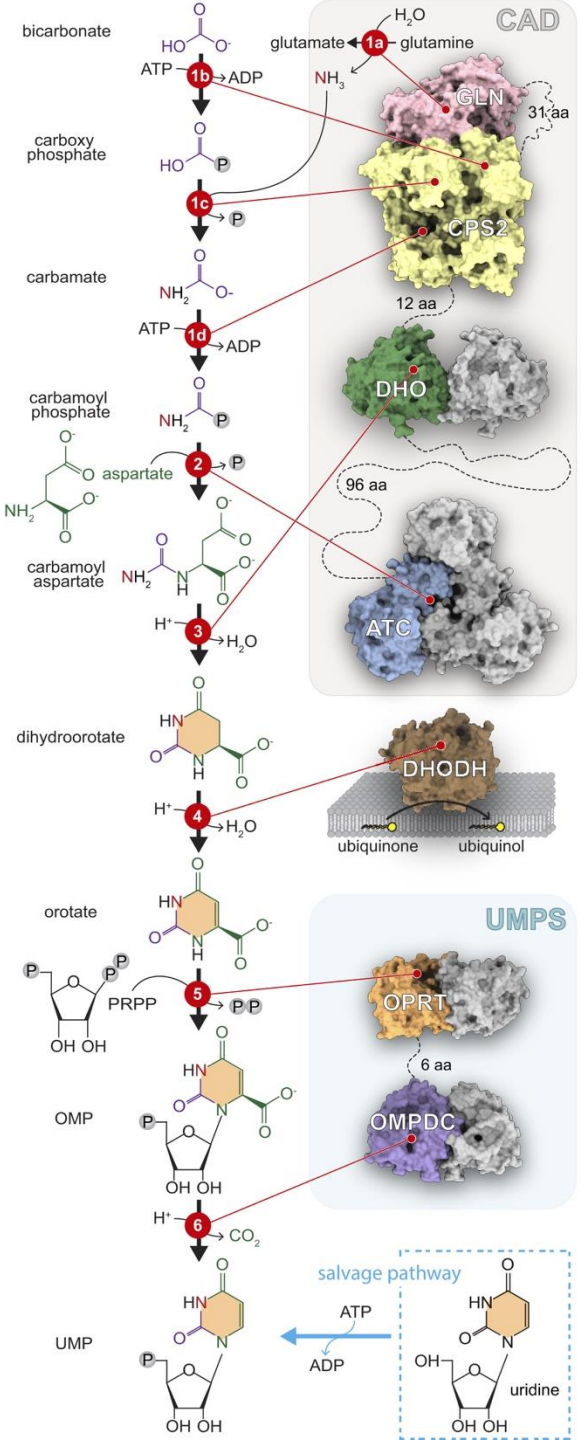
Structural insight into metabolic pathways in human disease

Lazarus Lab

NCCAT proposal for December 2024

Figure 1: CAD is the key enzyme for pyrimidine biosynthesis

- CAD catalyzes the first 3 steps of de novo pyrimidine biosynthesis
- In animals, first 3 steps are in one large, multifunctional protein
- Proposed to form 1.5 MDa hexameric units
- No structure for the first 2 domains (1400 amino acids)



Del Caño-Ochoa F, Ramón-Maiques S. Deciphering CAD: Structure and function of a mega-enzymatic pyrimidine factory in health and disease. 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 *Expi293 cells*

