Universitat Pompeu Fabra Barcelona

HUMAN MITOCHONDRIAL RNA POLYMERASE

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STRUCTURAL BIOLOGY

27/02/26



Image from Chimera of PDB code 3SPA

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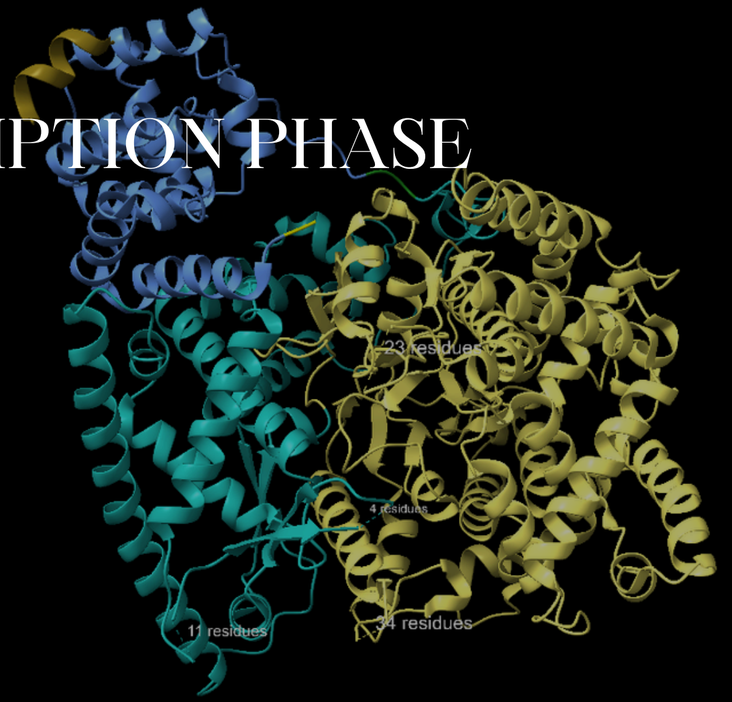


Image from Chimera of PDB code 3SPA

01 – INTRODUCTION

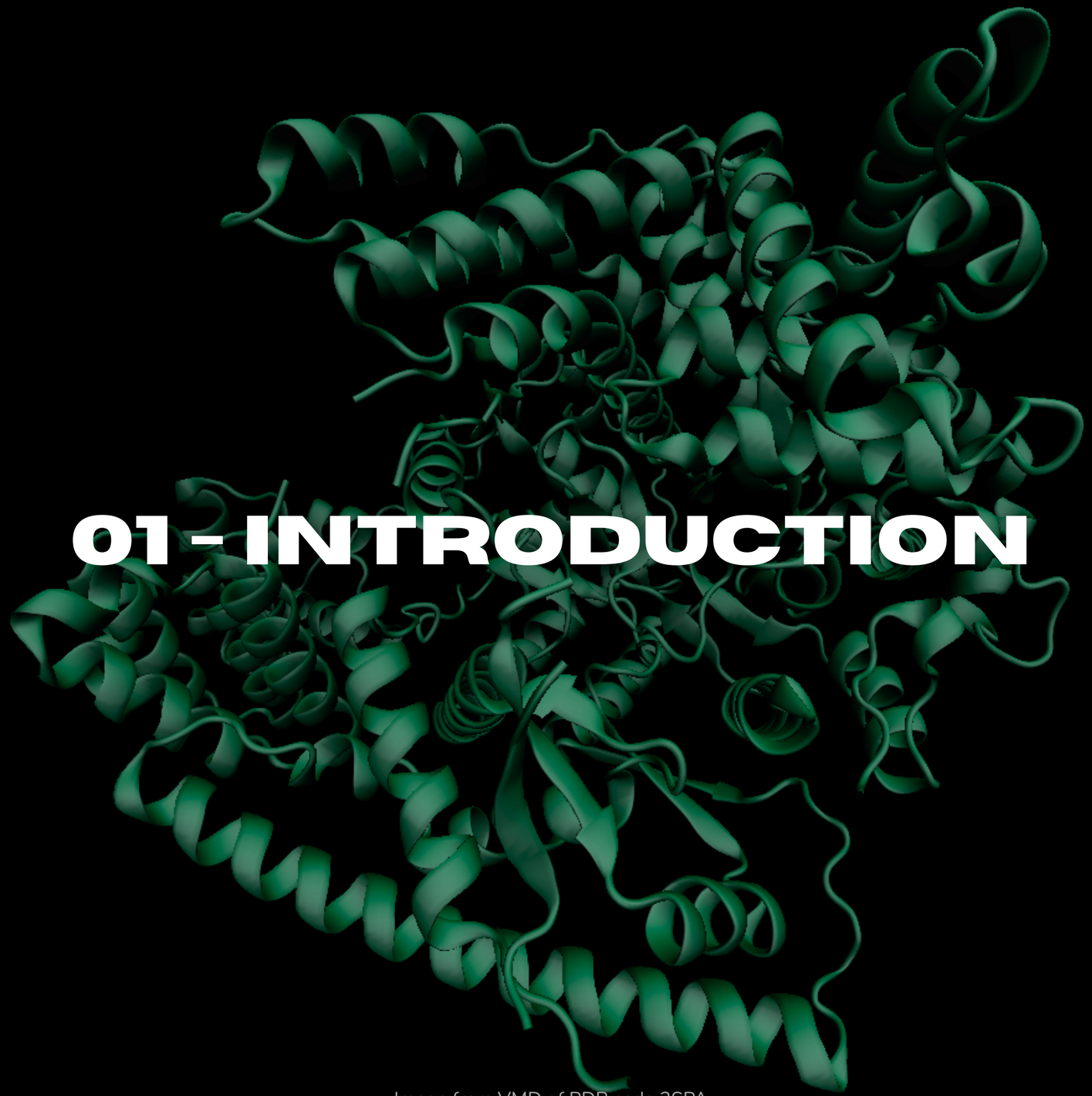


Image from VMD of PDB code 3SPA

RNA POLYMERASE IN EUKARYOTES

- RNA pol I ⟶ rRNA
- RNA pol II ⟶ mRNA and regulatory RNAs
- RNA pol III ⟶ tRNA and small RNAs
- POLRMT ⟶ mtDNA



