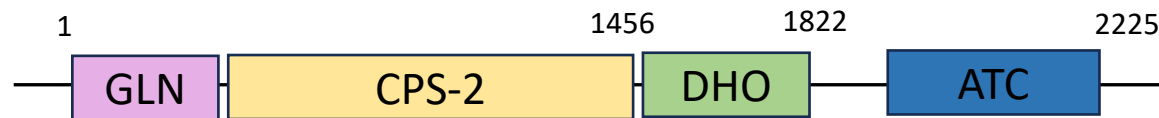


Structural Analysis of Metabolic Enzymes

Lazarus Lab

NCCAT proposal for January 2024

Figure 1: CAD is a fusion protein that participate in Pyrimidine synthesis



1-1456: glutaminase-dependent Carbamoyl phosphate synthetase

1457-1822: Dihydroorotase

1823-2225: Aspartate transcarbamoylase

CAD is a 1.5 MDa particle formed by hexameric association of a 240 kDa protein divided into different enzymatic domains, each catalyzing one of the initial reactions for de novo biosynthesis of pyrimidine nucleotides:

-glutaminase-dependent Carbamoyl phosphate synthetase

-Aspartate transcarbamoylase, and

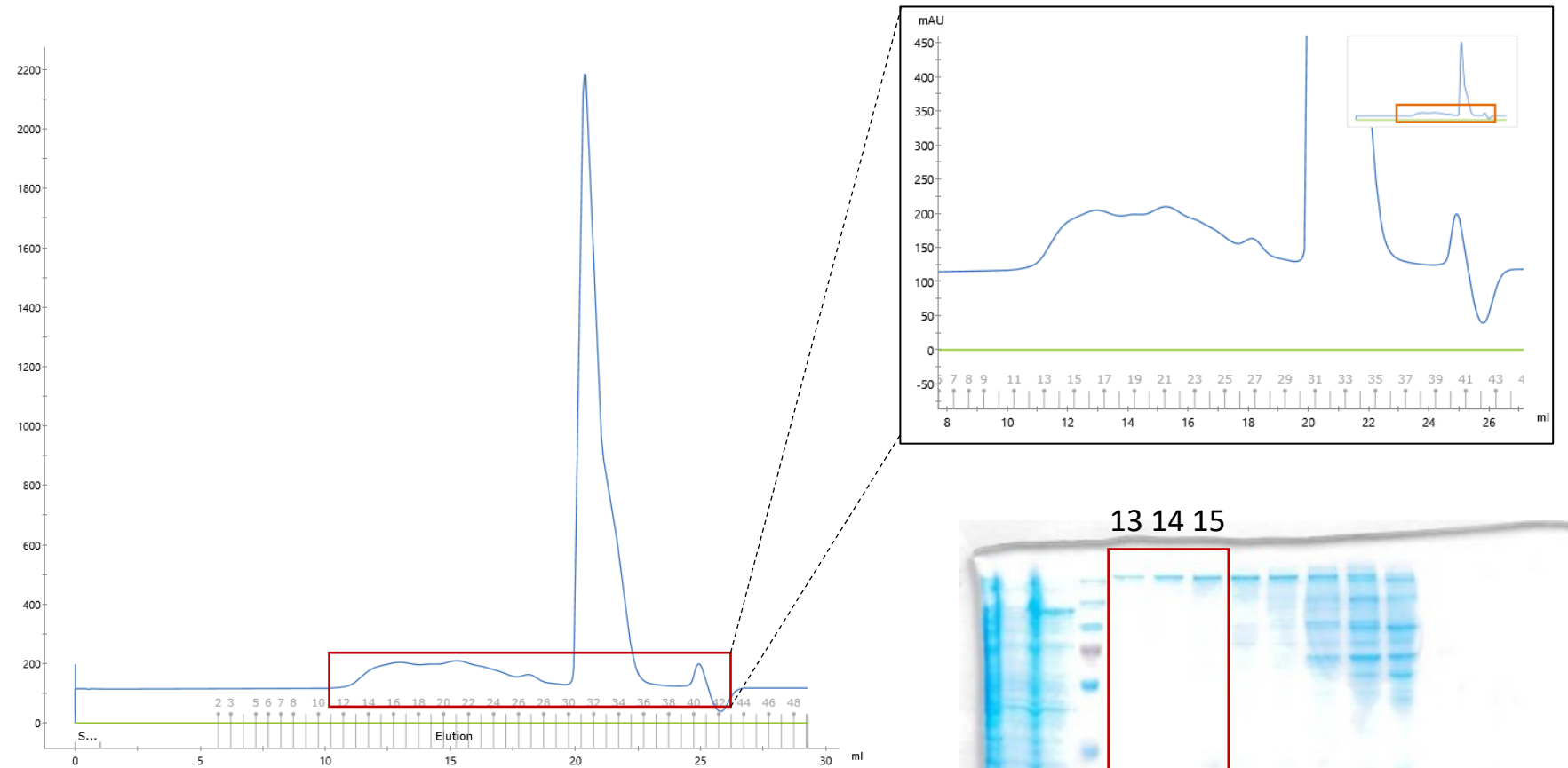
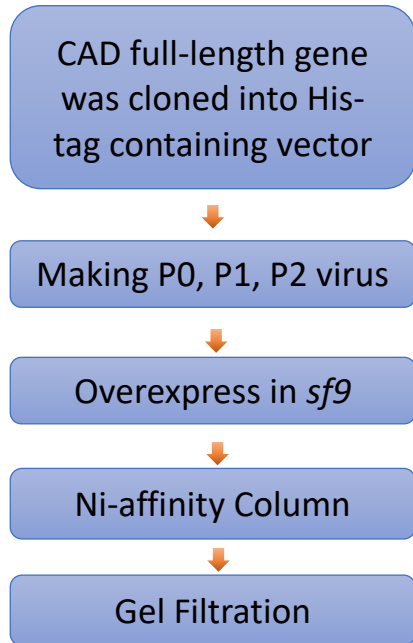
-Dihydroorotase.

