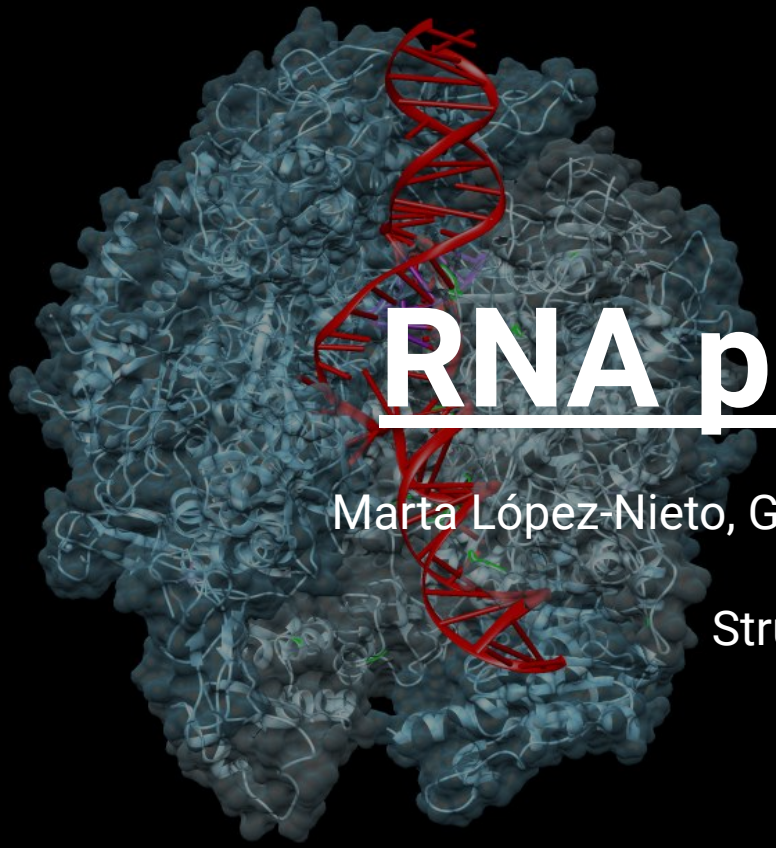
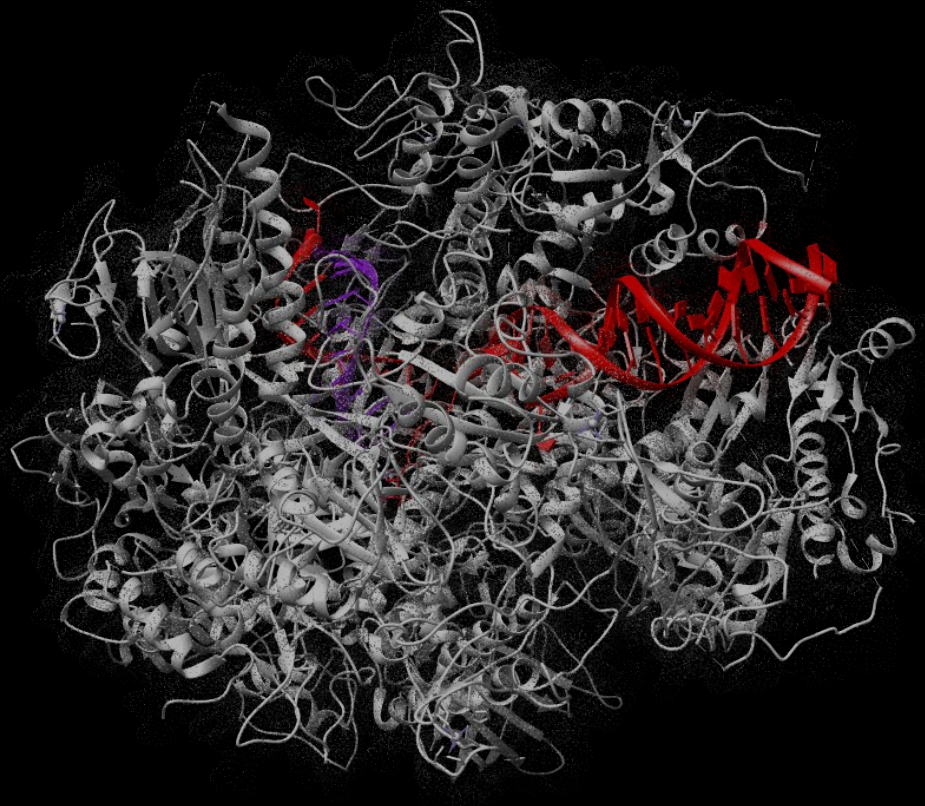


RNA polymerase

Marta López-Nieto, Gemma Nomdedéu and Pol Pérez

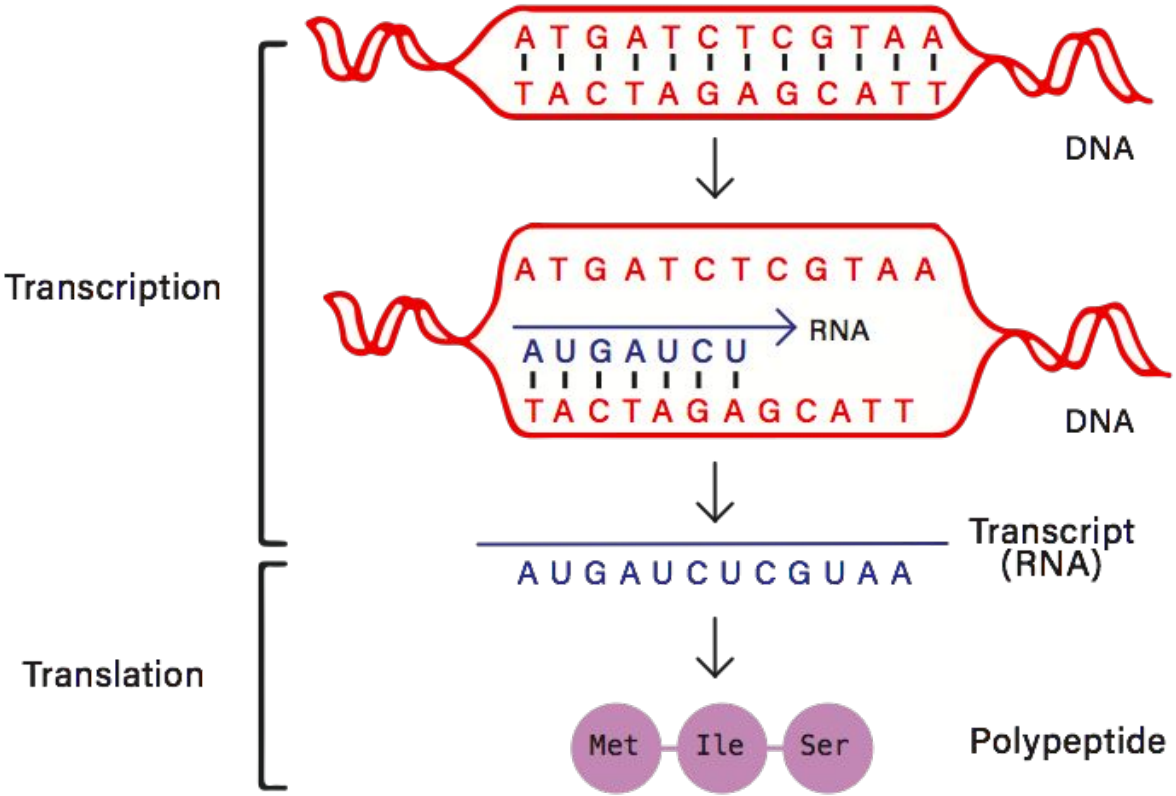
Structural Biology
2019-2020

SUMMARY

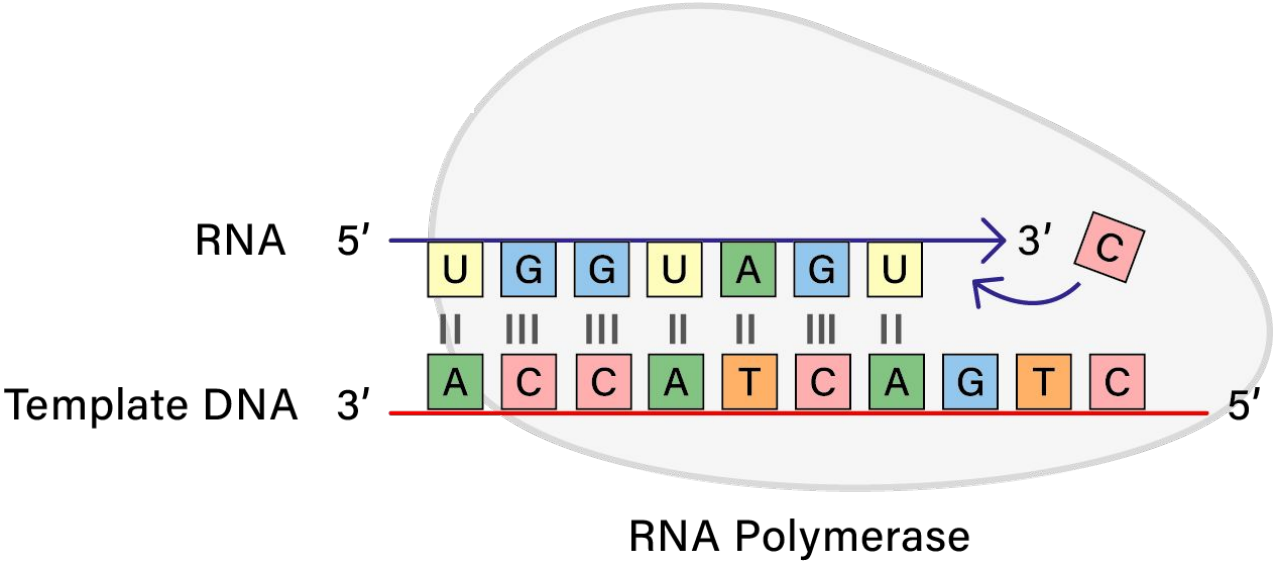


INTRODUCTION

TRANSCRIPTION



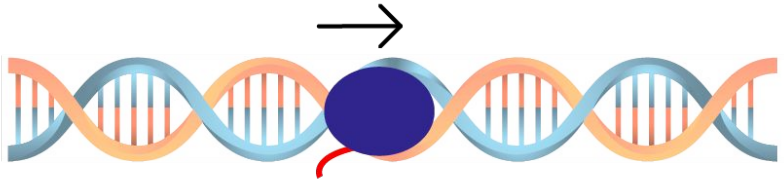
TRANSCRIPTION



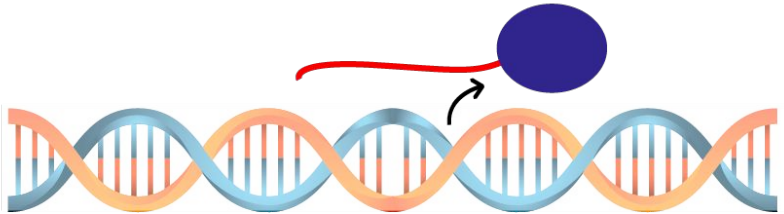
TRANSCRIPTION: Stages

Initiation

Elongation



Termination



RNA POLYMERASES I, II and III

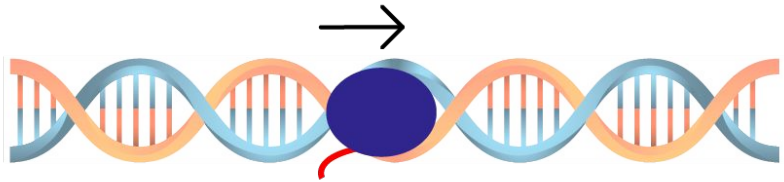
- DNA-dependent RNA polymerases
- *Bacteria* and *Archaea* → Only one RNA polymerase
- Present in all eukaryotes' **nucleus**

RNA polymerase I	**RNA polymerase II**	**RNA polymerase III**
rRNA	mRNA miRNA snRNA snoRNA	5S rRNA tRNA

AIM OF THE PROJECT

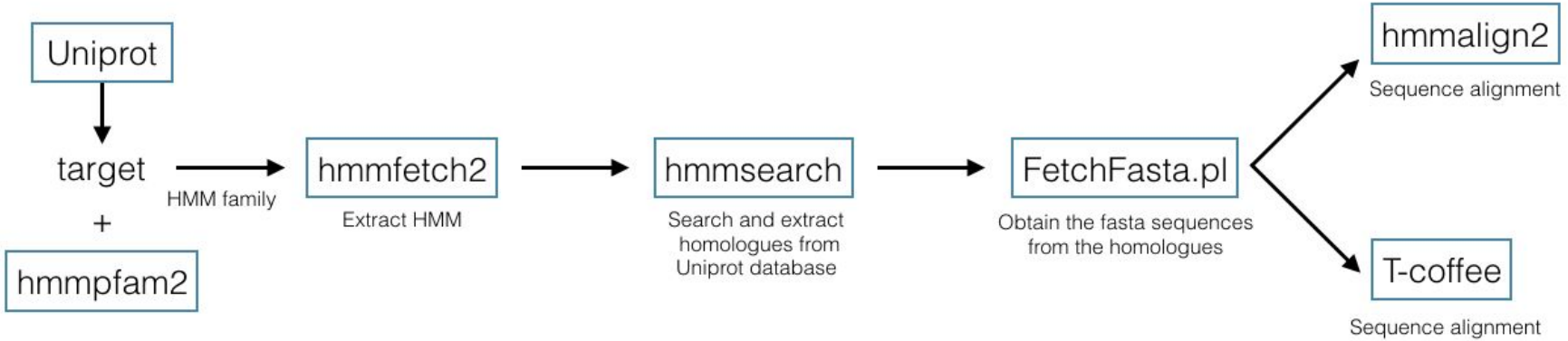
- RNA polymerase II and Elongation cycle

- The aim of this project is:
  - To identify the main structures involved in the elongation cycle.
  - To find the residues whose interactions are essential for the enzyme function.
  - To analyze their conservation among different species' genome.

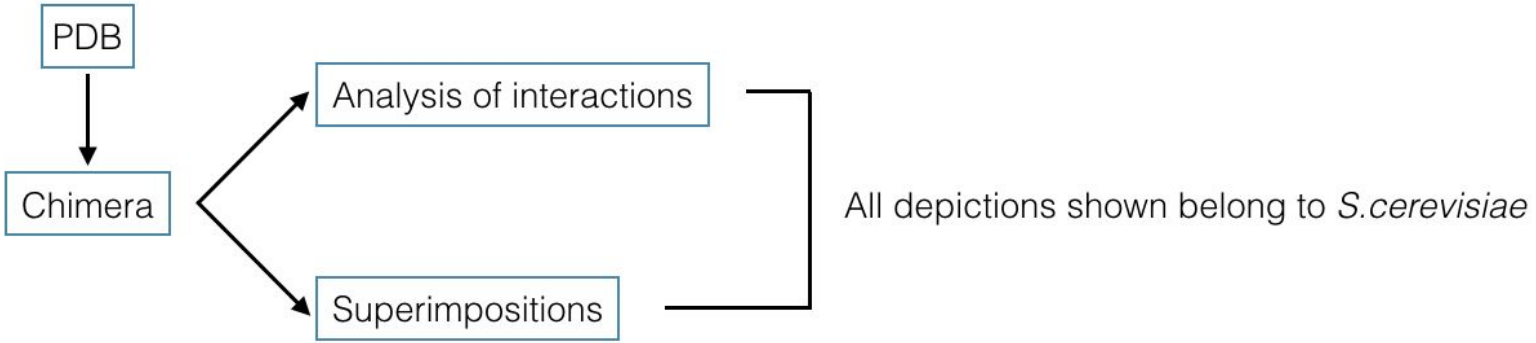


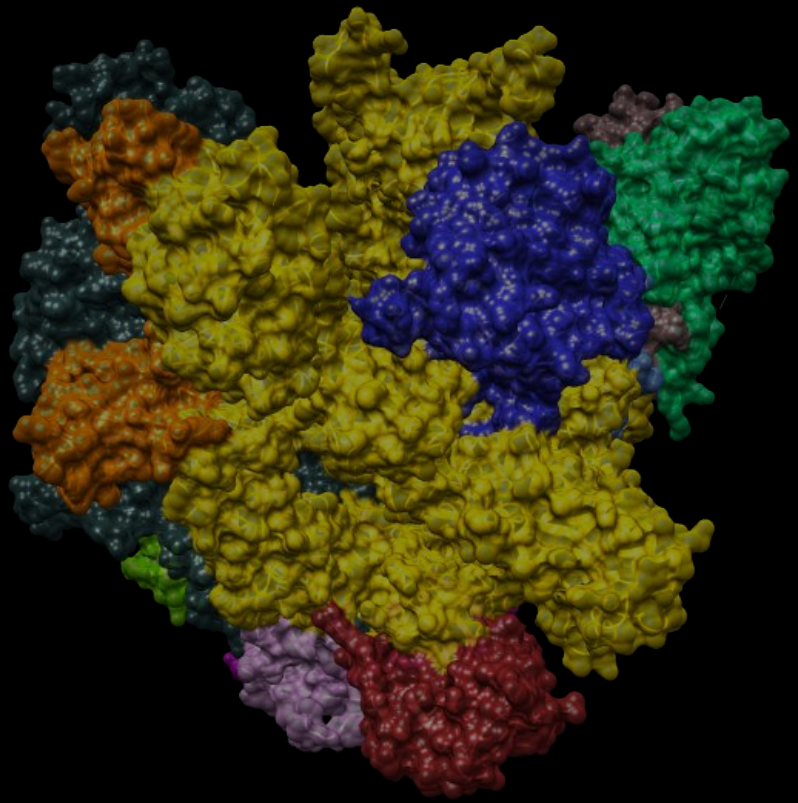
METHODS

Sequences



Structures

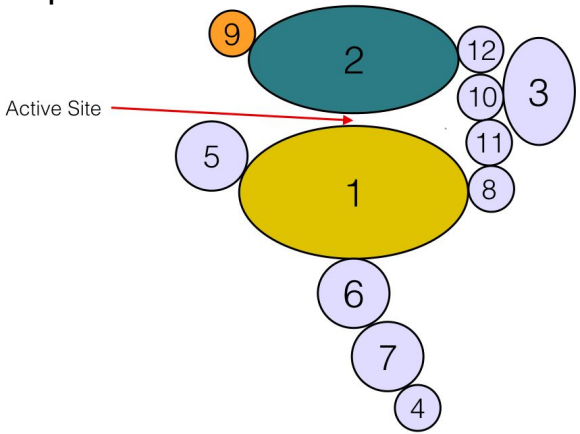


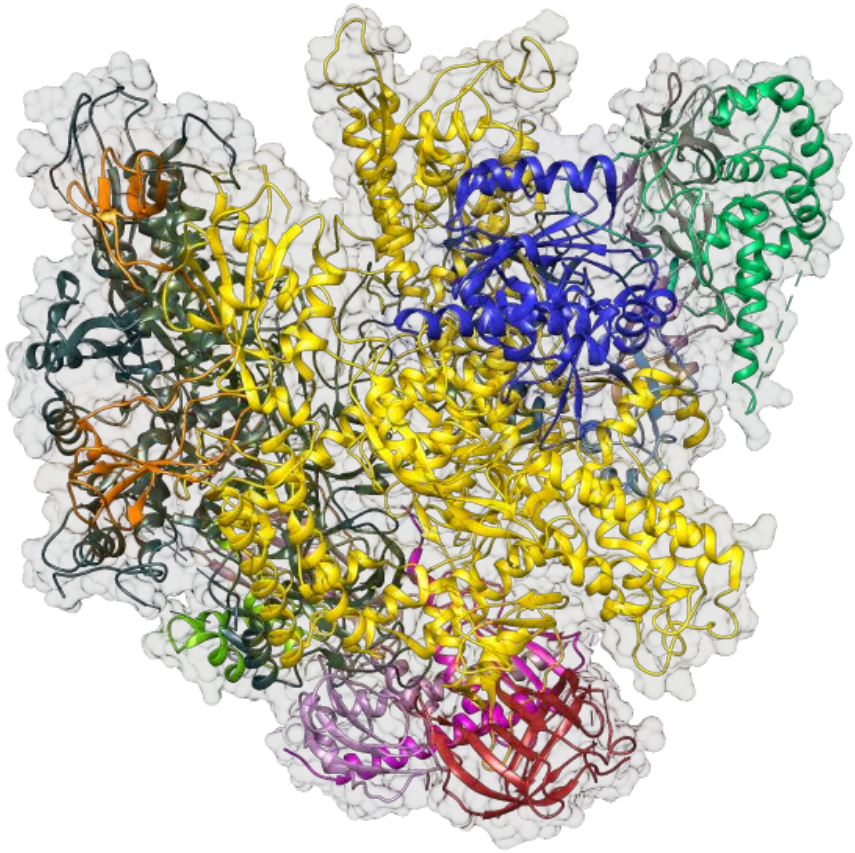


STRUCTURAL FEATURES OF RNA POLYMERASE II

RNA polymerase II

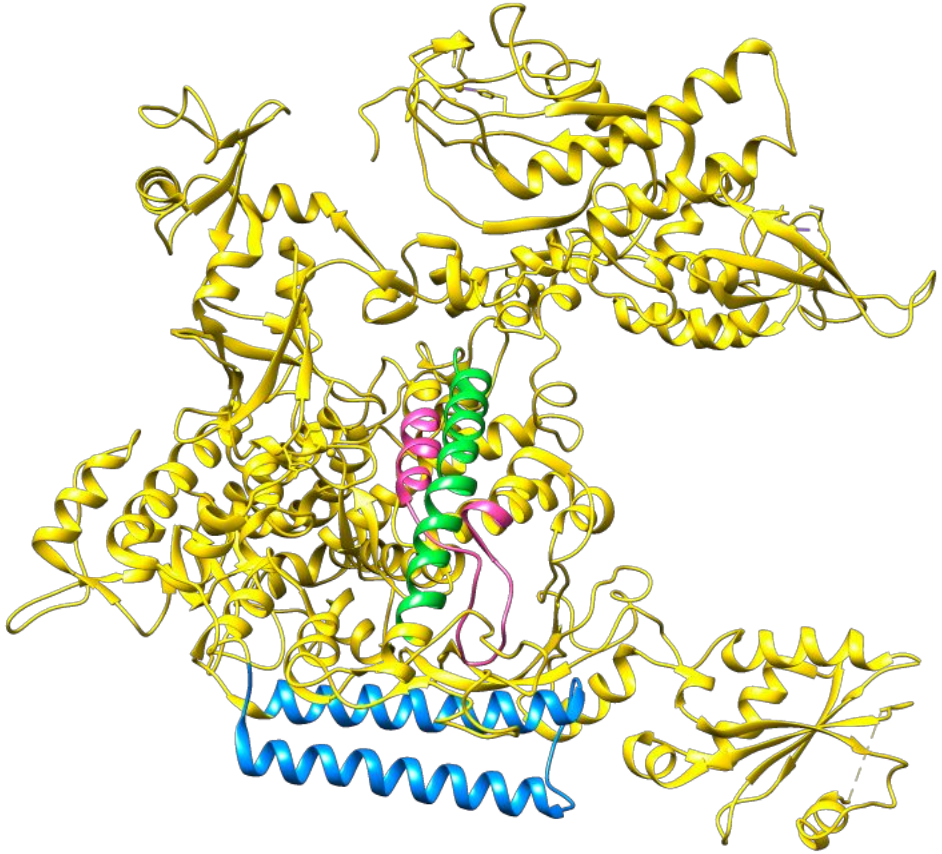
- 550 kDa
- 12 subunits (Rpb1-Rpb12)
  - Rpb1
  - Rpb2
  - Rpb9





