

Structural study of the influenza A virus RNA-dependent RNA polymerase

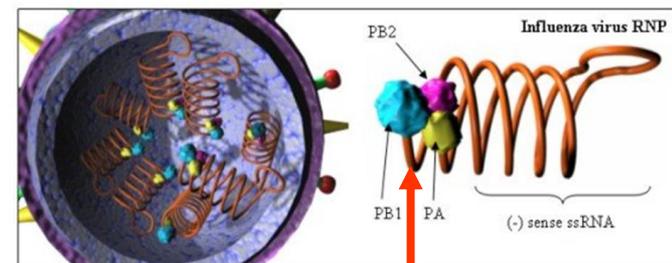
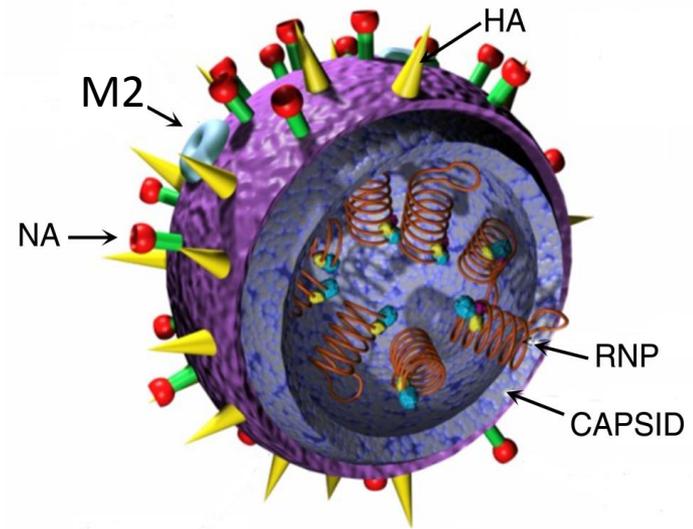


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Outline

- Background
- **Cryo-EM structure of PA, PB1 and PB2_N**
- The model for transcription and replication