Image from Canva

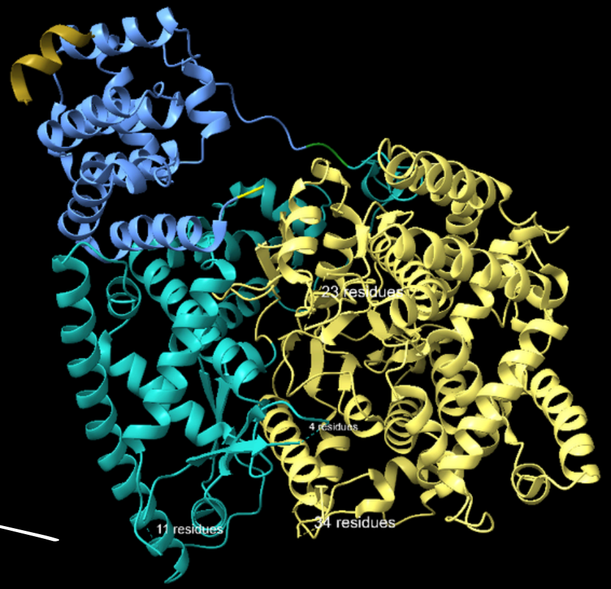


Image from Chimera of PDB code 3SPA

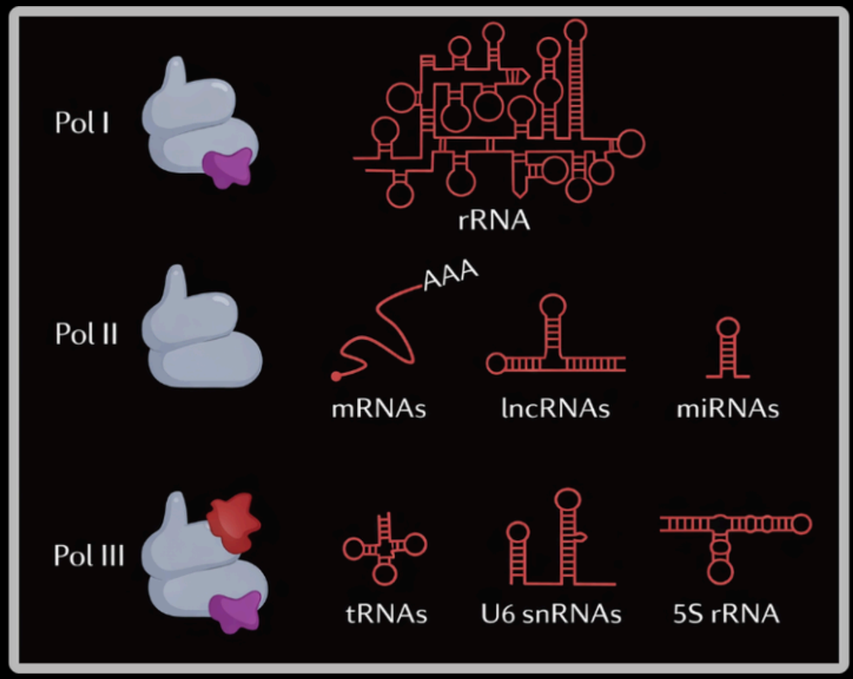
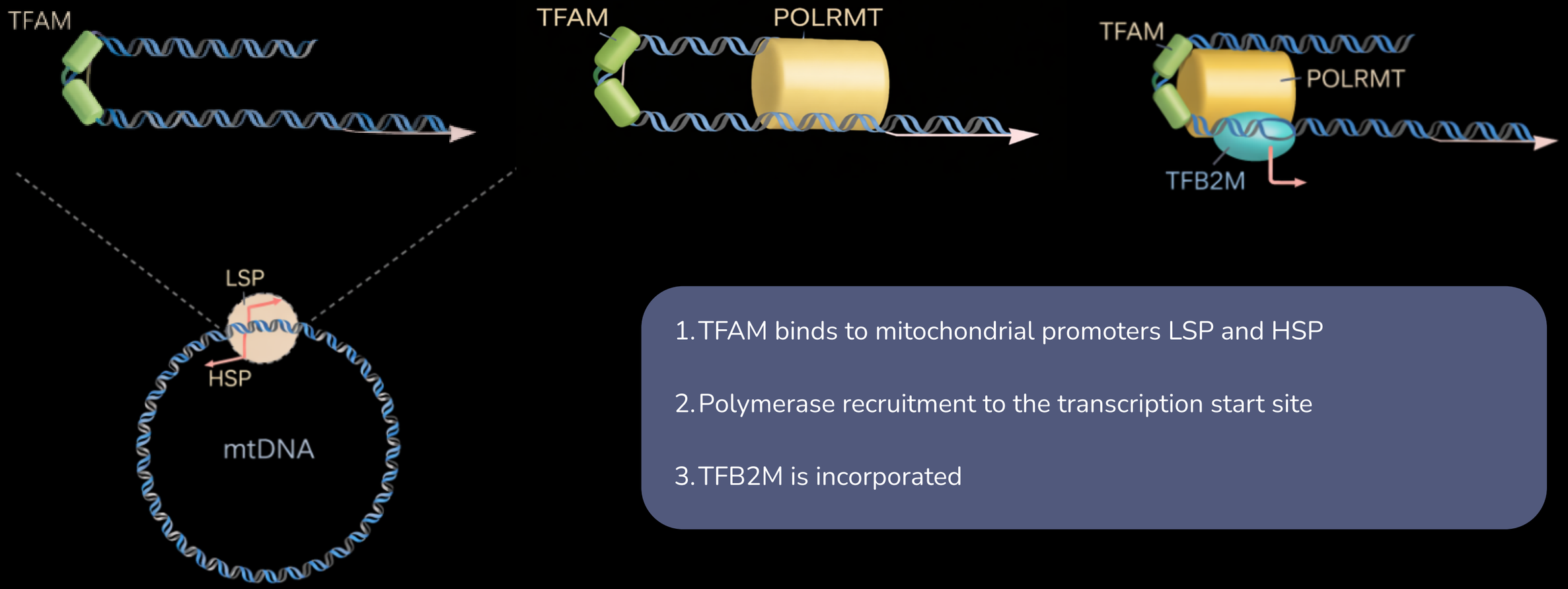


Figure adapted from: Jacobs RQ & Schneider DA, J. Biol. Chem (2024)

TRANSCRIPTIONAL CYCLE

INITIATION



1. TFAM binds to mitochondrial promoters LSP and HSP
2. Polymerase recruitment to the transcription start site
3. TFB2M is incorporated

Figure from: Quintero et al., Nat. Rev. Mol. Cell Biol. (2023)

TRANSCRIPTIONAL CYCLE

ELONGATION

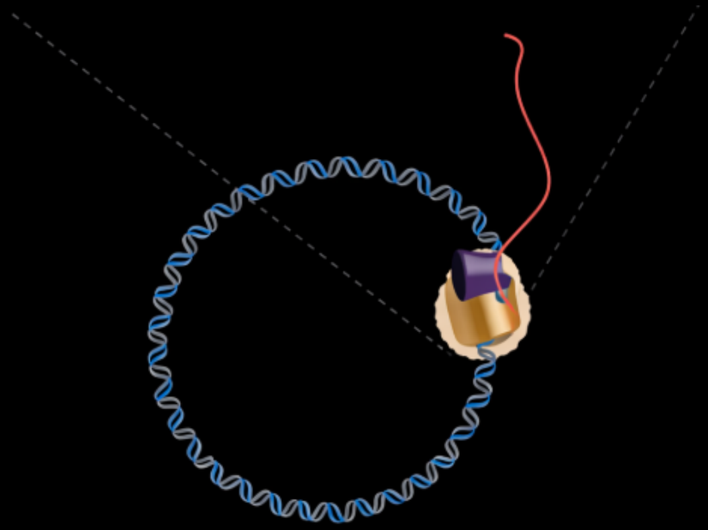


Figure from: Quintero et al., Nat. Rev. Mol. Cell Biol. (2023)

