Figures Captions for NCCAT Proposal, Lazarus Lab, January 2023.

Figure 1. Purification of full-length AASS and crosslinking of AASS complex. Left: We figured out how to purify the full-length 100 kDa protein in bacteria using a series of fusion tags, shown before and after tag cleavage on the gel. Right: we crosslinked the protein to maintain the tetrameric complex upon freezing, after initial data collection showed that only one domain was visible.

Figure 2. 2D class averages of crosslinked full-length AASS from data collected on a Krios. Initiallu, uncrosslinked AASS showed good density for the tetrameric core LOR domain, but no density for the SDH domain. With crosslinking, we can now see density for 2 of the 4 SDH domains.

Figure 3. AASS 3D reconstruction. Using the dataset above, we were able to refine a 3D reconstruction of AASS to better than 3Å resolution. There is clear density for the tetrameric LOR domain and 2 of the SDH domains of the protein.

Figure 4. Preliminary model of full-length AASS. Using our crystal structure of the LOR domain and another group's crystal structure of the SDH domain, we were able to construct a model of the full-length protein. Each chain is shown in a different color.

Figure 5. Micrograph from DDB1/DCAF16/FKBP12 complex on Krios microscope.

Figure 6. 2D class averages of DDB1 complex data. There is clear density for the trimeric beta propeller domain of DDB1

Figure 7. 3D reconstruction of DDB1-DCAF16 complex. We were able to obtain a high resolution structure of the complex (Left). However, most of DCAF16 is not visible. Only a helix-turn-helix (Upper Right) is visible and can be constructed in our model. Therefore, we have been able to crosslink the complex (Lower Right) and will collect data on the crosslinked complex.