CAD full-length gene was cloned into His-tag containing vector

↓

Making P0, P1, P2 virus

↓

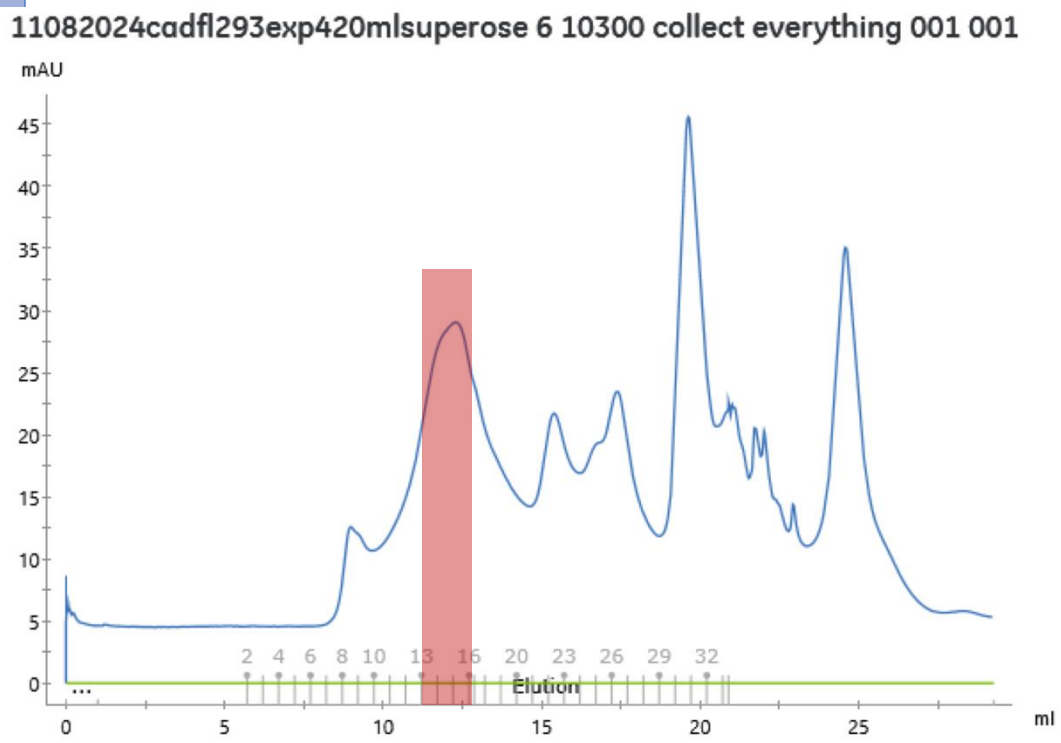
Express in *293*