We are interested in understanding the molecular mechanisms of CAD protein and as a possible therapeutic target for certain malignancies

Grande-García A, Lallous N, Díaz-Tejada C, Ramón-Maiques S.. Structure. 2014 Feb 4;22(2):185-98. doi: 10.1016/j.str.2013.10.016. Epub 2013 Dec 12. PMID: 24332717.

Del Caño-Ochoa F, Ramón-Maiques S. Protein Sci. 2021 Oct;30(10):1995-2008. doi: 10.1002/pro.4158. Epub 2021 Jul 22. PMID: 34288185; PMCID: PMC8442968.

Figure 2: CAD Protein was purified from *Sf9* cells



Fractions 13-15 were pooled for Cryo-EM

Figure 3: 2D classification of CAD

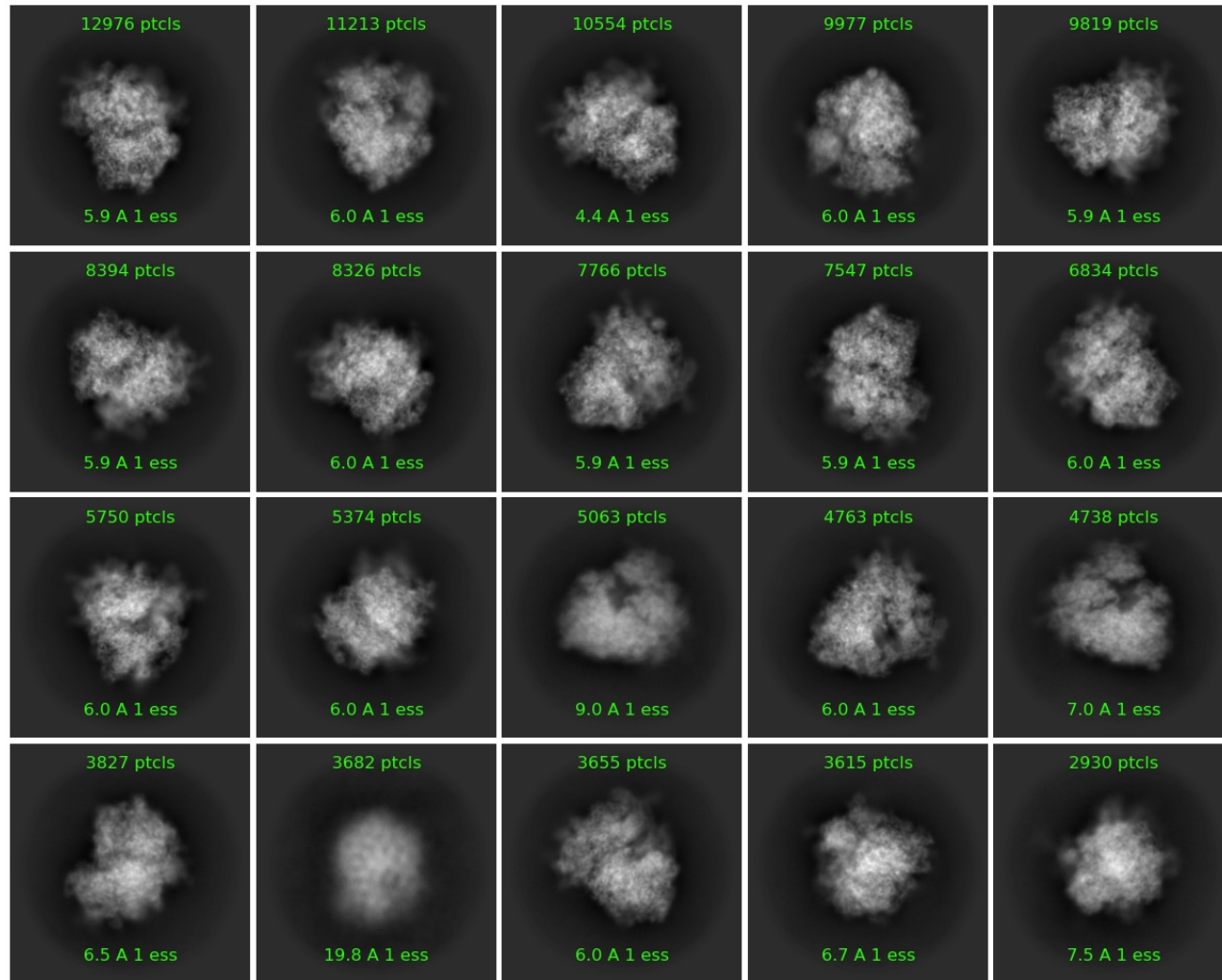


Figure 4: We are able to capture AASS with the substrates: NADPH and saccharopine