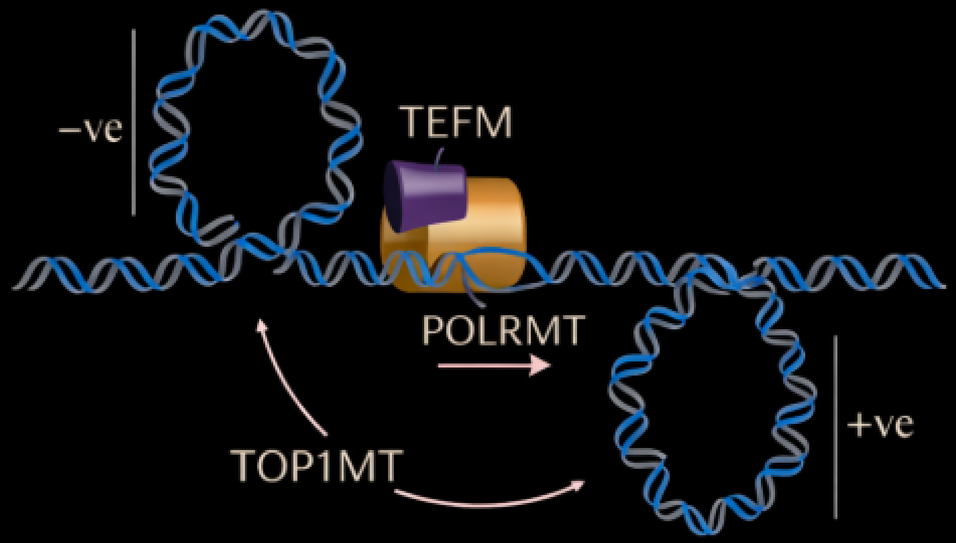


Figure from: Quintero et al., Nat. Rev. Mol. Cell Biol. (2023)

Elongation

Incorporation of ribonucleotides complementary to the DNA template strand

- Processive RNA synthesis (5' → 3')
- TEFM stabilizes POLRMT:
  - Prevents premature termination
  - Enables long transcript synthesis

TRANSCRIPTIONAL CYCLE

TERMINATION

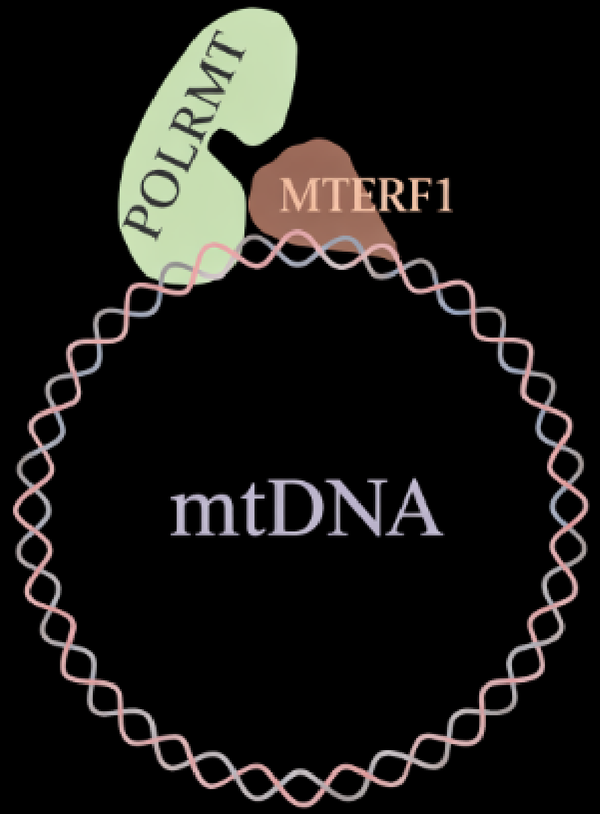


Figure from: Quintero et al., Nat. Rev. Mol. Cell Biol. (2023)

1. MTERF1 interacts with mitochondrial DNA and modulate the arrest of the transcription complex.
2. Synthesized RNA is released
3. Polymerase dissociates from the DNA

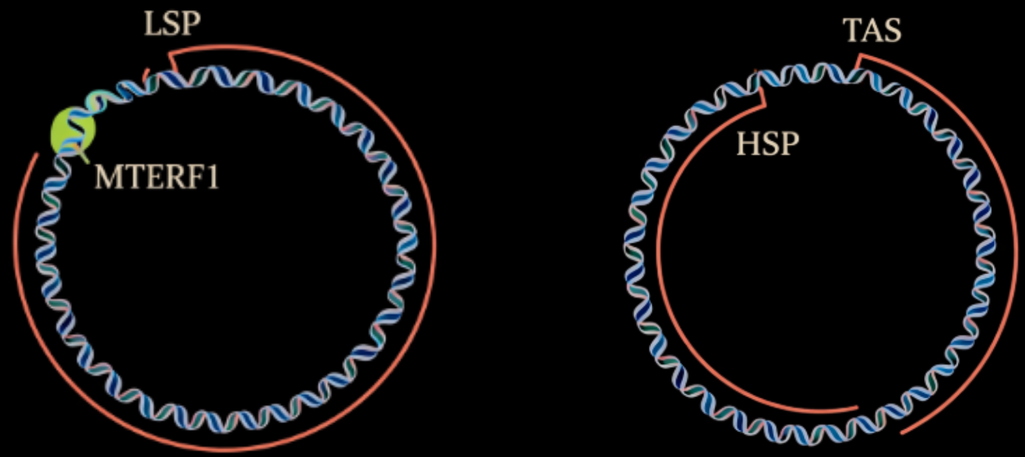


Figure from: Quintero et al., Nat. Rev. Mol. Cell Biol. (2023)