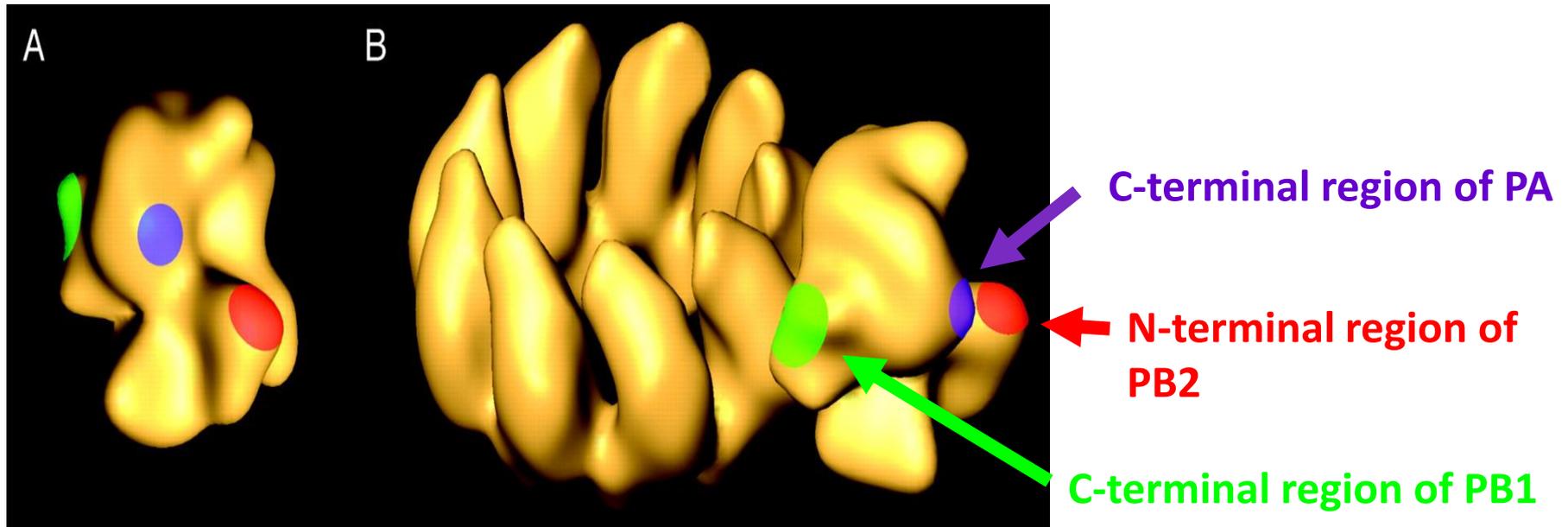


RNA polymerase



EM Structure of the Influenza Virus RNA Polymerase

Consists of three subunits: **PB1**, **PB2** and **PA**; MW: ~ 250KDa

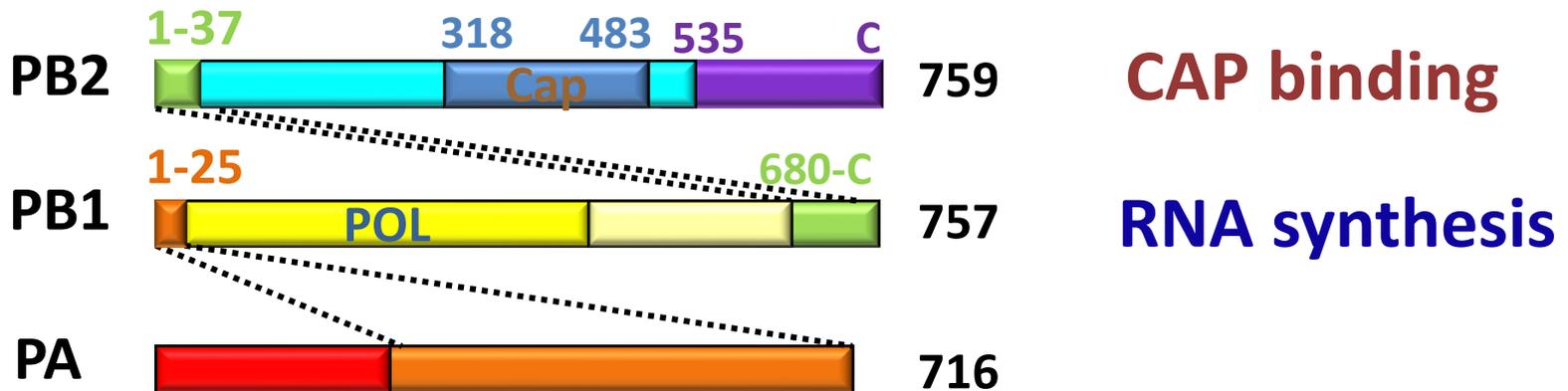


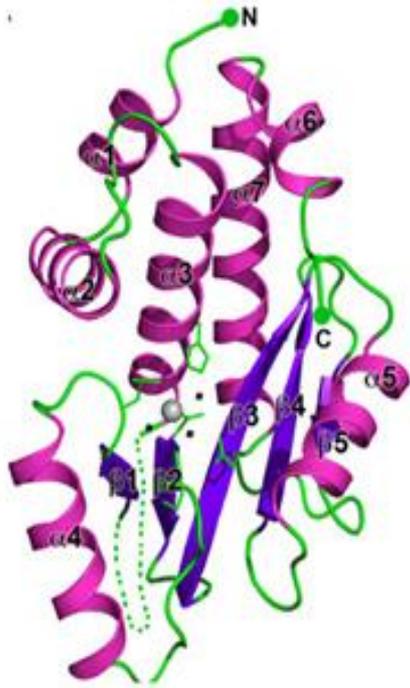
The functions of Influenza Virus RNA Polymerase

1. Replication: vRNA → cRNA → vRNA

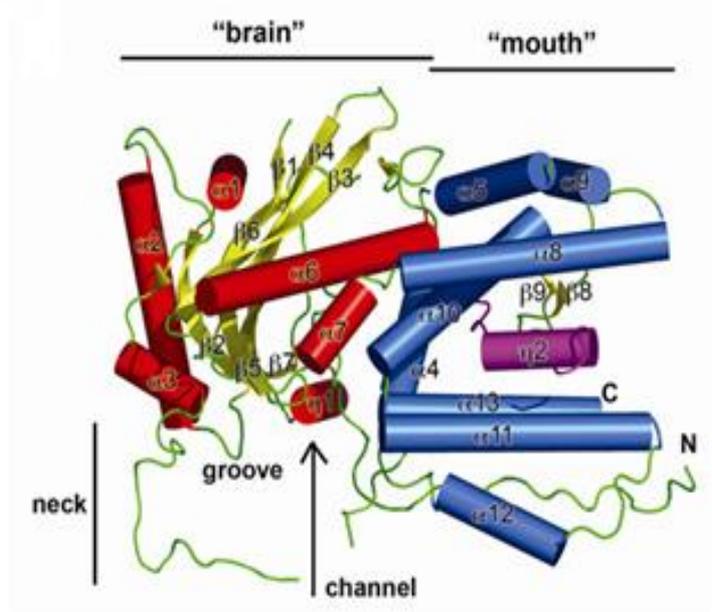
2. Transcription: vRNA → mRNA

RNA synthesis, Cap-binding, endonuclease, protease *etc*

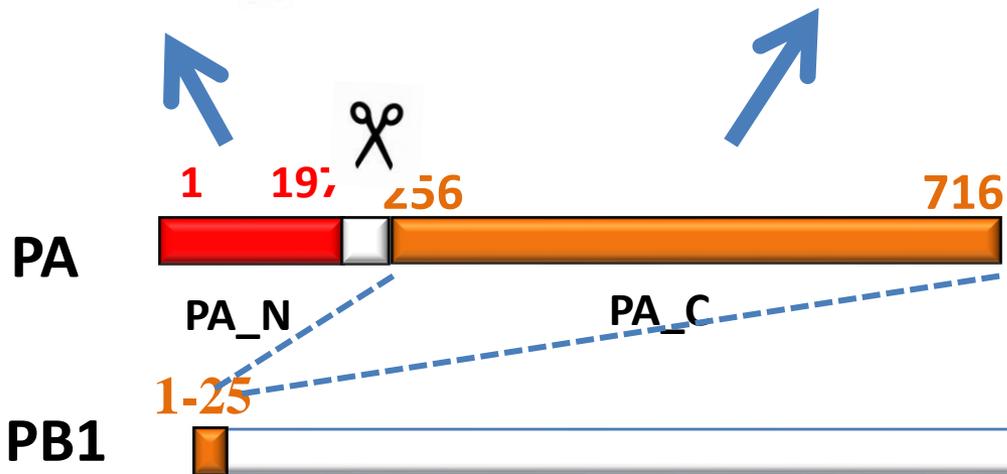


A

PA_N

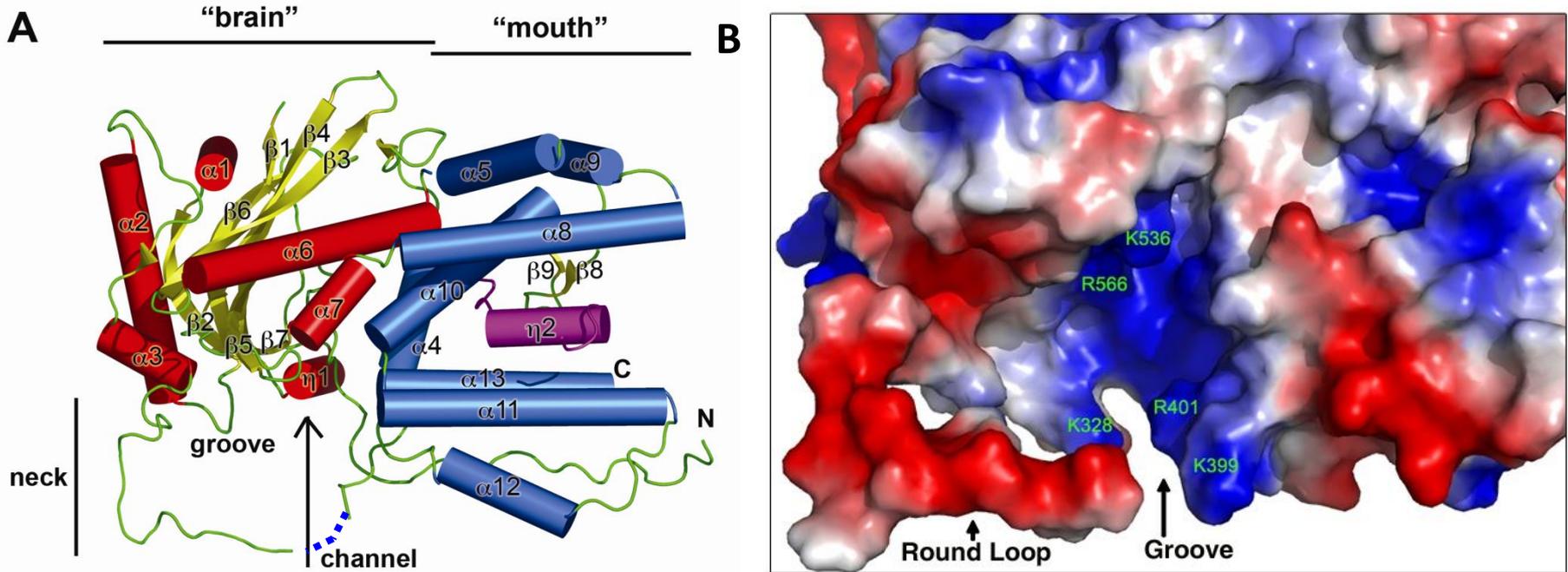
B

PA_C



(He X, et al, *Nature*, 2008)
 (Yuan et al, *Nature* 2009)