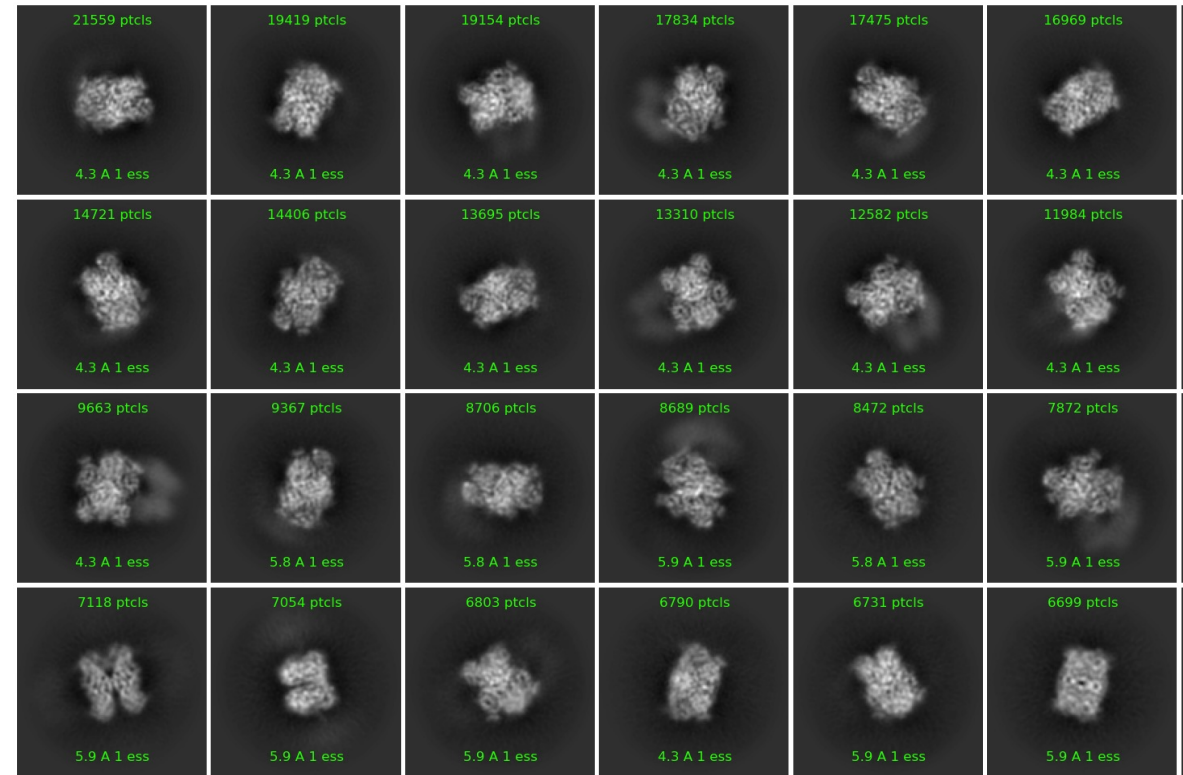
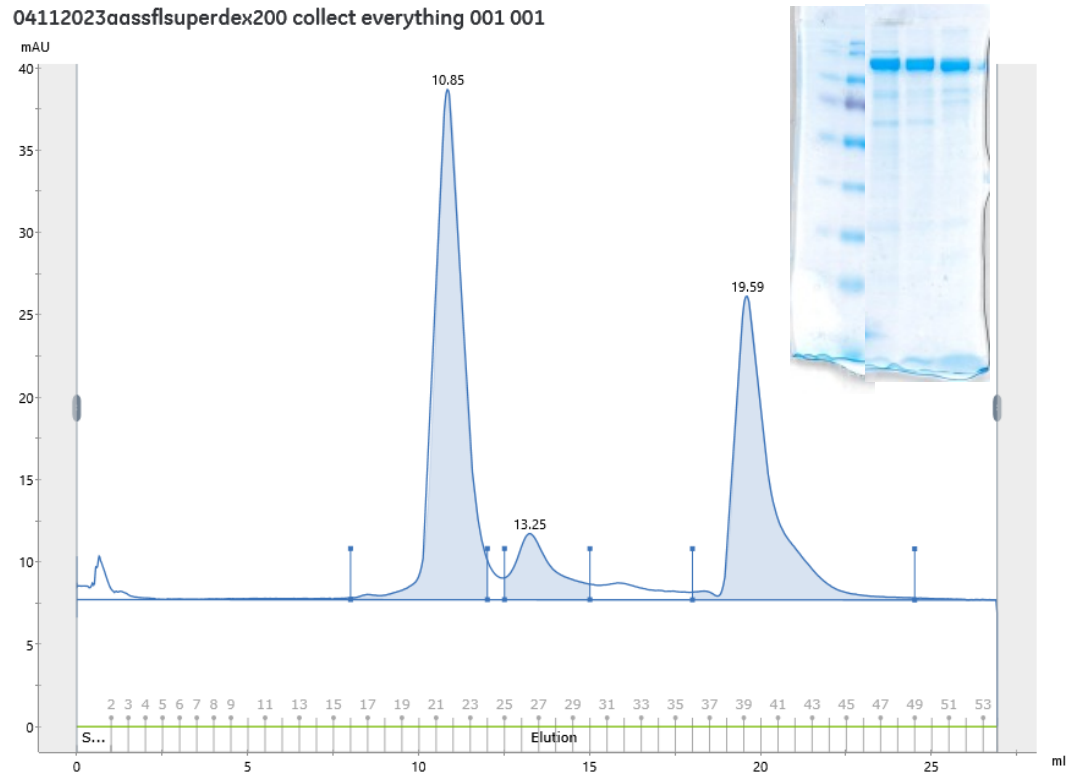