STRUCTURE

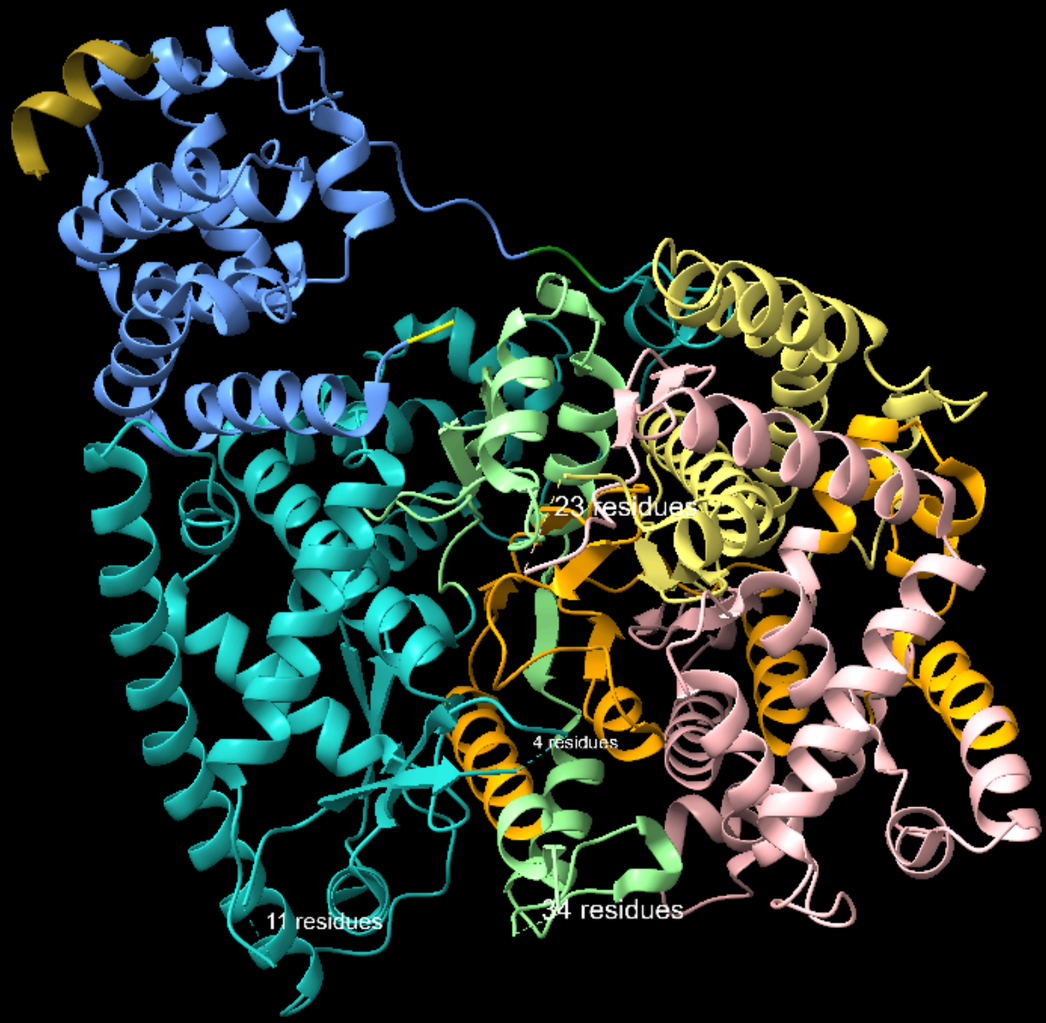
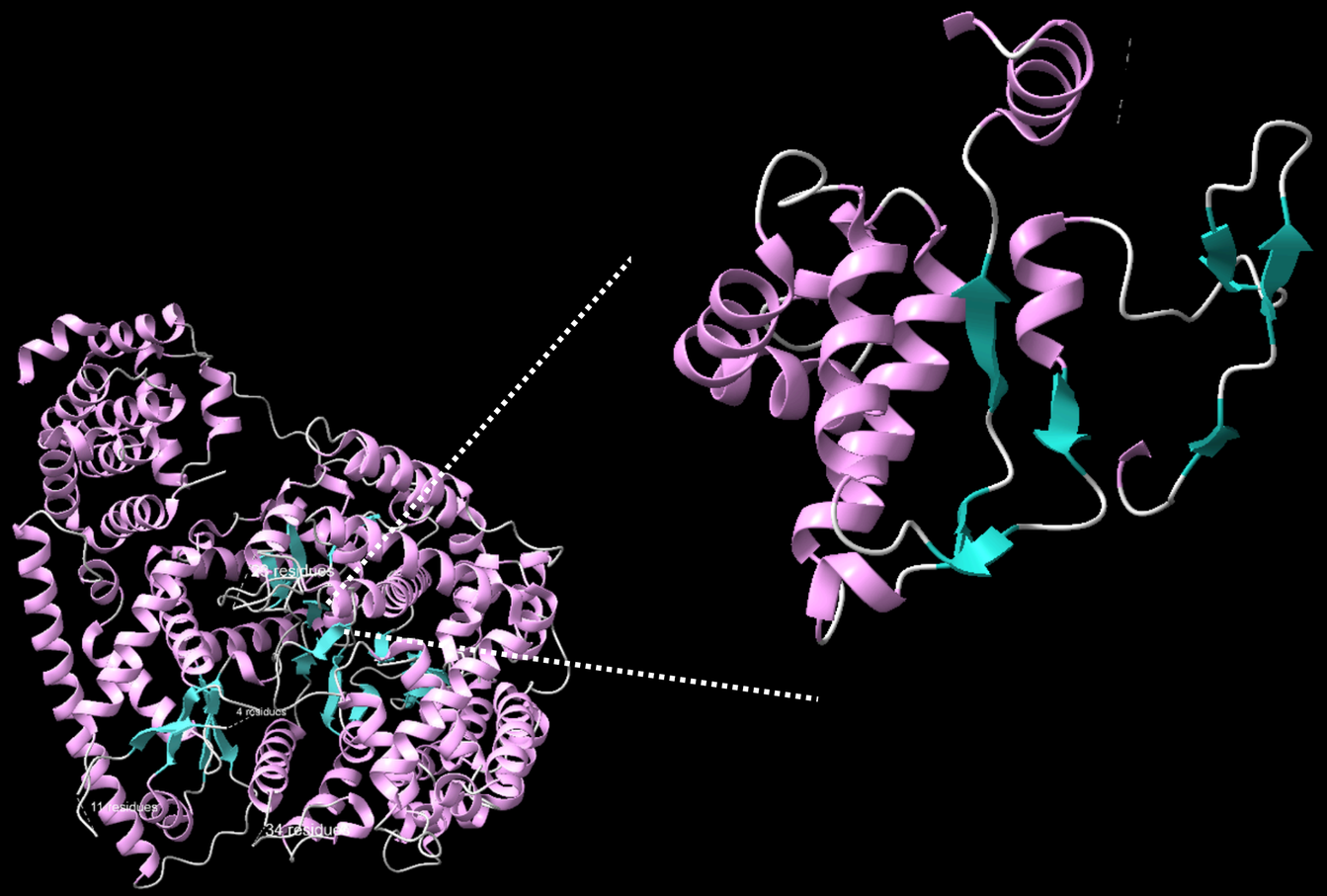


Image from Chimera of PDB code 3SPA

STRUCTURAL LINEAGE OF THE POLRMT CATALYTIC DOMAIN



Images from Chimera of PDB code 3SPA

class 1000003
Alpha and beta proteins (a+b)