A potential RNA-binding groove in PA subunit

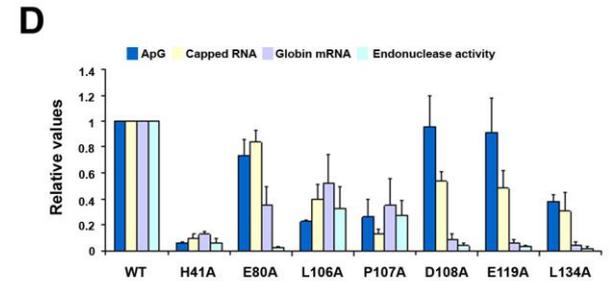
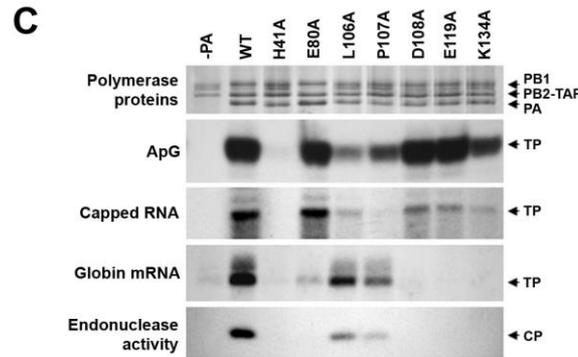
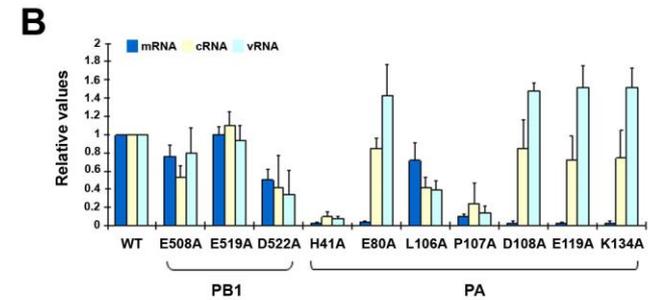
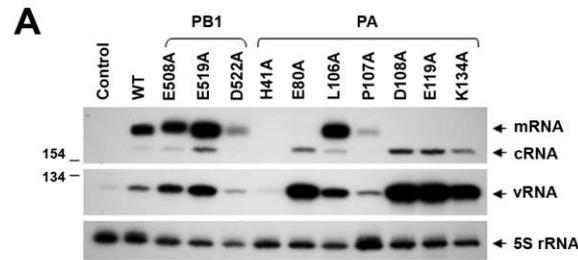
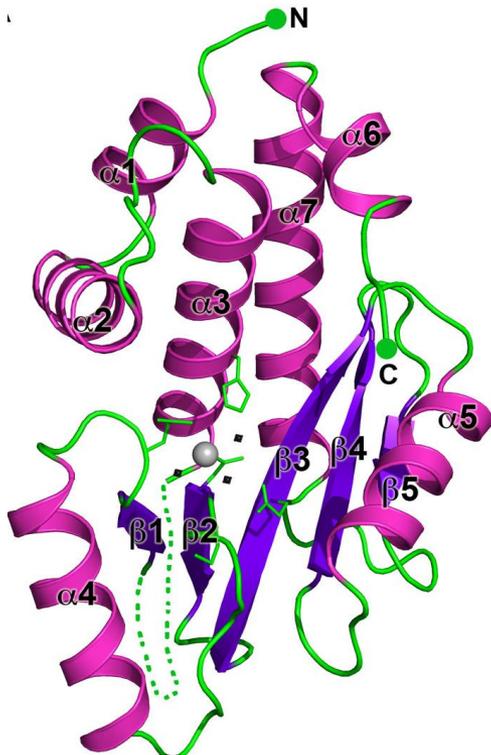


Conserved residues: K328, K539, R566 and K574.

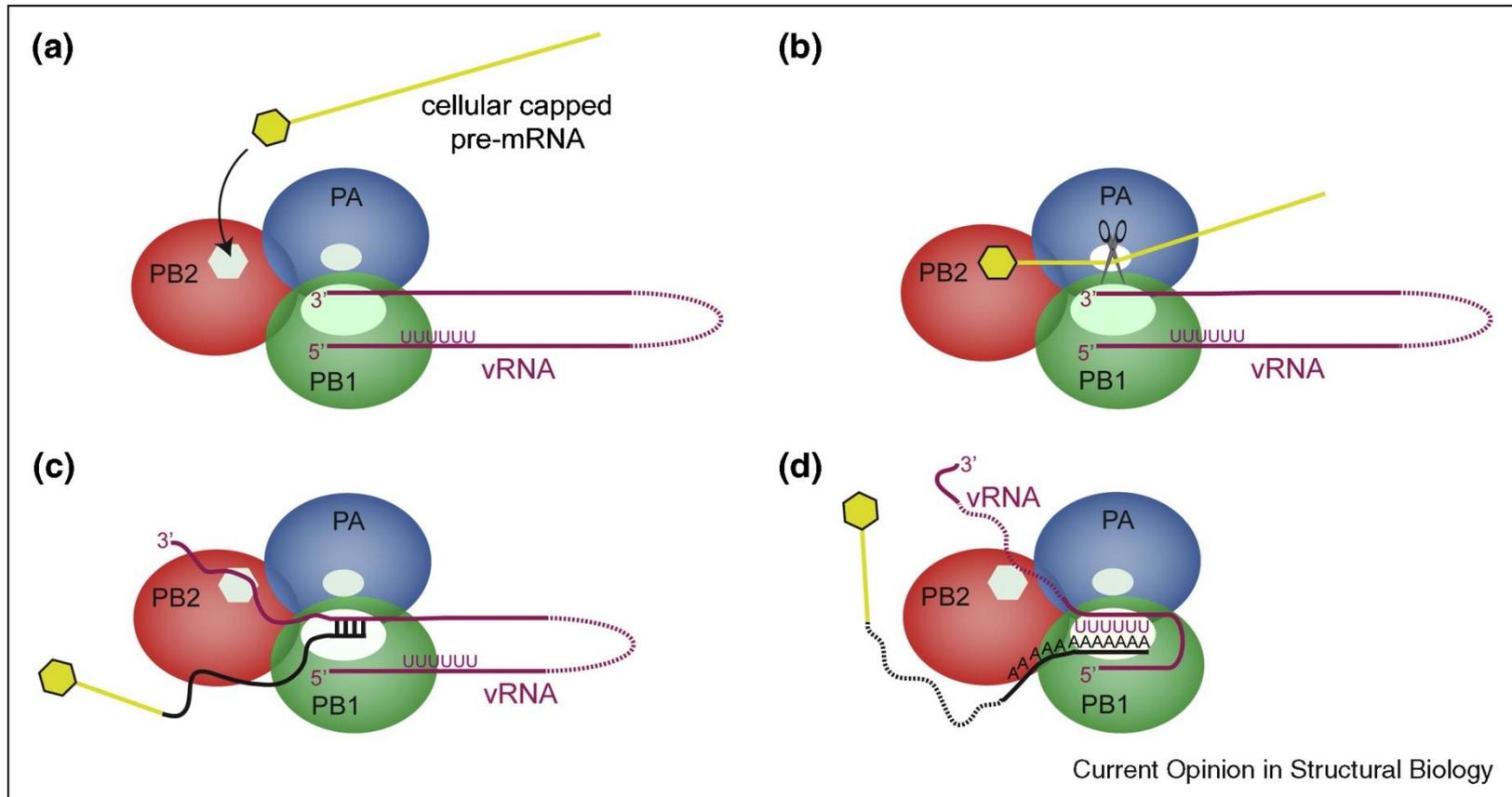
Mutation: K539A impairs cRNA and vRNA synthesis.