RNA polymerase II: Rpb1

- Largest subunit (220 kDa)

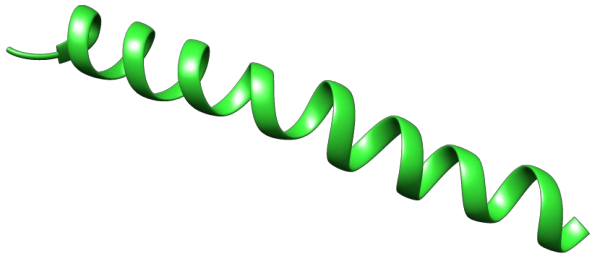


SCOP information:

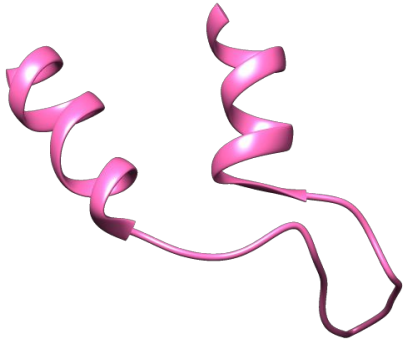
- **Class**: Multi-domains protein
- **Fold**: Beta and beta-prime subunits of DNA-dependent RNA polymerase
- **Superfamily**: Beta and beta-prime subunits of DNA-dependent RNA polymerase
- **Family**: RNA polymerase beta-prime

RNA polymerase II: Rpb1

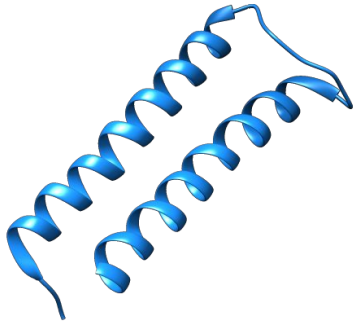
α-helix
(Bridge helix)



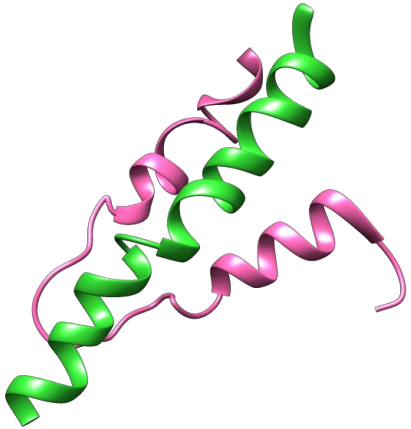
α-hairpin
(Trigger loop)



α-hairpin
(α20-α21)

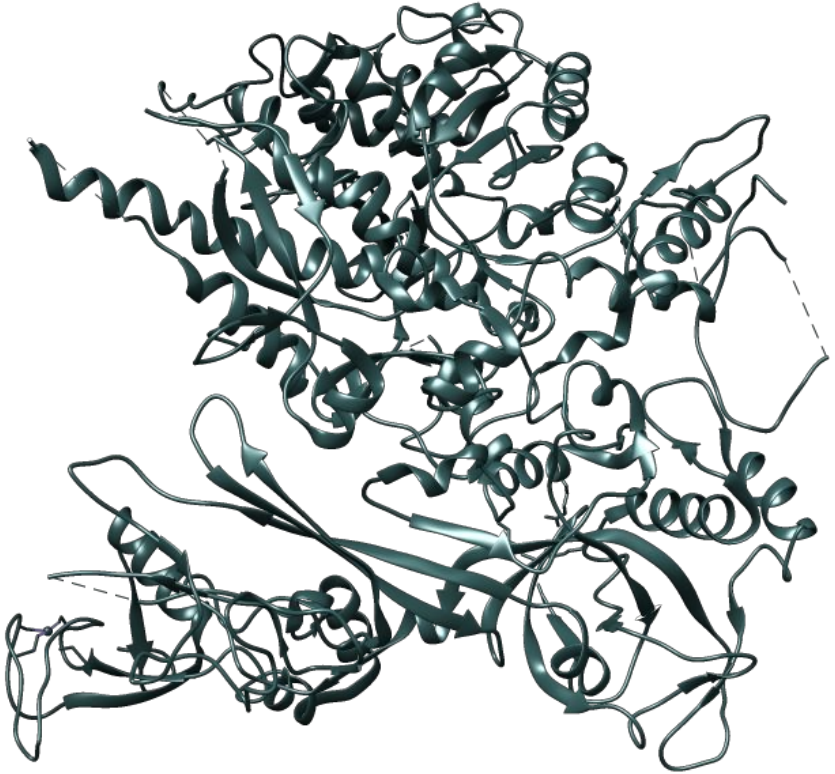


three helix
bundle (BH+TL)



RNA polymerase II: Rpb2

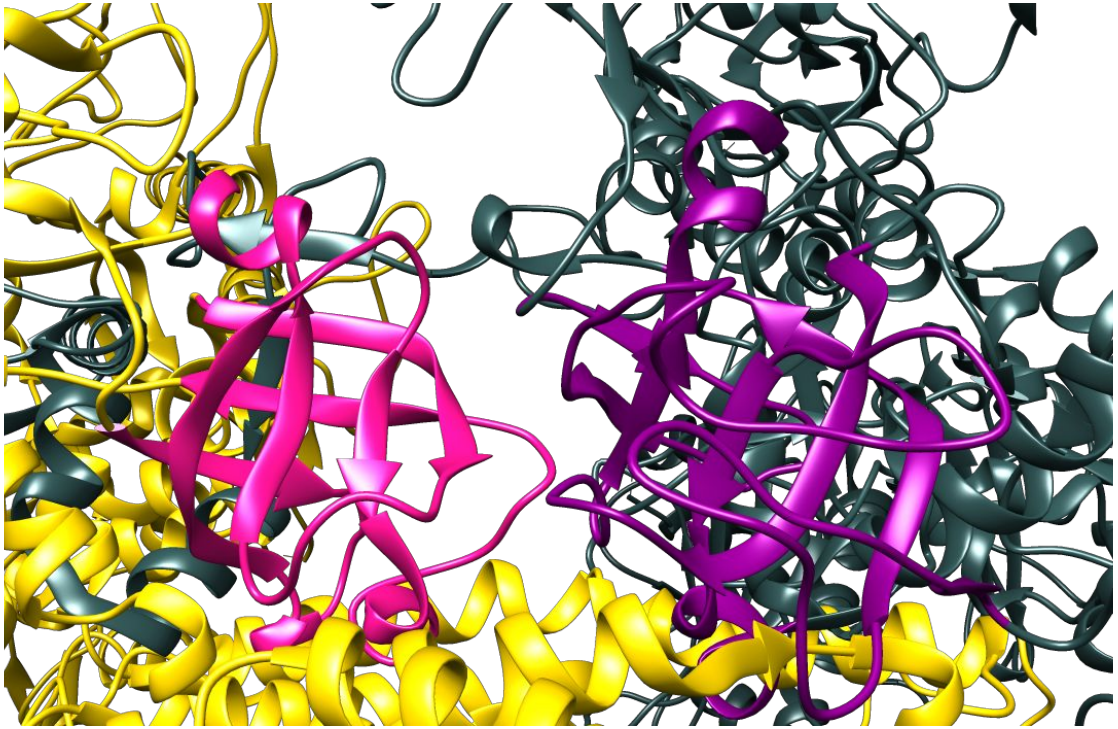
- 2nd largest subunit (140 kDa)

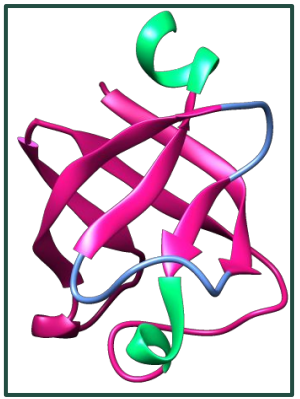


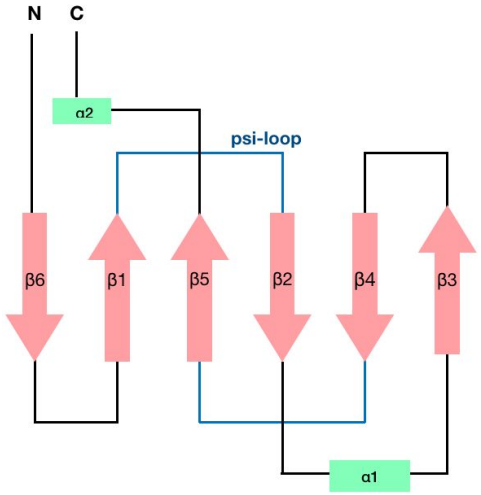
SCOP information:

- **Class**: Multi-domains protein
- **Fold**: Beta and beta-prime subunits of DNA-dependent RNA polymerase
- **Superfamily**: beta and beta-prime subunits of DNA-dependent RNA polymerase
- **Family**: RNA polymerase beta

RNA polymerase II: Rpb1 and Rpb2 double-psi β-barrels





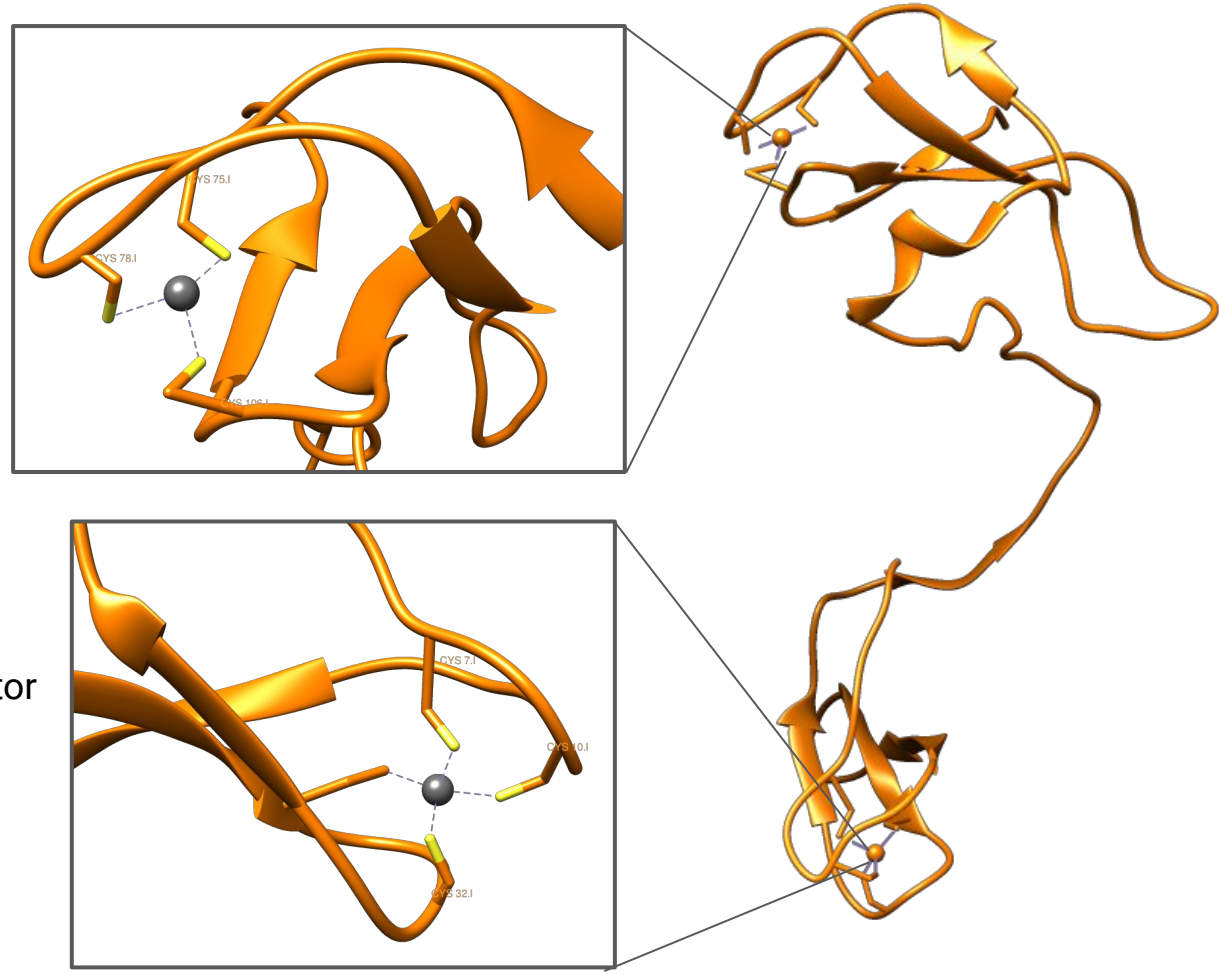


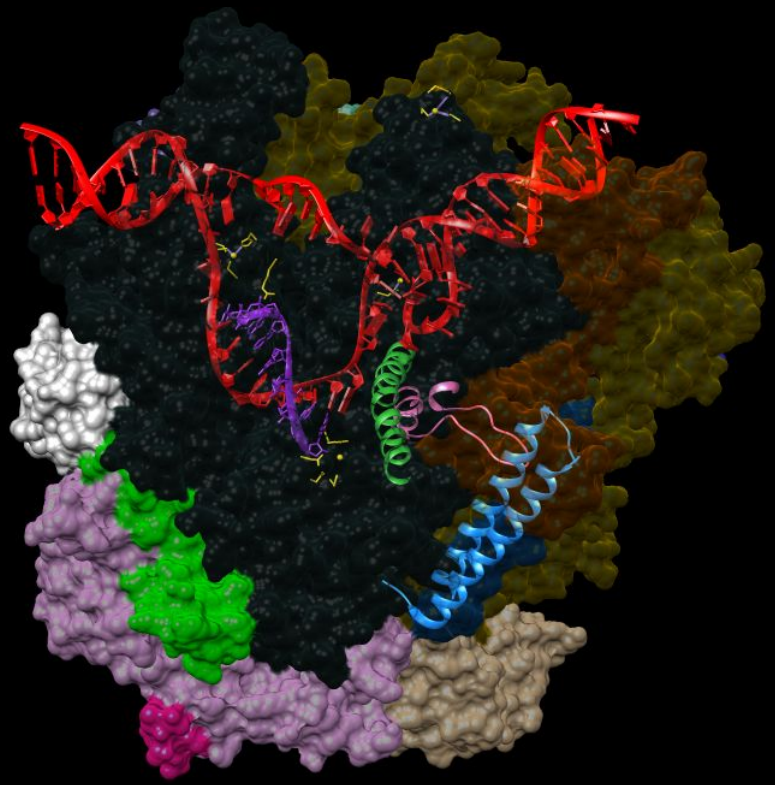
RNA polymerase II: Rpb9

- 14.5 kDa
- 2 Zn binding motifs

SCOP information:

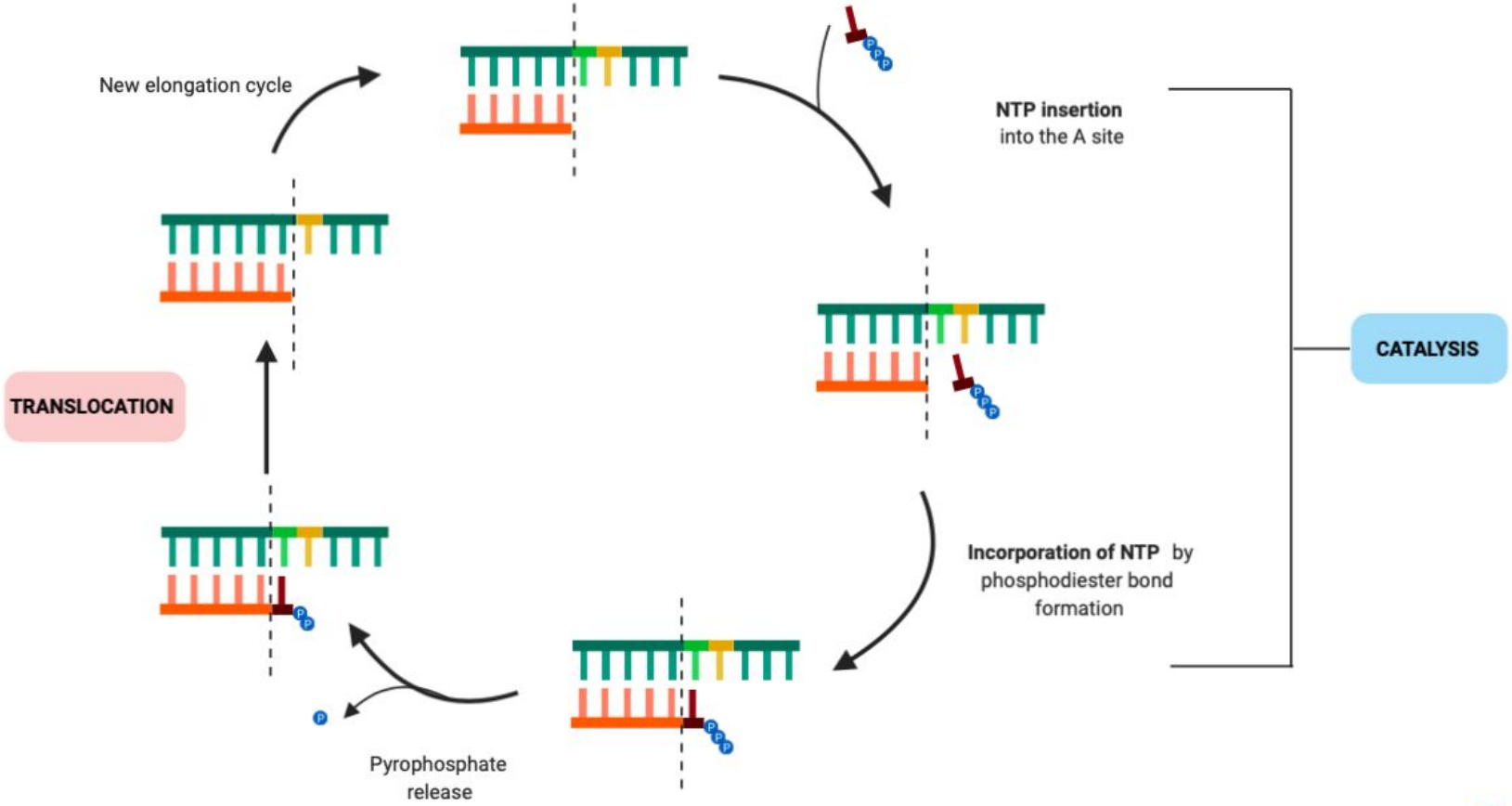
- **Class**: Small proteins
- **Fold**: Rubredoxin-like
- **Superfamily**: Zn beta-ribbon
- **Family**: Transcriptional factor domain