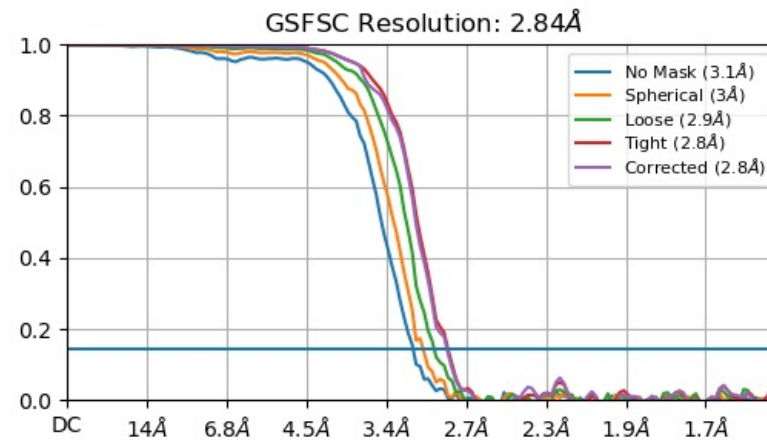
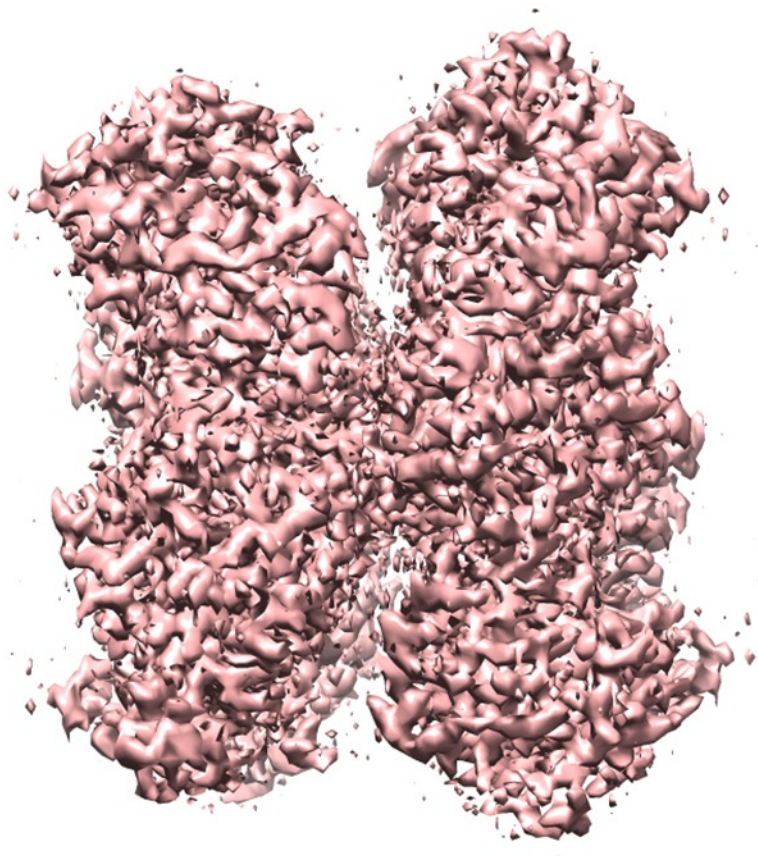


