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MOLECULAR MODELING OF HUMAN LINE-1 ORF2 PROTEIN

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Human LINE-1 ORF2p protein

ORF2p - open reading frame 2 protein

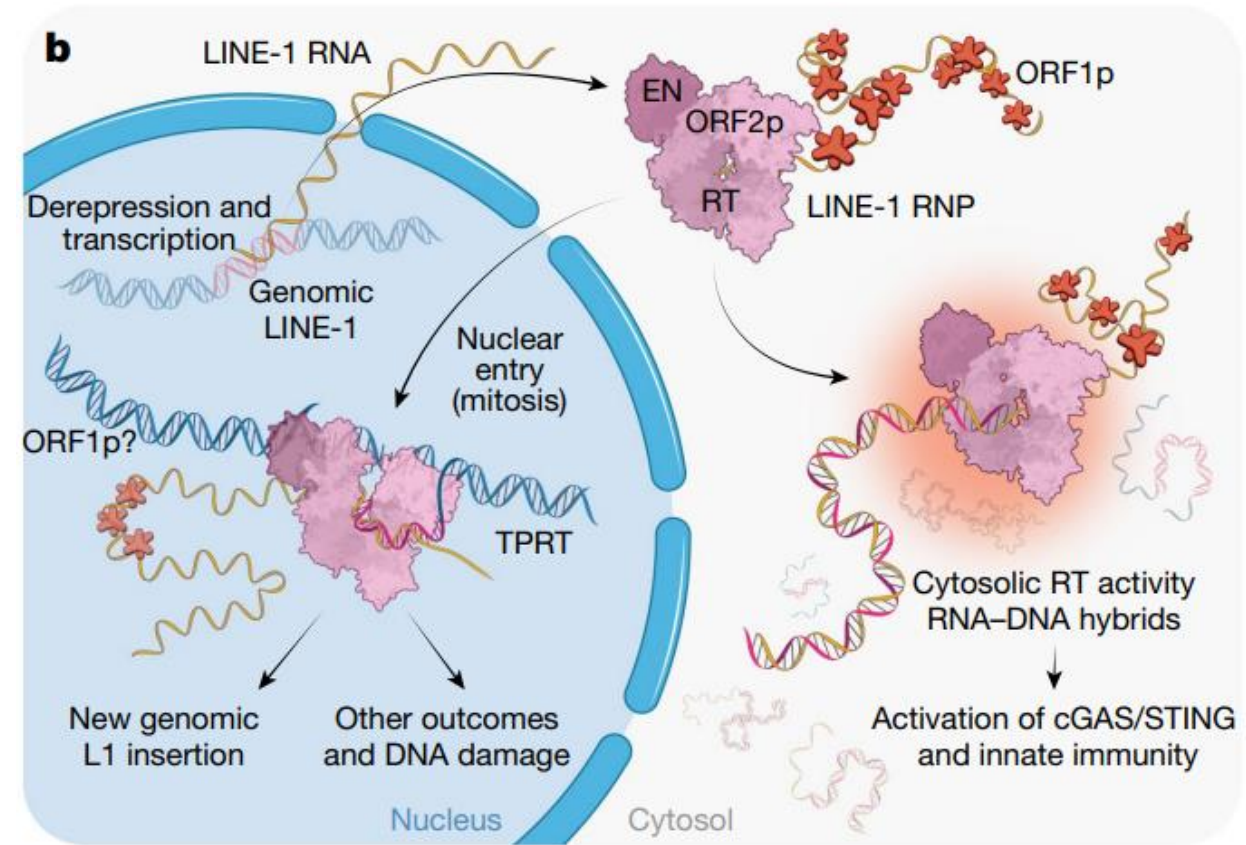
ORF2p is one of the LINE-1 retrotransposon proteins

Each person inherits about 100 polymorphic and fixed potentially active LINE-1s, a small subset of the approximately half a million inactive LINE-1 copies and fragments

Retrotransposons associated with aging, neurodegeneration, and cancer

Human LINE-1 encodes two proteins: ORF1p and ORF2p

ORF2p has a reverse transcriptase (RT) and endonuclease (EN) activity



Replication cycle of LINE-1

(Baldwin E.T. et al. Nature, 2024, 626, 194–206
<https://doi.org/10.1038/s41586-023-06947-z>)

Structure of ORF2p protein

ORF2p has a reverse transcriptase (RT) and endonuclease (EN) activity

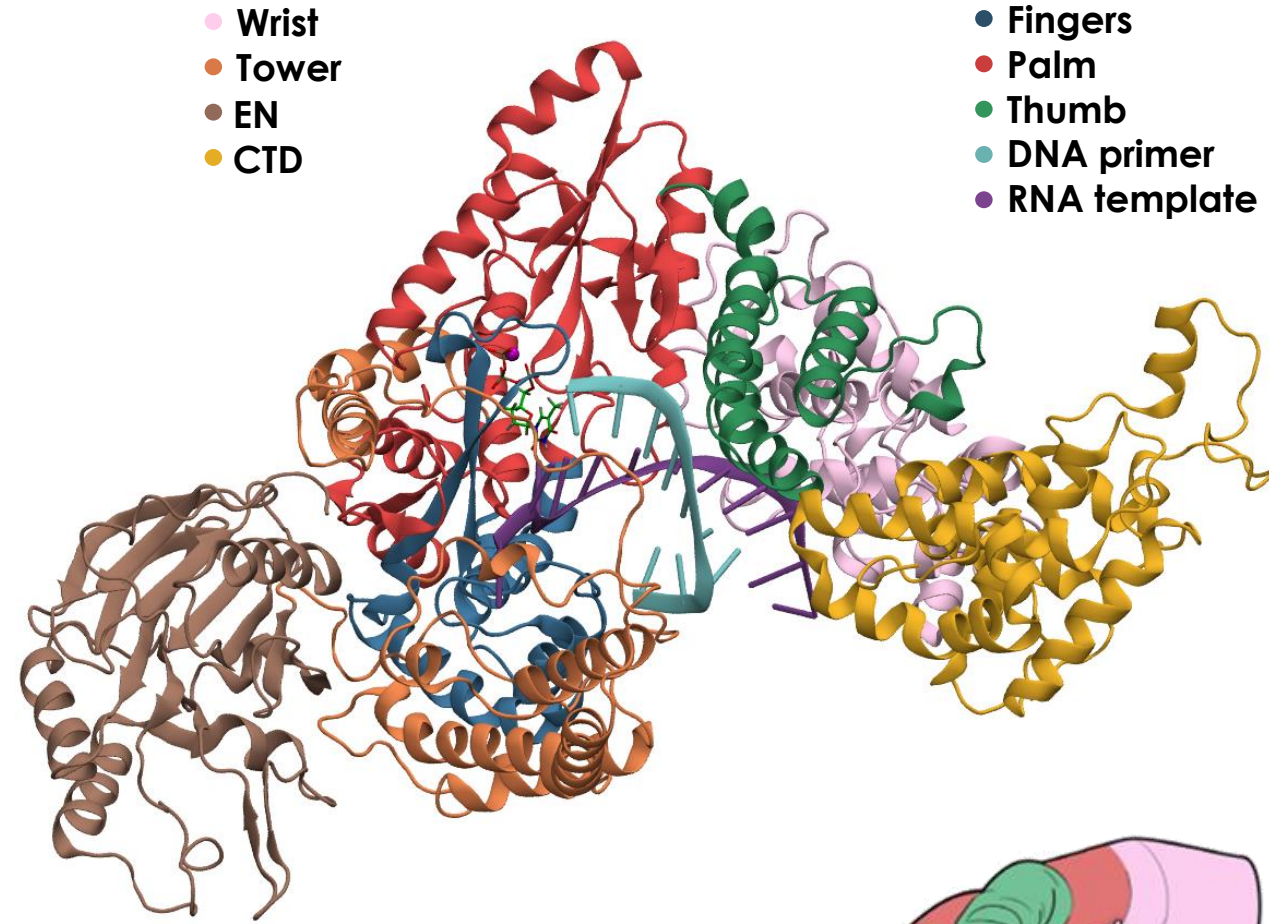
ORF2p has 7 domains:

