Figures Captions for NCCAT Proposal, Lazarus Lab, January 2024.

Figure 1: Summary of CAD protein, including a cartoon schematic. It is proposed to form a 1.5 megadalton hexameric complex and is upregulated in many cancers.

Figure 2. Purification of CAD from insect cells. We developed a purification strategy for the 250 kDa protein using a His-strep tag and a superose fplc column and obtained protein to good purity.

Figure 3. 2D classification of CAD. We obtained some preliminary 2D data from grids we froze with CAD protein, but the reconstruction does not match our expected size, so we optimized our purification strategy.

Figure 4. Purification of AASS with substrates. We purified AASS from insect cells and obtained good 2D data.

Figure 5. Electron density of AASS with substrates. We were able to solve a structure with the substrates. However, we only see density for the LOR core domain and not the SDH domain (see blurry SDH domain in Figure 4 class averages).

Figure 6. Structure of LOR domain of AASS tetramer with substrates.

Figure 7. Cartoon schematic of full-length protein.

Figure 8. Partial structure of full-length AASS. Resolution is poor for many parts of the protein, so we are not able to report a full-length structure.

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. Allosteric activator of AASS discovered in our high throughput screen. Looking for inhibitors of AASS, we discovered potent activators of the enzyme, likely through an allosteric conformational change.

Figure 6. Micrograph from DDB1/DCAF16/FKBP12 complex on Krios microscope.

Figure 7. 2D class averages of DDB1 complex data. There is clear density for the trimeric beta propeller domain of DDB1

Figure 8. 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.

Figure 9. Purification of DDB1-DCAF7 complex in insect cells. We are trying to learn how other DCAF proteins interact with DDB1.