Figures for NCCAT Proposal, Lazarus Lab

Figure 1. Purification of DDB1-DCAF16 complex. Using insect cells, we purified the large complex consisting of the scaffolding protein DDB1 and the E3 ligase-associated factor DCAF16, as seen on the Coomassie-stained gel. The proteins coelute as a homogenous peak on a superdex200 increase column, as seen in the chromatogram here.

Figure 2. Purification of FKBP12. We purified bacterially expressed FKBP12 to high purity and homogeneity. We then used the molecular glue KB02-SLF which is known to be a covalent targeter of DCAF16 and of FKBP12 to assemble the 3-protein complex.

Figure 3. Collection of DDB1-DCAF16-KB02-FKB12 complex on the Titan Krios 3 at NYSBC. We collected a full data set on the complex on the Krios and obtained nice micrographs as shown here.

Figure 4. 2D classification of the DDB1 complex. The staff at NYSBC automated processing and classification of our dataset. We can see good resolution images from the data that also bear the 3-fold symmetry of DDB1 itself.

Figure 5. 3D reconstruction of the DDB1 complex. Our initial solving of the data suggests that the full complex might be visible, but the resolution is too low for us to make out all the members of the complex.

Figure 6. Purification of AASS. AASS is a 100 kDa bifunctional metabolic enzyme that is thought to exist as a tetramer. We expressed the protein in bacteria and purified it by ion exchange to obtain high-quality protein, as seen in this Coomassie gel of the ion exchange fractions.

Figure 7. Negative staining of AASS. After purifying the protein, we performed negative staining experiments on the protein at our institutional electron microscope. The protein behaved well by negative staining.

Figure 8. AASS data collection on Titan Krios 3 from NYSBC. Using our NYSBC Krios time, we screened grids with AASS and collected a series of micrographs shown here, which indicate decent resolution. We did not have enough time to collect a full dataset.

Figure 9. 2D classification of AASS. Using our screening micrographs, we were able to do some initial 2D classification, which indicated the tetrameric structure. However, we were unable to conclude more without collecting more data.