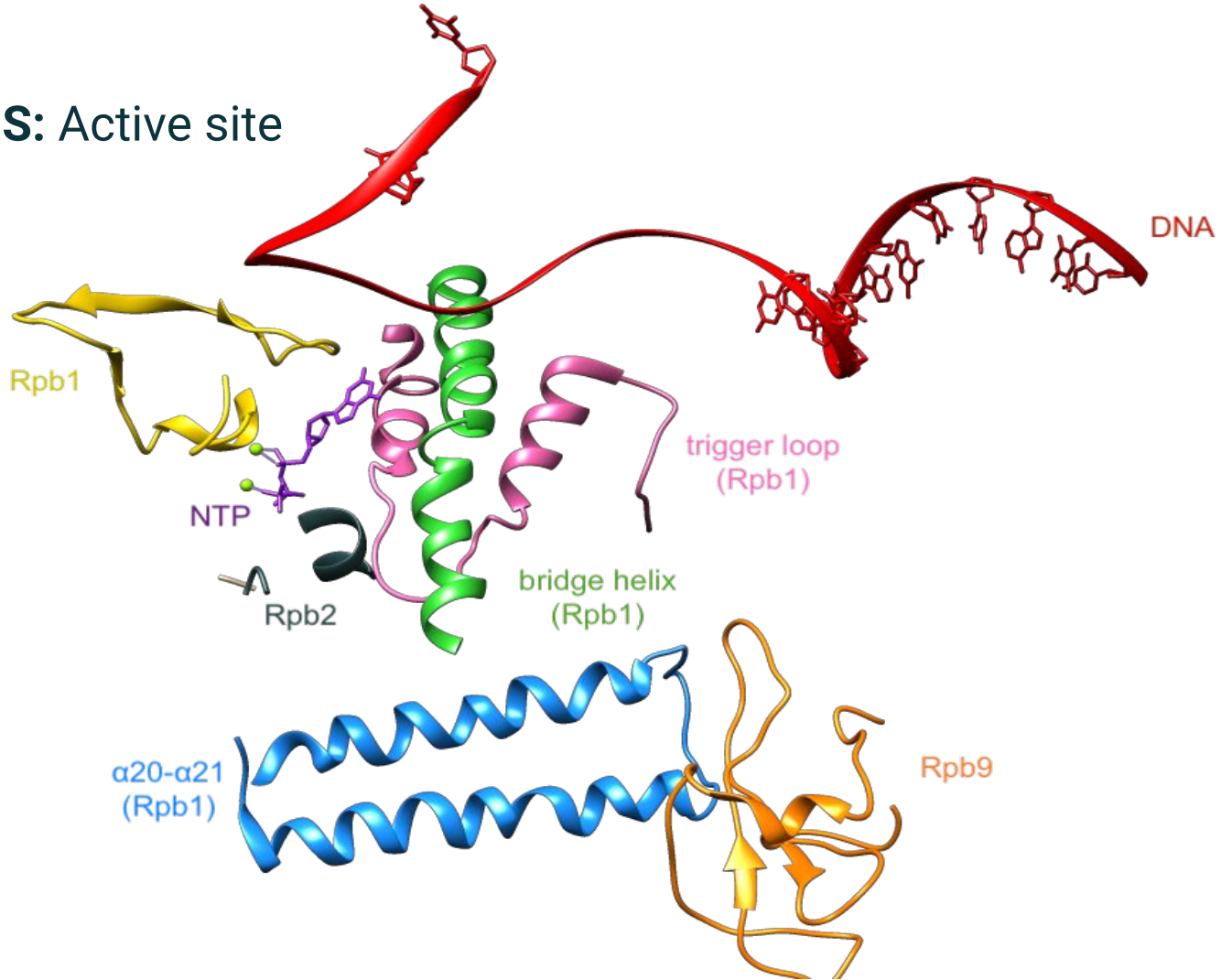


THE ELONGATION CYCLE

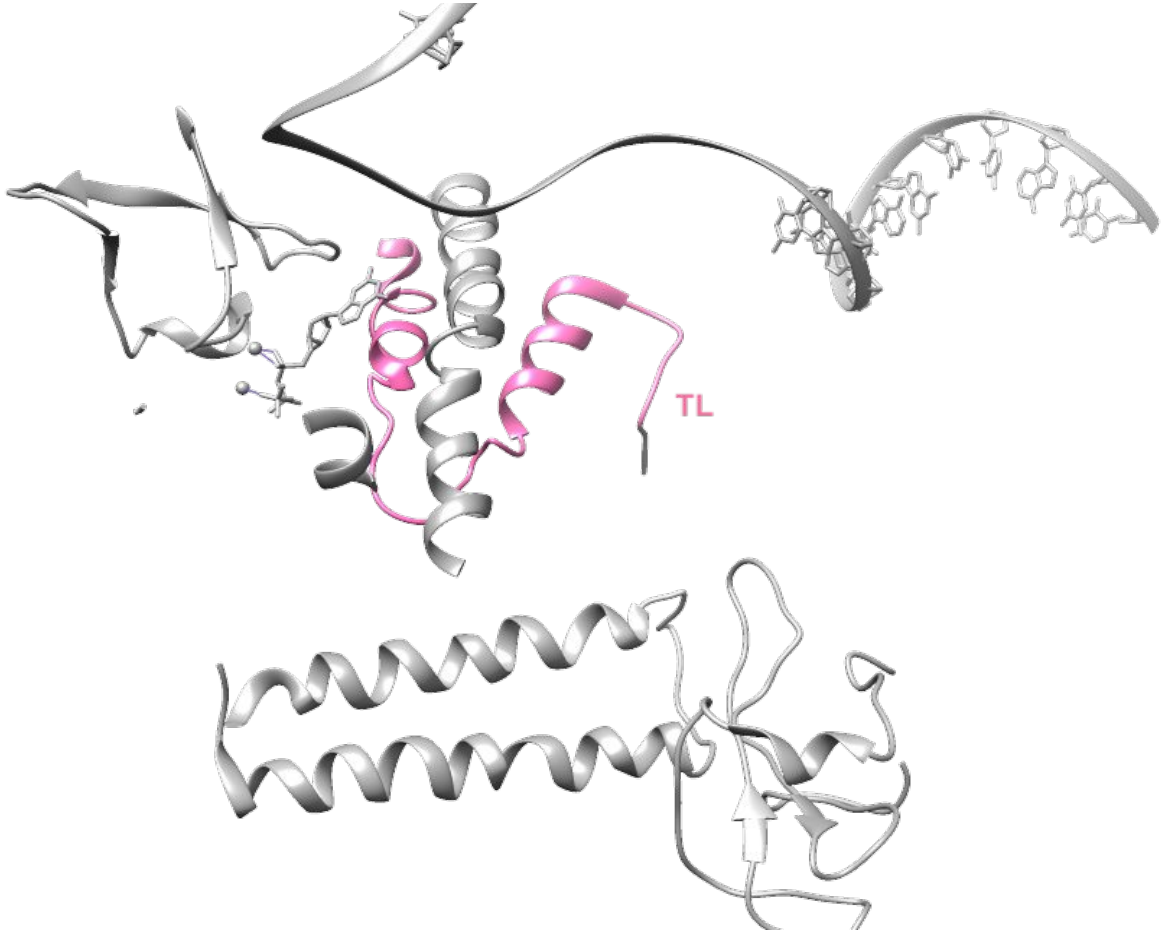
ELONGATION CYCLE



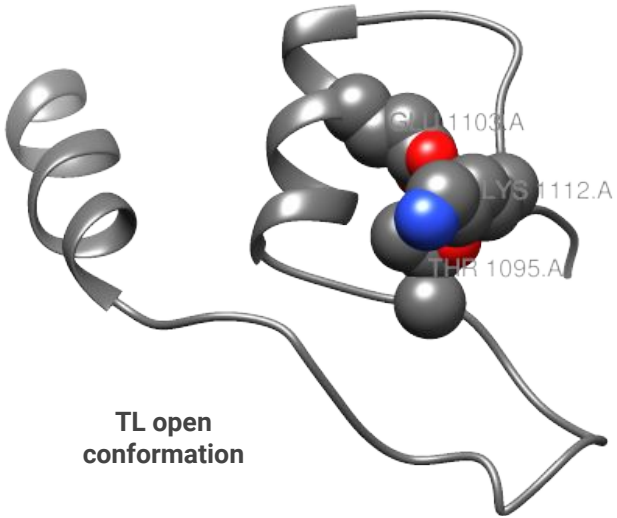
CATALYSIS: Active site

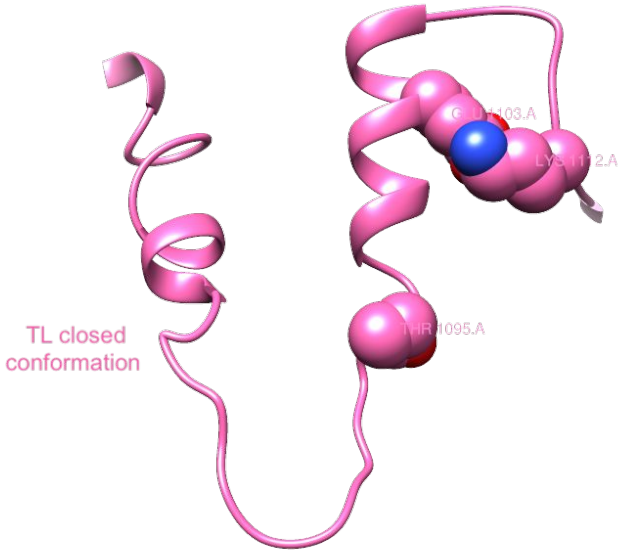


CATALYSIS: Trigger loop (TL) mobility

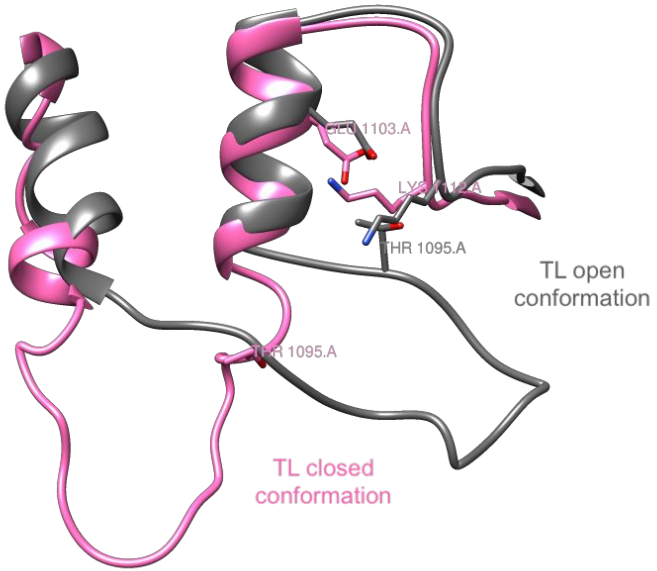


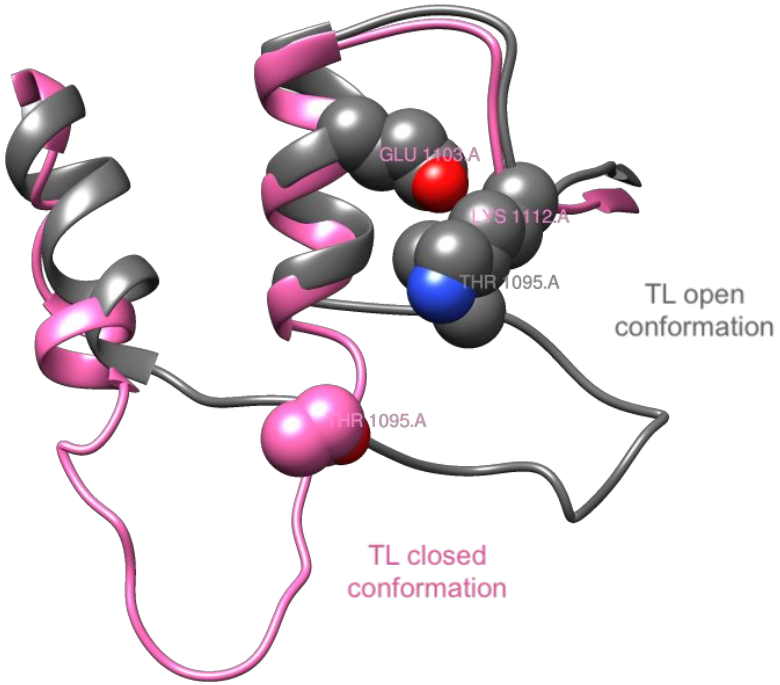
CATALYSIS: Trigger loop (TL) mobility





CATALYSIS: TL mobility (superimposition)

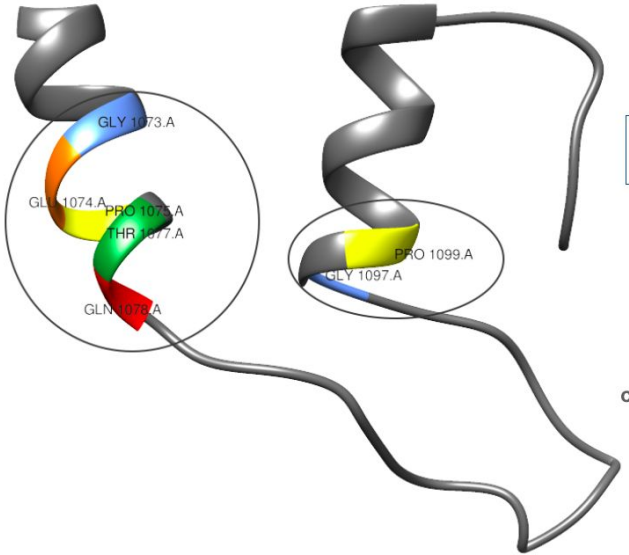




RMSD = 0.953 A

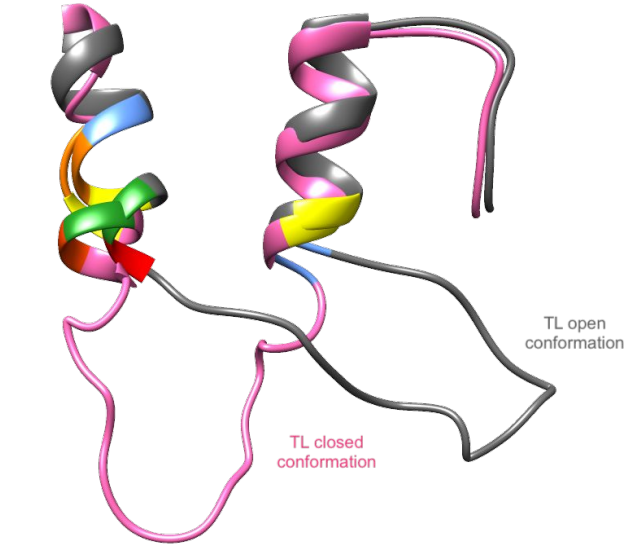
CATALYSIS: Hinge regions allow trigger loop mobility

Hinge region motif (H1):
GEPXTQ



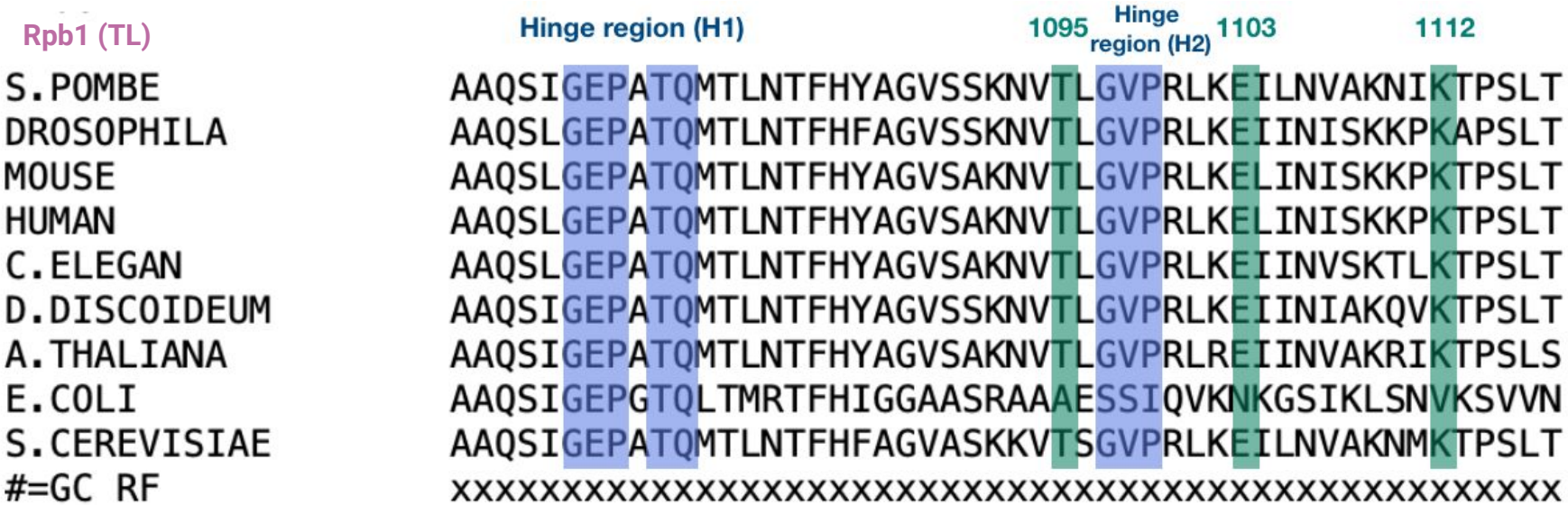
TL open conformation

Hinge region motif (H2):
GXP

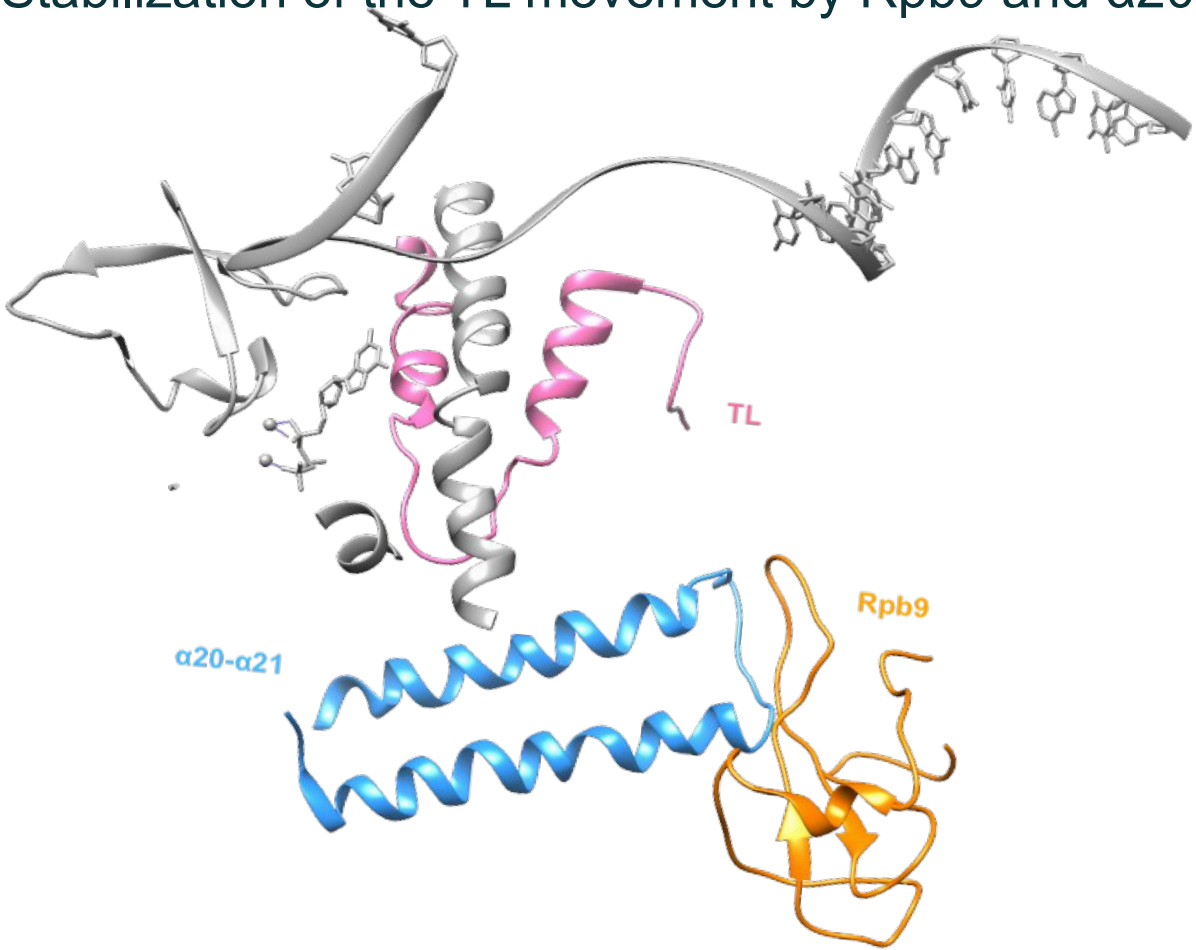


RMSD = 0.953 A

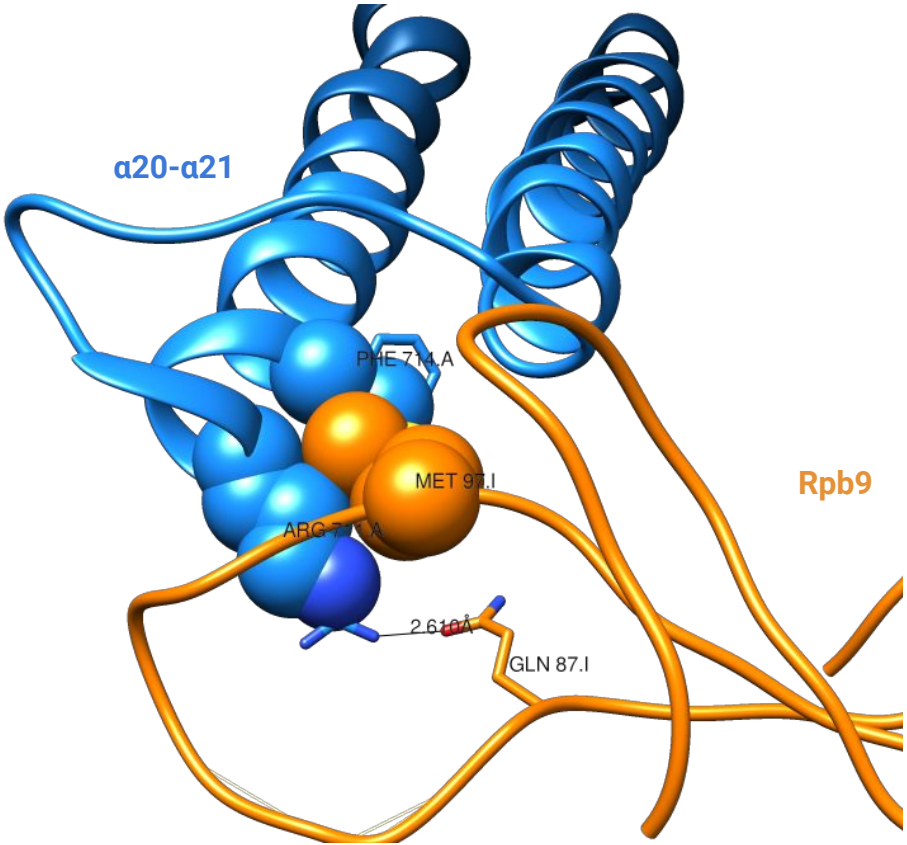
CATALYSIS: residues involved in TL movement are conserved