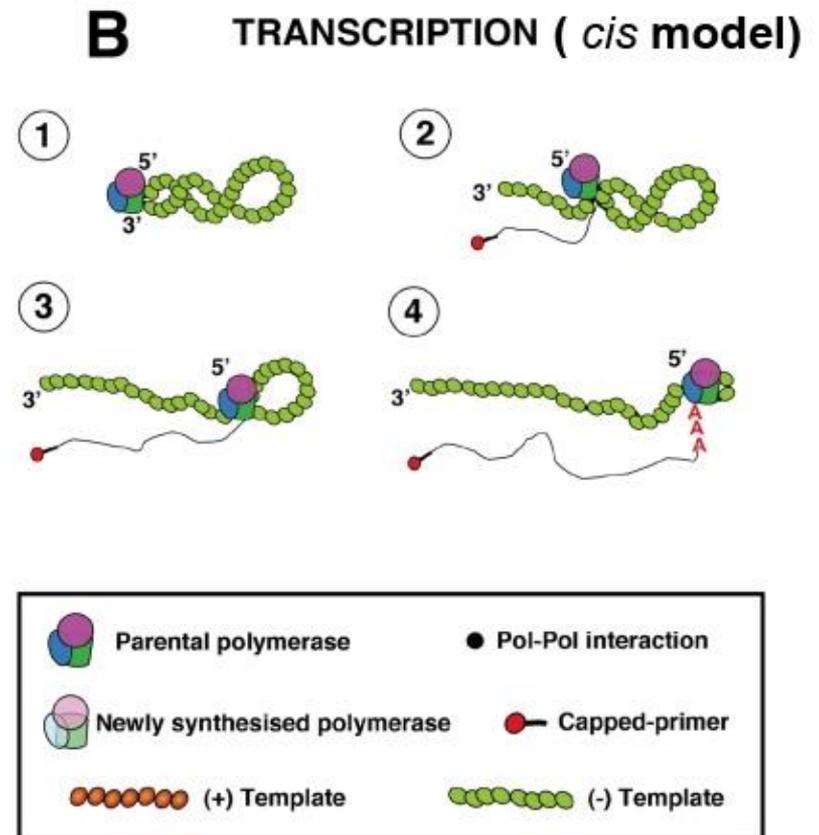
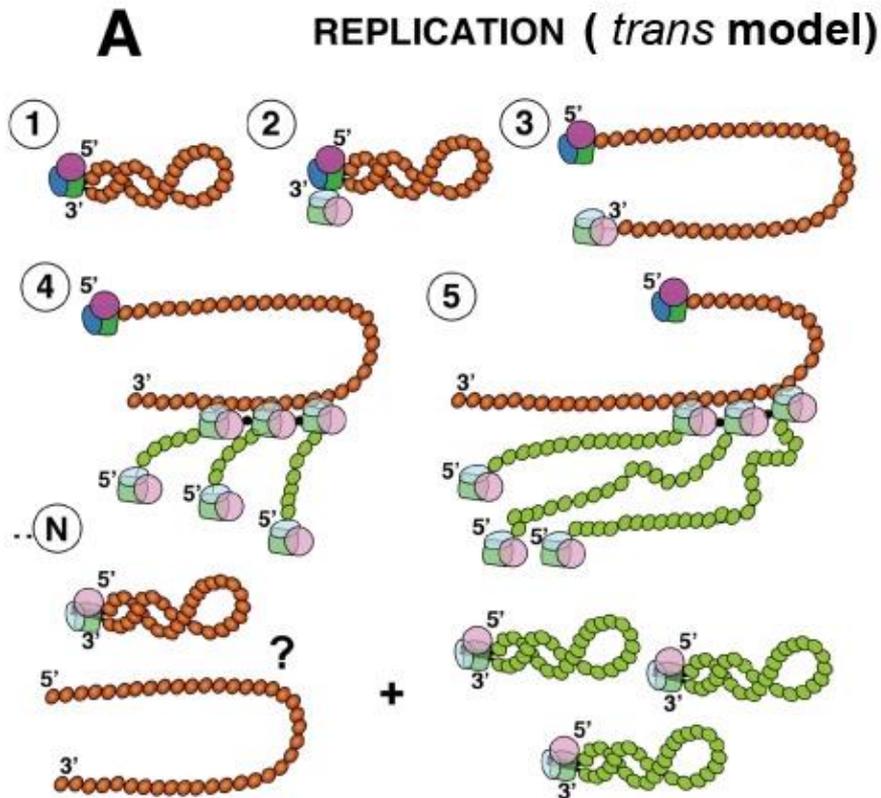
PA is the endonuclease activity subunit