fold 2000014
Ferredoxin-like

superfamily 3001272
Nucleotide cyclase/DNA polymerase palm domain-like

family 4002801
T7 RNA polymerase

N-TERMINAL DOMAIN

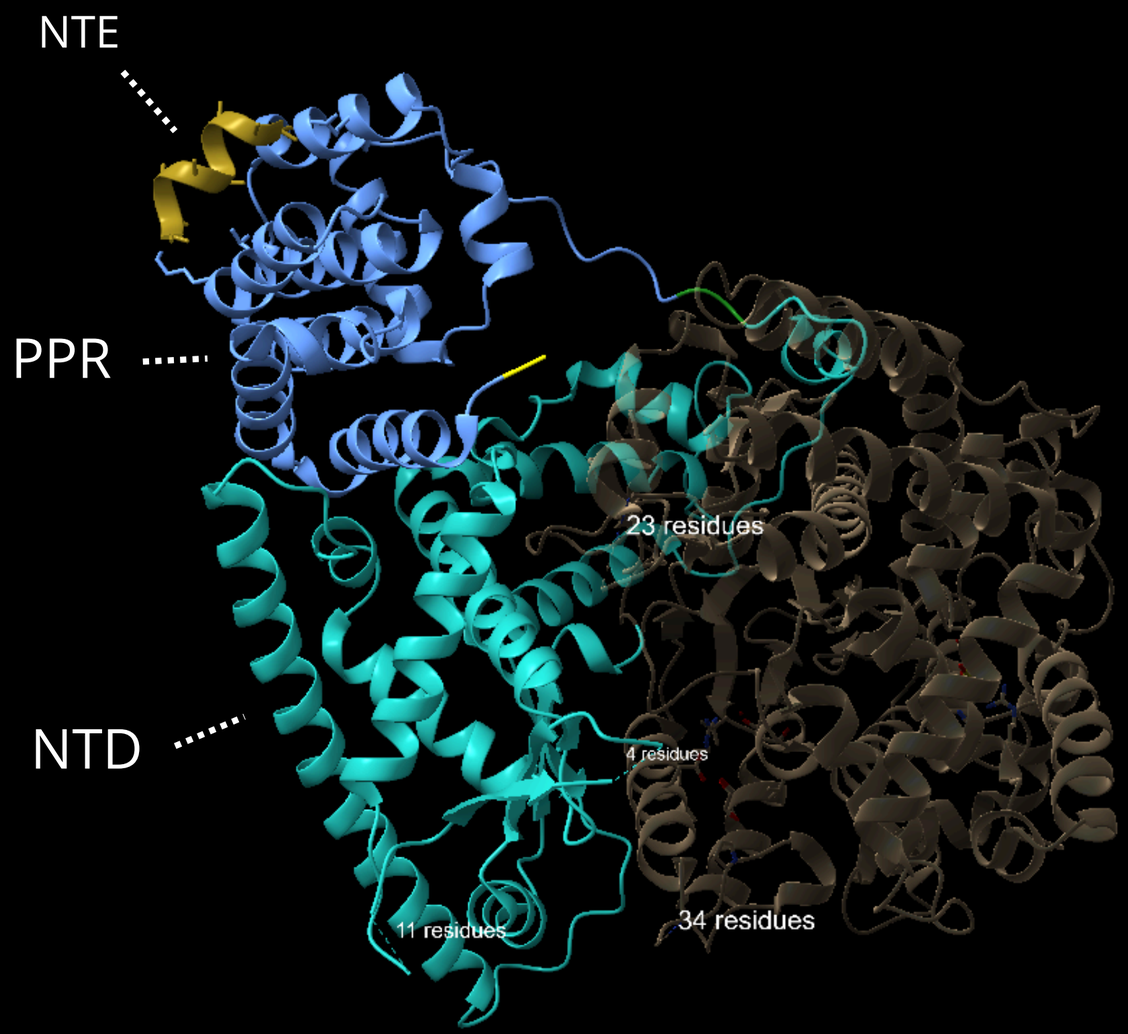


Image from Chimera of PDB code 3SPA

N-terminal region (NTD)

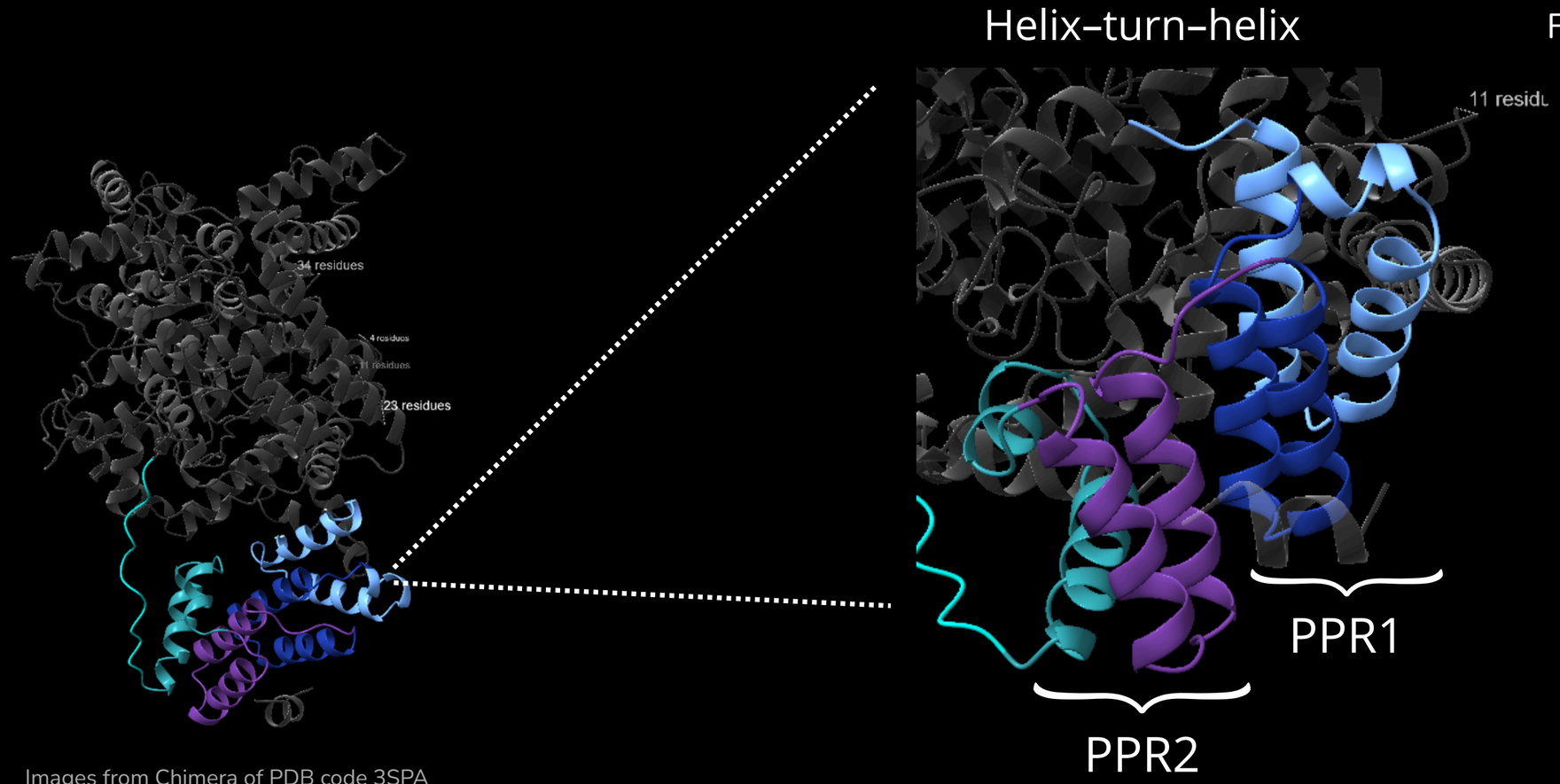
→ Promoter recognition

→ Early stages of transcription

N-terminal extension (NTE)

- **S**tructurally flexible and intrinsically disordered.
- Stable conformation only upon interaction with TFAM.

PENTATRICOPEPTIDE REPEAT DOMAIN (PPR)



Images from Chimera of PDB code 3SPA

Functions:

- Guide and stabilize the nascent RNA as it emerges from the active site.
- Prevents RNA–DNA rehybridization and reduces the likelihood of R-loop formation.
- Provides an interaction platform for elongation factors (TEFM).