ORF2p core:

- Tower (residues 239-439)
- Fingers (residues 440-557)
- Palm (residues 558-775)
- Thumb (residues 776-862)
- Wrist (residues 863-1060)

Endonuclease domain (EN) (residues 1-238)

Carboxy-terminal domain (CTD) (residues 1061-1275)



ORF2p structure

PDB ID: 8C8J

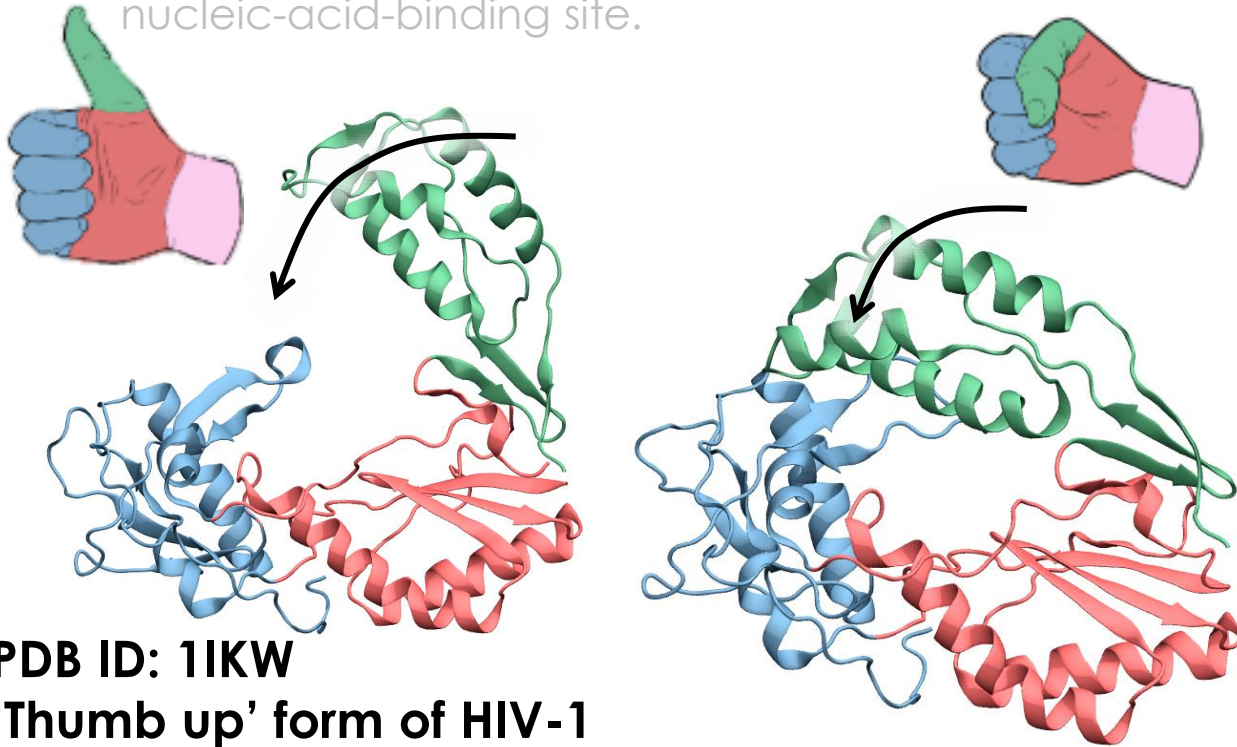


('hand scheme' from Baldwin E.T. et al. Nature, 2024, 626, 194–206)

Conformation of ORF2p protein

Conformation of reverse transcriptase (RT): 'thumb up' and 'thumb down'

