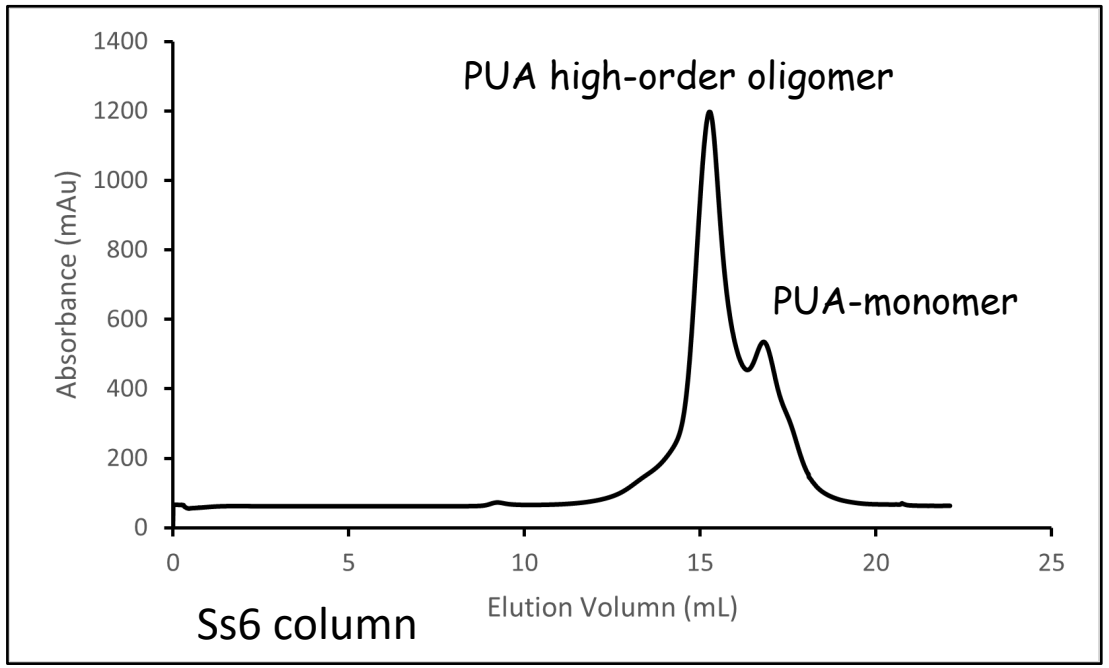
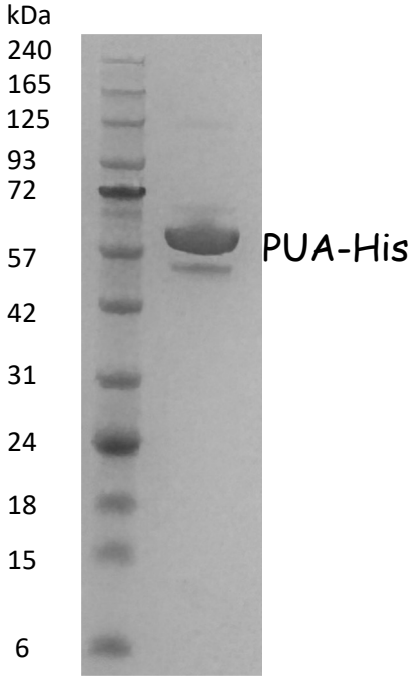
PUAlike-Calcineurin-CE+HAD project

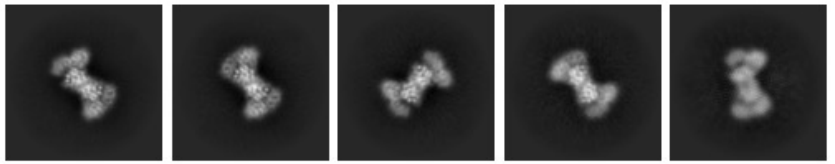
PUA can exist as a monomer or a high-order oligomer on SEC