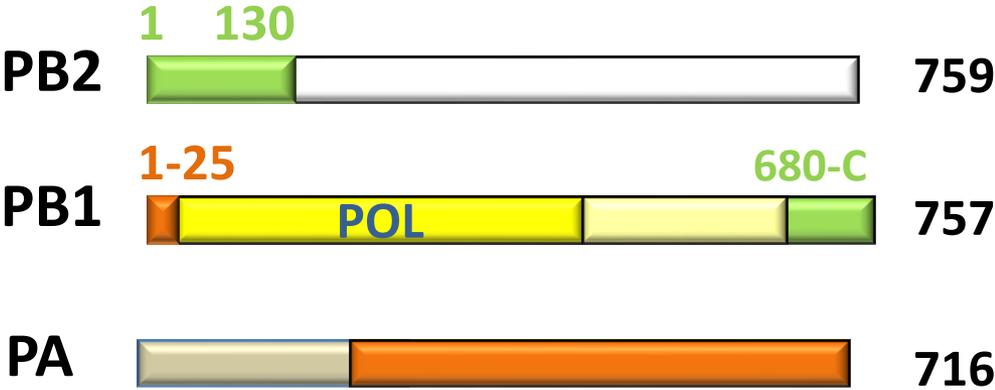
Model for the transcription (Cis-model)