CATALYSIS: Stabilization of the TL movement by Rpb9 and α20-α21

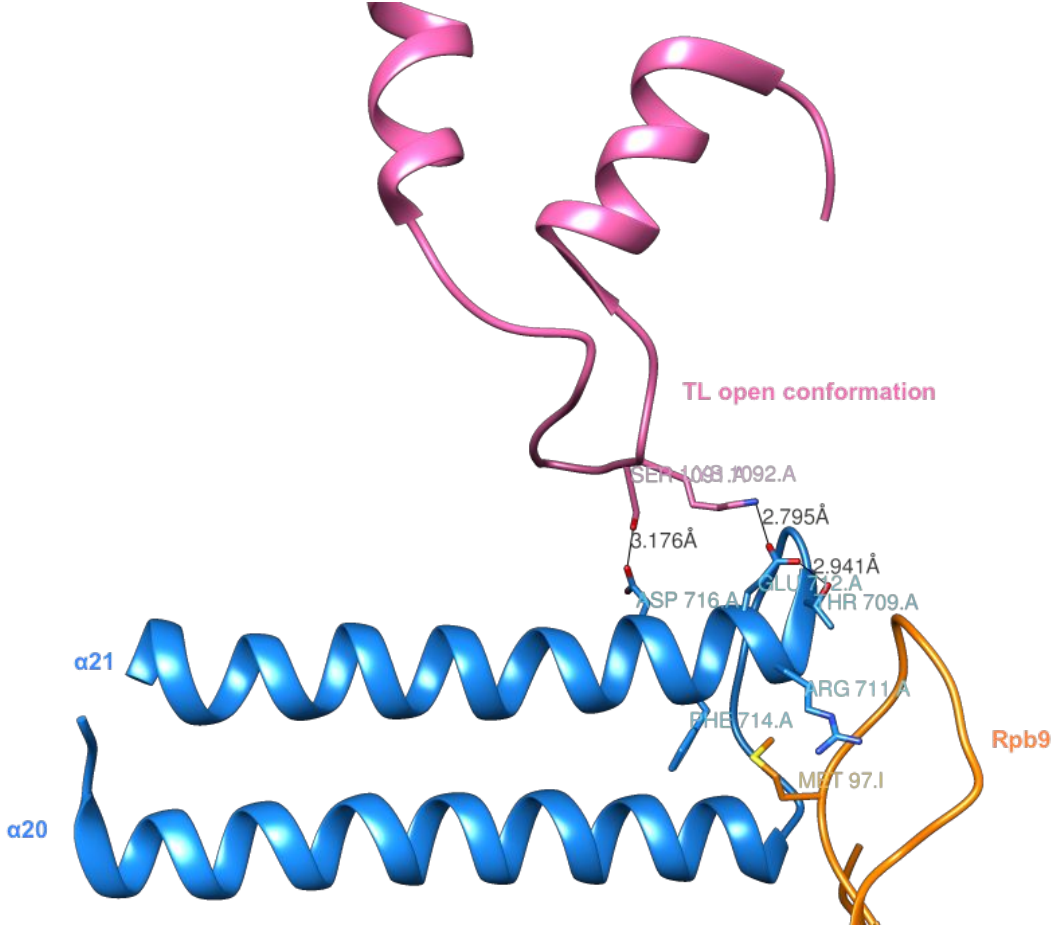


CATALYSIS: Rpb9 stabilizes α20 and α21 from Rpb1

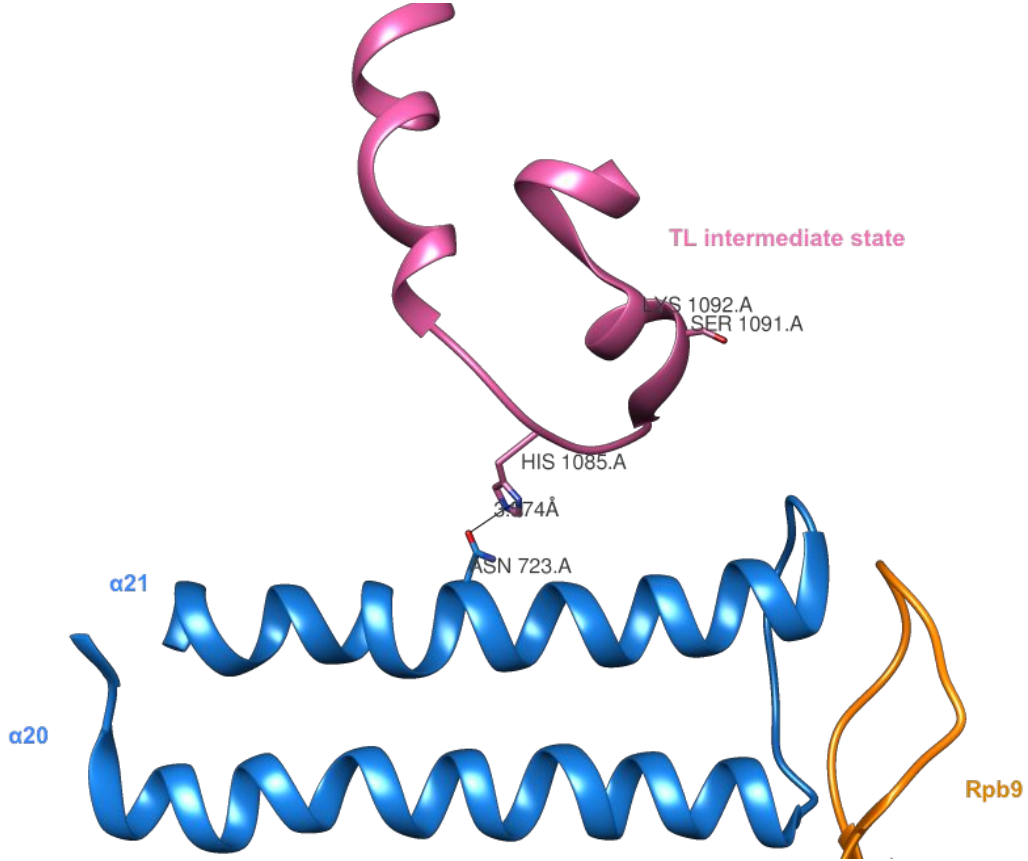



Rpb9
87
97
HUMAN KITHEVDELTQIIADVSQDPTLPRTEDHPCQKCGHKEAVFFQSHSARAEDAMRLYYVCTA
PIG KITHEVDELTQIIADVSQDPTLPRTEDHPCQKCGHKEAVFFQSHSARAEDAMRLYYVCTA
MOUSE KITHEVDELTQIIADVSQDPTLPRTEDHPCQKCGHKEAVFFQSHSARAEDAMRLYYVCTA
BOVIN KITHEVDELTQIIADVSQDPTLPRTEDHPCQKCGHKEAVFFQSHSARAEDAMRLYYVCTA
DROSOPHILA KIMHEIDELTHIVPDVISDPTLPRTEDHACPKCSHREAVFFQAQTRRAEEEMRLYYVCTN
S.POMBE ELQSSNVENTTVSHDASTDPTLPR-SDKECPRCHQHEAVFYQTHSRRGDTMMTLIYVCVH
S.CEREVISIAE ELITNIGETAGVVQDIGSDPTLPR-SDRECPKCHSRENVFFQSQQRRKDTSMVLFFVCLS
:: . * : : * ****** .*: * :* :* **:*:: * : * * :**
Rpb1
711
714
S.POMBE evtrtvkearrqvaecIQDAQH-NRLKPEPGMTLRESFEAKVSRILNQAR
D.DISCOIDEUM kvtltissaknqvkelIIKAQN-KQFECQPGKSVIETFEQKVNQVLNKAR
C.ELEGANS diqntirkakqdvvdvIEKAHN-DDLEPTPGNTLRQTFENKVNQILNDAR
MOUSE diqntikkakqdvievIEKAHN-NELEPTPGNTLRQTFENQVNRILNDAR
HUMAN diqntikkakqdvievIEKAHN-NELEPTPGNTLRQTFENQVNRILNDAR
DROSOPHILA eiqqaikkakddvinvIQKAHN-MELEPTPGNTLRQTFENKVNRILNDAR
ARCHEA rieahlrnaearvdqlIEAYEN-GELEPLPGRSLEETLEMKIMQVLGEAR
S.CEREVISIAE eitetiaeakkkvldvTKEAQA-NLLTAKHGMTLRESFEDNVVRFLNEAR
#=GC RF xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx

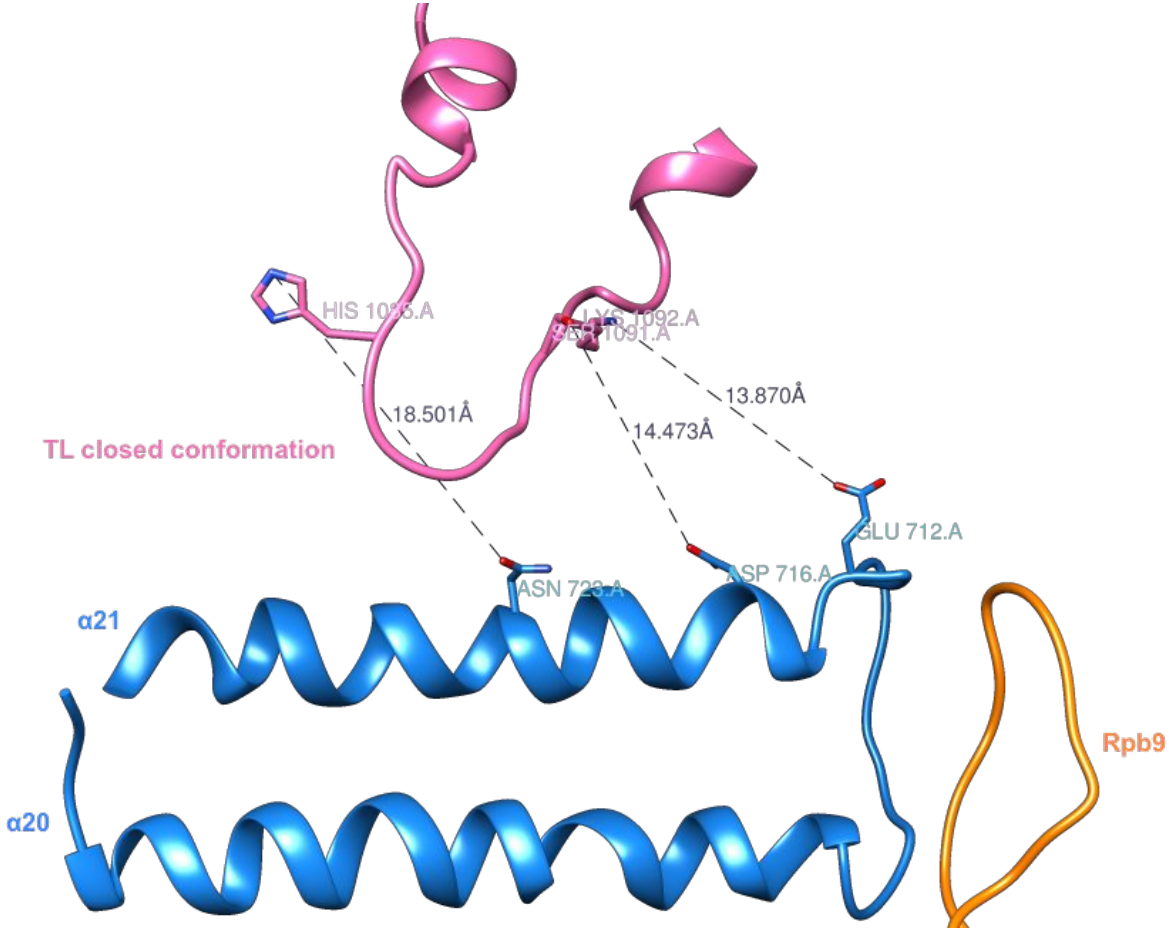
CATALYSIS: α20 and α21 stabilize TL movement



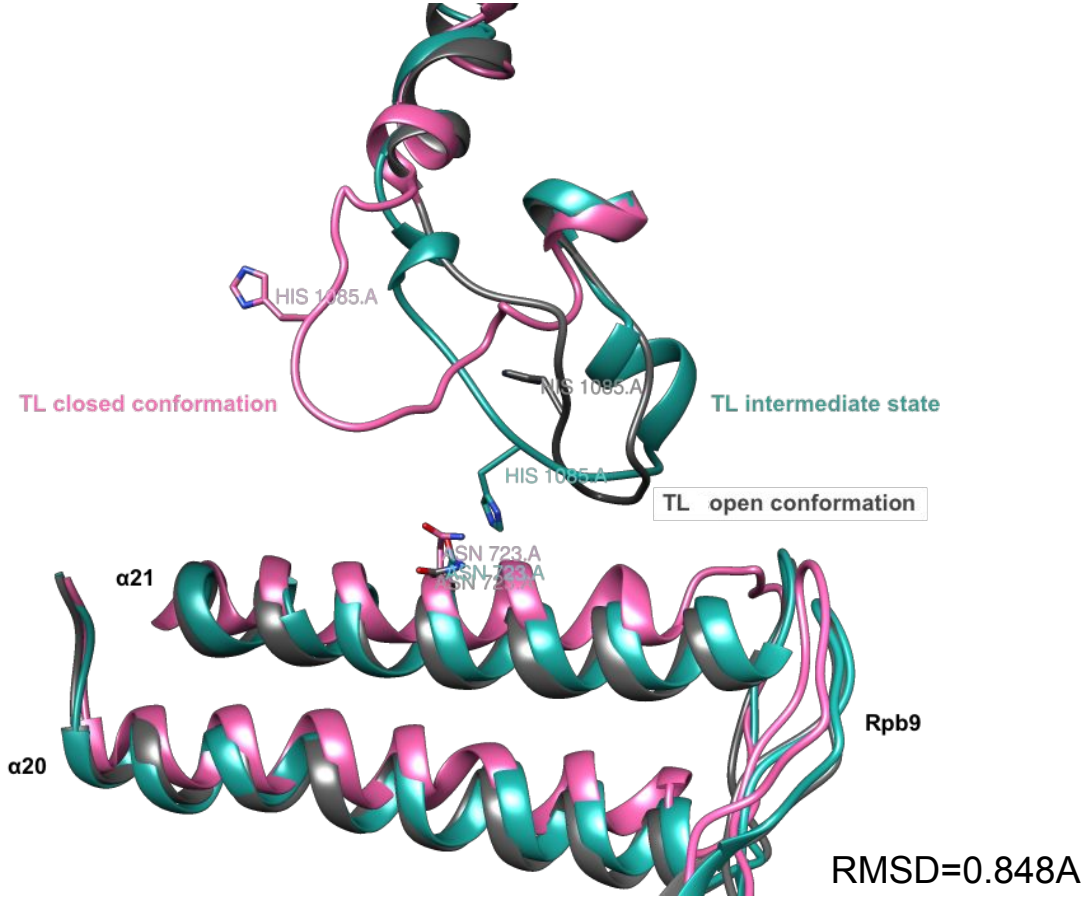
CATALYSIS: α20 and α21 stabilize TL movement



CATALYSIS: α20 and α21 stabilize TL movement



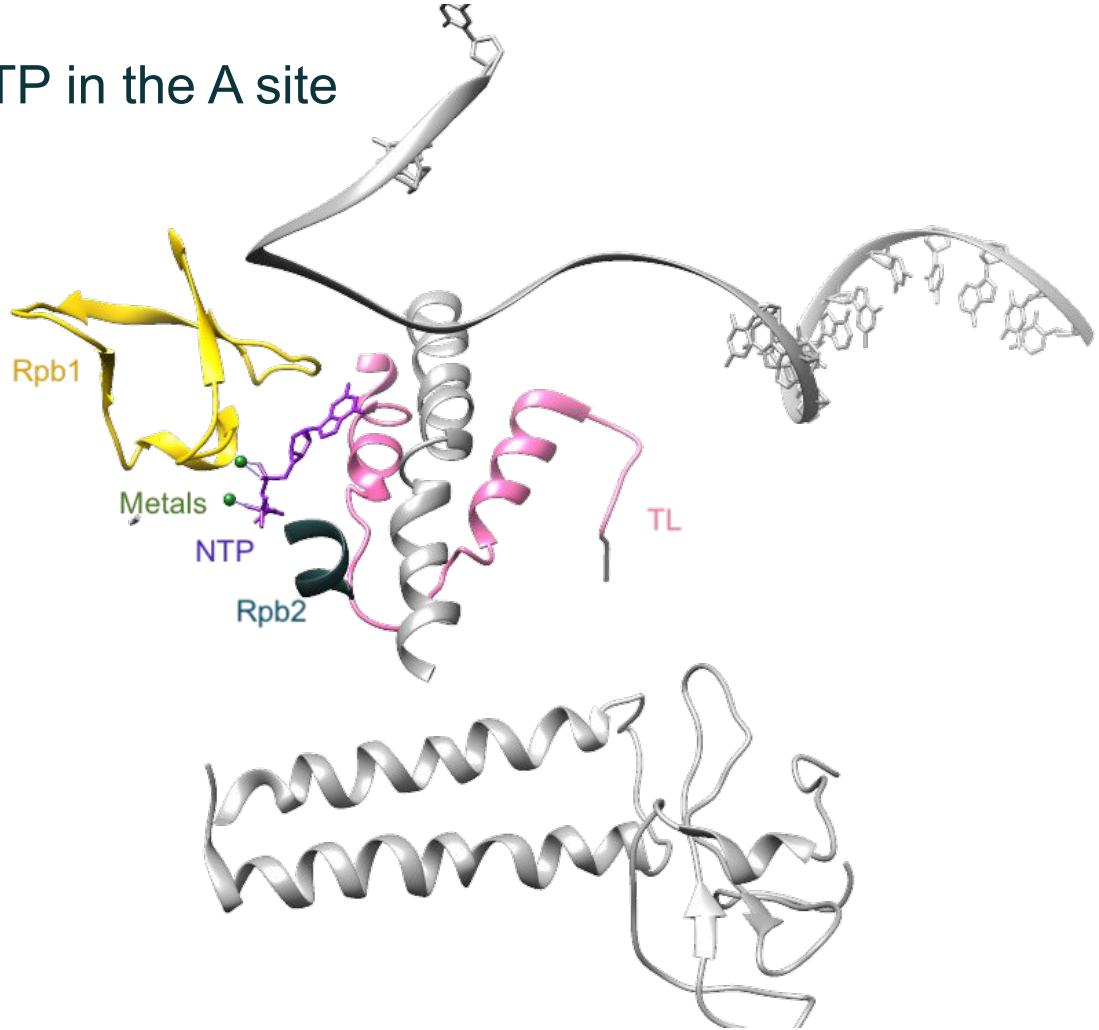
CATALYSIS: α20 and α21 stabilize TL movement (superimposition)