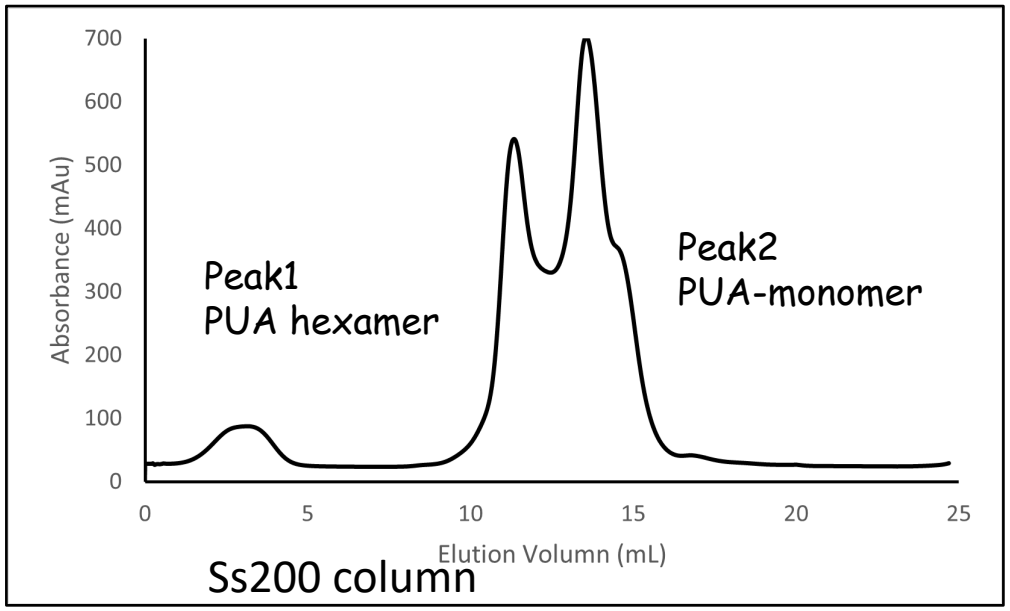
PUA high-order oligomer: Molecular weight ~360 kDa
prepare cryo-EM sample.