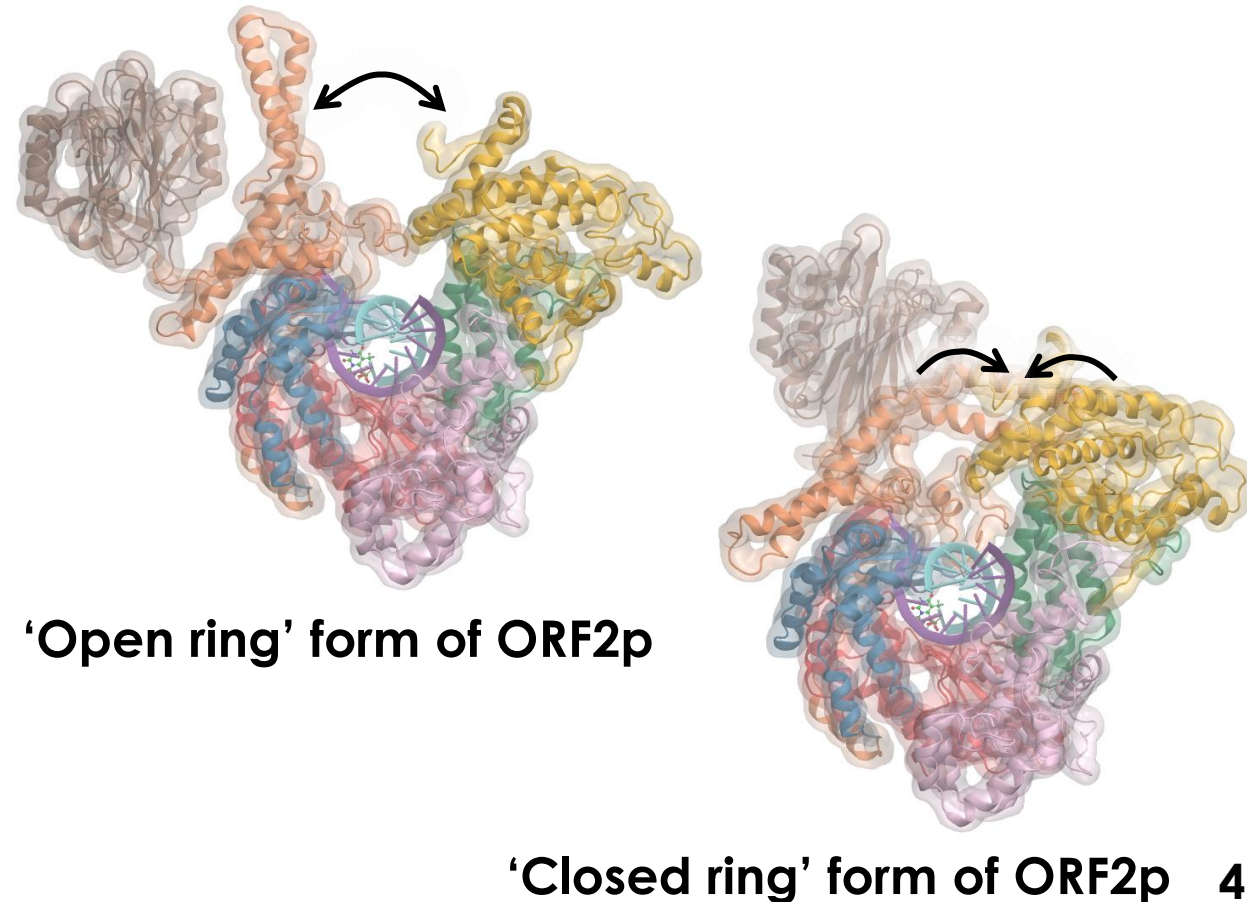
Apo viral RTs assumed an inactive 'thumb down' conformation, in which the thumb occupied the nucleic-acid-binding site.



(‘hand scheme’ from Baldwin E.T. et al. Nature, 2024, 626, 194–206)

Conformation of ORF2p: 'open ring' and 'closed ring'

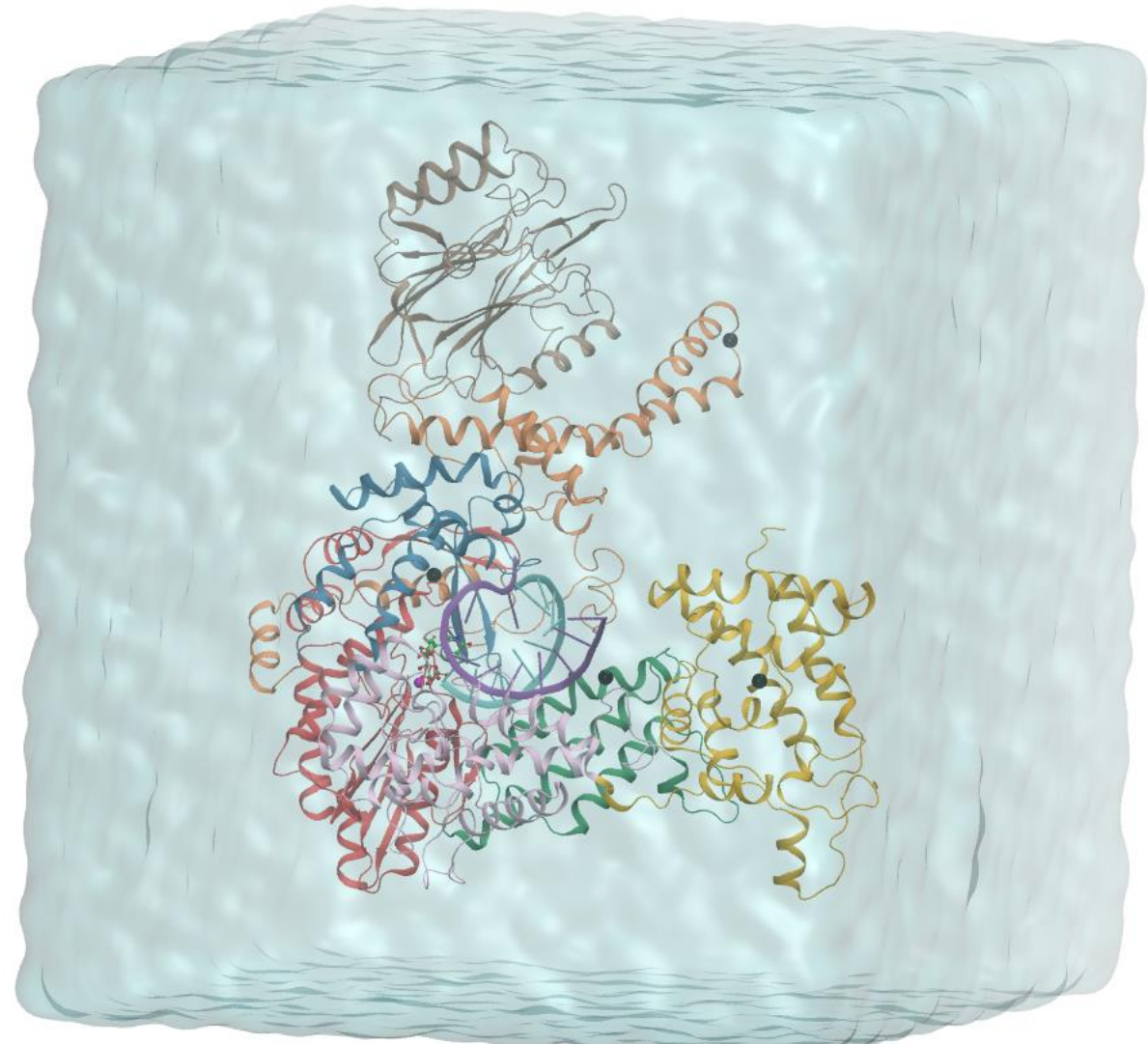
Closed ring state represent a predominant conformation when ORF2p is bound to messenger RNA. Open ring state may be involved in retrotransposition.