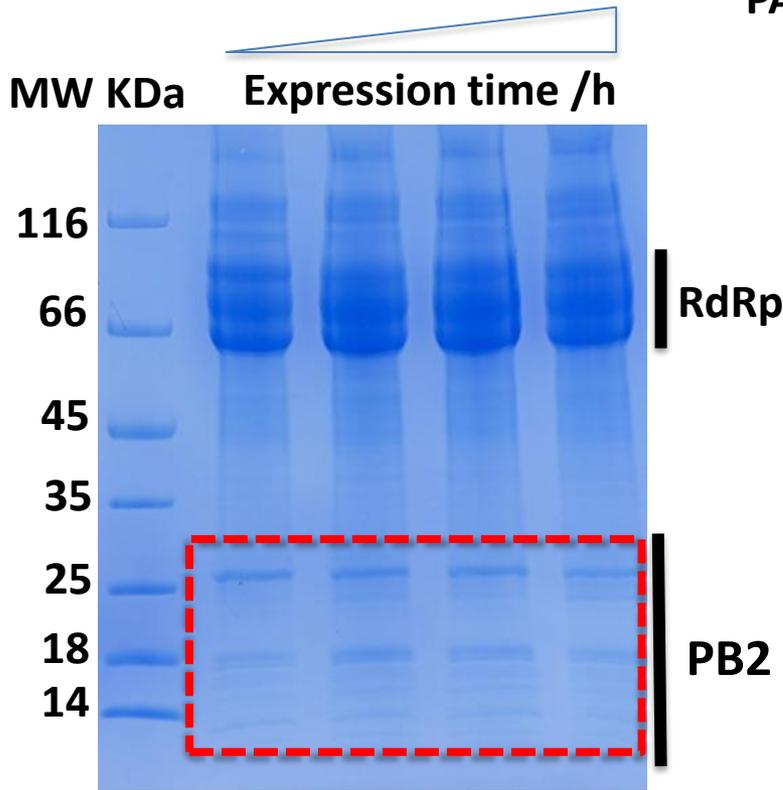
Replication/Transcription Model



Cryo-EM Structure of the influenza RdRP sub-complex

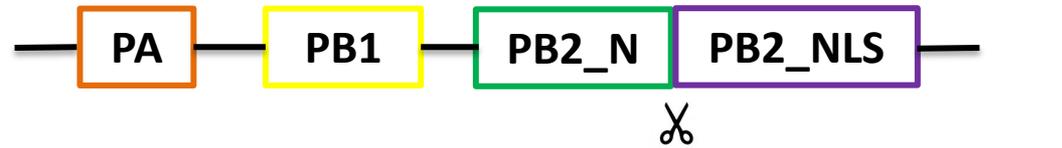


N-terminal mapping of PB2

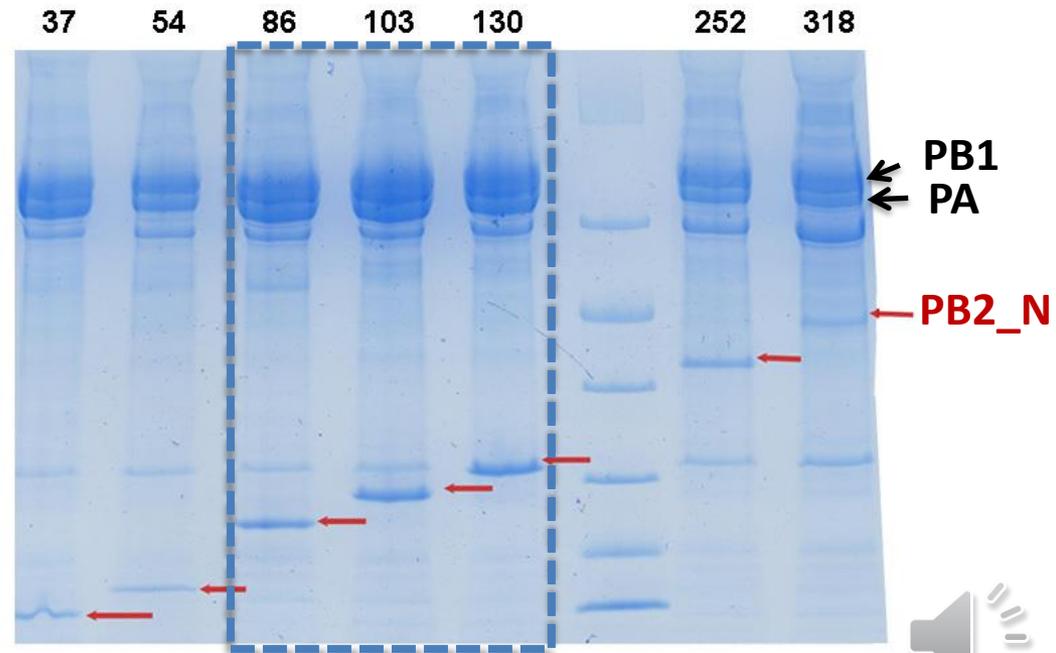


The degradation of PB2

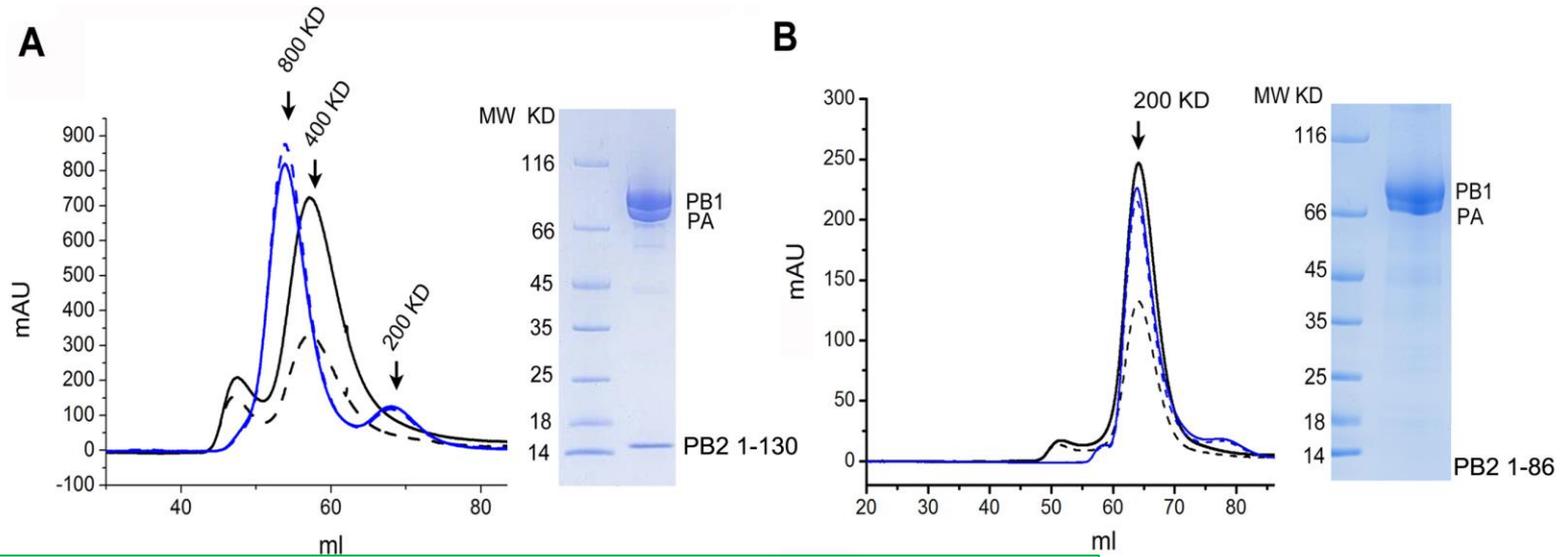
PA, PB1 and PB2_N form a stable RdRp sub-complex



Stable fragment PB2 1-130



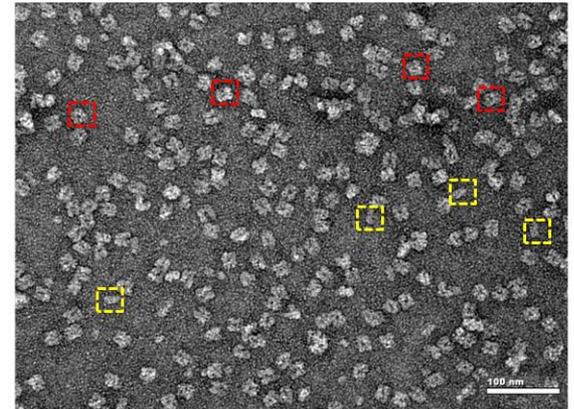
oligomer State transition of the Sub-complexes



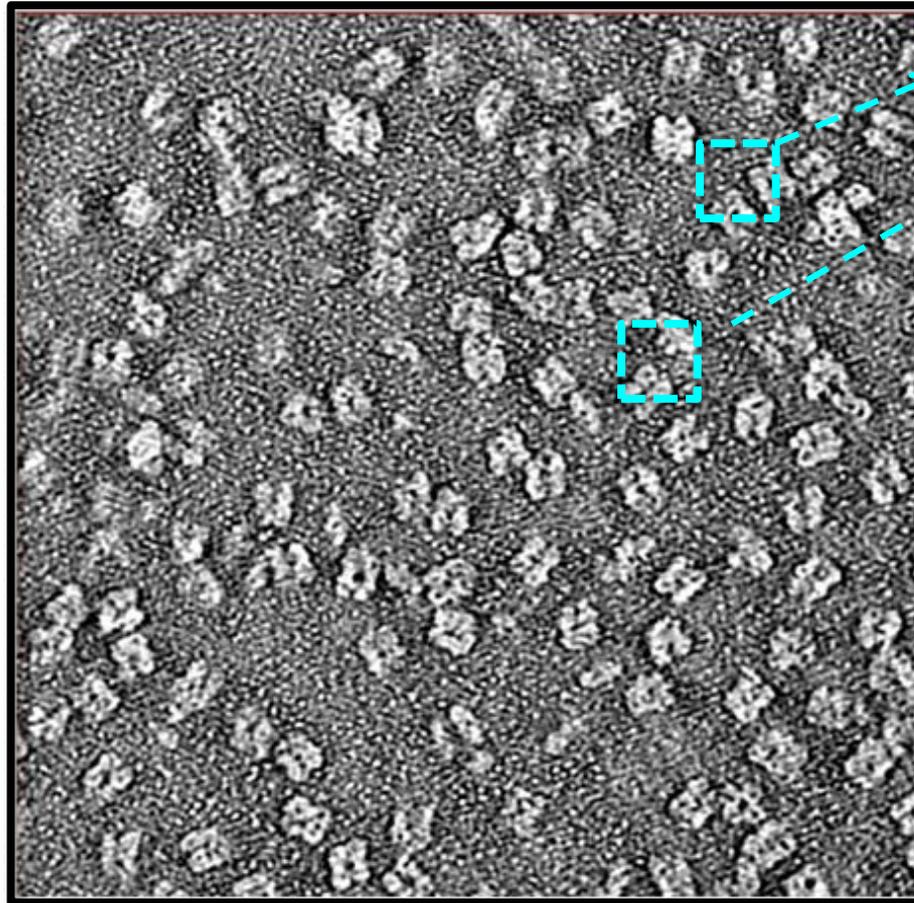
1. The sub-complex exists as a dimer in solution.
2. The promoter induces a dimer-timer transition.
3. PB2 86-130 mediates the dimer formation.
4. dimer conformation is necessary tetramer formation.