PUA-monomer: Molecular weight ~60 kDa
Crystal screening



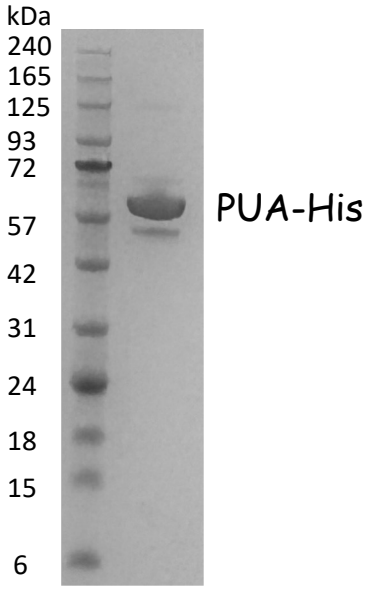
preferred orientation problem

The PUA hexamer and monomer are in equilibrium

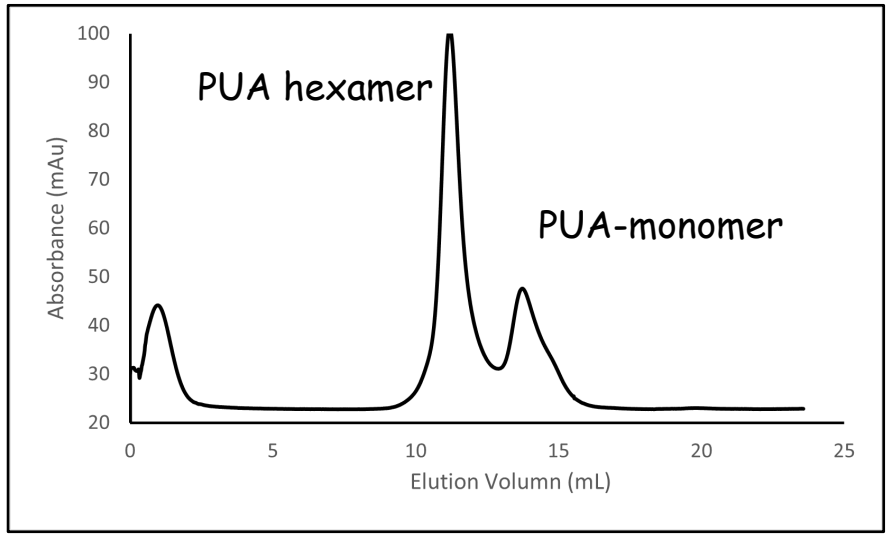


Ss200 column

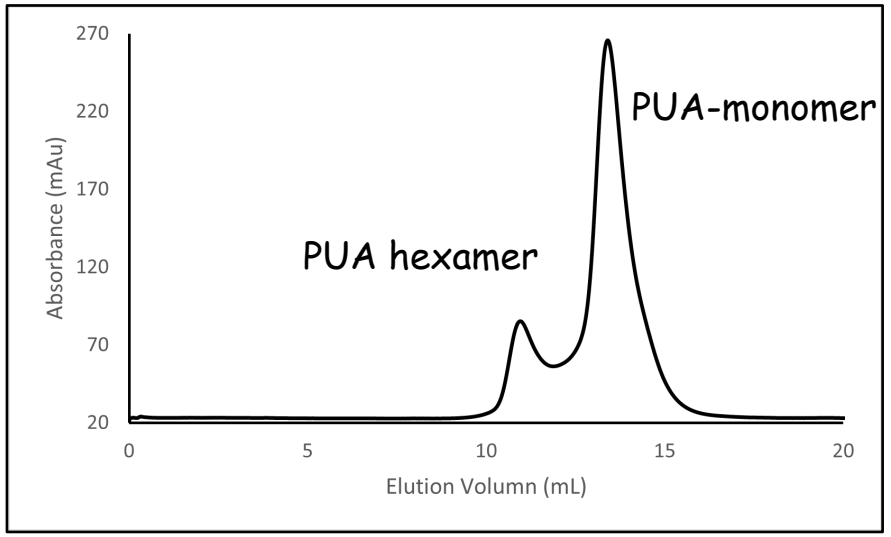