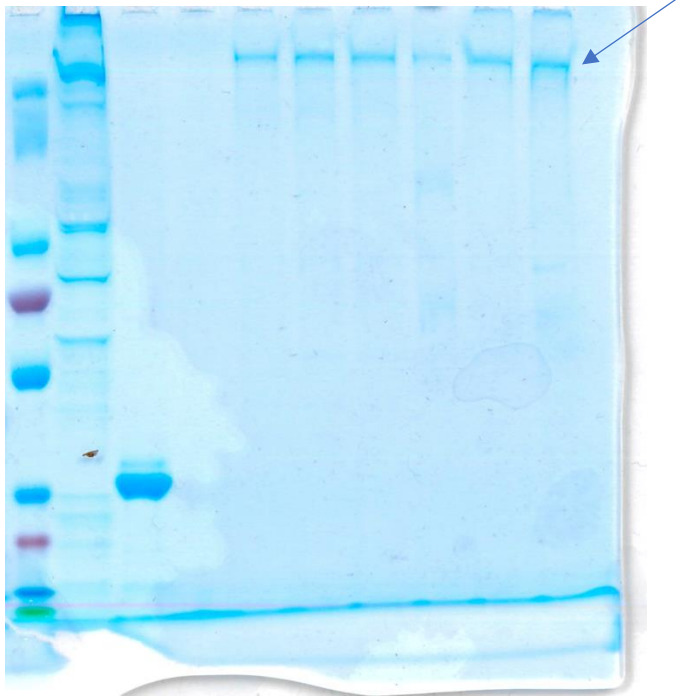
↓

Ni-affinity Column

↓

Superose 6 Gel Filtration





Full-length cad

Figure 3: CAD micrograph

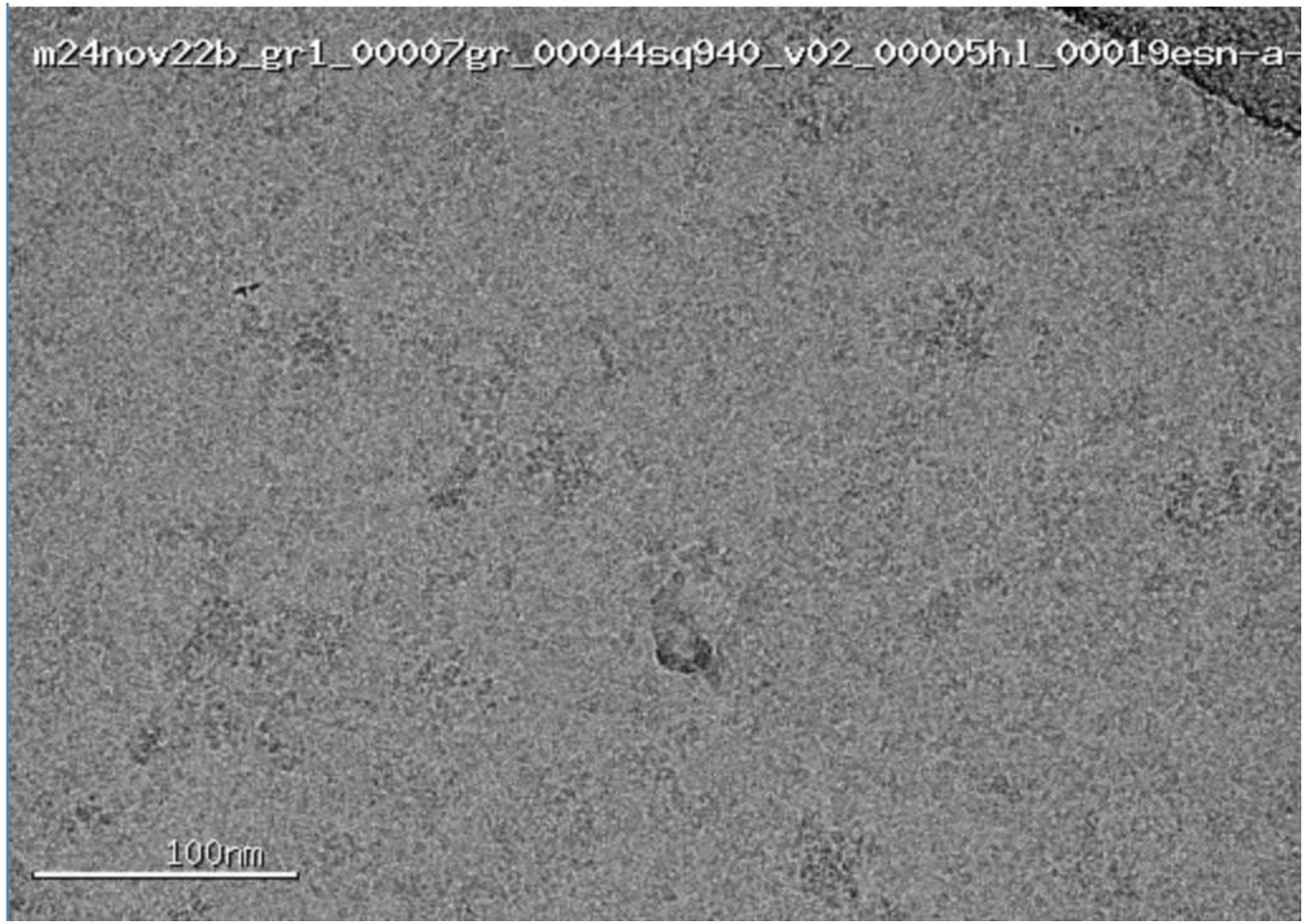