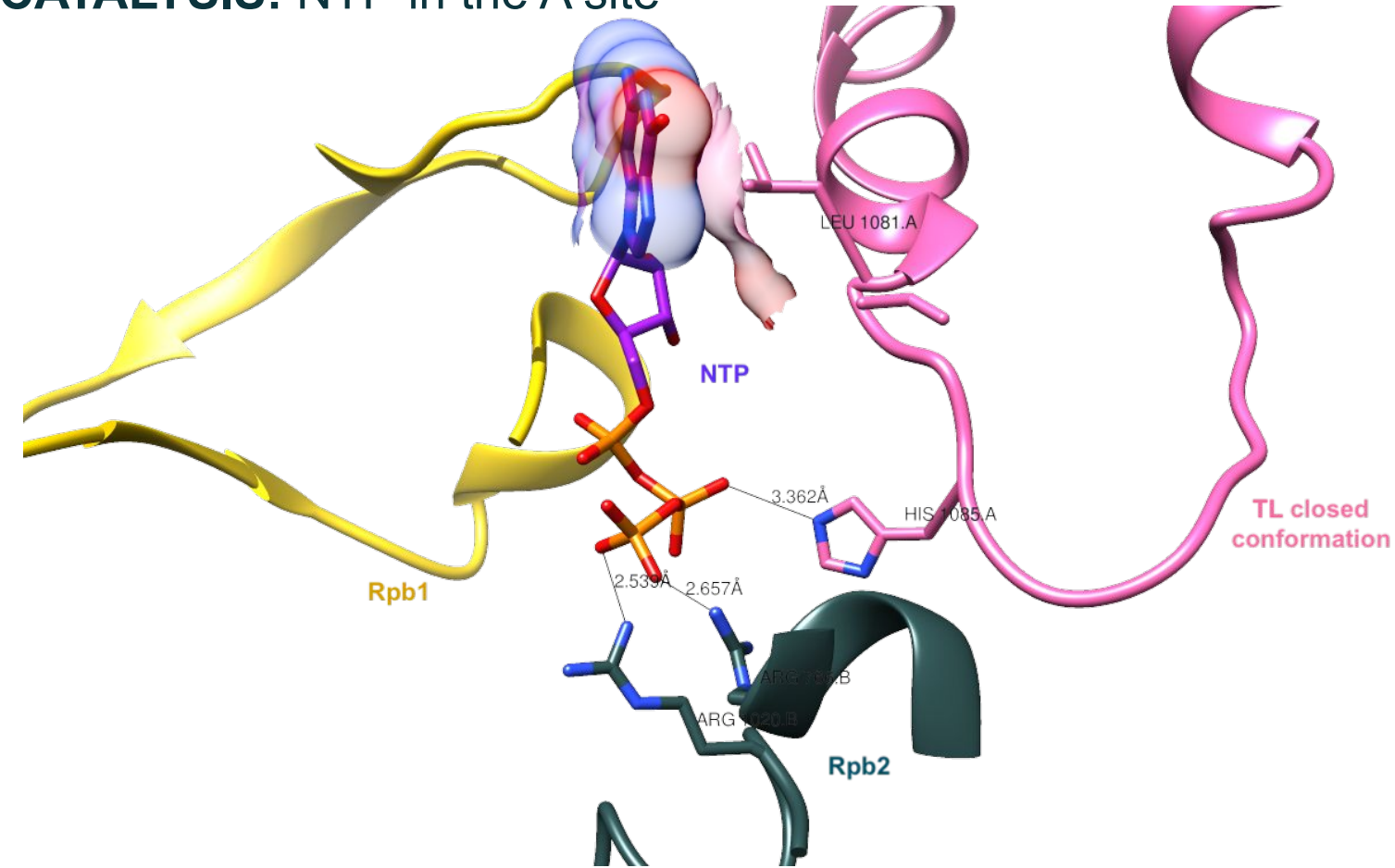
RMSD=0.848A

CATALYSIS: conservation of residues that stabilize TL movement

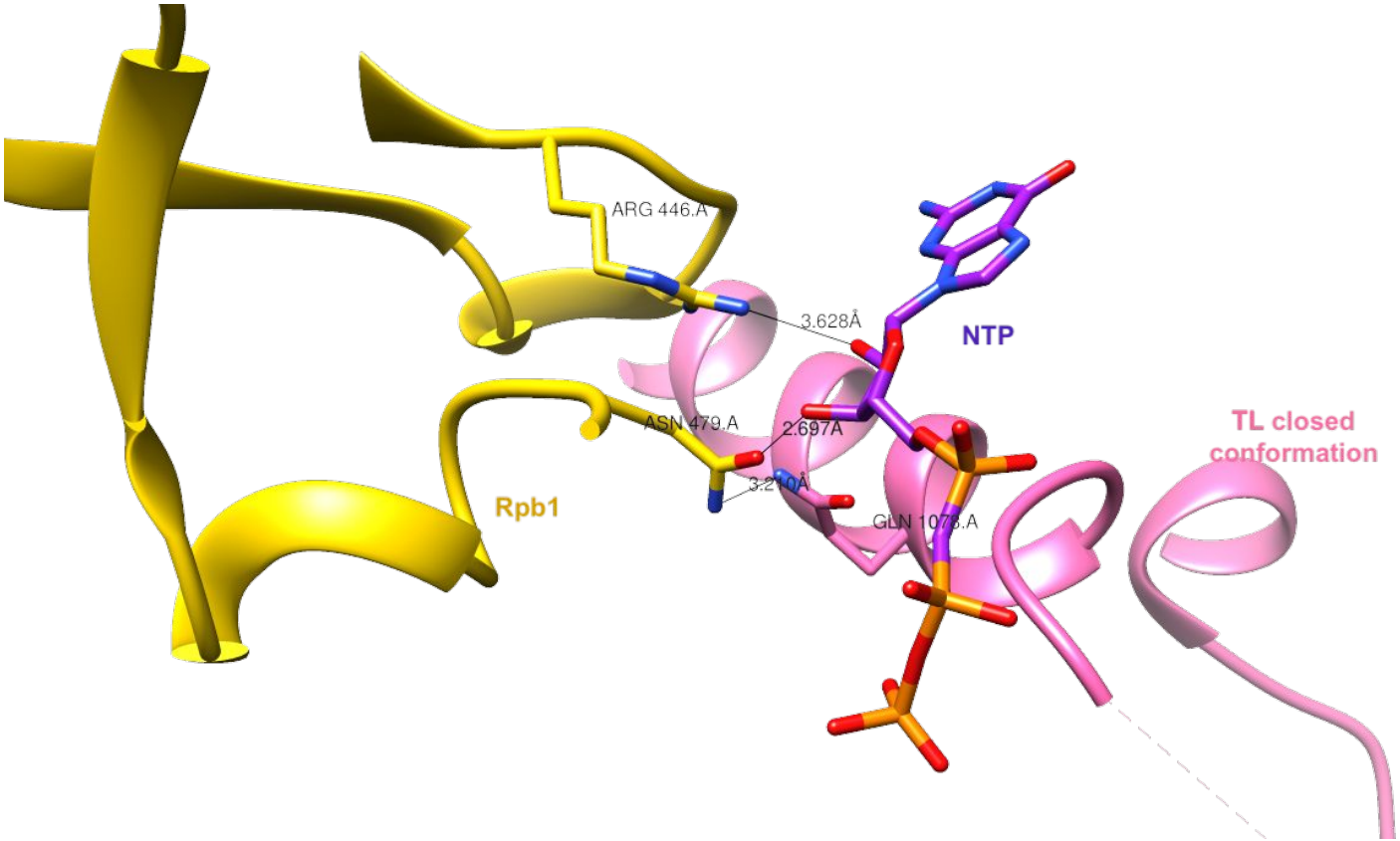
CATALYSIS: NTP in the A site



CATALYSIS: NTP in the A site



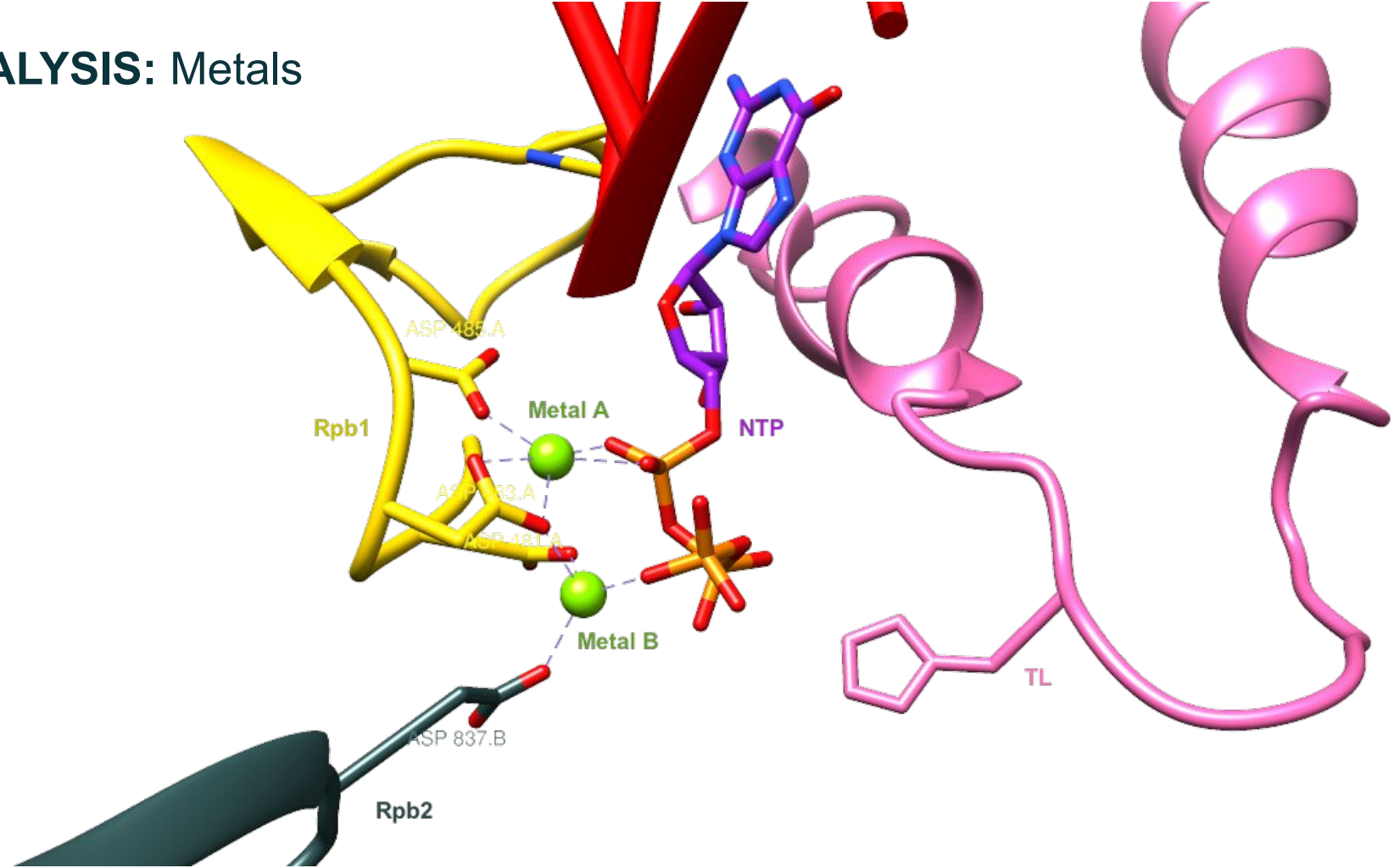
CATALYSIS: NTP in the A site



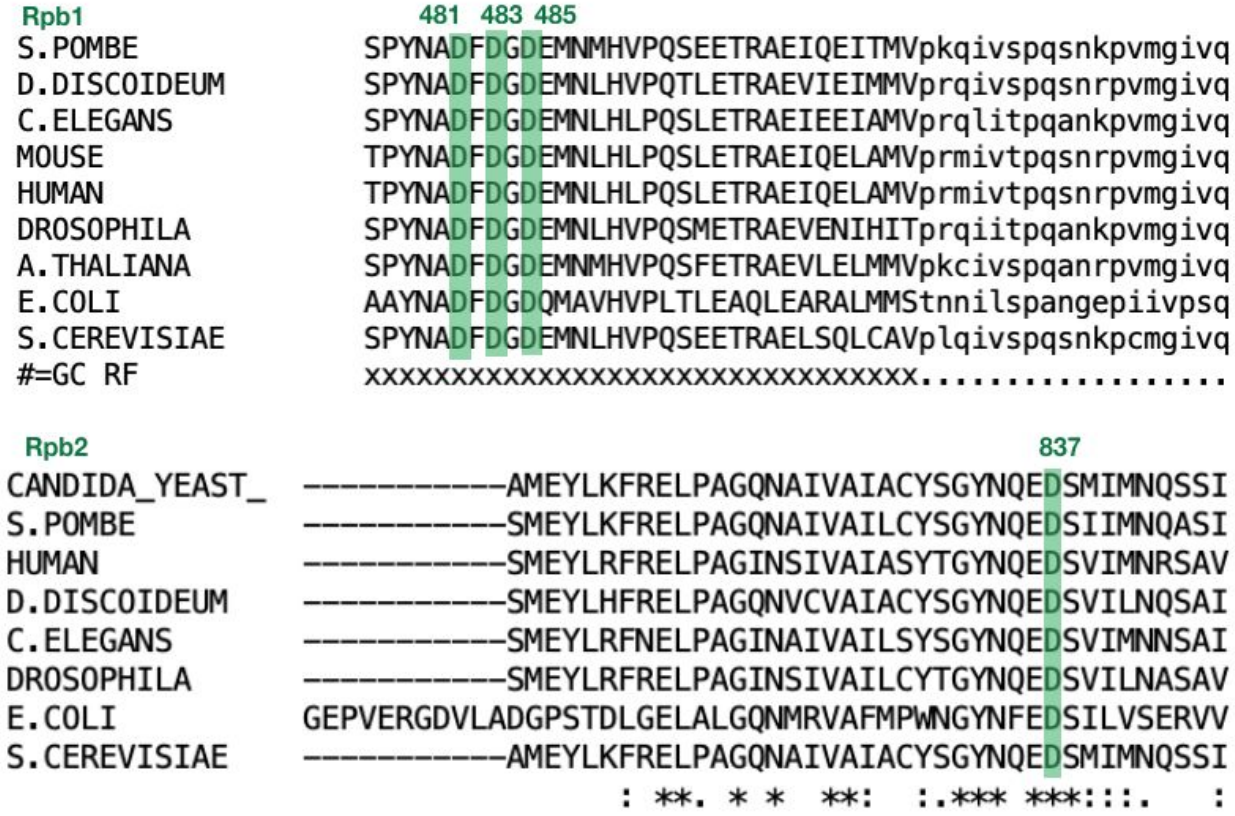
CATALYSIS: Residues that contact the NTP are conserved

CATALYSIS: Residues that contact the NTP are conserved

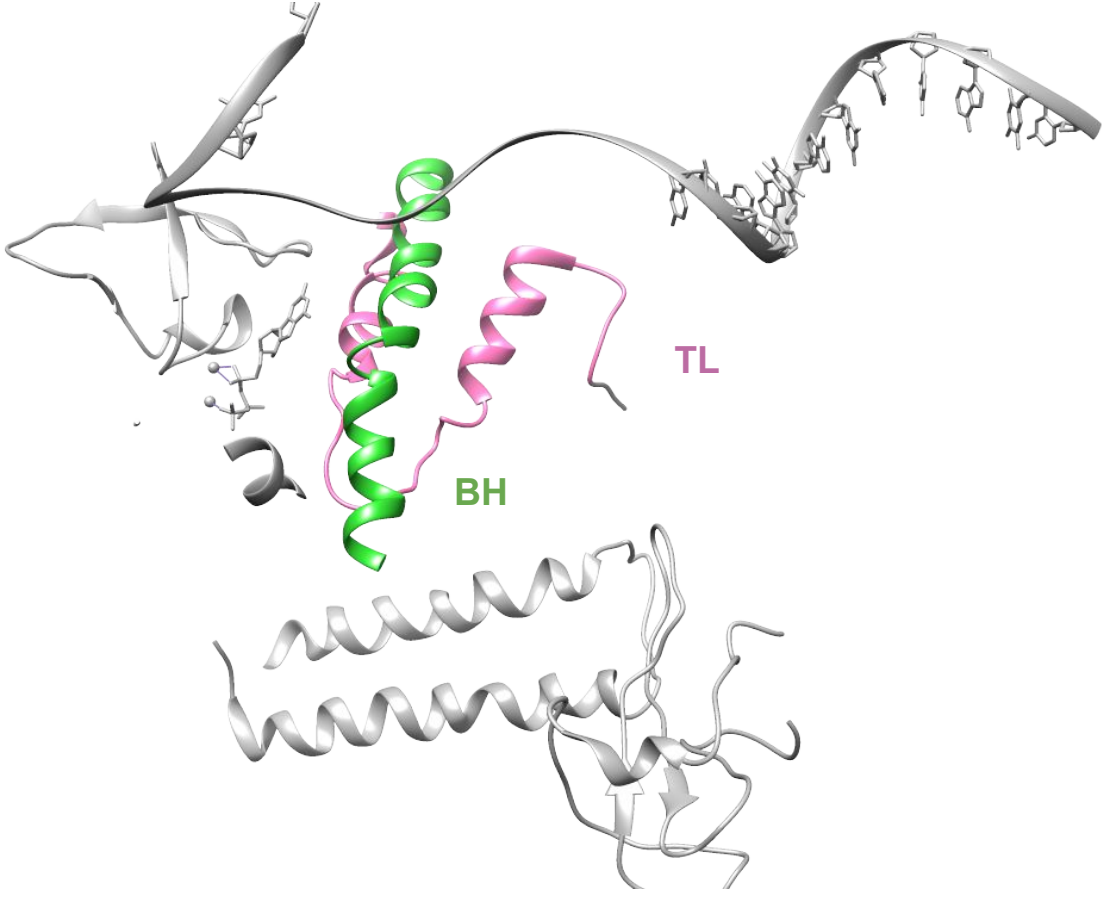
CATALYSIS: Metals



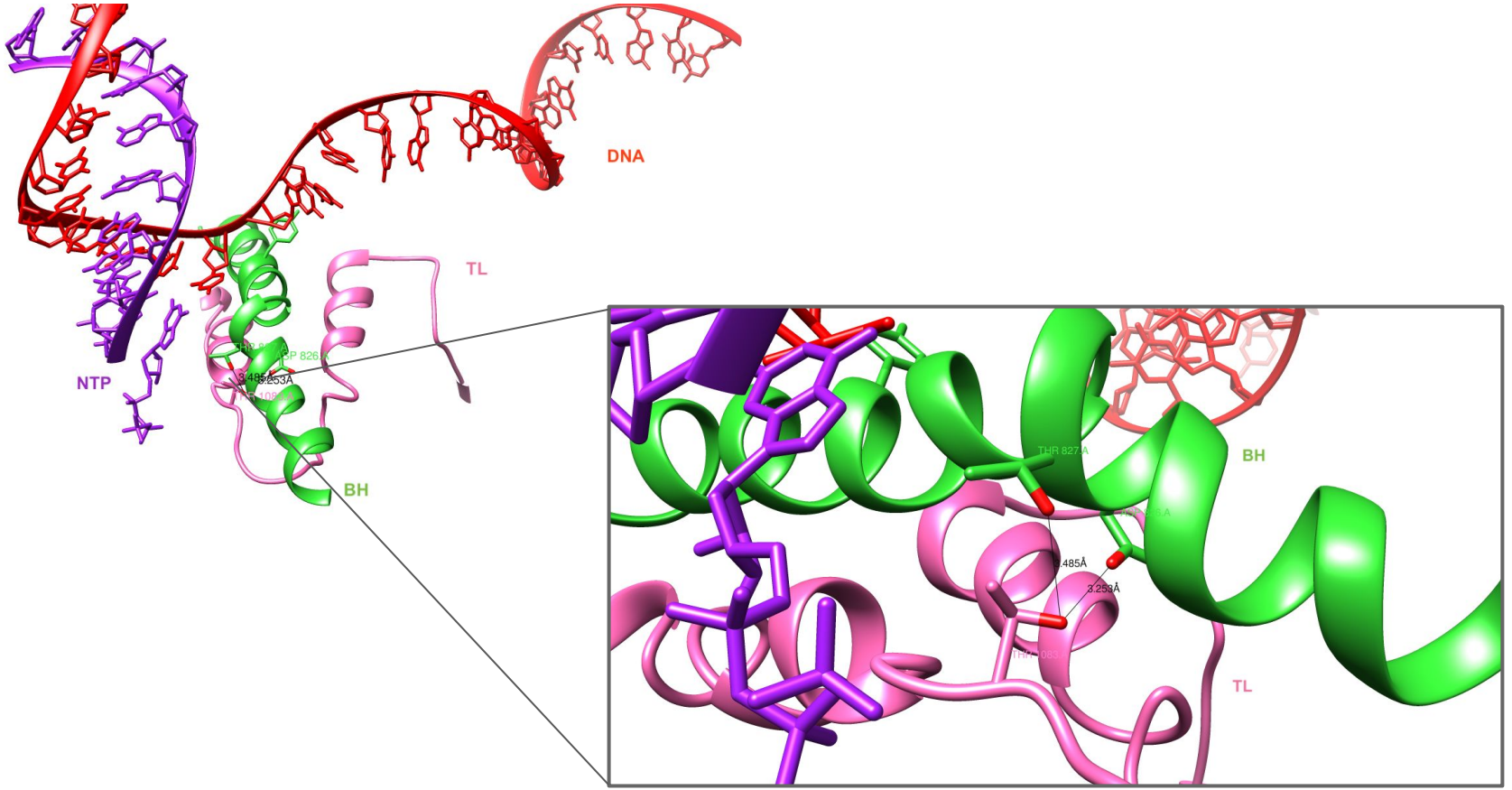
CATALYSIS: Residues that contact Mg^{2+} are conserved



TRANSLOCATION: key role of bridge helix (BH)