1st SEC



PUA-His



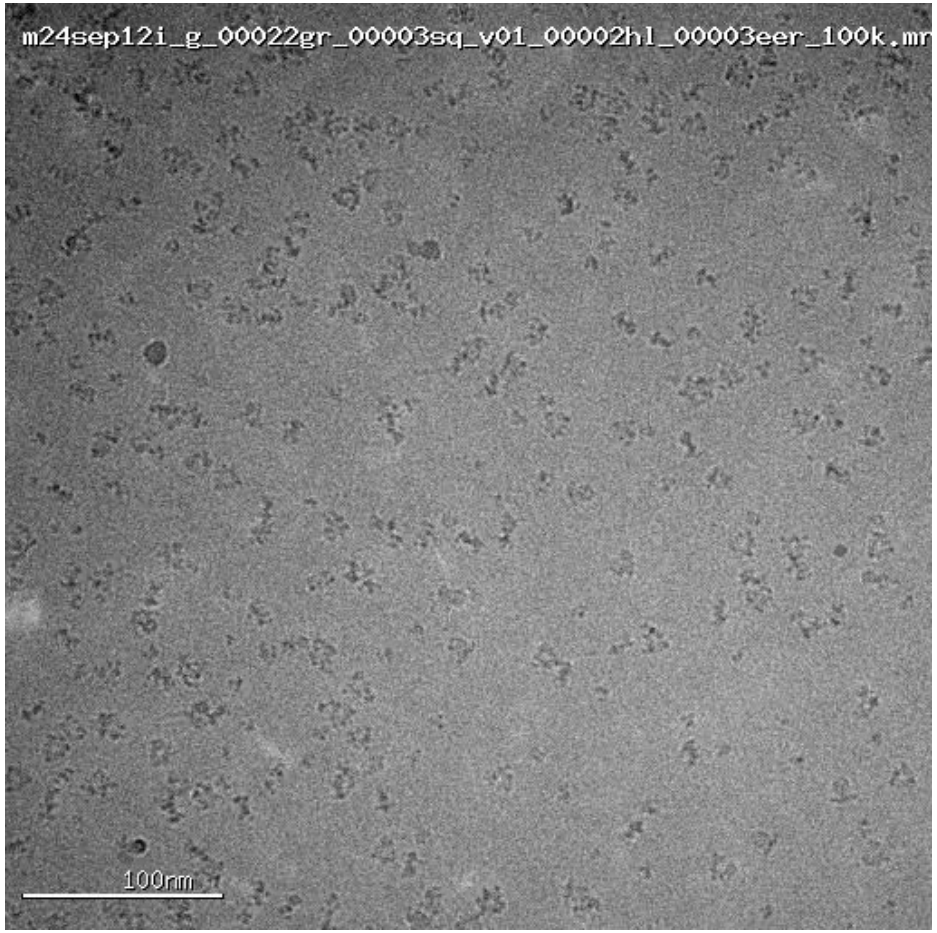
Peak1 2nd SEC



Peak2 2nd SEC

PUA sample was added FOM to improve the preferred orientation

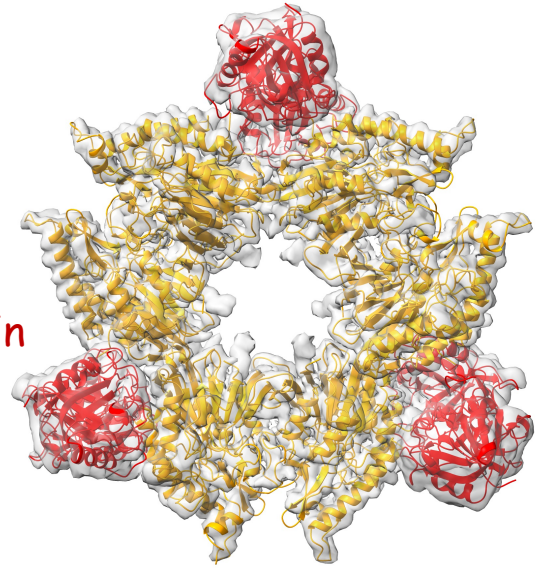
PUA: 0.5 mg/mL
Only the side view

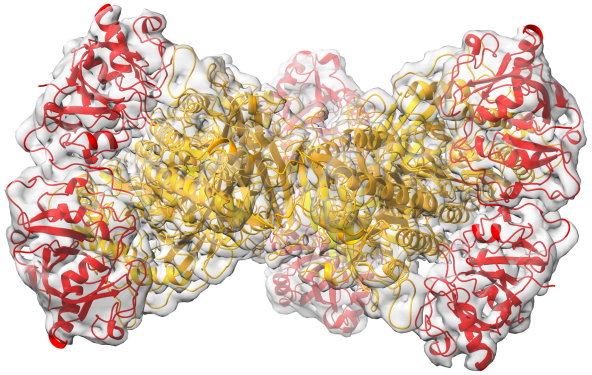