Aims of research

Study of dynamical behavior of different ORF2p complexes

1. Conformation connected with reverse transcriptase activity
'thumb up' vs 'thumb down'
2. Conformation connected with retrotransposition activity
'closed ring' vs 'open ring'



ORF2p complex with RNA template, DNA primer and thymidine triphosphate in water box

PDB ID: 8C8J

System size ~ 283.000 atoms

Molecular modeling

Classical molecular dynamic

NAMD package:

NPT ensemble (P = 1 atm, T = 300 K)

Timestep: 2 fs

Time length: 500 ns * 8

ORF2p structures:

PDB ID: 8C8J

CHARMM force field

Water – TIP3P force field

'Open ring' (o) and 'closed ring' (c) states –
from CryoEM (Baldwin E.T. et al. Nature, 2024,
626, 194–206)

Preparation:

Water box equilibration:

Classical MD with fixed atoms

NPT ensemble (P = 1 atm, T = 300 K)

Timestep: 2 fs

Time length: 5 ns

4*2 structures:

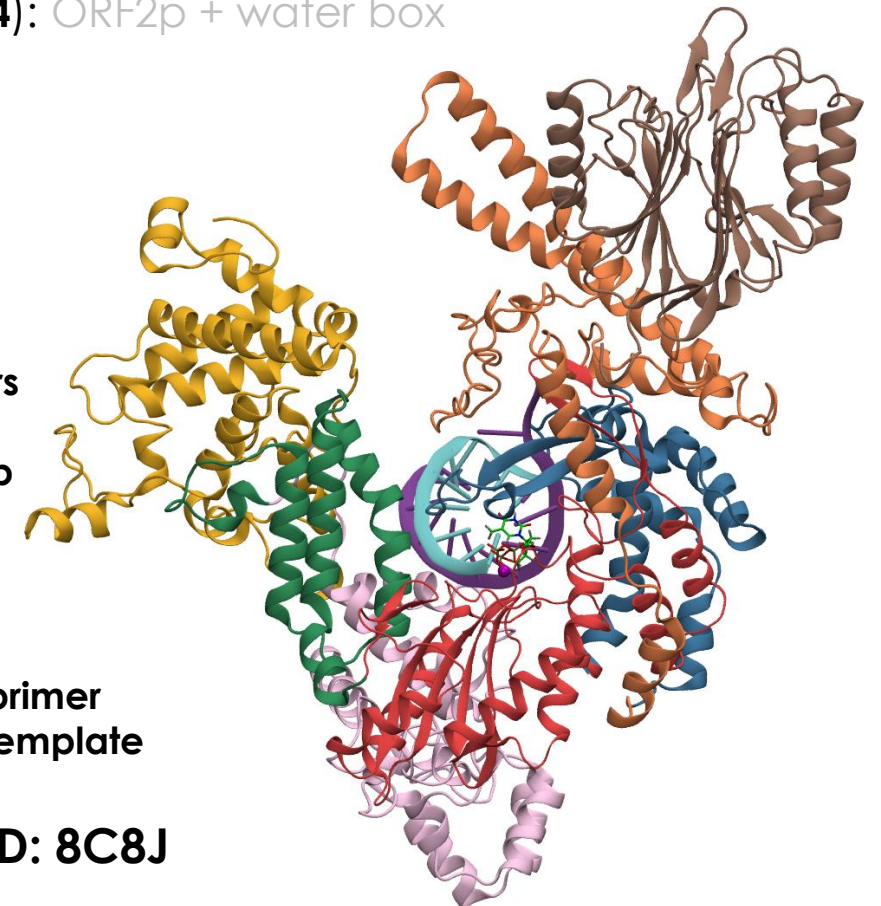
All-form (1): ORF2p + RNA template + DNA primer +
thymidine triphosphate (TTP) + water box

DNA-form (2): ORF2p + RNA template + DNA primer +
water box

RNA-form (3): ORF2p + RNA template + water box

Apo-form (4): ORF2p + water box

- Fingers
- Palm
- Thumb
- Wrist
- Tower
- EN
- CTD
- DNA primer
- RNA template



PDB ID: 8C8J

Classical molecular dynamics

Molecular dynamics trajectories:

