

Figures Captions for NCCAT Proposal, Lazarus Lab, December 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 Expi293 mammalian 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. Micrograph of CAD from a Krios session. There are some large complexes but also some smaller particles, indicating heterogeneity in the sample.

Figure 4. 2D classification of CAD. We obtained some preliminary 2D data from grids we froze with CAD protein, but the 2D classifications are not of sufficient quality yet.

Figure 5: NMNAT1 catalyzes the synthesis of NAD⁺. It was found to be a dependency for several malignancies.

Figure 6: NMNAT1 forms a hexamer at 198 kDa, as shown in x-ray crystal structures.

Figure 7. We were unable to solve NMNAT1 with inhibitor bound due to the preferred orientation. Shown here are the 2D class averages, a 3D reconstruction, as well as protein purification images.

Figure 8. We solved the structure of NMNAT1 with inhibitor bound in the presence of detergent. Using a detergent, we were able to obtain a structure.

Figure 9. In the presence of a new inhibitor, we still obtained preferred orientation issues for NMNAT1, even in the presence of detergent. This is likely due to the new inhibitor and high DMSO content.

Figure 10. 2D class averages of NMNAT1 with new inhibitor, showing severe preferred orientation.