Figure 4: CAD 2D class averages

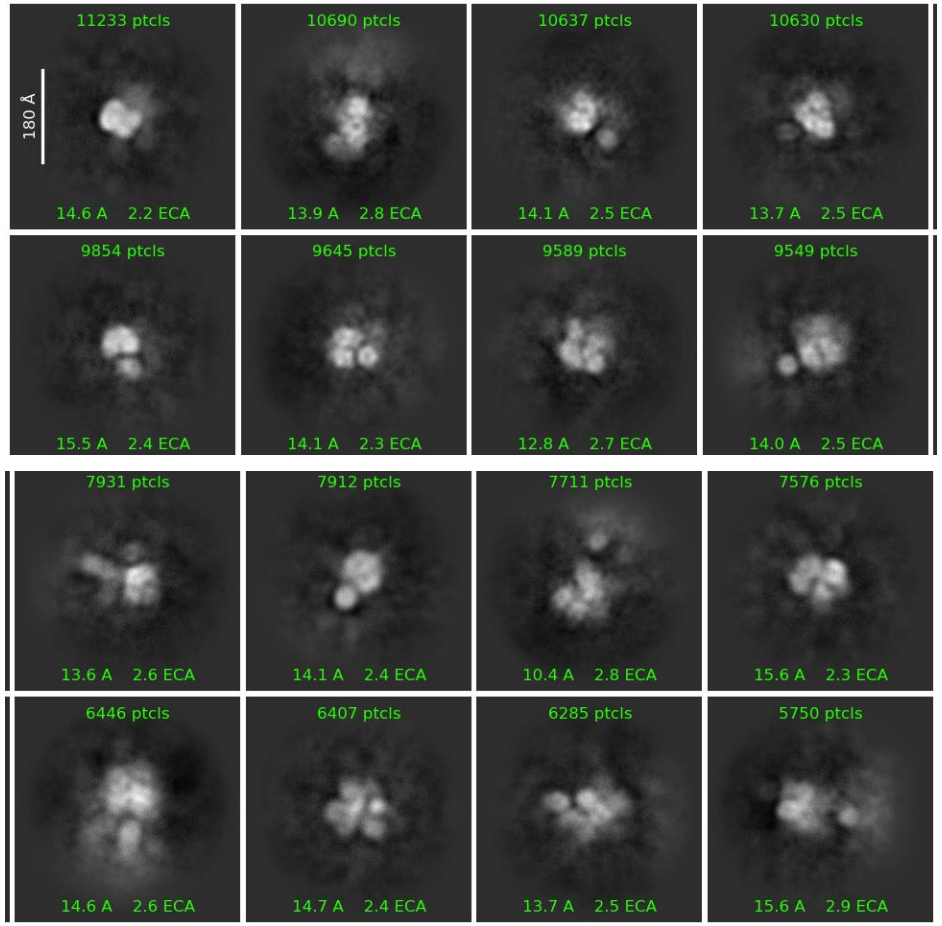


Figure 5: NMNAT1 catalyzes the synthesis of NAD+

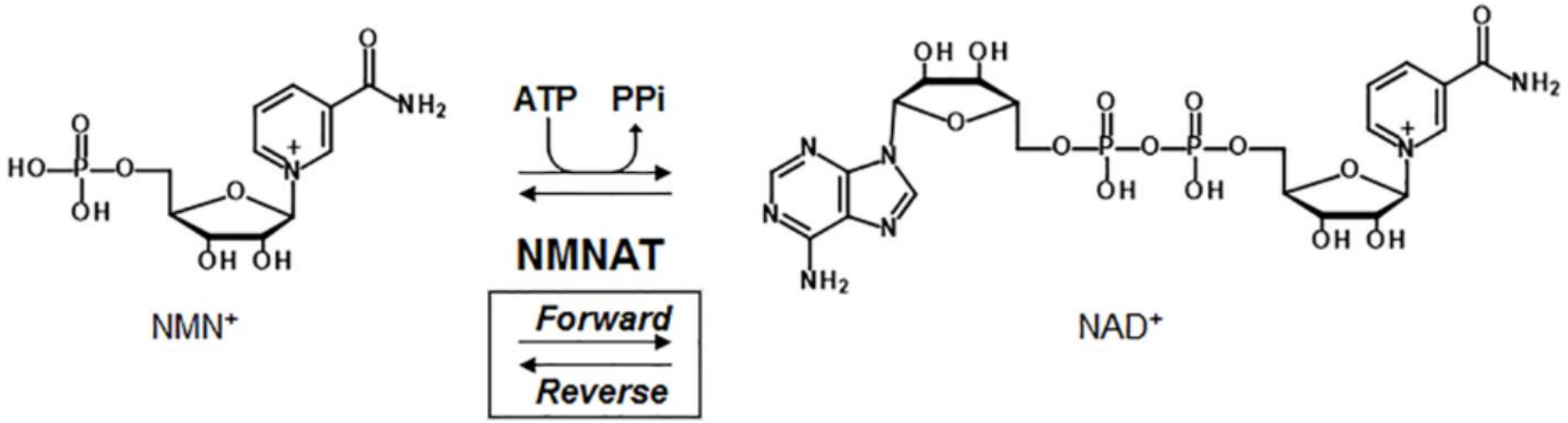