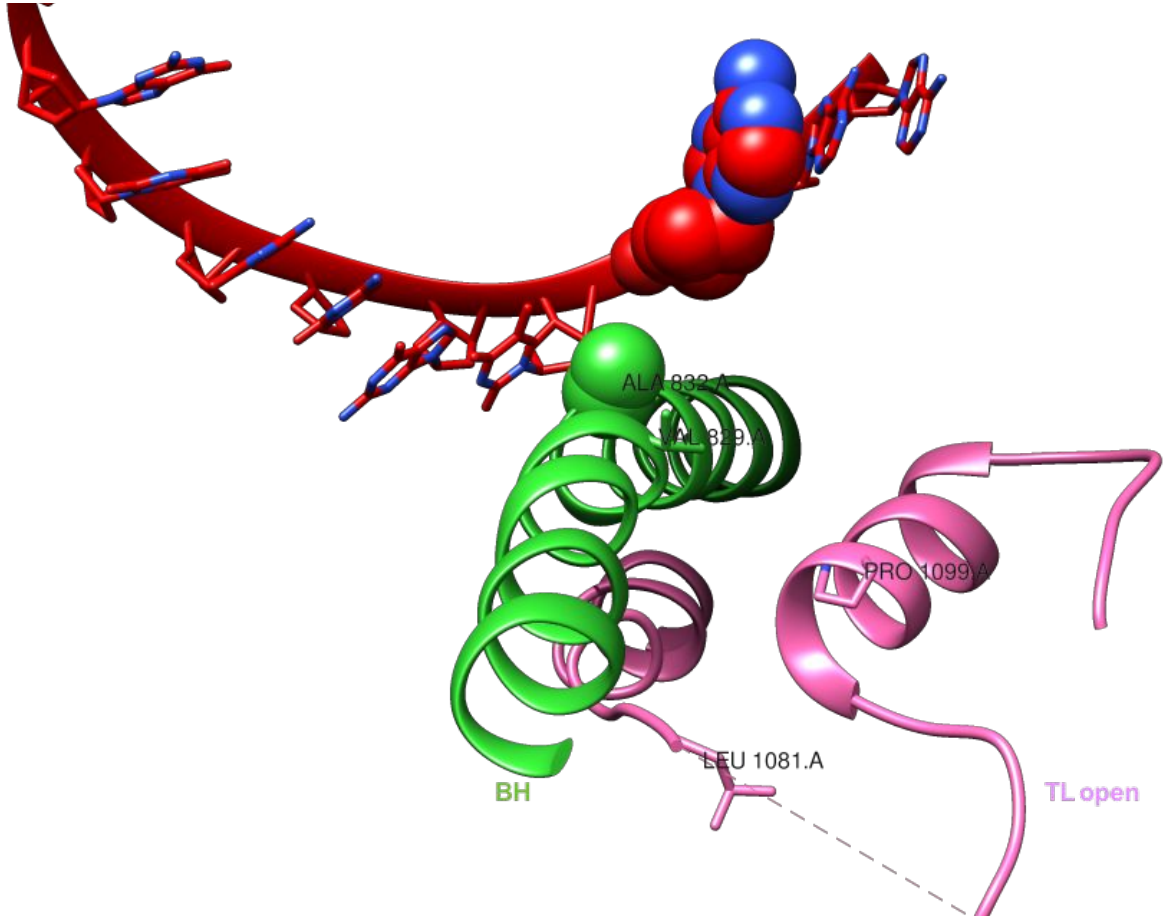


TRANSLOCATION: BH and TL interaction

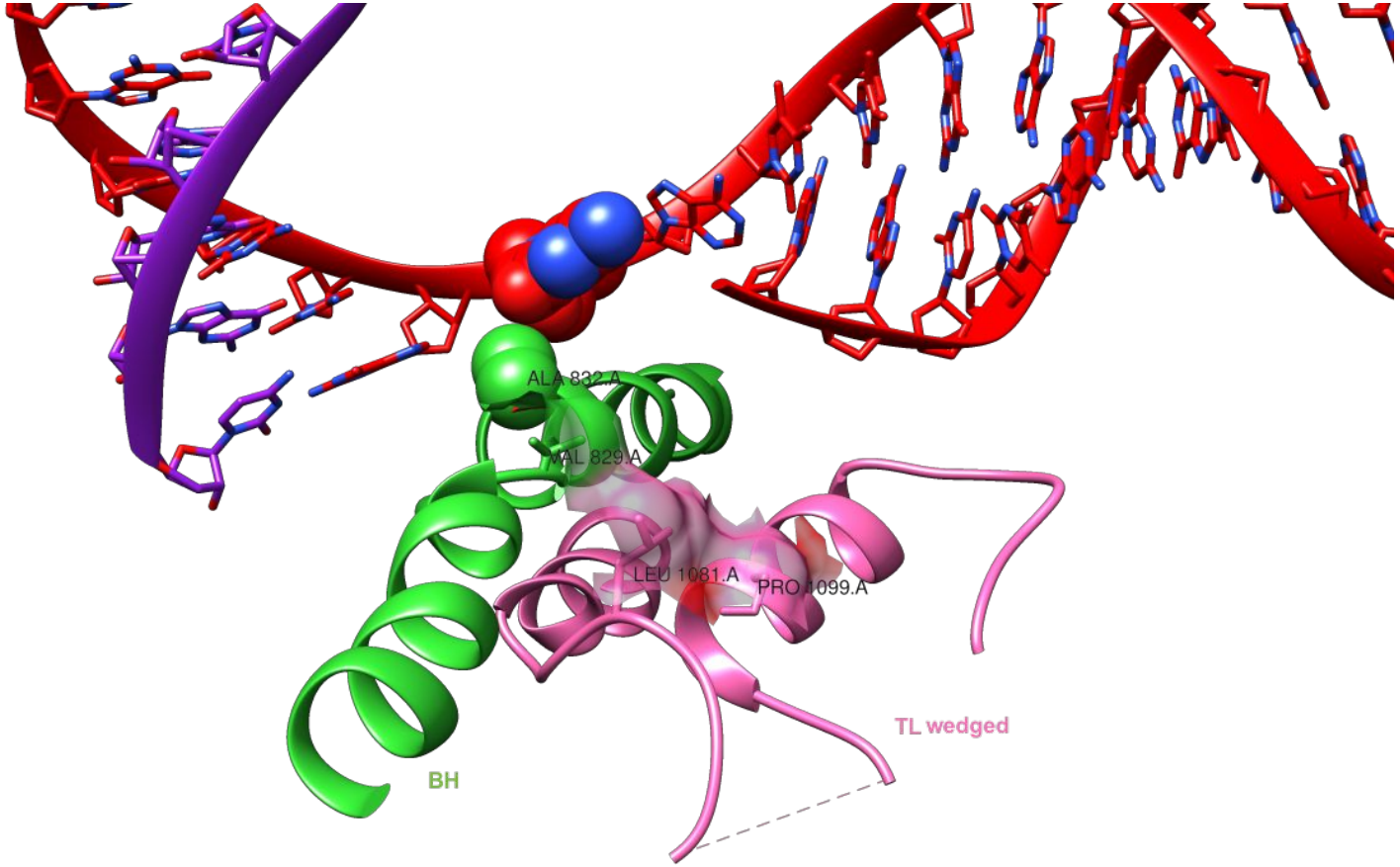


TRANSLOCATION: conservation of residues involved in BH and TL interaction

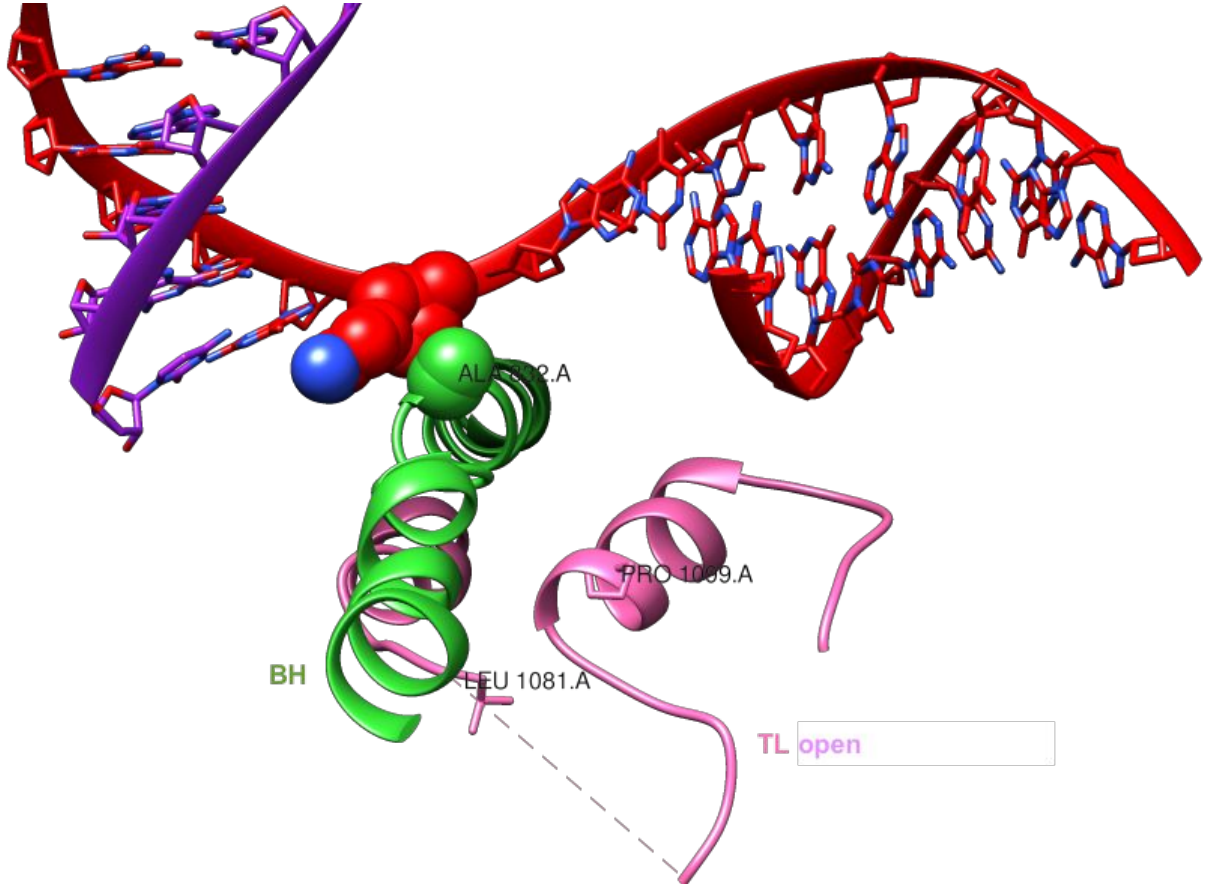
TRANSLOCATION: Pre-translocation



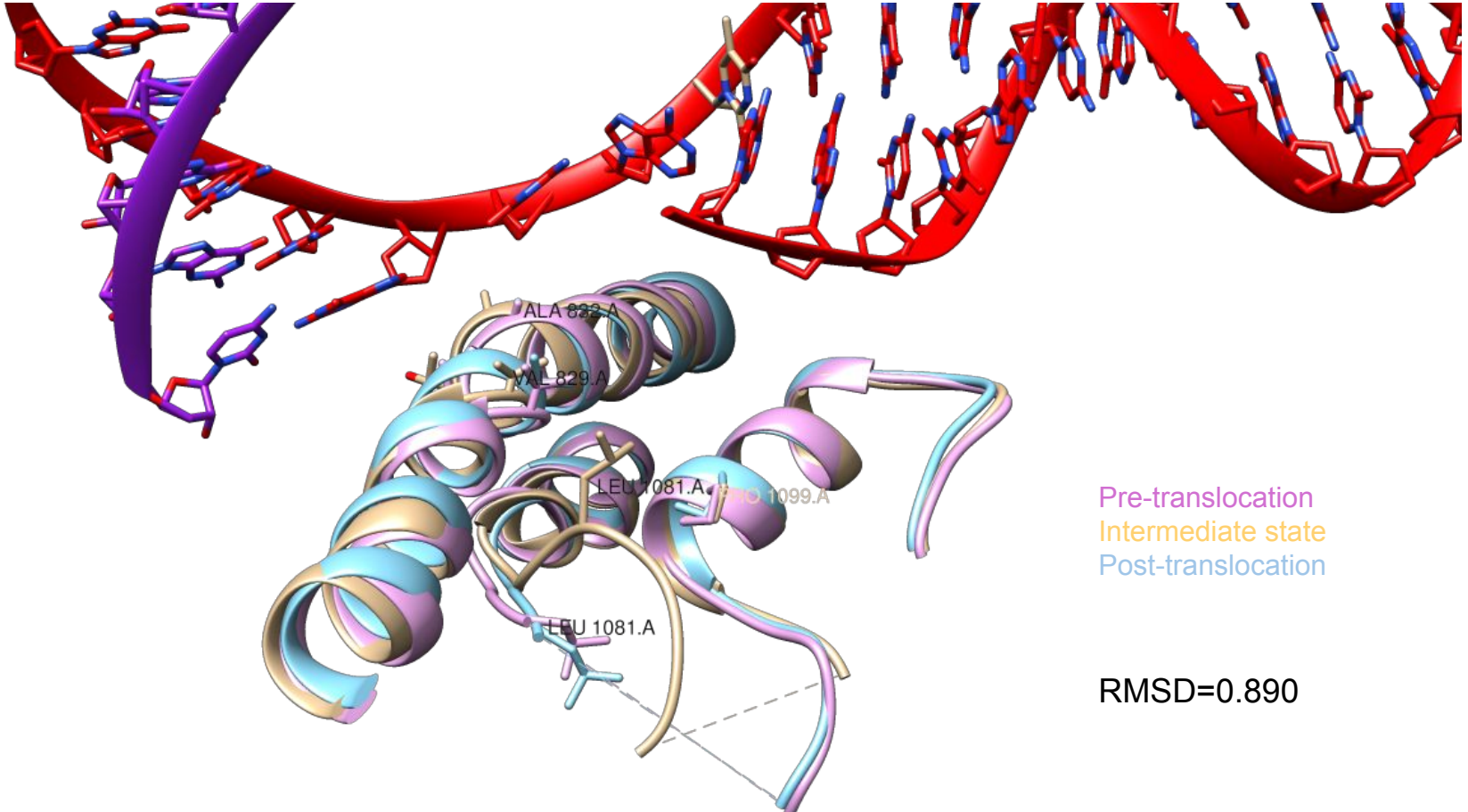
TRANSLOCATION: Intermediate state



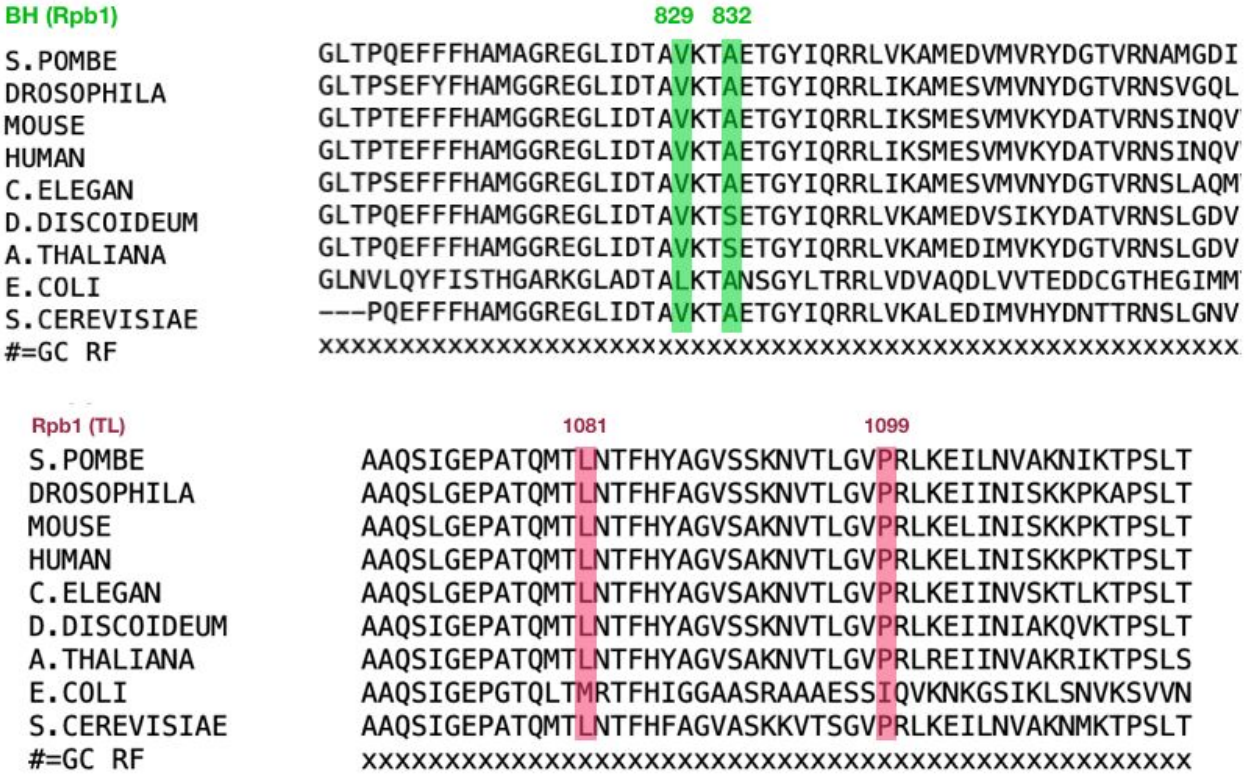
TRANSLOCATION: Post-translocation



TRANSLOCATION: Superimposition of the three states

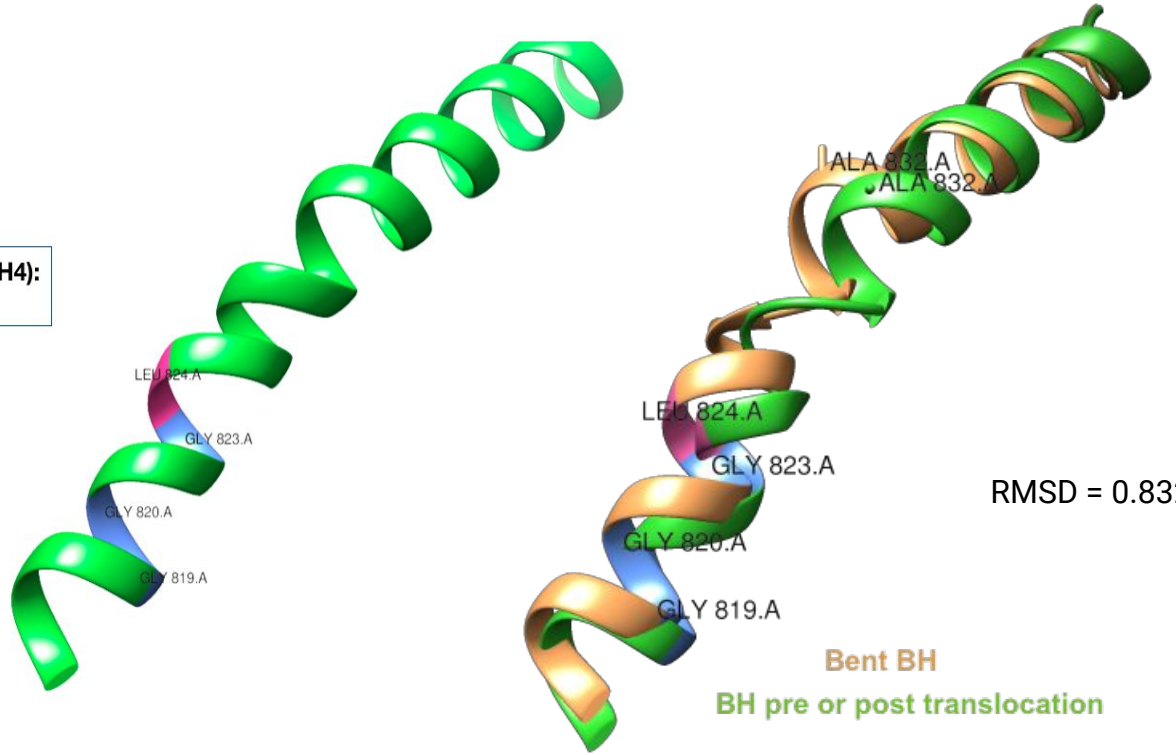


TRANSLOCATION: conservation of key residues



TRANSLOCATION: Hinge regions of BH

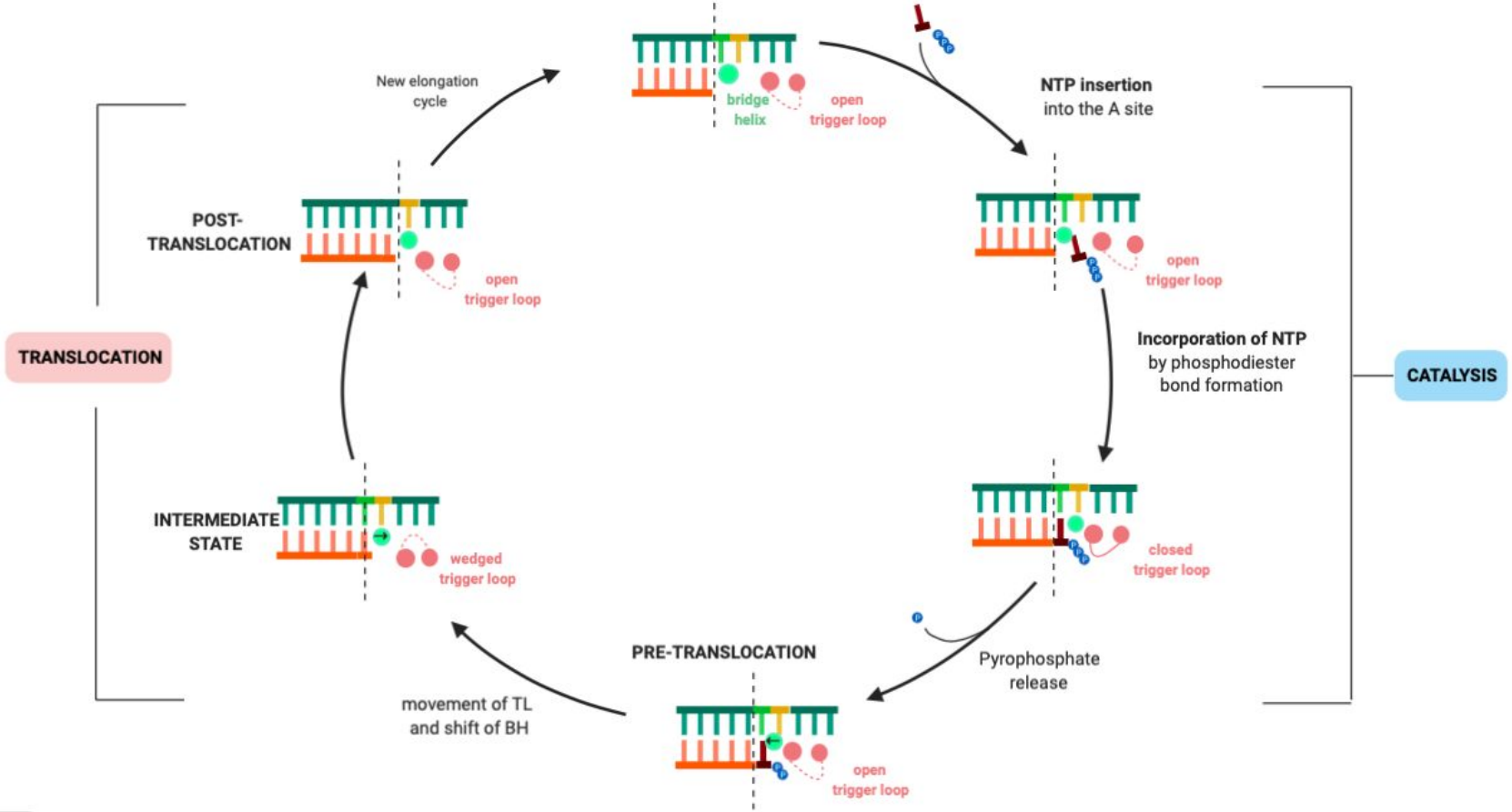
Hinge region motif (H3/H4):
GGXGL

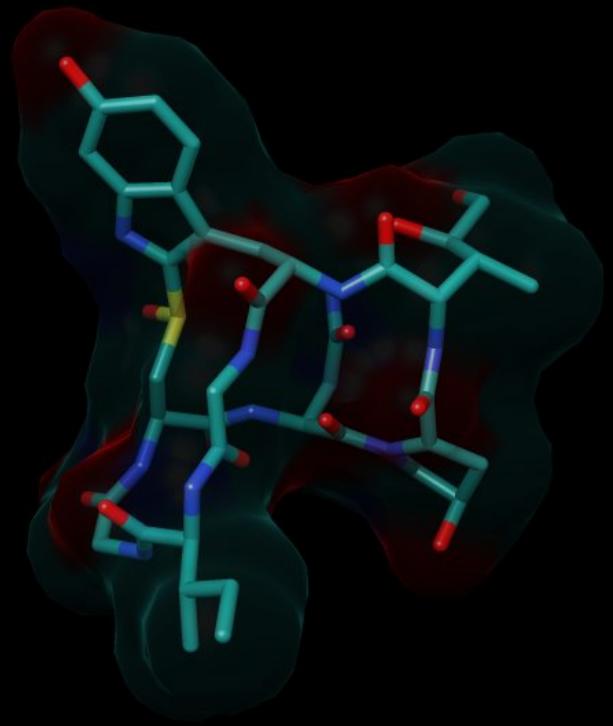


RMSD = 0.832

TRANSLOCATION: Hinge regions of BH

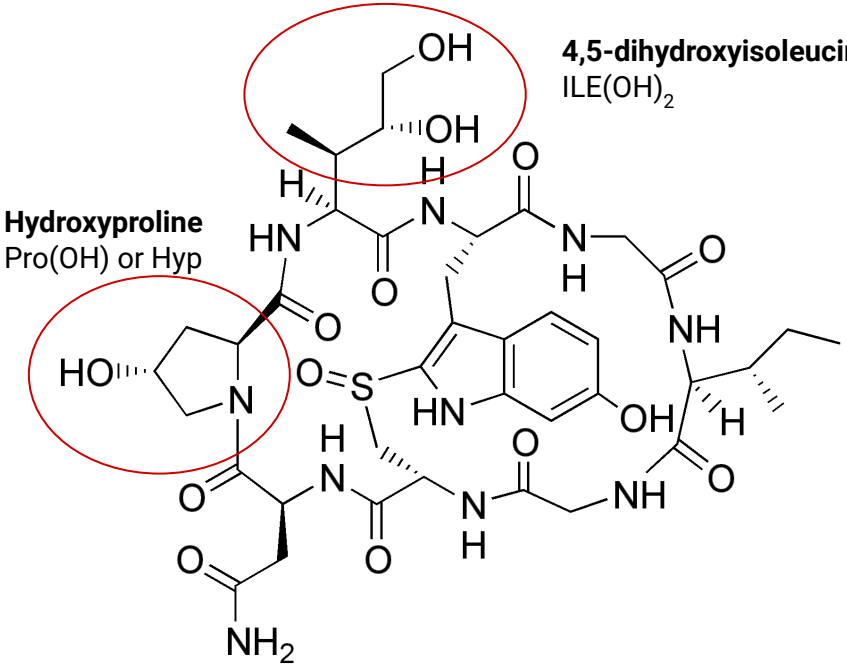
ELONGATION CYCLE: summary

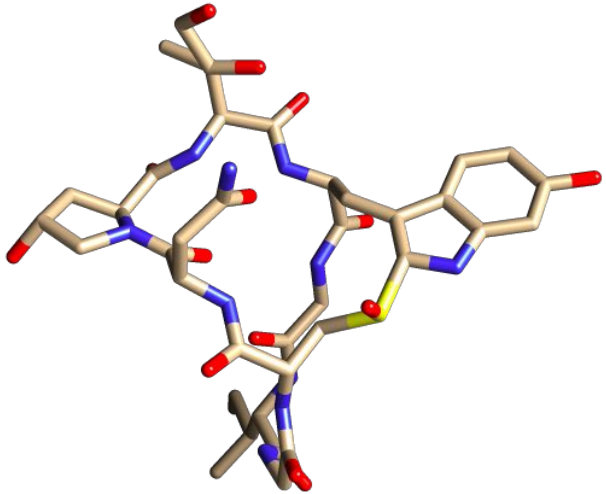




INHIBITOR: α-AMANITIN

INHIBITOR: α-amanitin

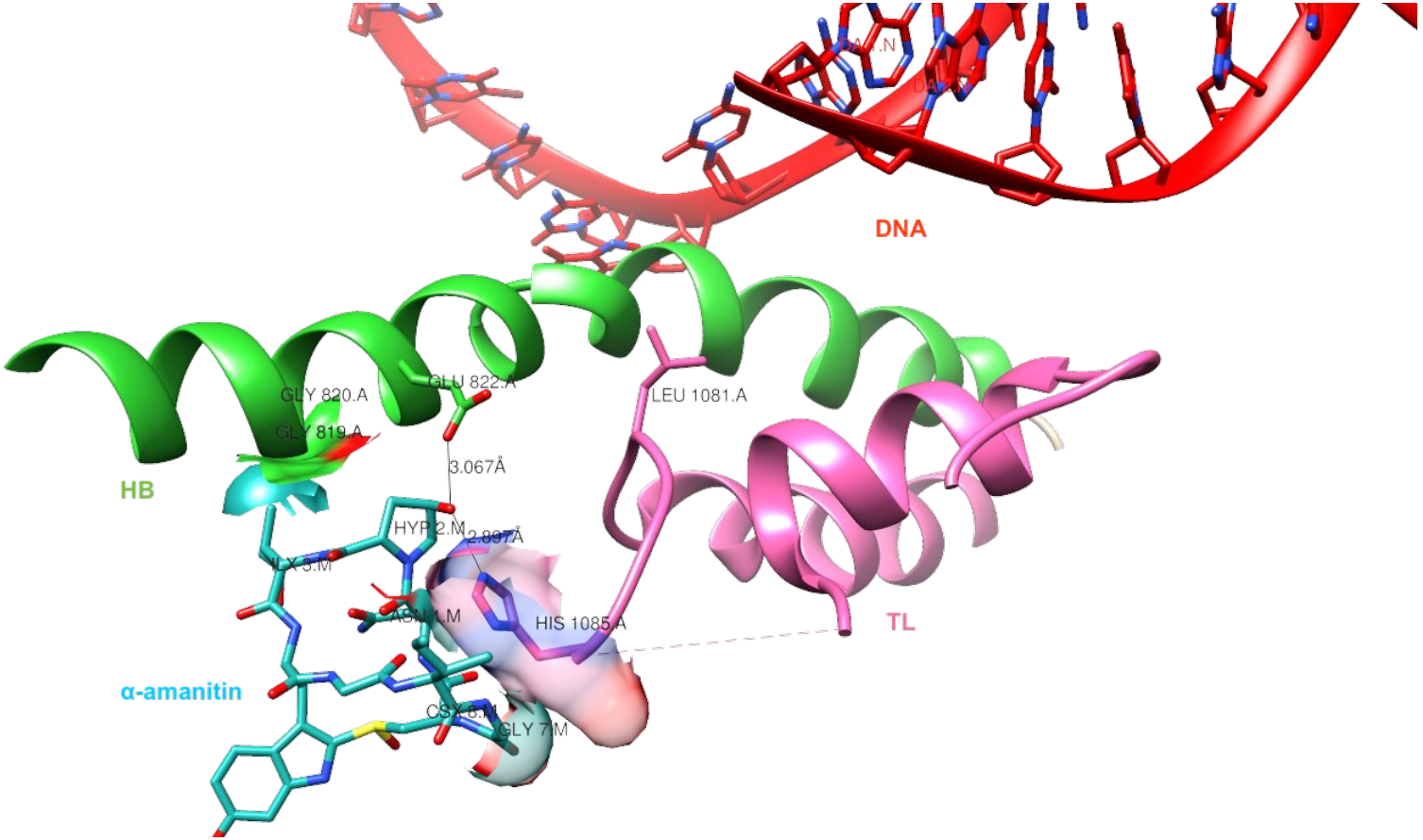


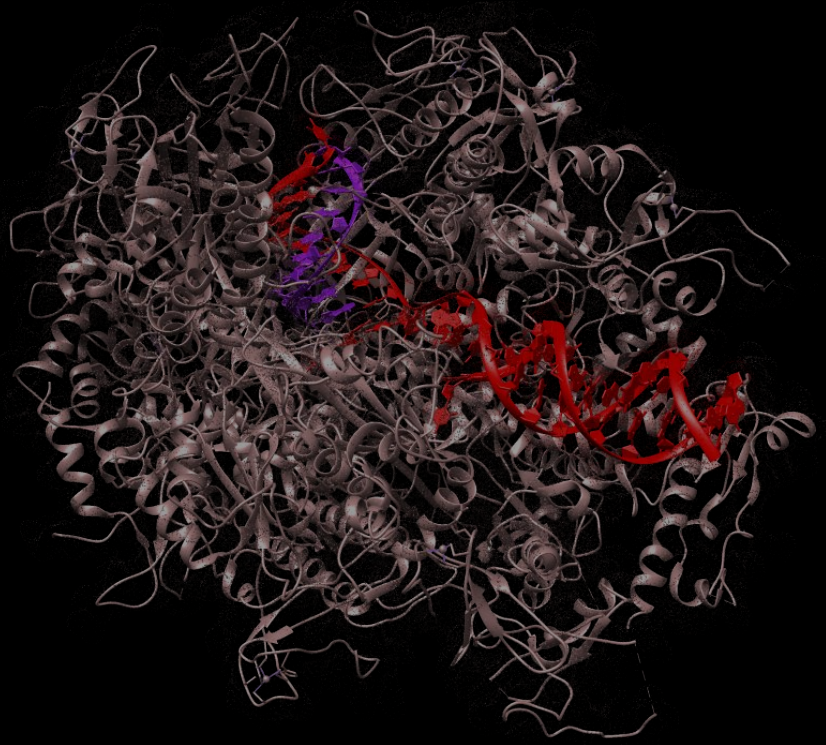


Death cap
(*A. phalloides*)

Destroying angel
(*A. bisporigera*)

INHIBITOR: α-amanitin binds to the active site of RNA pol II

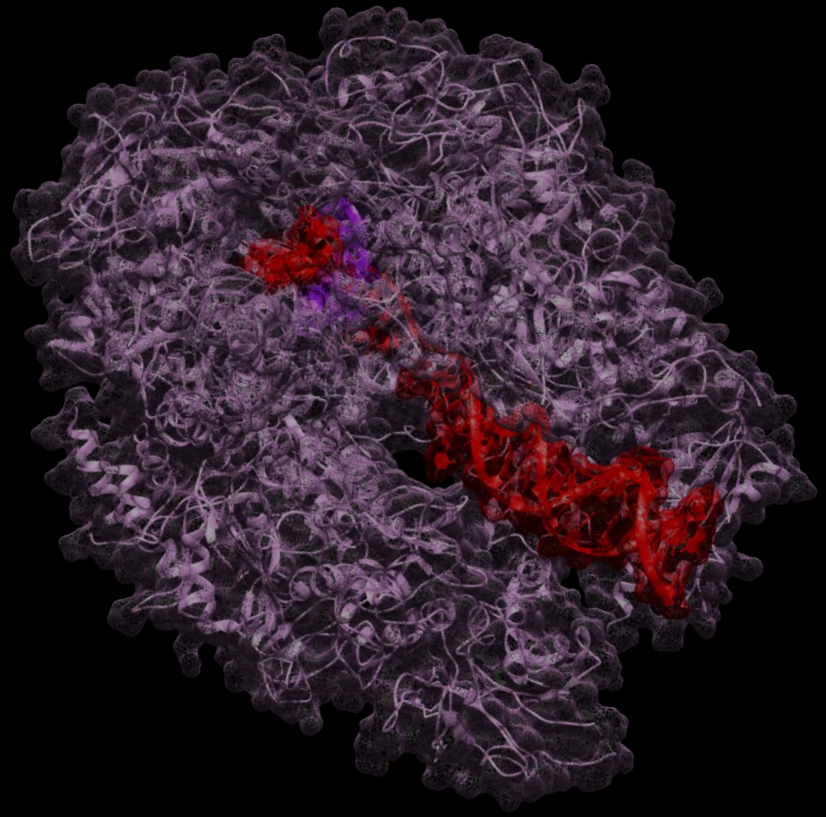




CONCLUSIONS

CONCLUSIONS

1. Transcription is an event that is **conserved** among all species and cell types.
2. There are different RNA polymerases but in eukaryotes, only **RNA polymerase II** transcribes protein coding genes.
3. RNA polymerase II is a multisubunit complex: subunits **Rpb1**, **Rpb2** and **Rpb9** form and/or stabilize the active site of the enzyme.
4. The **elongation cycle** is the process in which NTPs are added to the new RNA strand. It is divided in two phases: **catalysis** and **translocation**.
5. In **catalysis**, the **TL** has to **move** from open conformation to close conformation. In the active site, the **NTP** establishes interactions with the TL, other residues from Rpb1 and Rpb2.
6. In **translocation**, the TL moves from closed to open conformation and adopts an intermediate **wedged state**. The **BH bends** to move the next DNA nucleotide towards the active site.
7. **Amanitin** inhibits transcription by **blocking the TL** in the **wedged state** and not allowing translocation.
8. **Sequence alignments** show high conservation in key residues involved in transcription.



THANK YOU

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MULTIPLE CHOICE QUESTIONS

1. **Which of the following structures is formed when the trigger loop (TL) and the bridge helix (BH) are together?**
   a. TIM barrel
   b. Rossman folding
   c. Polymerase like folding
   d. Three helix bundle
   e. Greek key

2. **Select all the correct statements:**
   1. Rpb2 is the largest subunit of RNA polymerase II
   2. Rpb1 and Rpb2 share similar SCOP information
   3. Bridge helix and trigger loop can be found in subunit Rpb9
   4. RNA polymerase II is composed by 12 subunits

   a) 1, 2, 3
   b) 1, 3
   c) 2, 4
   d) 4
   e) 1, 2, 3, 4

MULTIPLE CHOICE QUESTIONS

1. **In RNA polymerase II, catalysis and translocation:**