All-form

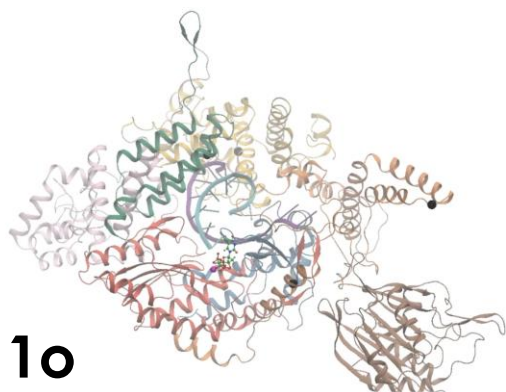
DNA-form

RNA-form

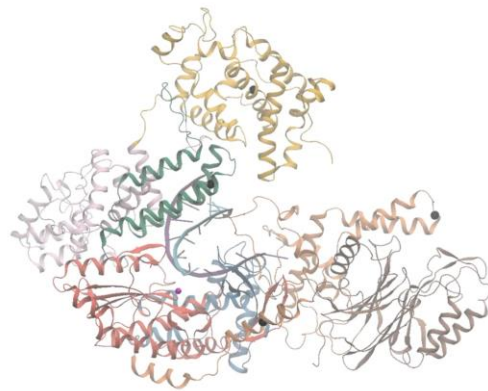
Apo-form

Open ring

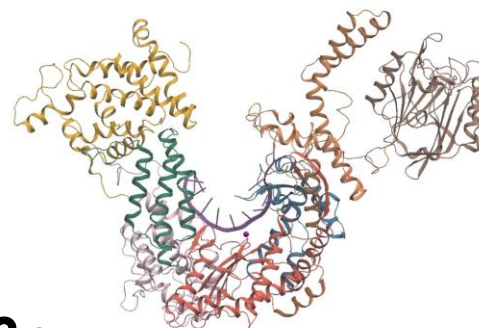
1o



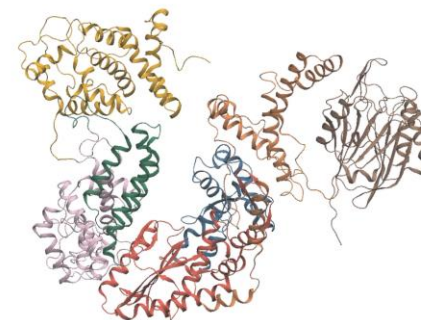
2o



3o

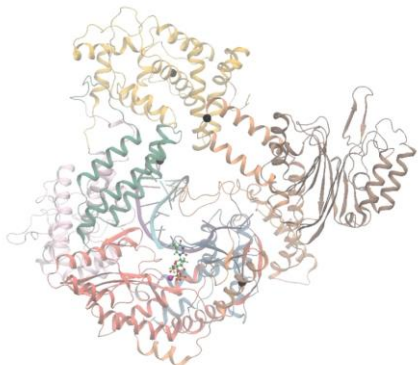


4o

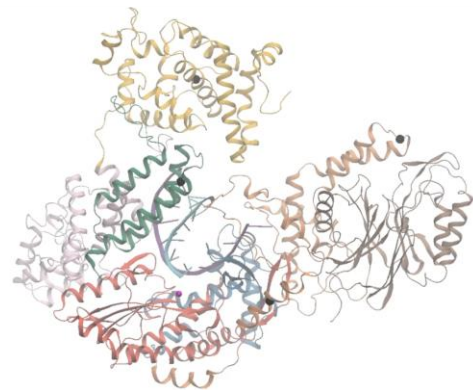


Closed ring

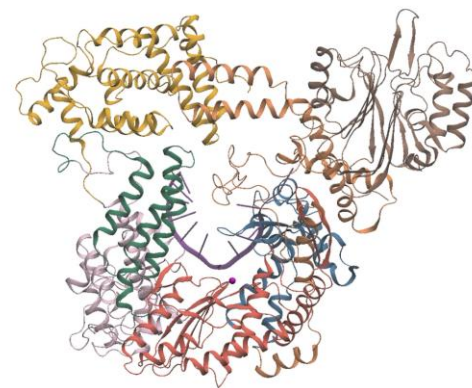
1c



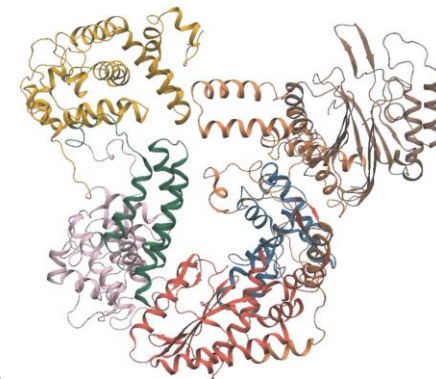