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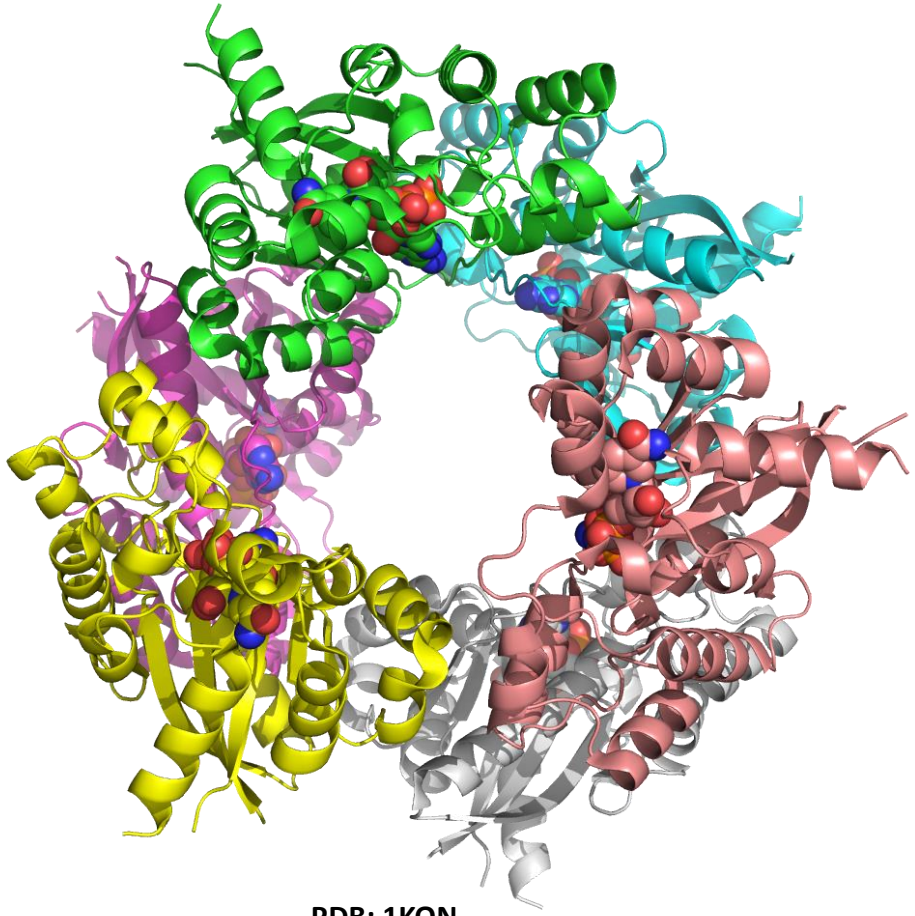
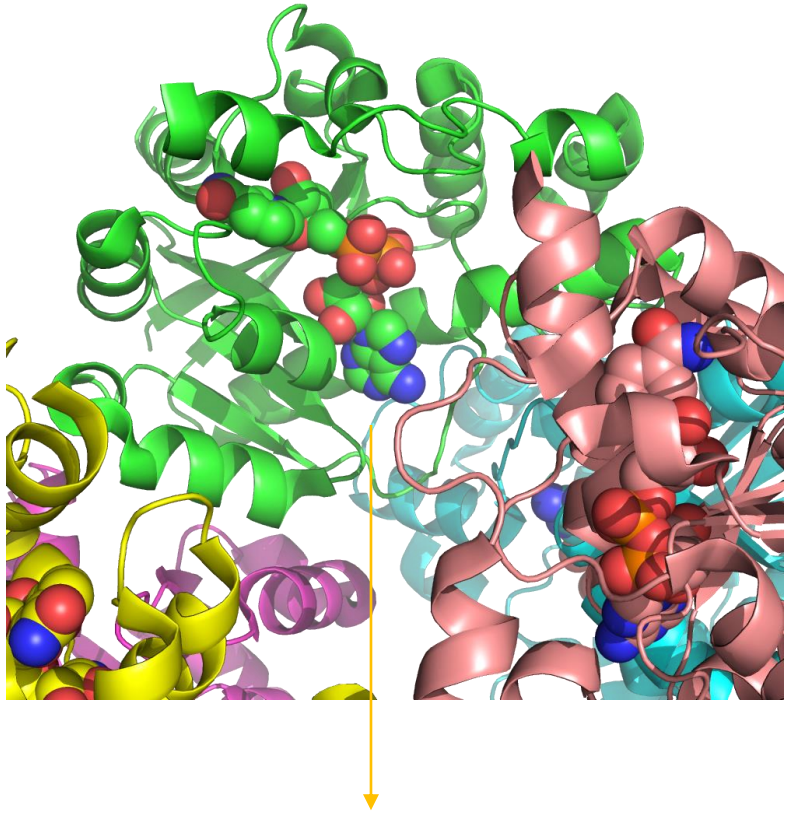
Figure 6: NMNAT1 forms a hexamer at 198 kDa