Figure 5: We are able to capture AASS with the substrates-NADPH and saccharopine



Fitted structure with NADPH
and Saccharopine

Figure 6: We obtained the first structure of AASS with NADP and saccharopine, but can only see LOR

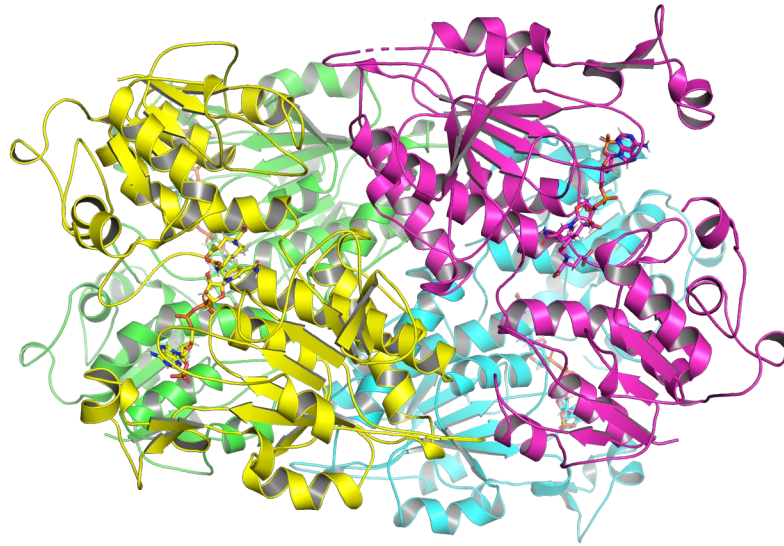
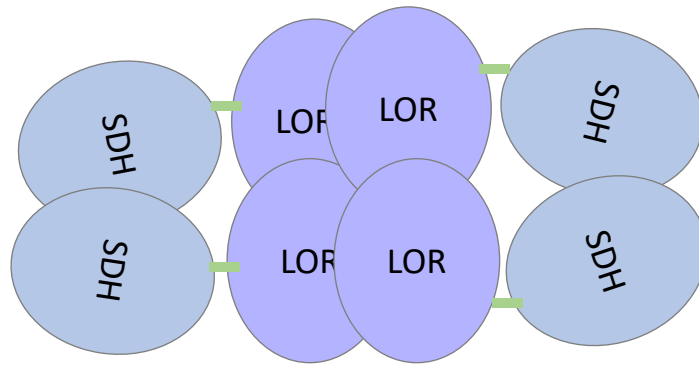