2c



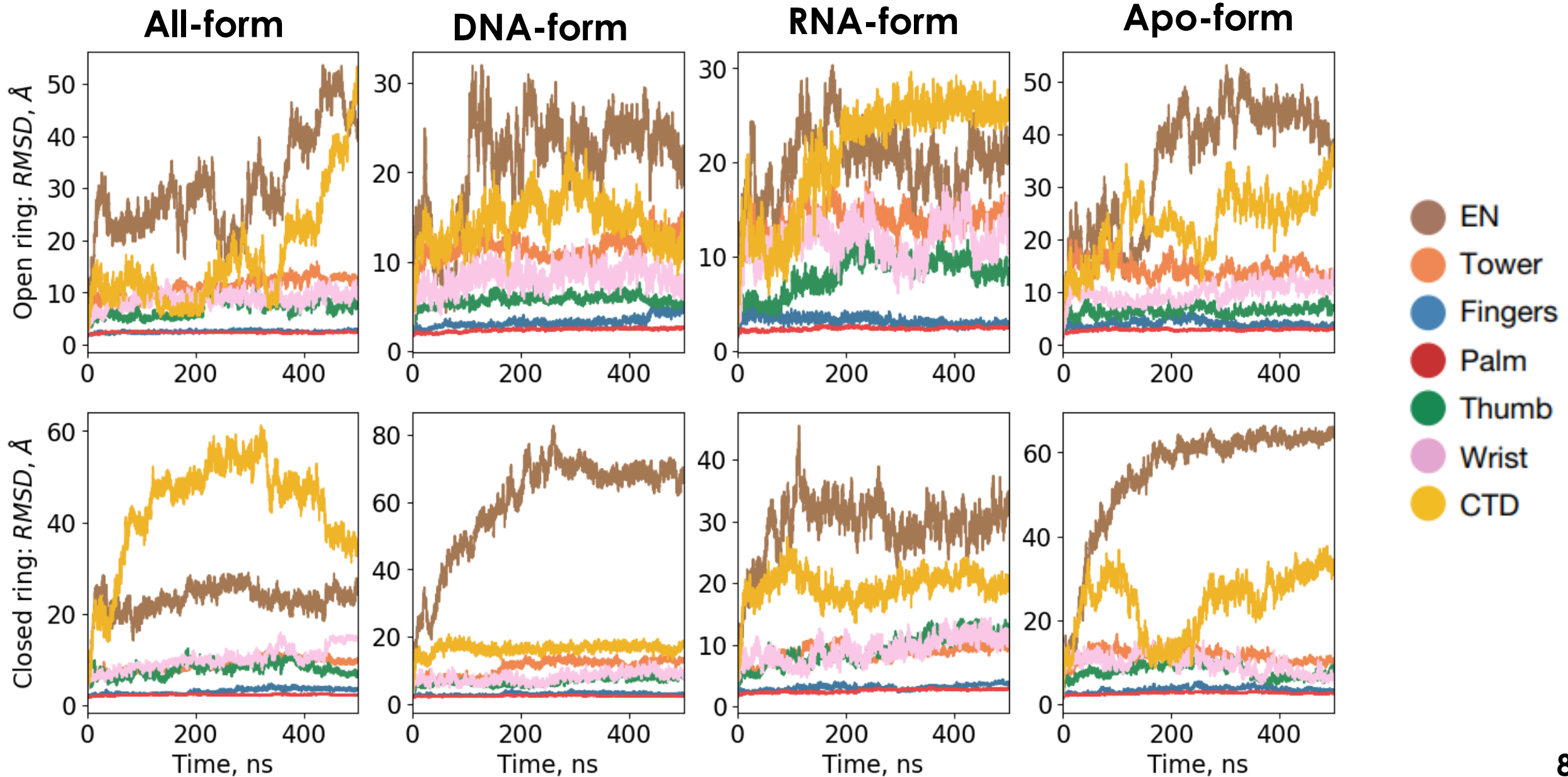
3c



4c



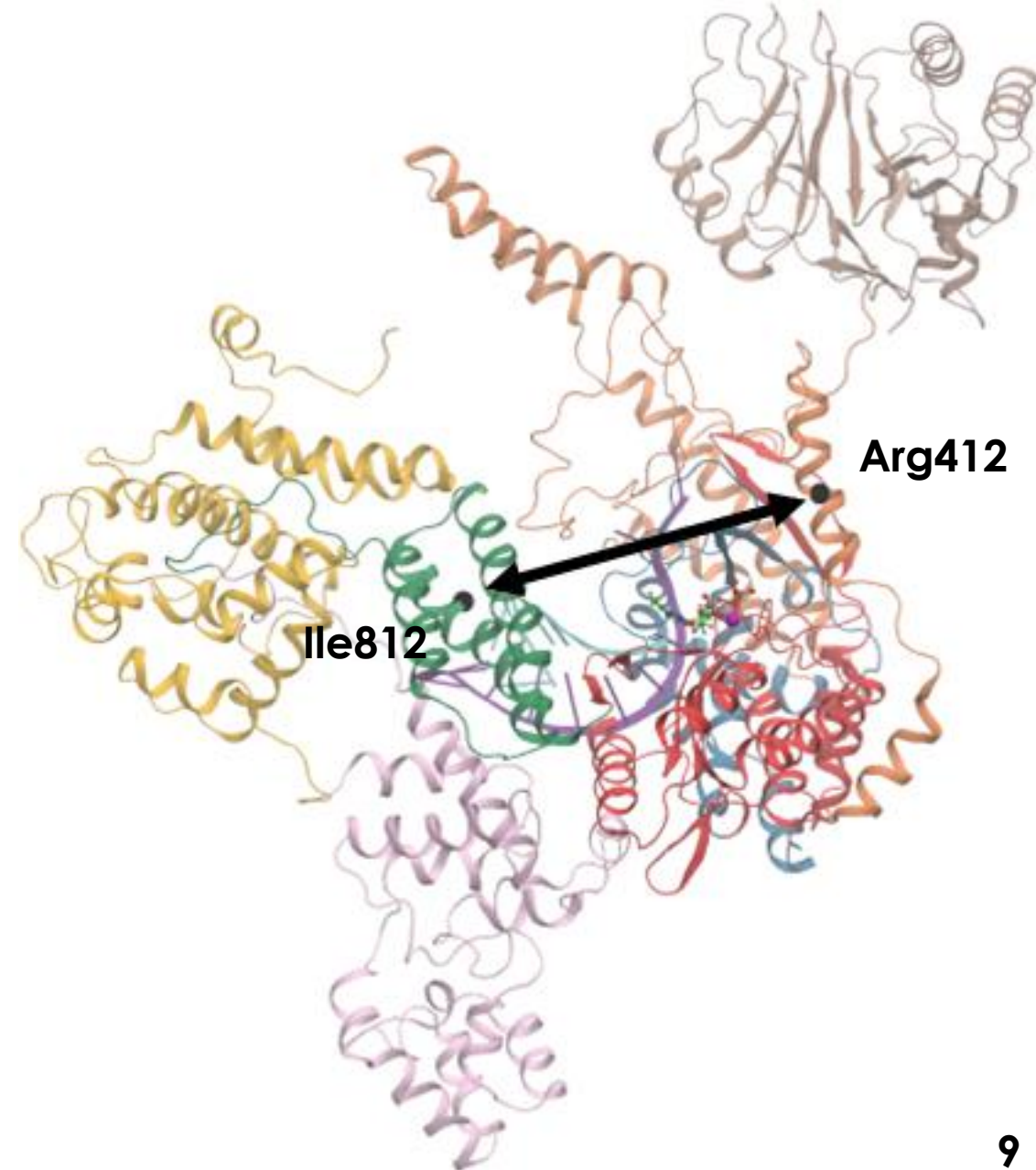
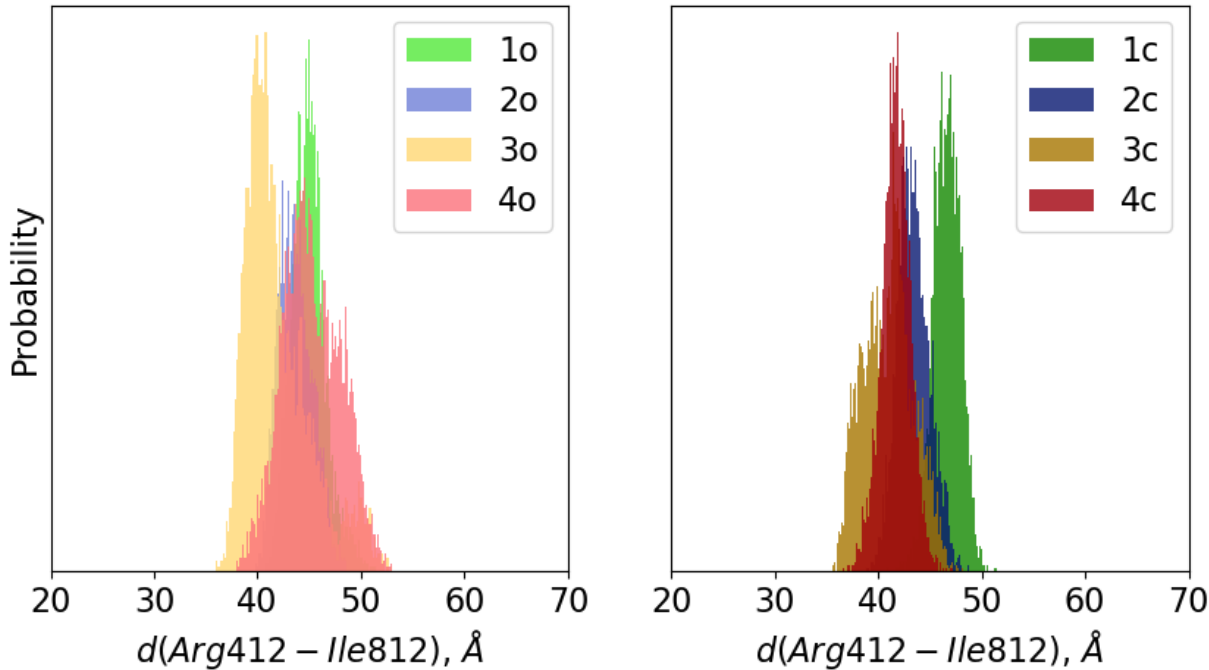
RMSD of domains (Aligned at Palm domain)



'Thumb up' vs 'Thumb down'

Distance between Ca atoms:
Arg412 – center of Tower helix (404-409)
Ile812 – top of the Thumb domain

In HIV-1:
 $d \approx 40\text{\AA}$ – 'thumb up', $d \approx 20\text{\AA}$ – 'thumb down'