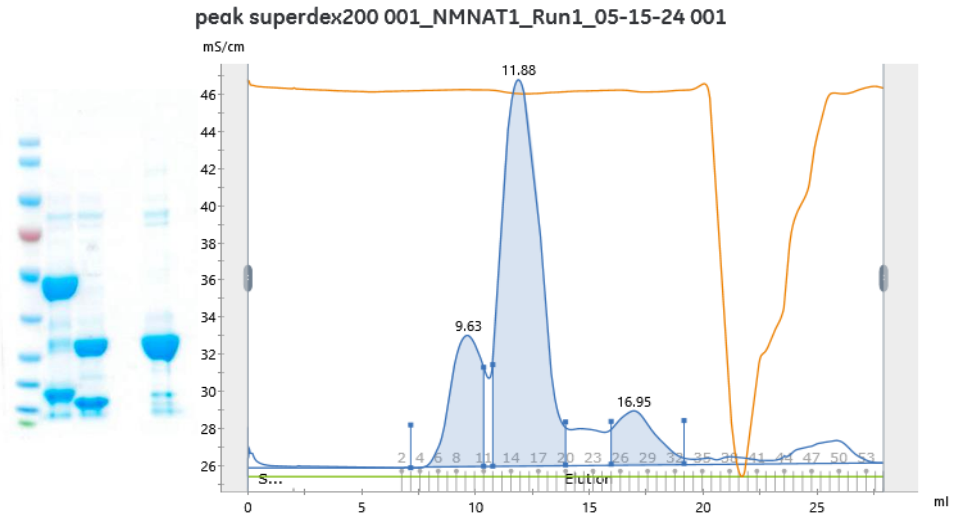
PDB: 1KQN

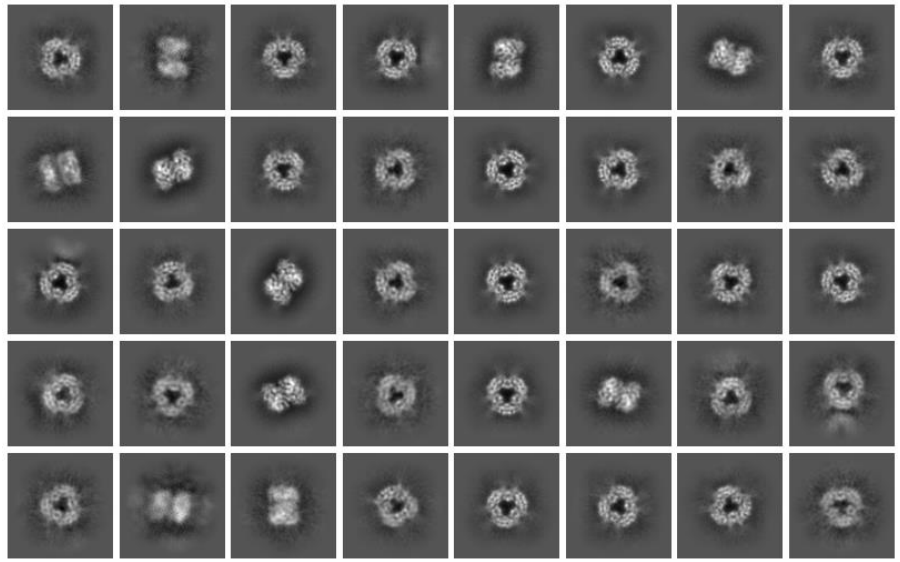


NAD

Zhou T, Kurnasov O, Tomchick DR, Binns DD, Grishin NV, Marquez VE, Osterman AL, Zhang H. Structure of human nicotinamide/nicotinic acid mononucleotide adenylyltransferase. Basis for the dual substrate specificity and activation of the oncolytic agent tiazofurin. J Biol Chem. 2002 Apr 12;277(15):13148-54. doi: 10.1074/jbc.M111469200. Epub 2002 Jan 11. PMID: 11788603.

Figure 7: We were unable to solve NMNAT1 with our first inhibitor bound due to preferred orientation