N-TERMINAL DOMAIN

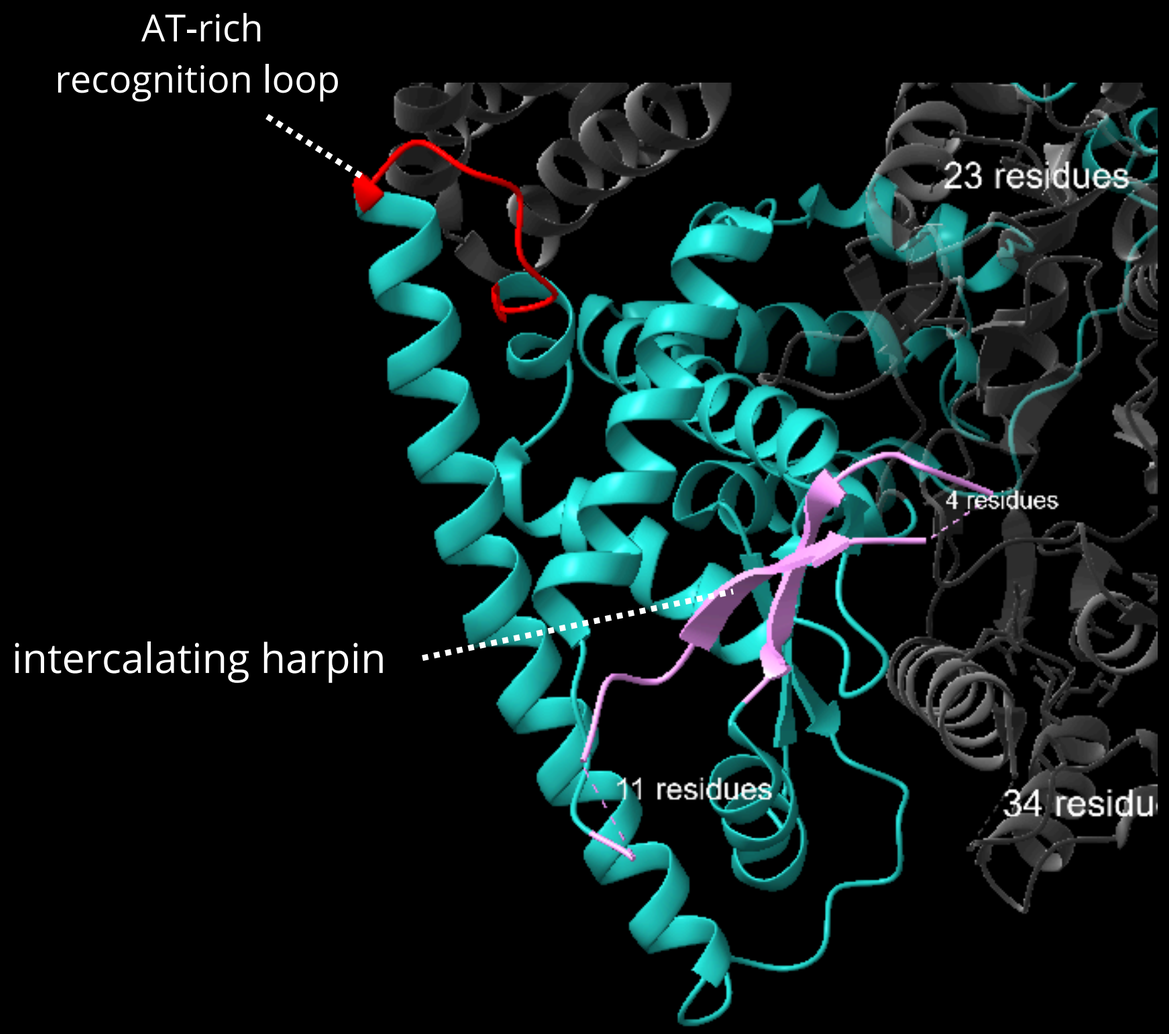


Image from Chimera of PDB code 3SPA

AT-rich recognition loop: participates in promoter sequence recognition (LSP and HSP).

Intercalating harpin: separates the two DNA strands and stabilizes the transcription bubble during initiation