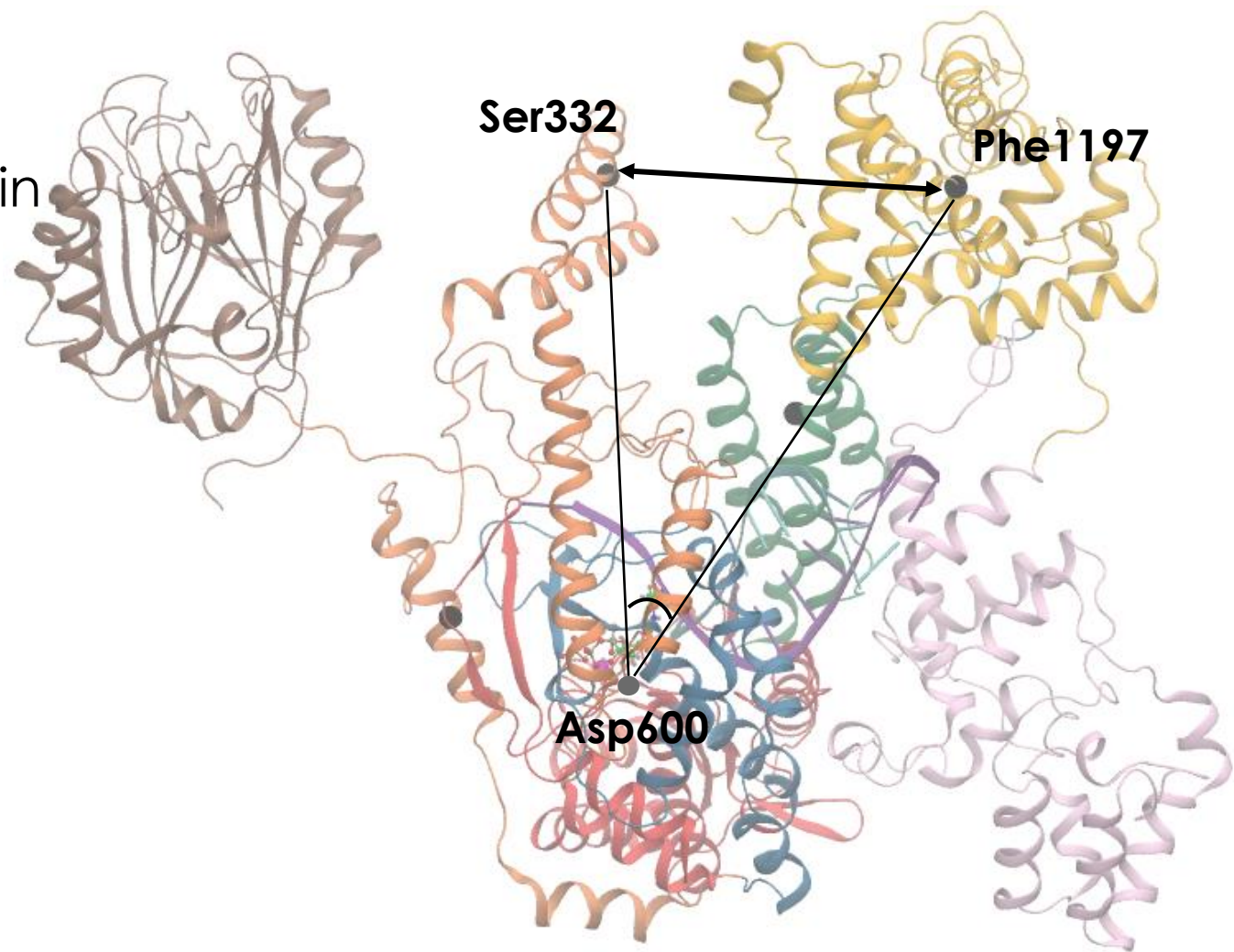
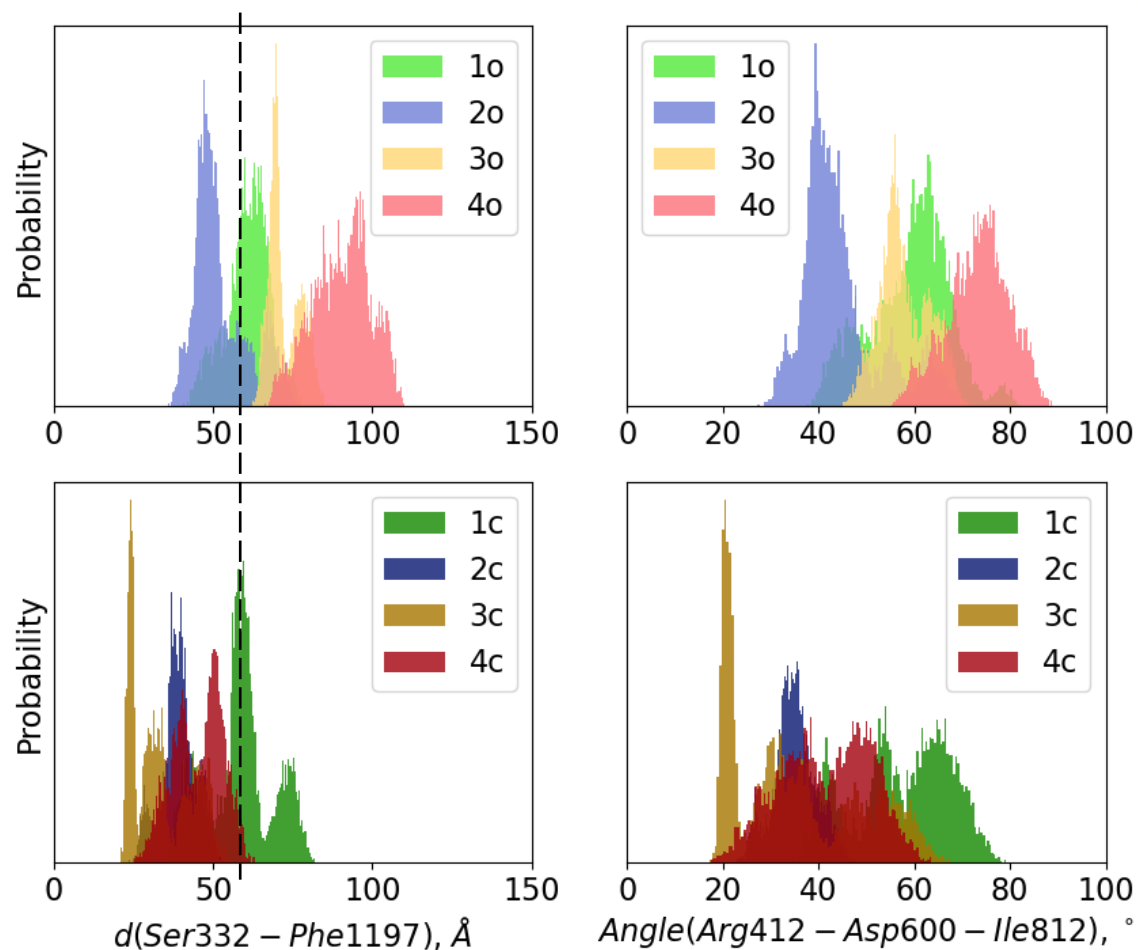
'Open ring' vs 'Closed ring'

Distance and angle between Ca atoms:

Phe1197 – center of CTD helix (404-409)