Figure 7: AASS forms a tetramer under Cryo-EM



Core
Domain

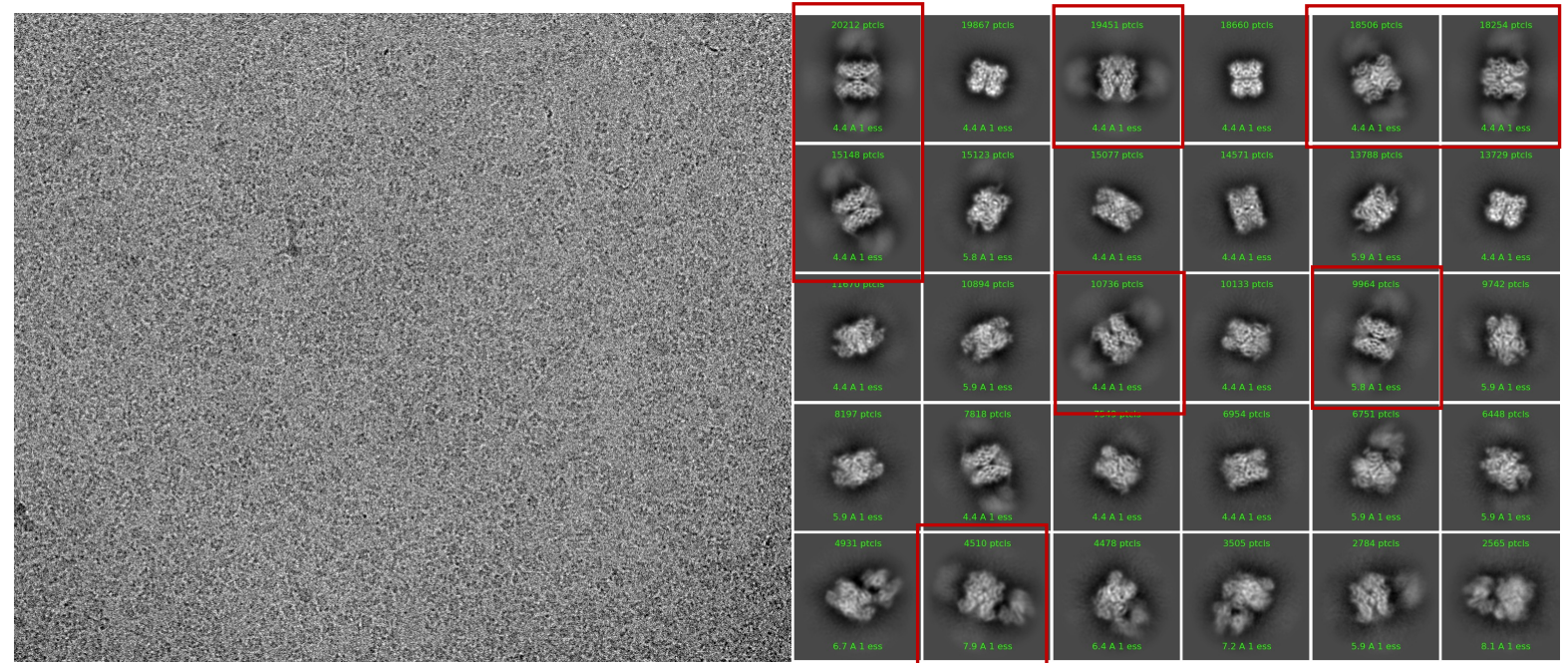
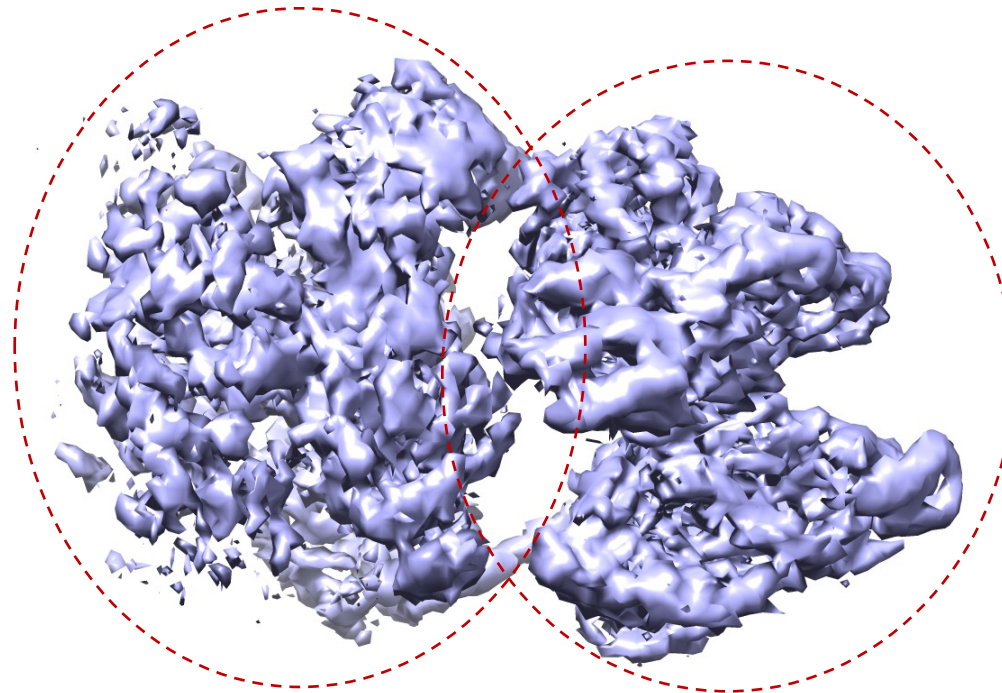