PUA: 6 mg/mL + 0.5 mM FOM
Different views

PUA-Calcineurin-CE hexamer with two different conformations

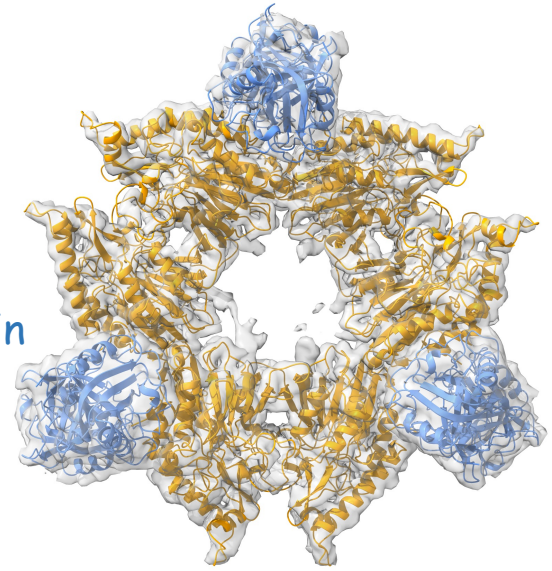
PUA-like domain

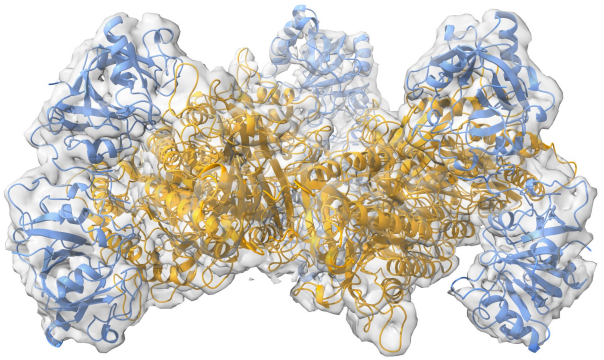




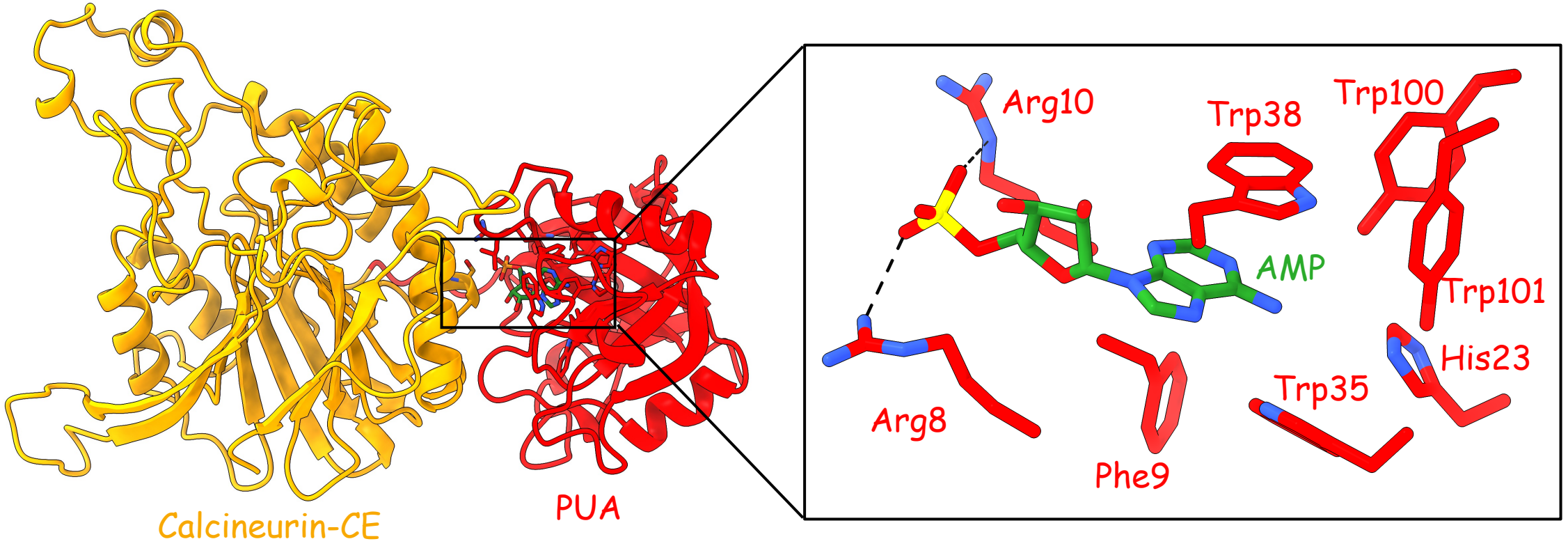