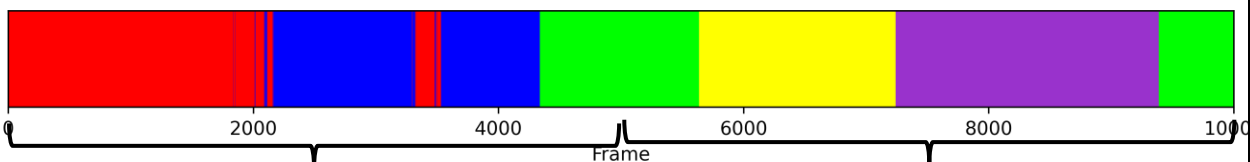
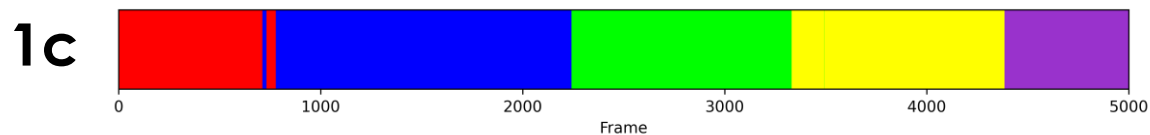
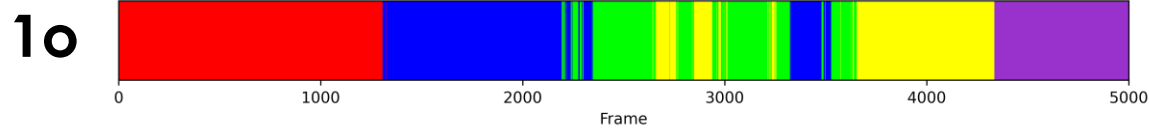
Asp600 – catalytic residue in Palm domain

Ser332 – top of the Tower domain



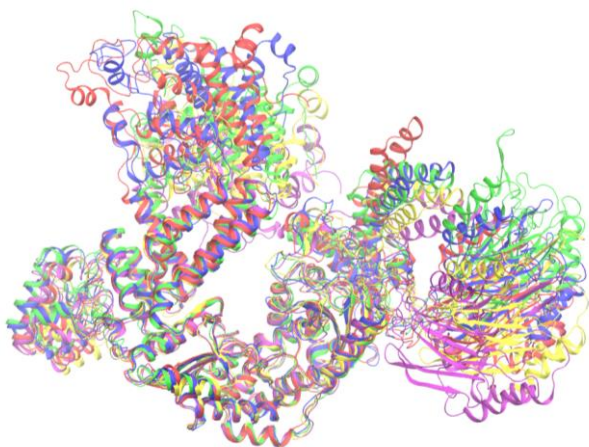
TTClust (Clustering by RMSD of CTD and Tower domains)

All-form

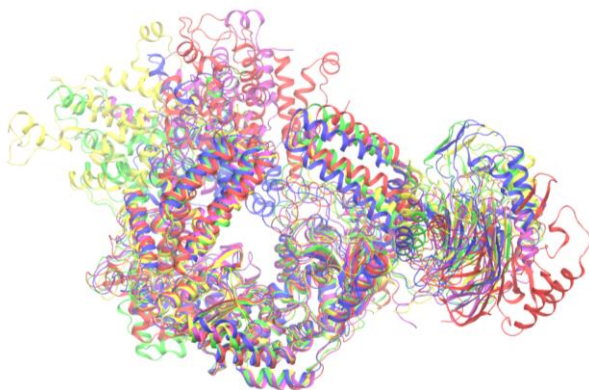


1o

1c

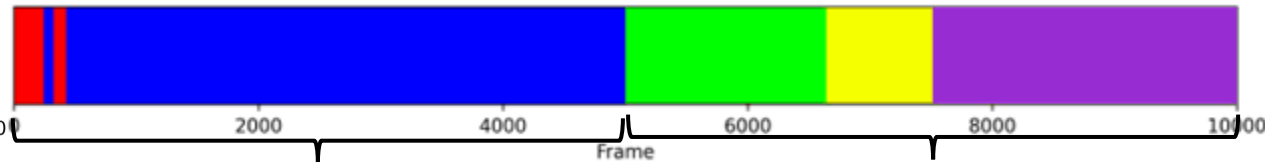
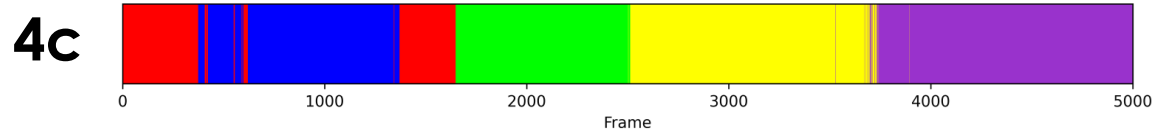
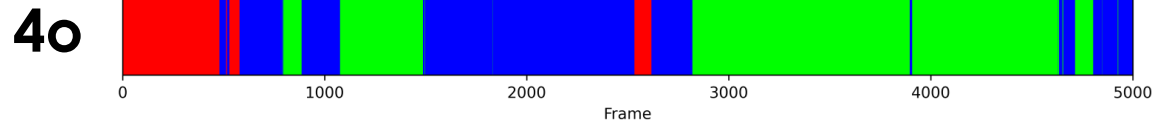


1o



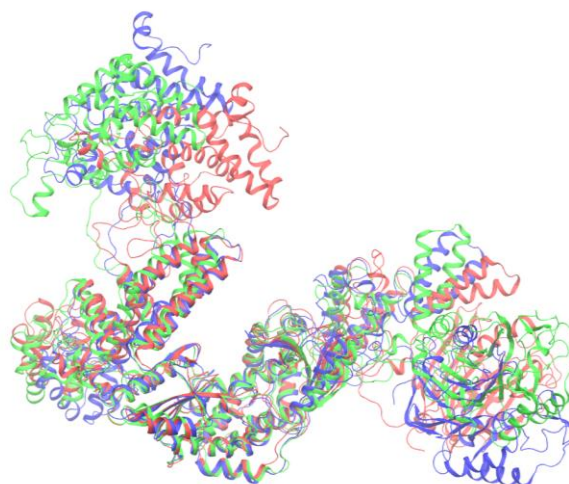
1c

Apo-form

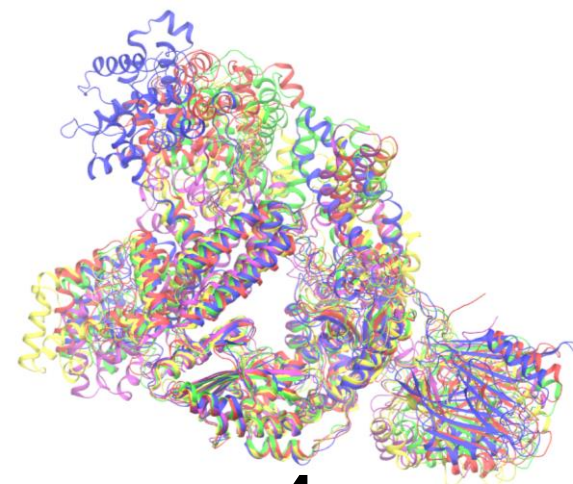


4o

4c



4o



4c

Conclusions

1. Reverse transcriptase domain of ORF2p occurs in active 'thumb up' conformation regardless of the shape of the complex throughout the molecular dynamics simulation;
2. Apo-form of ORF2p is more flexible as other complexes. Open ring conformation of ORF2p ternary complex tends to more stable closed ring conformation.



Thanks for attention!