krios without the assistance of Cs-corrector or phase-plate imaging, from which a 3D reconstruction has reached better than 4Å resolution (preliminary data in Figure 1).

In order to achieve atomic resolution in *Bs*Csp3 structure elucidation, we plan to utilize Cs-corrector coupled with phase-plate imaging in cryo-EM data collection. Such a dataset will also facilitate the validation of our current theoretical model and algorithm for Z-contrast enhanced cryo-EM image analysis. This NCCAT GUP2 proposal will proceed in two steps:

Step 1: Utilize Chameleon to produce uniform and thin-ice cryo grids.

One challenge that we have encountered in the pilot study is to make consistently uniform, thin-ice cryo-grids for large dataset acquisition. We plan to experiment on Chameleon to produce grids of optimal quality (ice thickness, monodispersed particle distribution and density, *etc.*) for high-resolution *Bs*Csp3 structure determination.

Step 2: Apply VPP and Cs-corrector in Cu(I)-bound *Bs*Csp3 cryo-EM data collection.

Our pilot study has confirmed the technical feasibility of cryo-EM structure study on Cu(I)-*Bs*CSP3 – we have sufficient amount of protein specimen at high purity for data collection, and the small protein particles are clearly identifiable in ice even without using phase plates. Acquiring additional large datasets with the assistance of VPP and Cs-corrector imaging will enable us to

1) elucidate the interaction of Cu-ions with *Bs*Csp3 protein at atomic resolution;

2) compare and characterize Z-contrast enhancement under various cryo-EM imaging conditions.

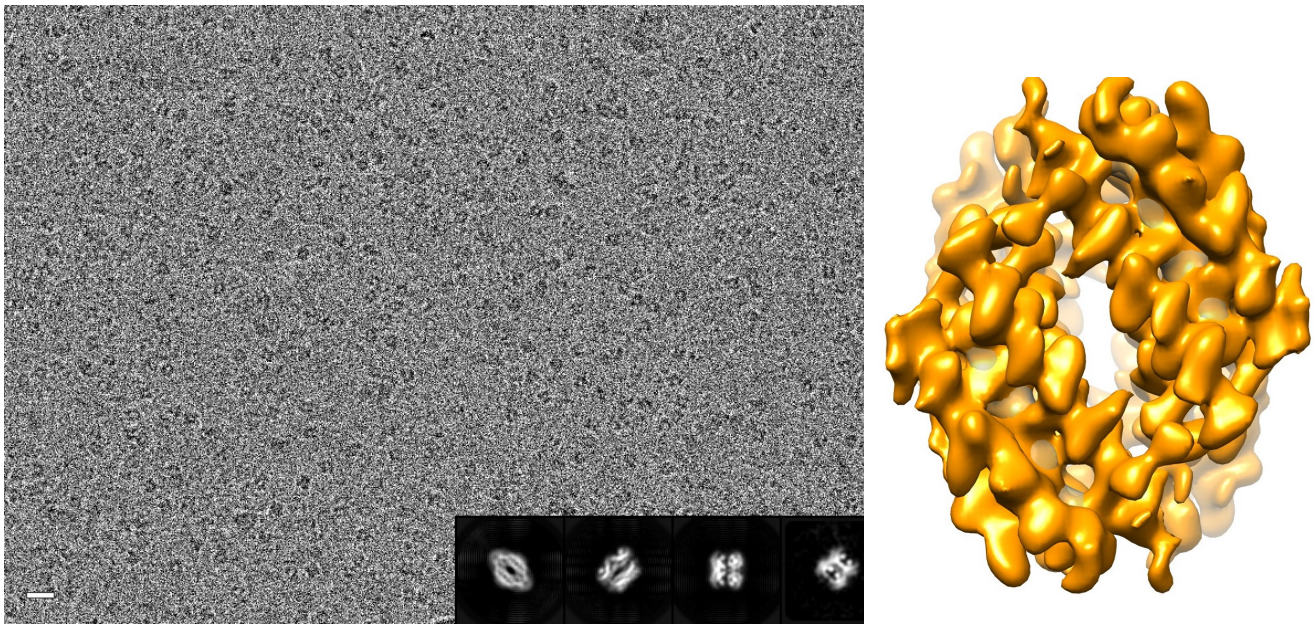

Figure 1: ***L***) Cryo-EM Image of *Bs*Csp3, 300 KeV, 1.6 µm defocus, *without* phase-plate imaging (scale-bar: 10nm) and example class averages. ***R***) A preliminary 3D reconstruction.