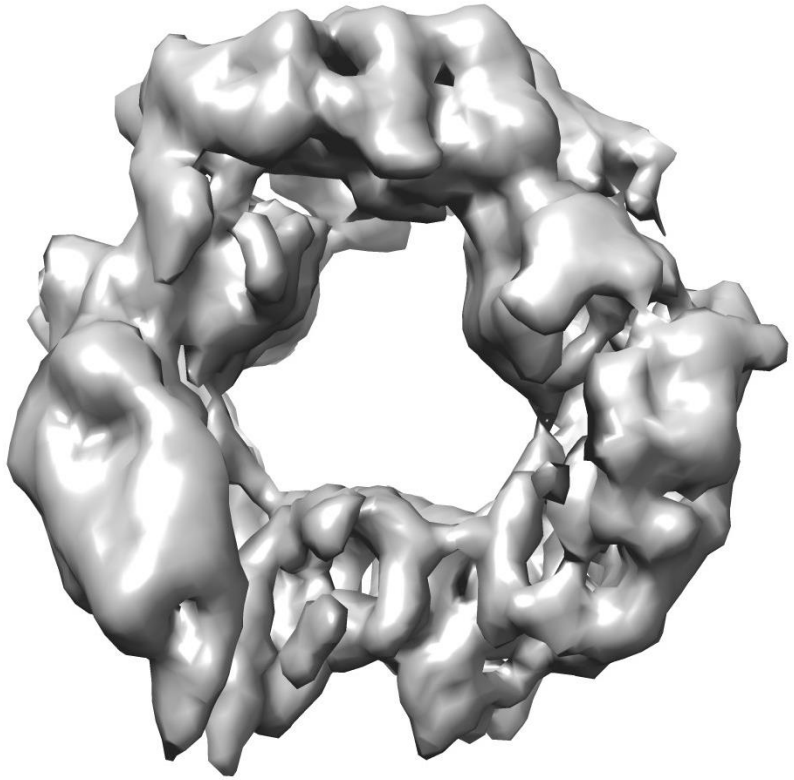
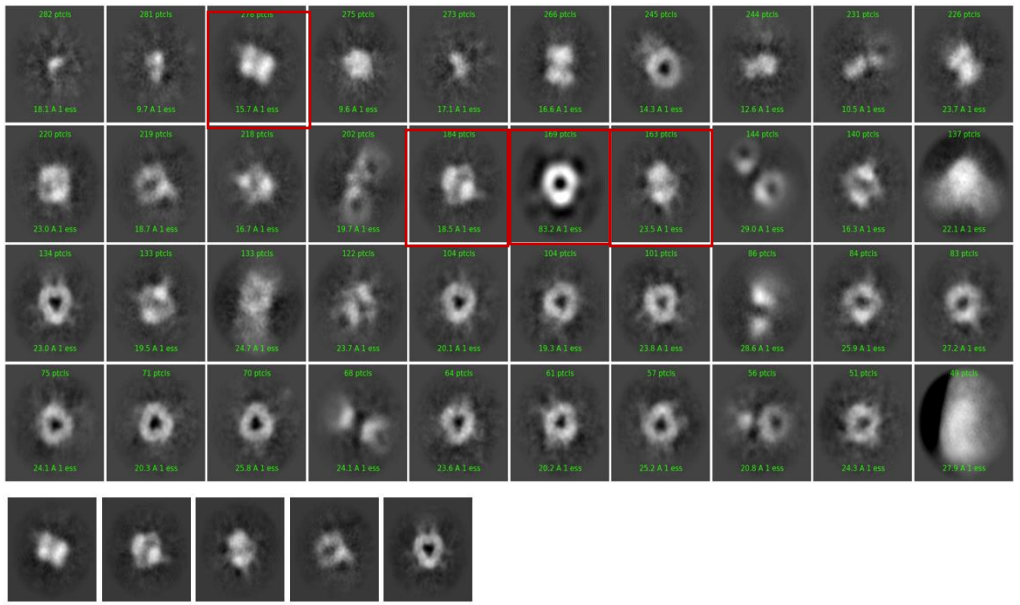


Figure 8: We solved the structure of NMNAT1 with one inhibitor bound, by using detergent



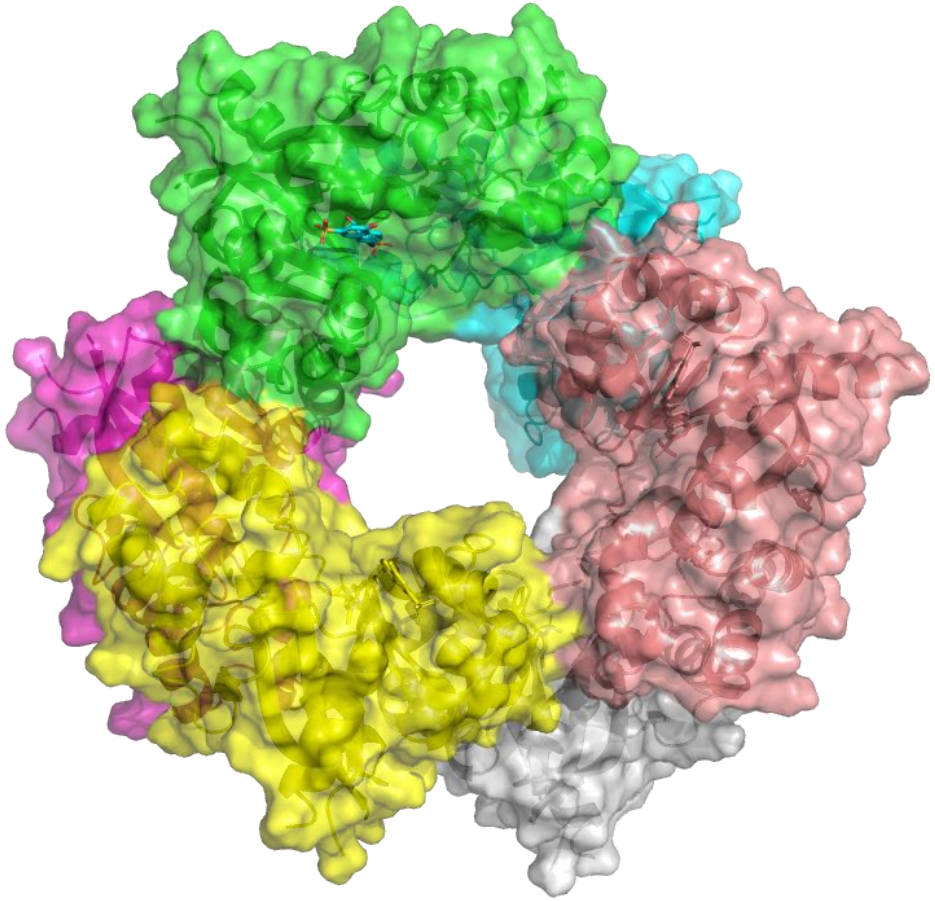


Figure 9: With new inhibitors we have a preferred orientation even in the presence of detergent