Calcineurin-CE domain

PUA-like domain

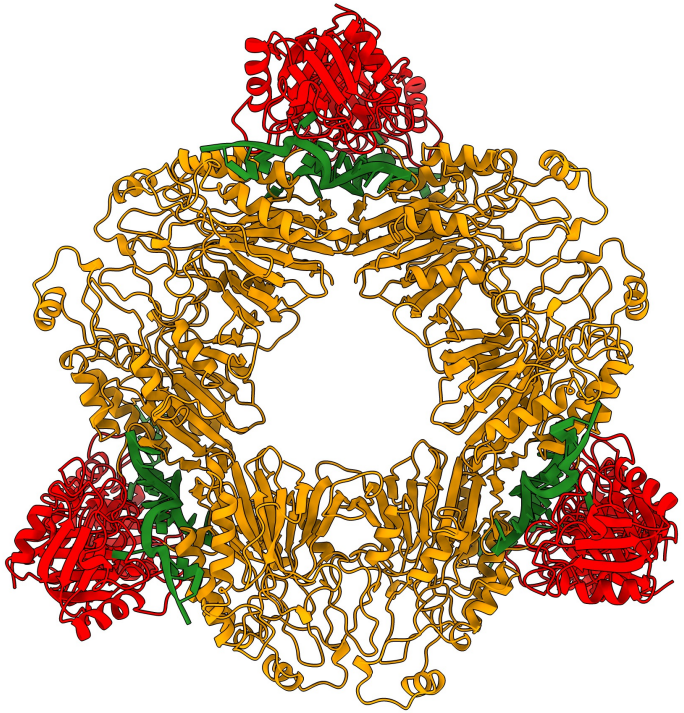


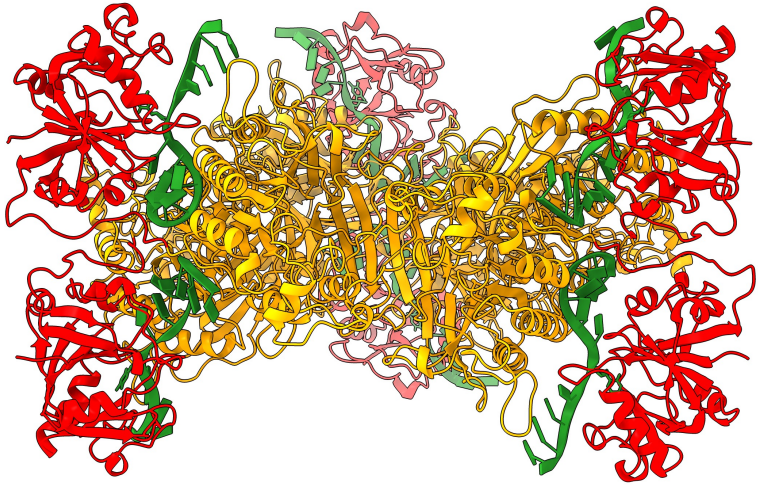


Interactions between PUA and AMP

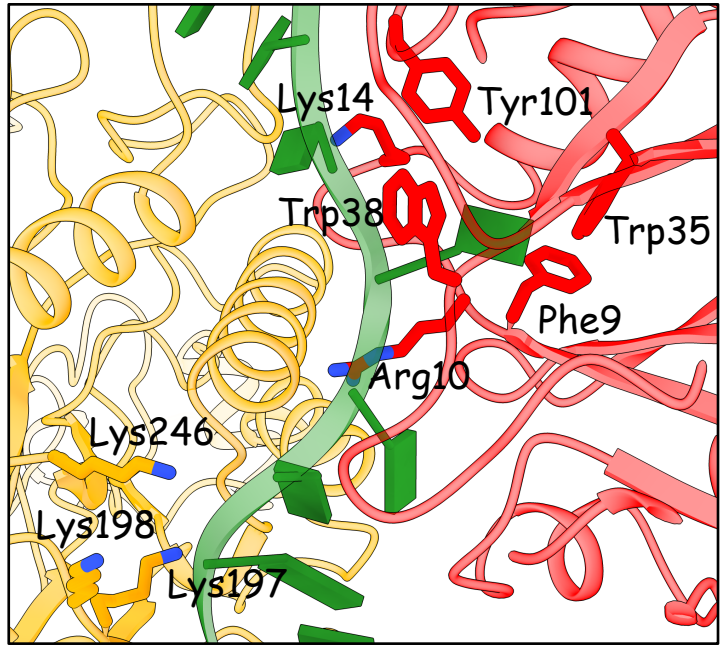


PUA-Calcineurin-CE hexamer with 5mC-RNA (Alphafold3 predicted model)