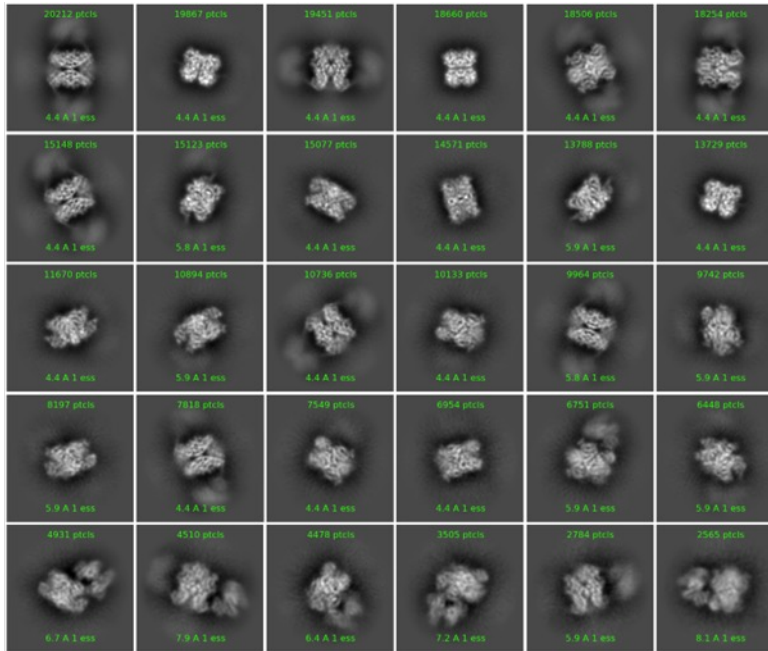


Figure 8: We are trying to capture full length AASS



LOR Domain

SDH Domain