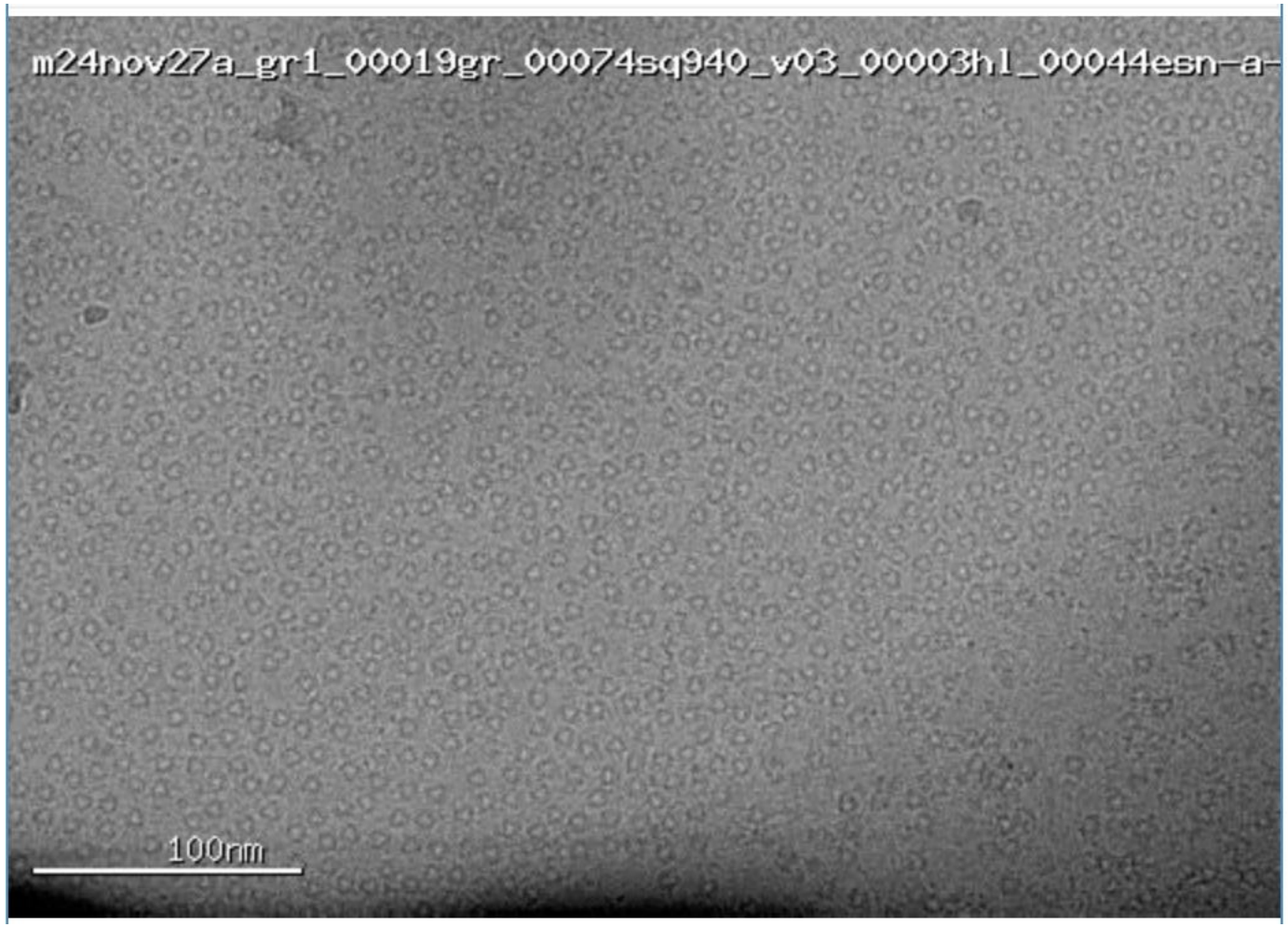


Figure 10: 2D class averages of NMNAT1 with new inhibitor showing preferred orientation