Negative staining EM analysis:

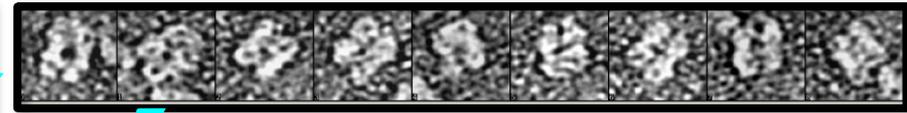
Tetrameric sub-complex I (as red squares shown) and minority dimeric sub-complex I (as yellow squares shown)



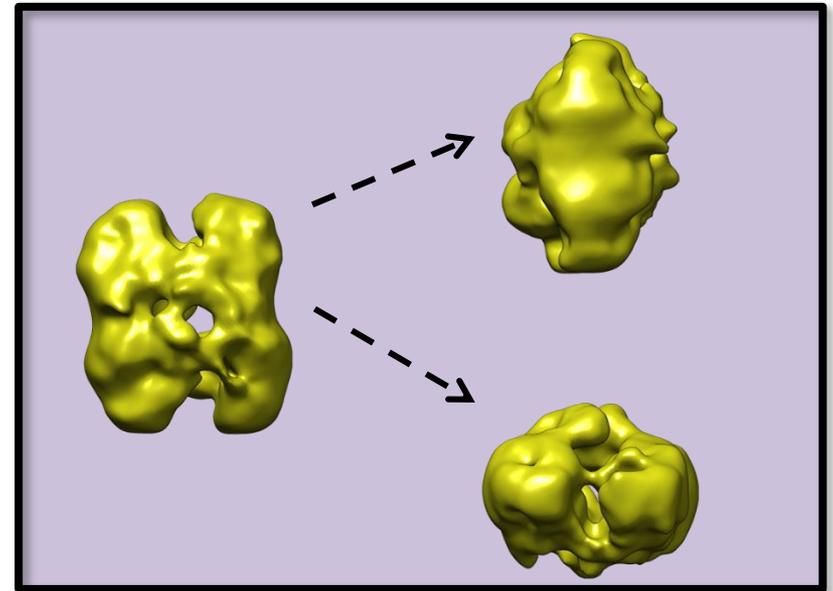
Initial model generation by tomographic sub-volume averaging