C-TERMINAL DOMAIN

PALM DOMAIN

- Contains the catalytic active site

FINGER DOMAIN

- Positions the incoming rNTP
- Contributes to nucleotide selection and fidelity

THUMB DOMAIN

- Stabilizes the DNA–RNA hybrid
- Maintains processivity during elongation

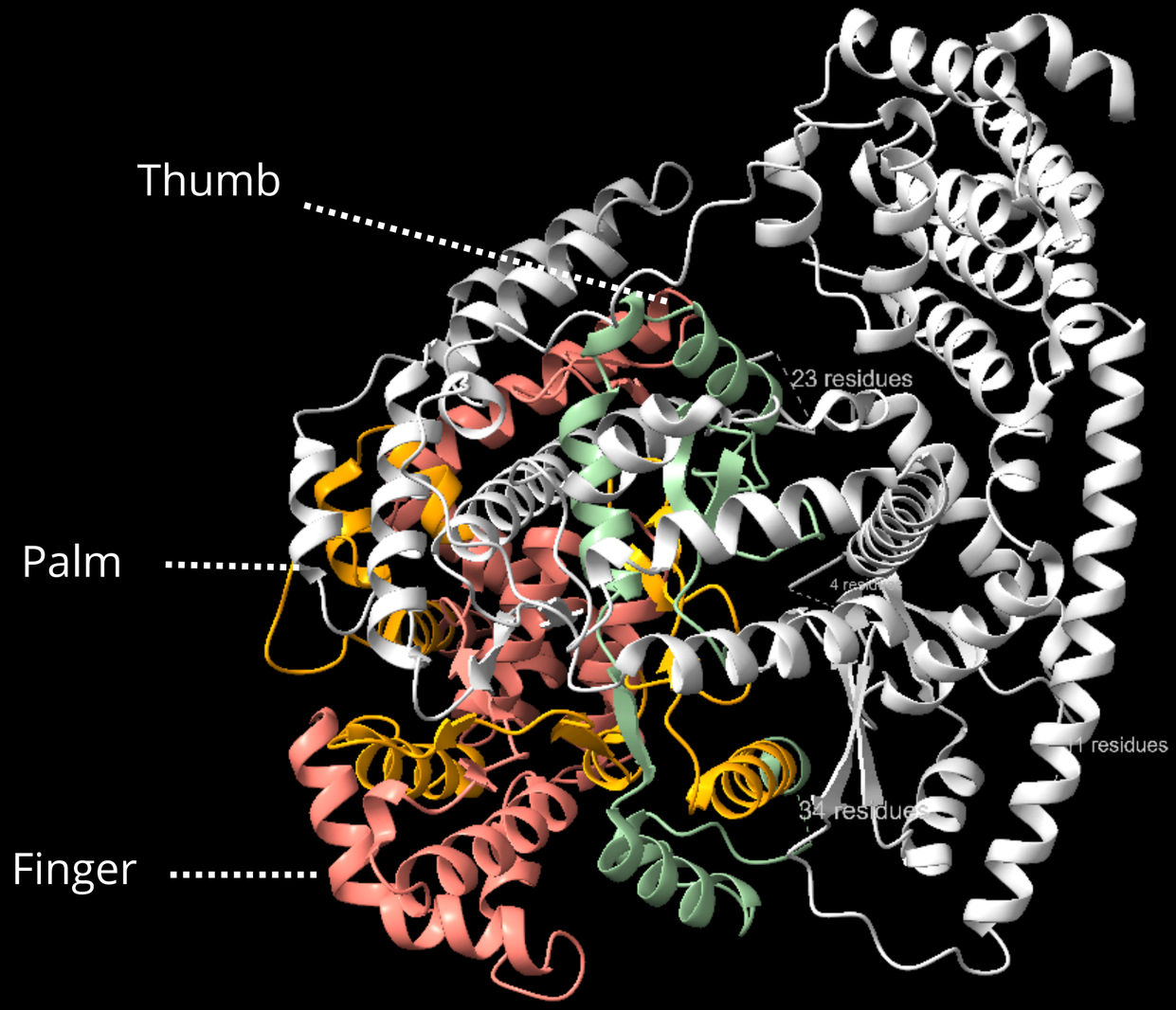
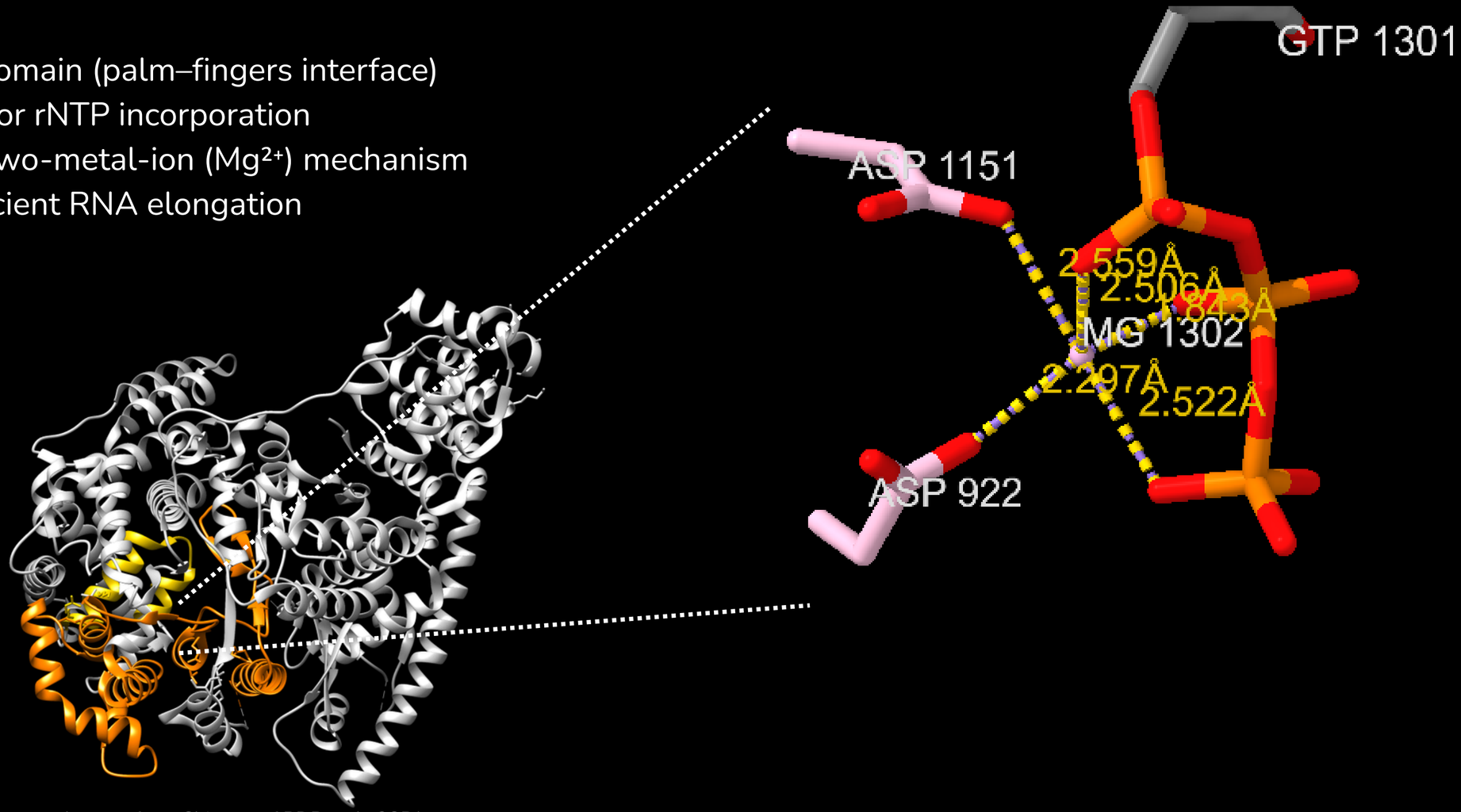


Image from Chimera of PDB code 3SPA

PALM DOMAIN

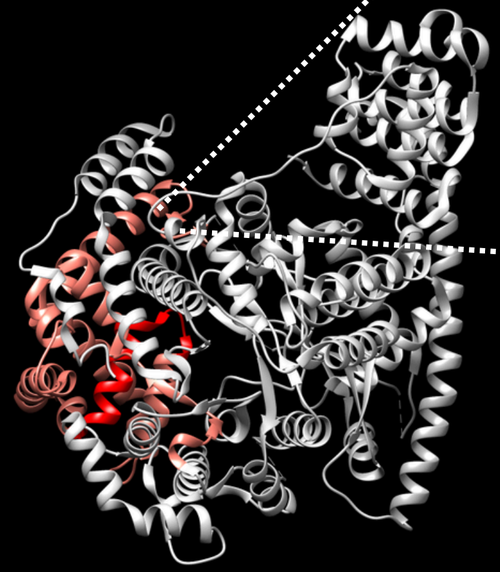
Active site:

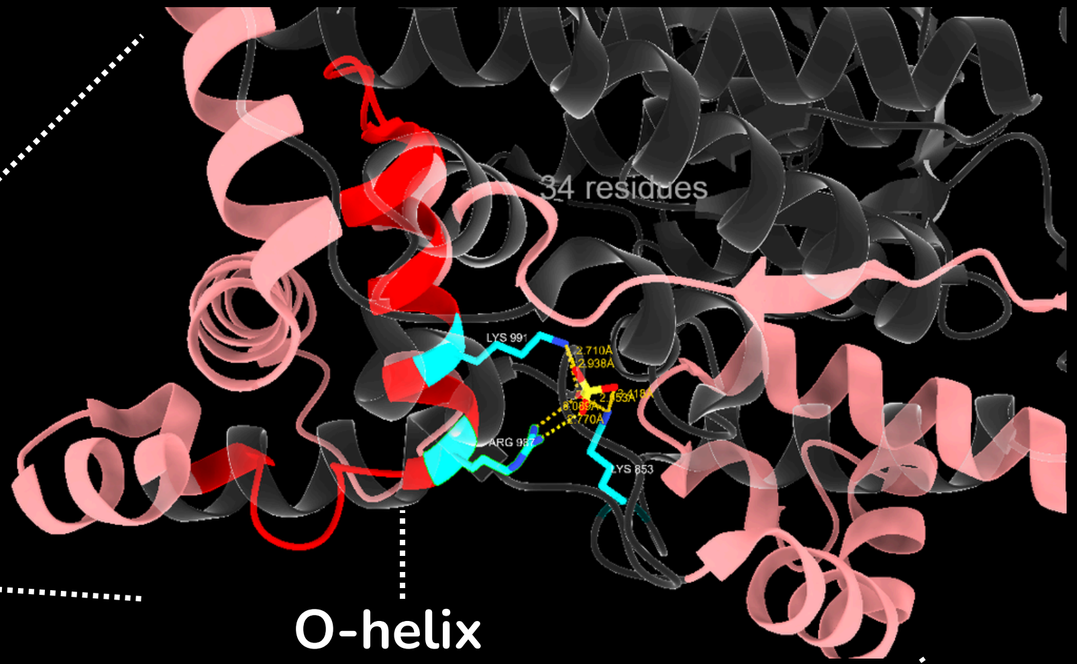
- Located in the palm subdomain (palm–fingers interface)
- Forms a catalytic pocket for rNTP incorporation
- Supports the conserved two-metal-ion (Mg^{2+}) mechanism
- Ensures accurate and efficient RNA elongation