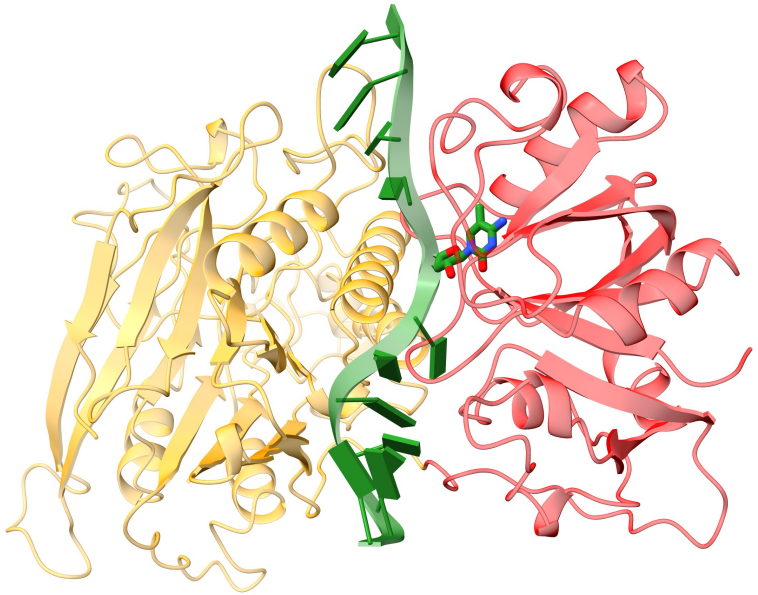


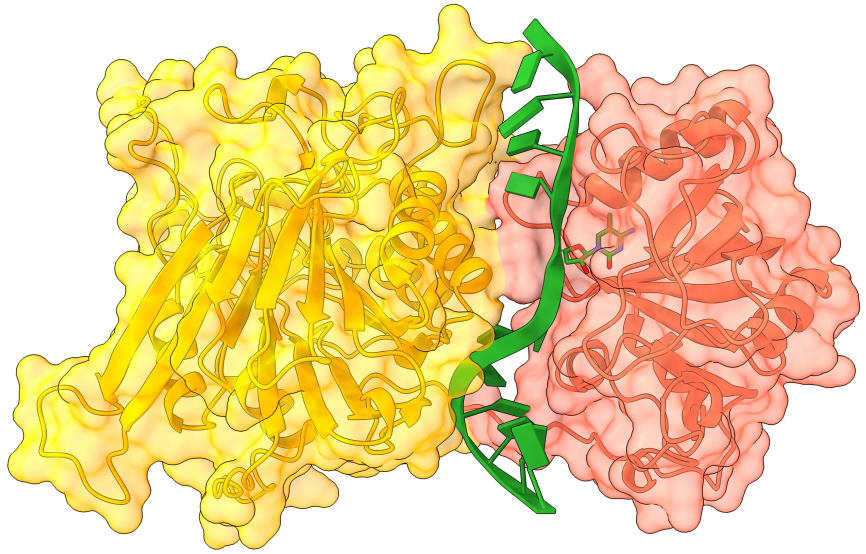


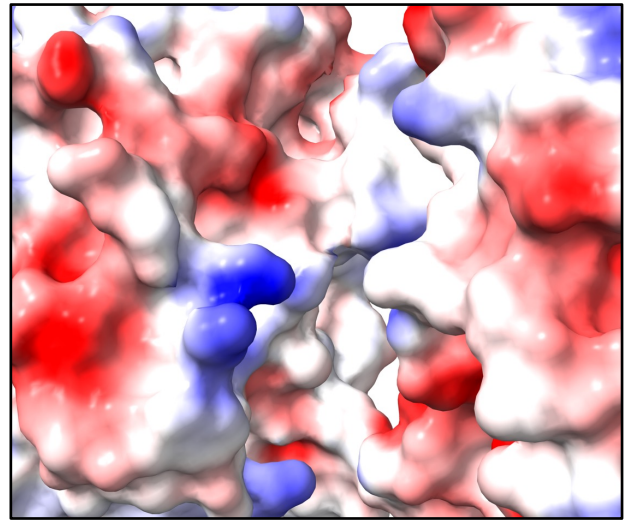
m5C-RNA
PUA-like domain
Calcineurin-CE domain