   a. **Require coordination of the main two structures involved in the processes (bridge helix and trigger loop).**
   b. They are independent processes so can happen simultaneously.
   c. In translocation, the NTP is incorporated to the RNA.
   d. Translocation is different from catalysis because it does not involve any structures from the active site.
   e. All of the above.

2. **Select the correct statements about the metals of active site**
   a. Both metals are always present in the active site
   b. Metal A is always present while metal B is present only when NTP is in the active site.
   c. These metals are both zinc ions.
   d. More polymerases have 2 metals in the active site
      a) 1,2,3
      b) 1,3
      c) 2,4
      d) 4
      e) 1,2,3,4

MULTIPLE CHOICE QUESTIONS

1. **When the NTP is incorporated into the forming RNA strand:**
   a. Trigger loop is in its open conformation
   b. Trigger loop is in its closed conformation
   c. Trigger loop is in its wedged conformation
   d. TL can have all the different conformations
   e. None of the statements above are correct

2. **Select the false statement:**
   a. Rpb9 subunit contacts directly with TL
   b. Rpb9 subunit modulates the TL movement
   c. alfa20-alfa21 from Rpb1 are important for the mobility of the TL
   d. The TL mobility is allowed by the hinge regions which provide flexibility to the structure
   e. alfa20-alfa21 interact with Rpb9 by VDW and hydrogen bond interactions.

MULTIPLE CHOICE QUESTIONS

1. **In the wedged conformation of the trigger loop (TL), translocation:**
   a. It's over.
   b. It's being carried out.
   c. It has not started yet.
   d. All of the above.
   e. None is correct.

2. **In translocation of DNA to begin another elongation cycle:**
   a. The nucleotide is pulled to the active site by a hydrogen bond.
   b. The DNA moves independently to arrive to the active site.
   c. The DNA is translocated by a transcription factor.
   d. When the bridge helix bends, it forms multiple hydrogen bonds with the following nucleotides.
   e. When the bridge helix bends, an alanine can contact the dNTP through VDW interactions and can push it to the active site.

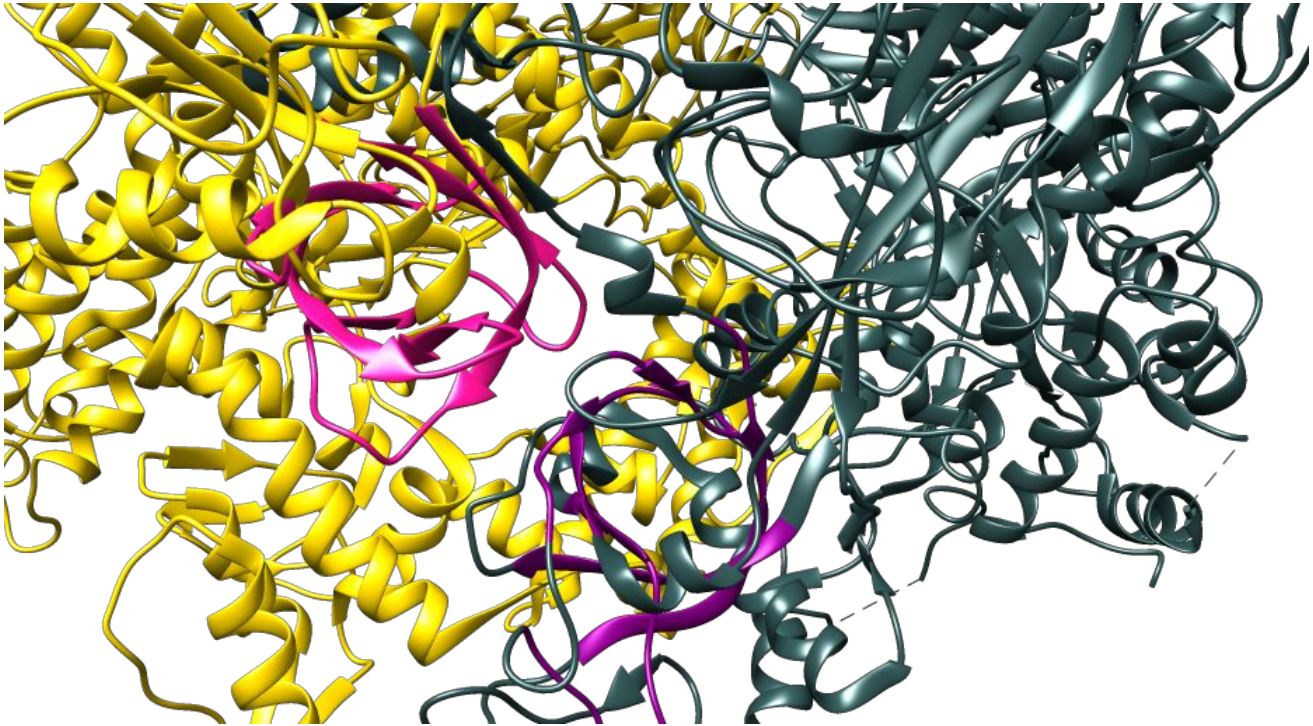
MULTIPLE CHOICE QUESTIONS

1. **About the hinge regions of the bridge helix:**
   a. They are regions that provide flexibility to the structure.
   b. Their sequence is highly conserved among species.
   c. Their residues cannot make hydrogen bonds.
   d. They allow the bridge helix bending.
   e. All of the above.

2. **About the inhibitor alpha-amanitin:**
   a. Alpha-amanitin binds to the enzyme through the modified amino acids hydroxyproline and dihydroxyisoleucine.
   b. Trigger loop movement is blocked when alpha-amanitin interacts with the enzyme
   c. The two above are correct
   d. Alpha-amanitin binds to the surface of the enzyme and causes a conformational change that blocks transcription
   e. All the above are correct

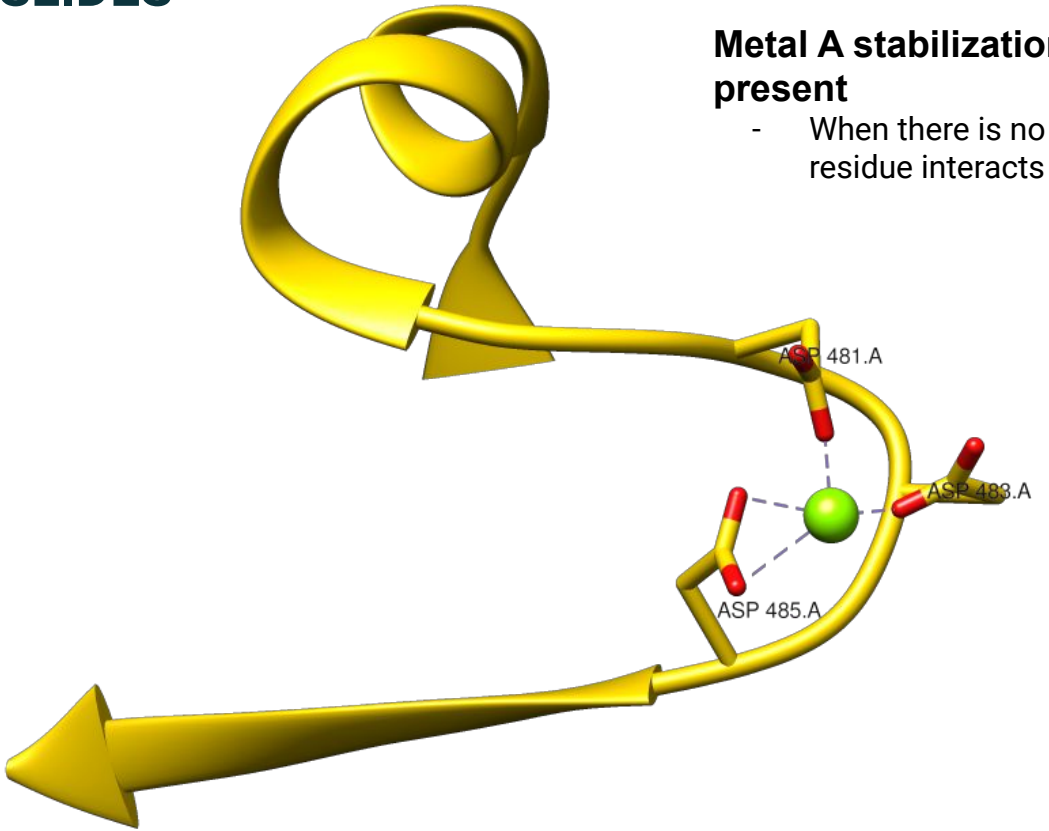
EXTRA SLIDES

EXTRA SLIDES



Double psi barrel
A different view.