Tomographic reconstruction



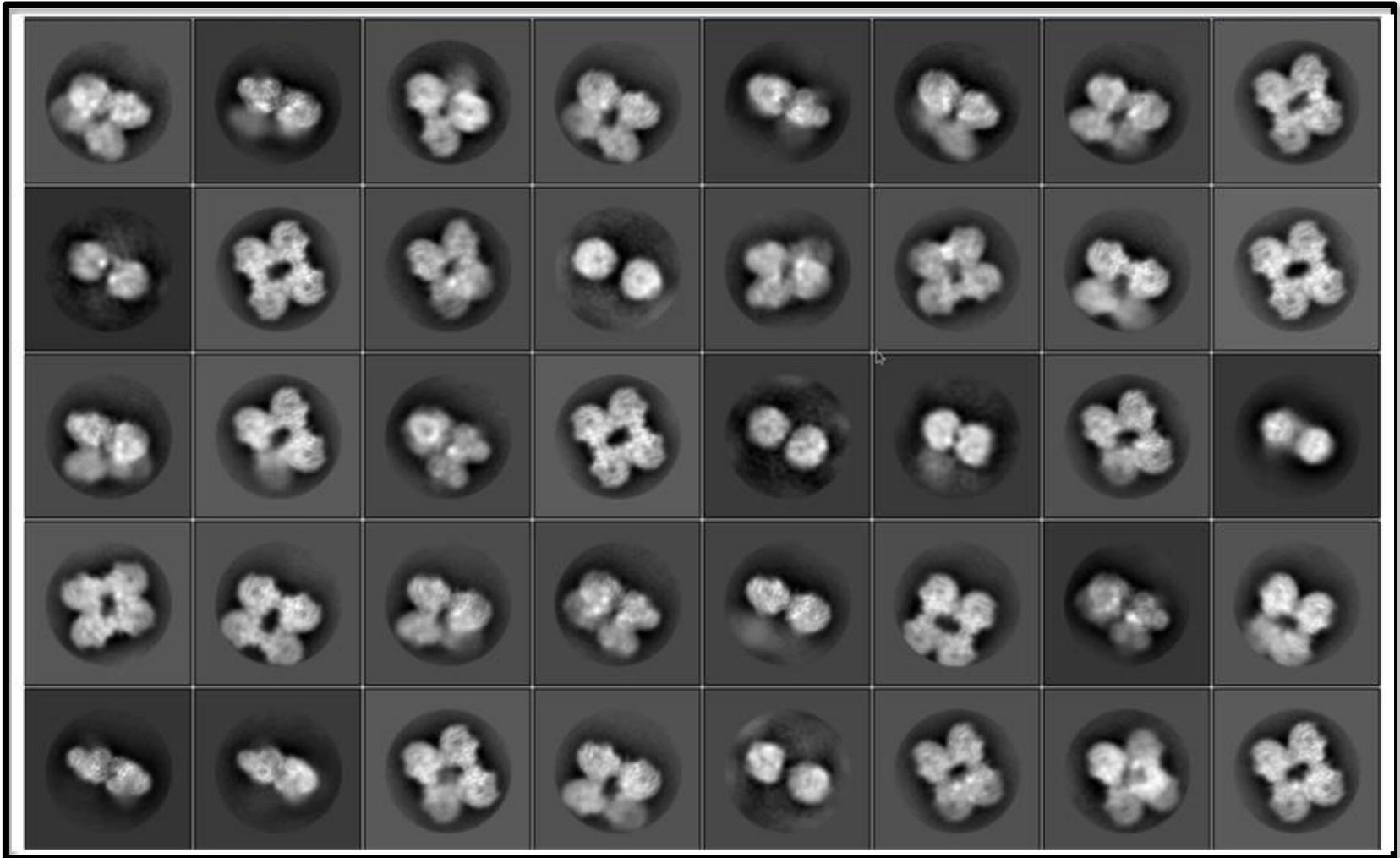
sub-volume averaging



Different views of initial model



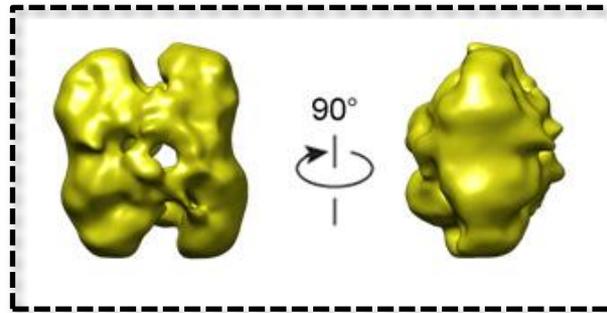
High resolution cryo-EM analysis



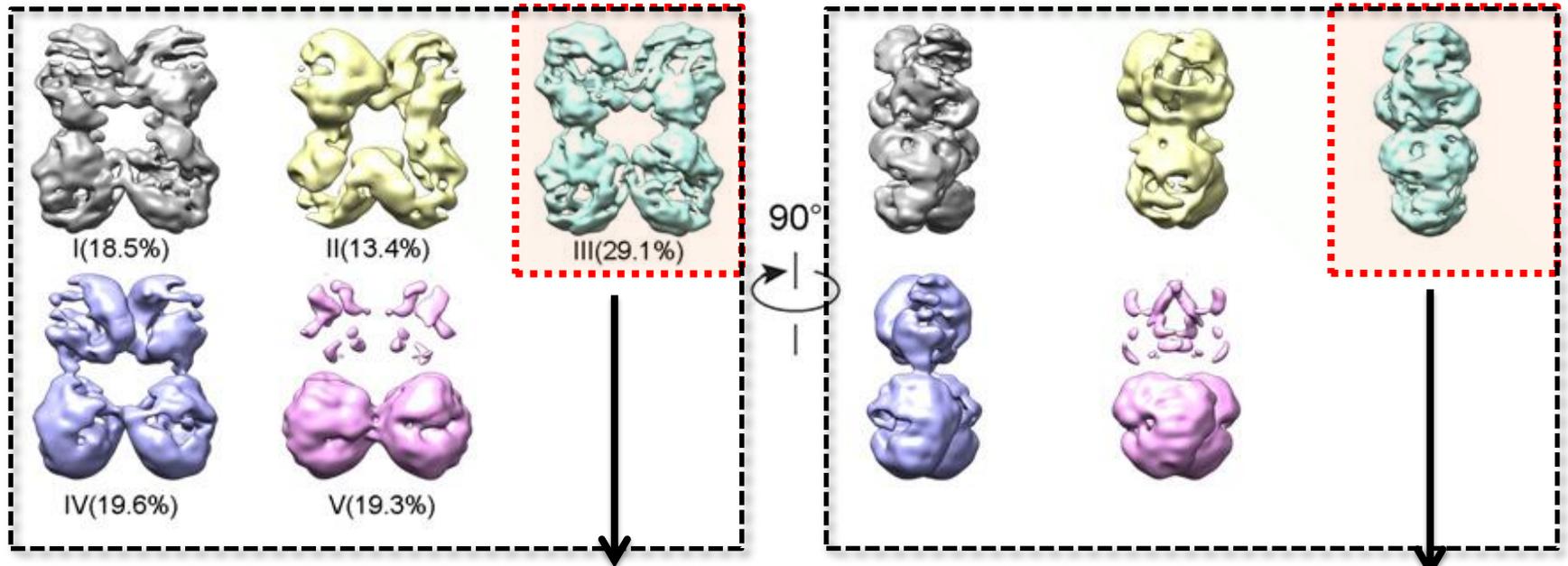
Typical good reference-free 2-D class averages from the single particle images. **clear secondary-structural element features**



3D classification of the cryo-EM data sets.
~160,000 particles

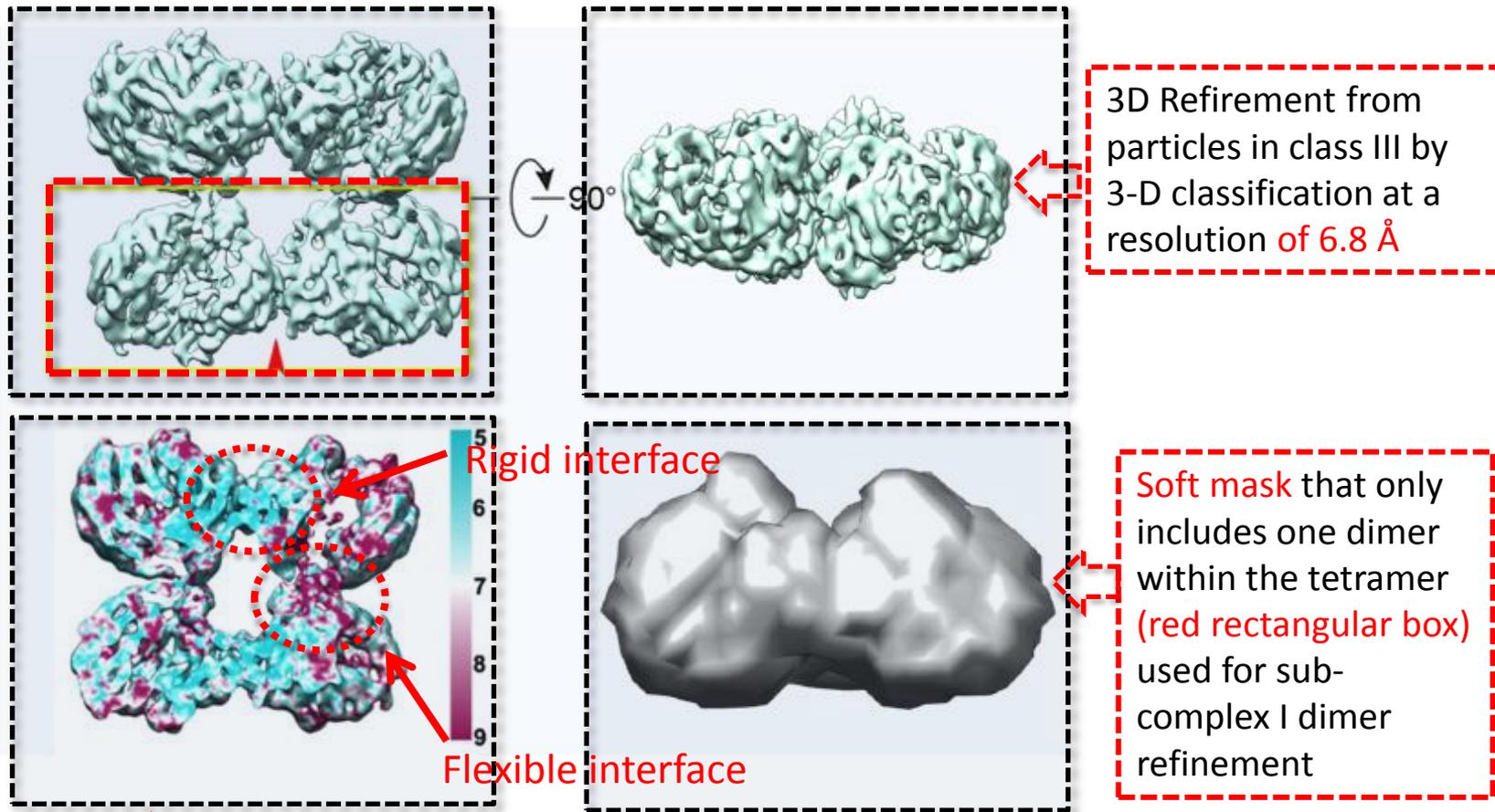


3D classification with C2 symmetry



Class III For next step 3D auto refinement





Tetramer 3D auto refinement

ResMap rendering of the 3-D density map filtered at 6.8 Å resolution. The map was cut in the middle to show the resolution distribution inside the molecule, which shows clear resolution difference between the rigid and flexible interaction interfaces. So we generate a soft mask used for sub-complex I dimer refinement.