Electrostatic properties of the PUA-Calcineurin-CE and RNA interaction region







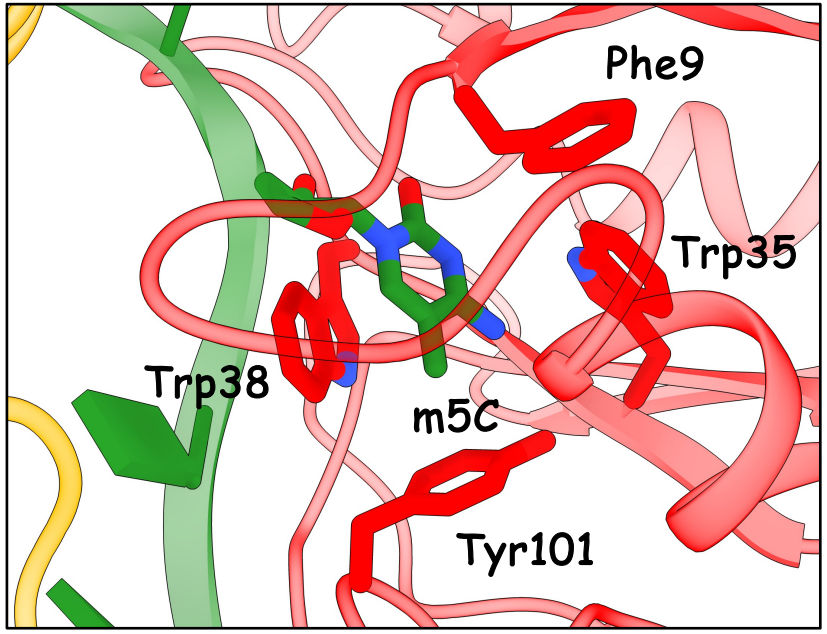
PUA

m5C-RNA

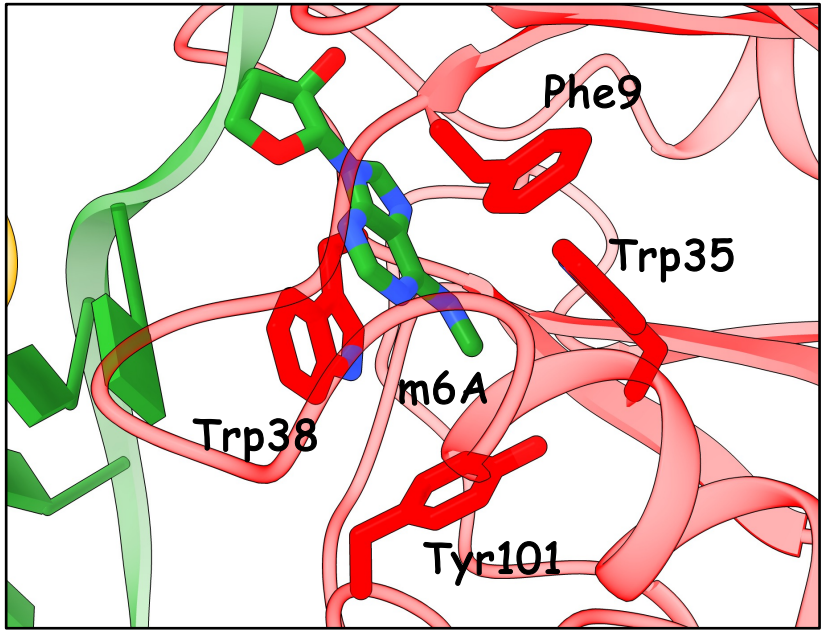
Calcineurin-CE

AlphaFold3 Model of PUA-Cal + m5C-RNA/m6A-RNA

PUA-Cal: 6; m5C-RNA/m6A: 6



m5C-RNA



m6A-RNA

Aromatic cage on the PUA domain can capture both
flipped out m5C and m6A modifications at ssRNA level