EXTRA SLIDES

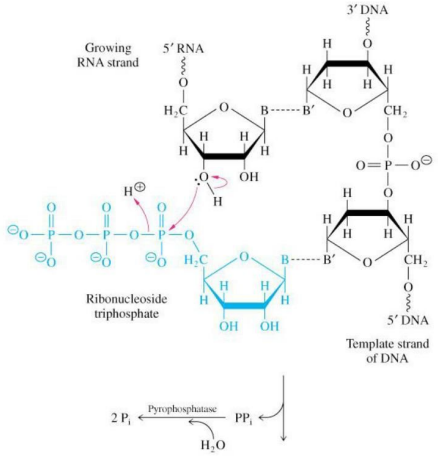


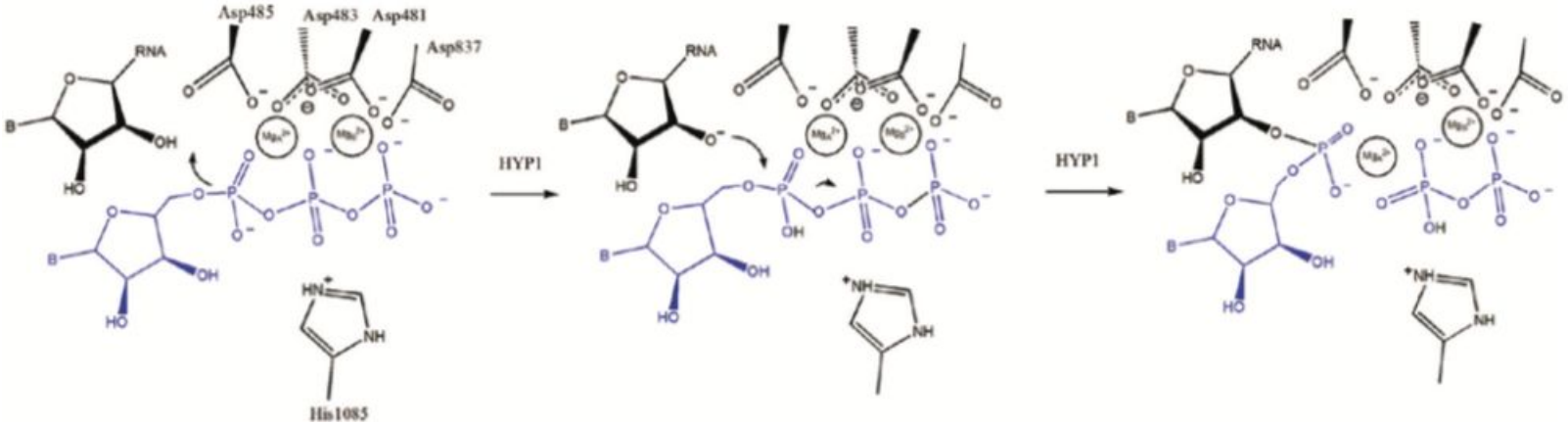
Metal A stabilization when NTP is not present

- When there is no NTP, a new aspartic acid residue interacts with the magnesium atom.

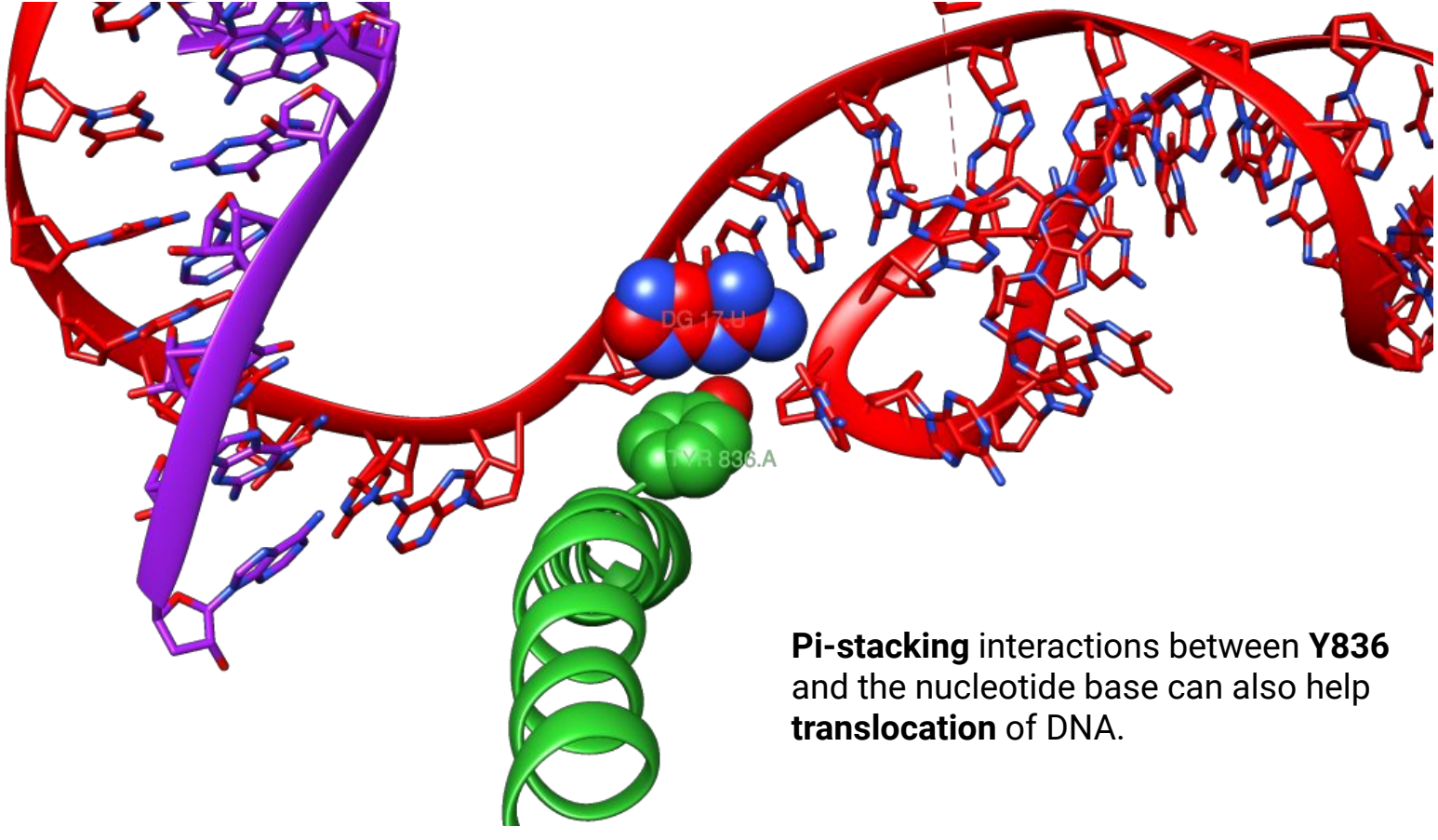
EXTRA SLIDES

- RNA polymerase reaction





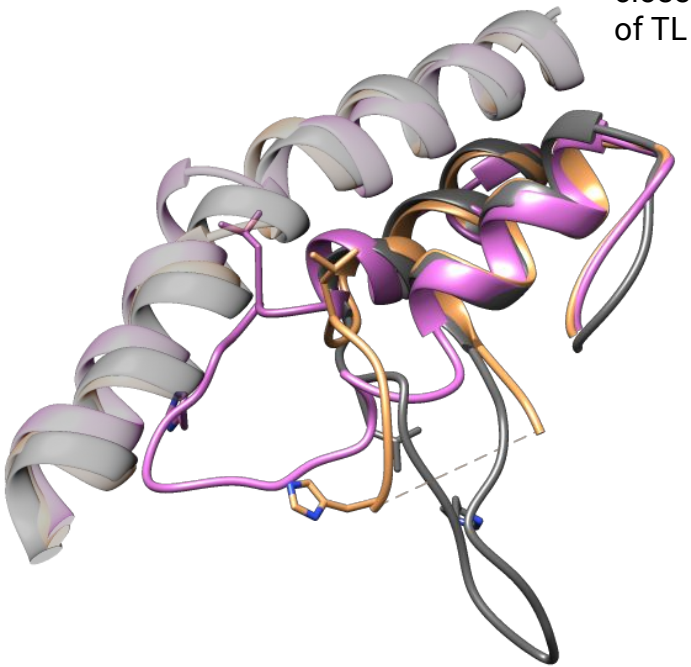
EXTRA SLIDES



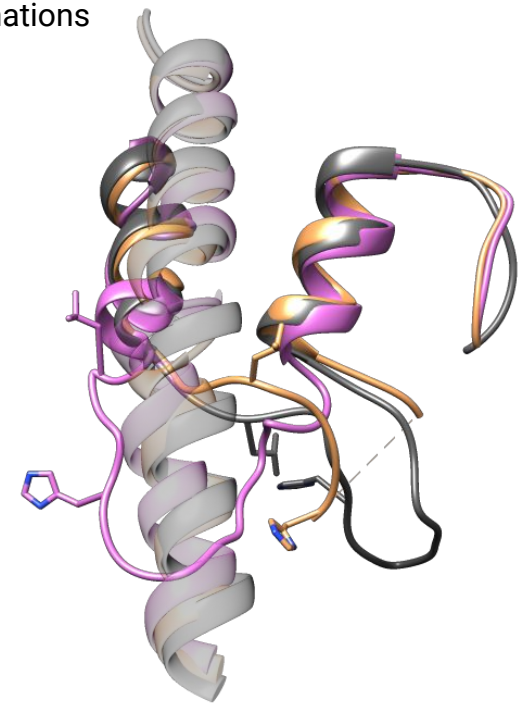
Pi-stacking interactions between **Y836** and the nucleotide base can also help **translocation** of DNA.

EXTRA SLIDES

TL movement during translocation. A closer look to the different conformations of TL in translocation.

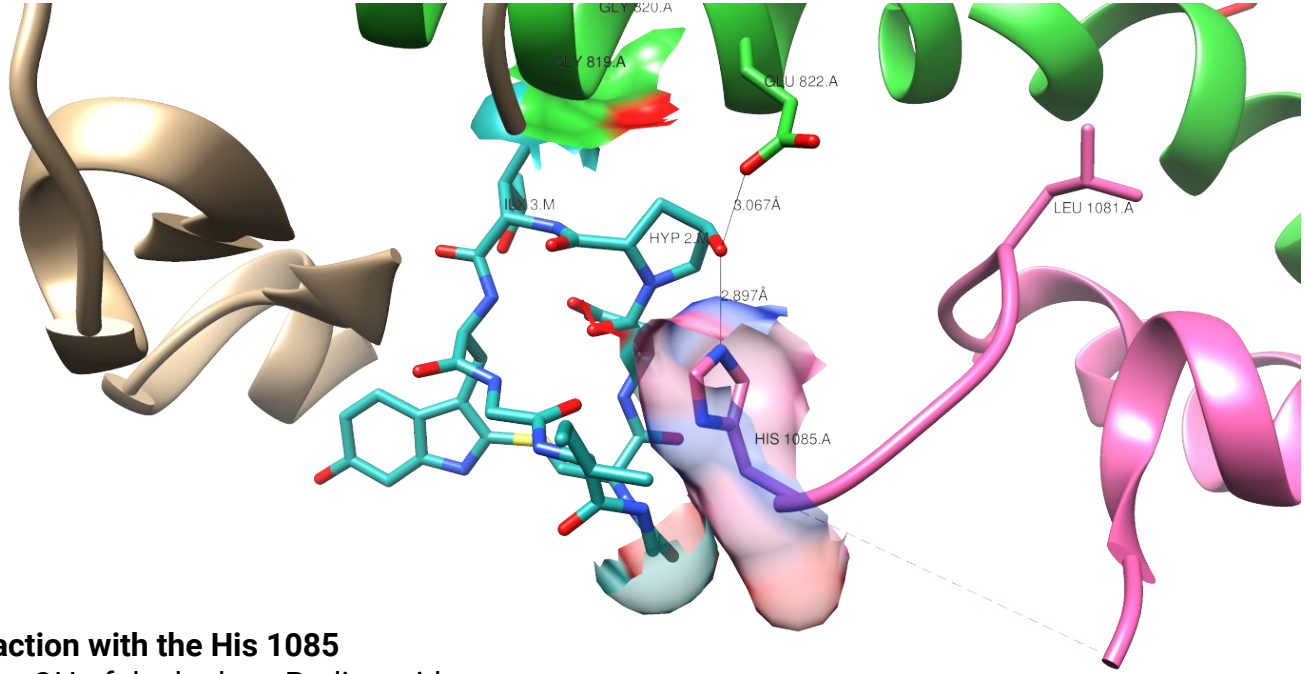


TL closed
TL wedged
TL open



RMSD= 0.826 A

EXTRA SLIDES

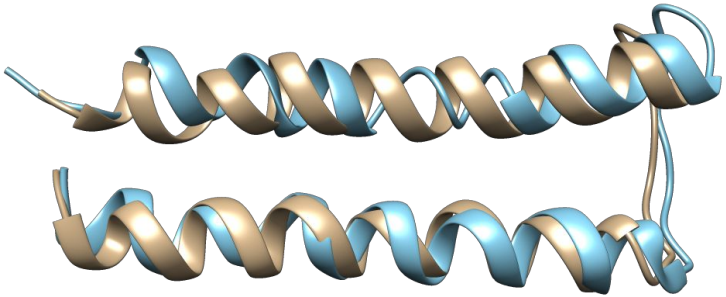


Amanitin interaction with the His 1085

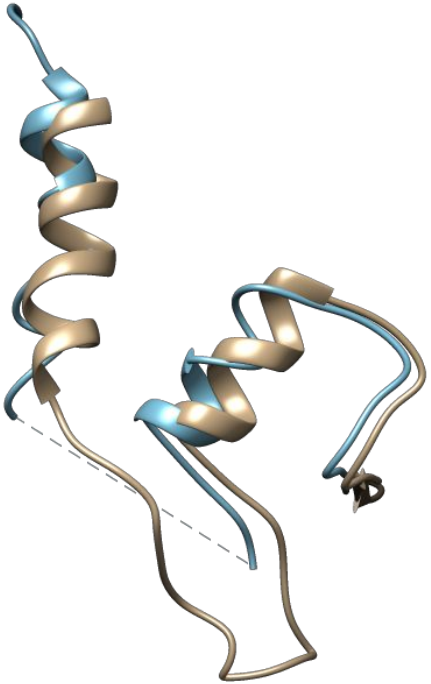
Interaction of the OH of the hydroxyProline with:

- the NH of the His
- the NH2 of the Asp

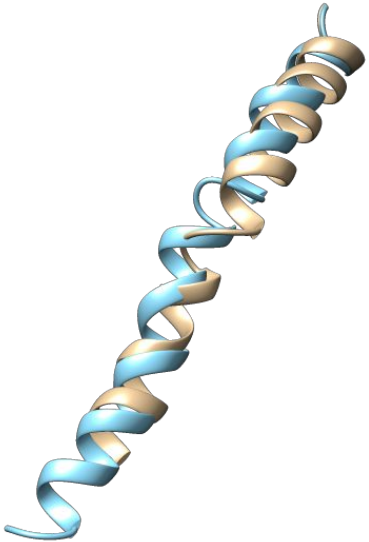
EXTRA SLIDES: superimposition human/S.cerevisiae



alfa20-21



Trigger Loop

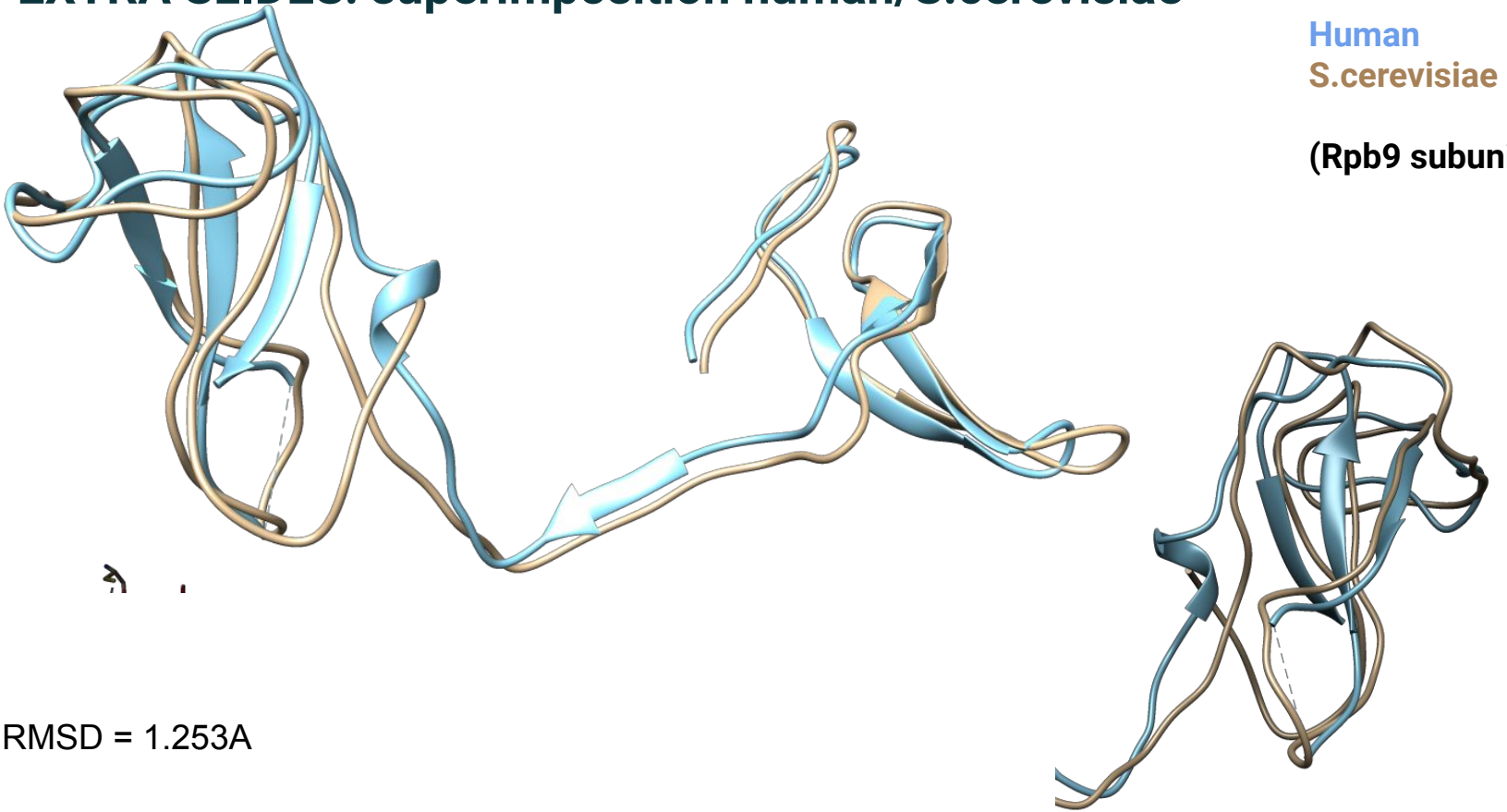


Bridge Helix

RMSD=1.288 A

Human
S.cerevisiae

EXTRA SLIDES: superimposition human/S.cerevisiae



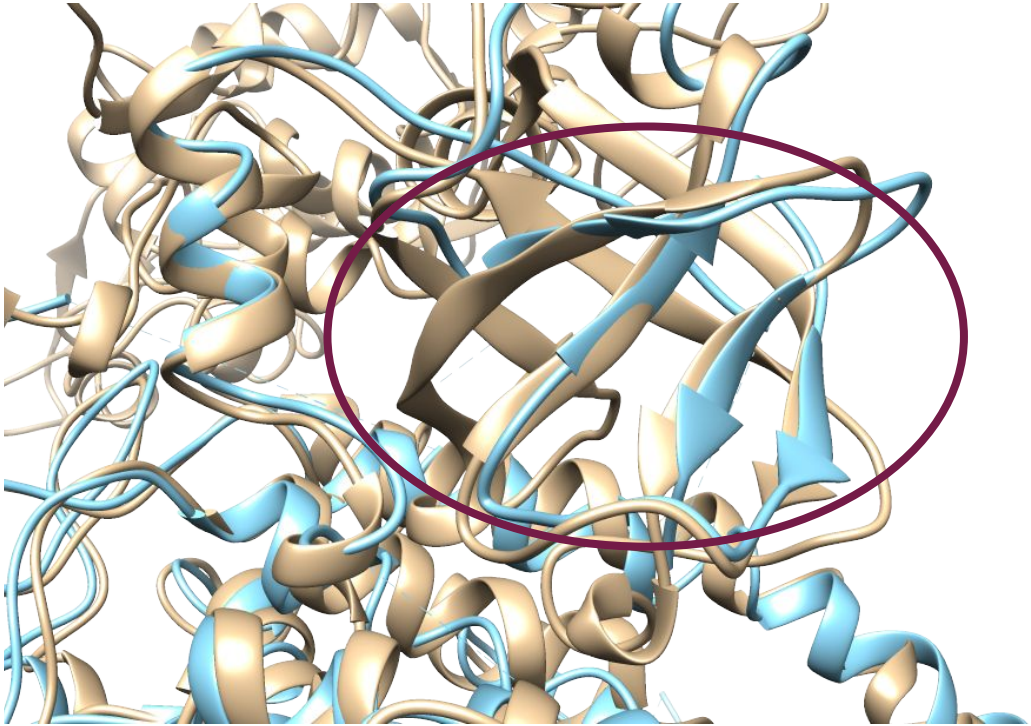
Human
S.cerevisiae

(Rpb9 subunit)

RMSD = 1.253A

EXTRA SLIDES: superimposition human/S.cerevisiae

Human
S.cerevisiae



RMSD = 1.288A
Human pdb is cut, some beta sheet are not present, it may be due to the crystallization process