Images from Chimera of PDB code 3SPA

FINGERS: O-HELIX



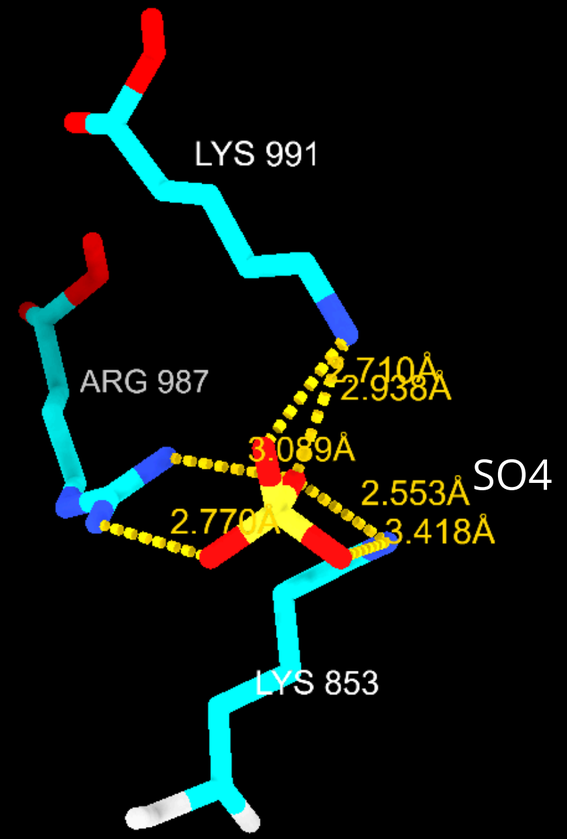


O-helix

↓

O-helix coordinates a sulfate ion through basic residues such as R987, K991

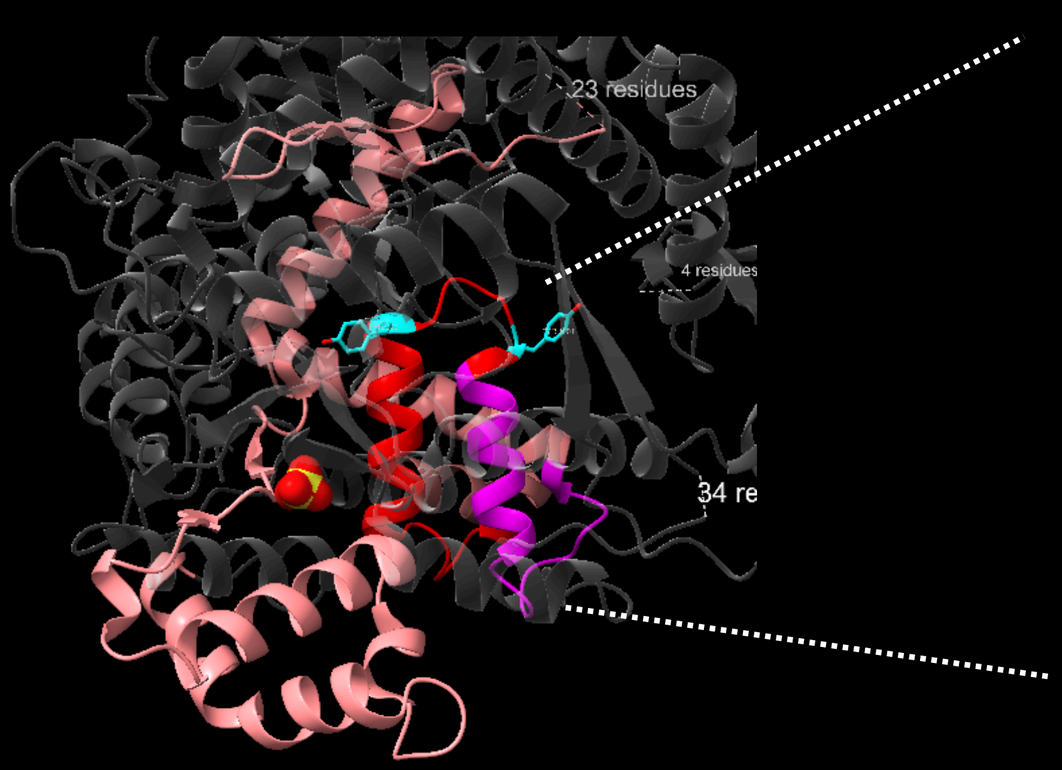
Fingers

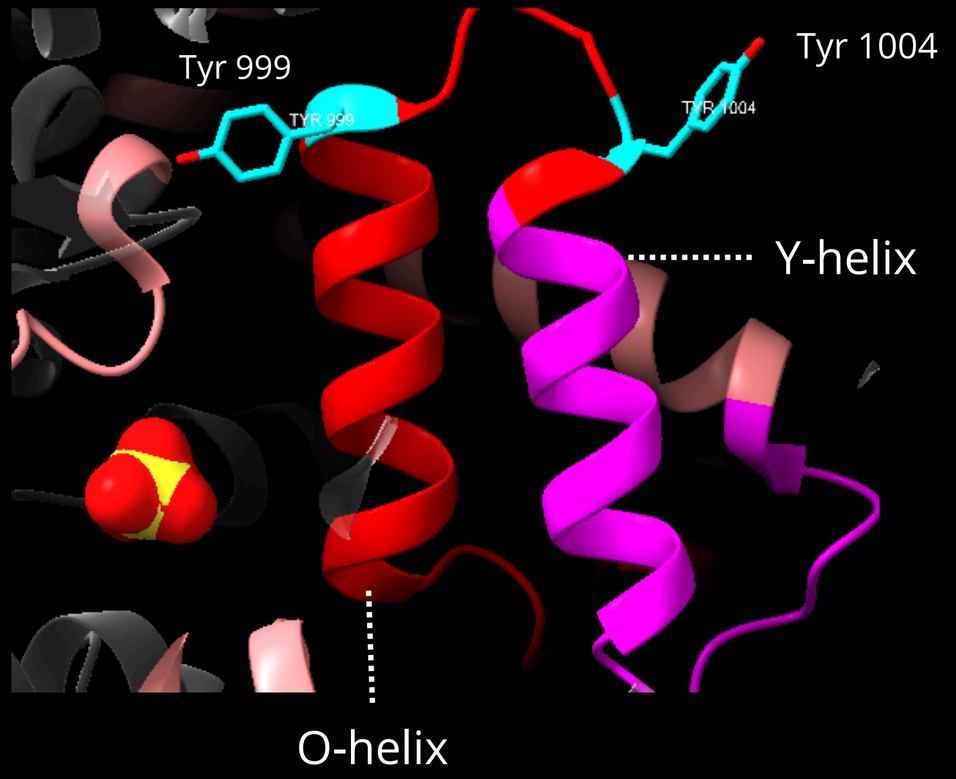


Images from Chimera of PDB code 3SPA

FINGERS: Y-HELIX

- Y999 (O-helix) and Y1004 (Y-helix) partially occlude NTP entry
- Contributes to transitions between open and tight conformations



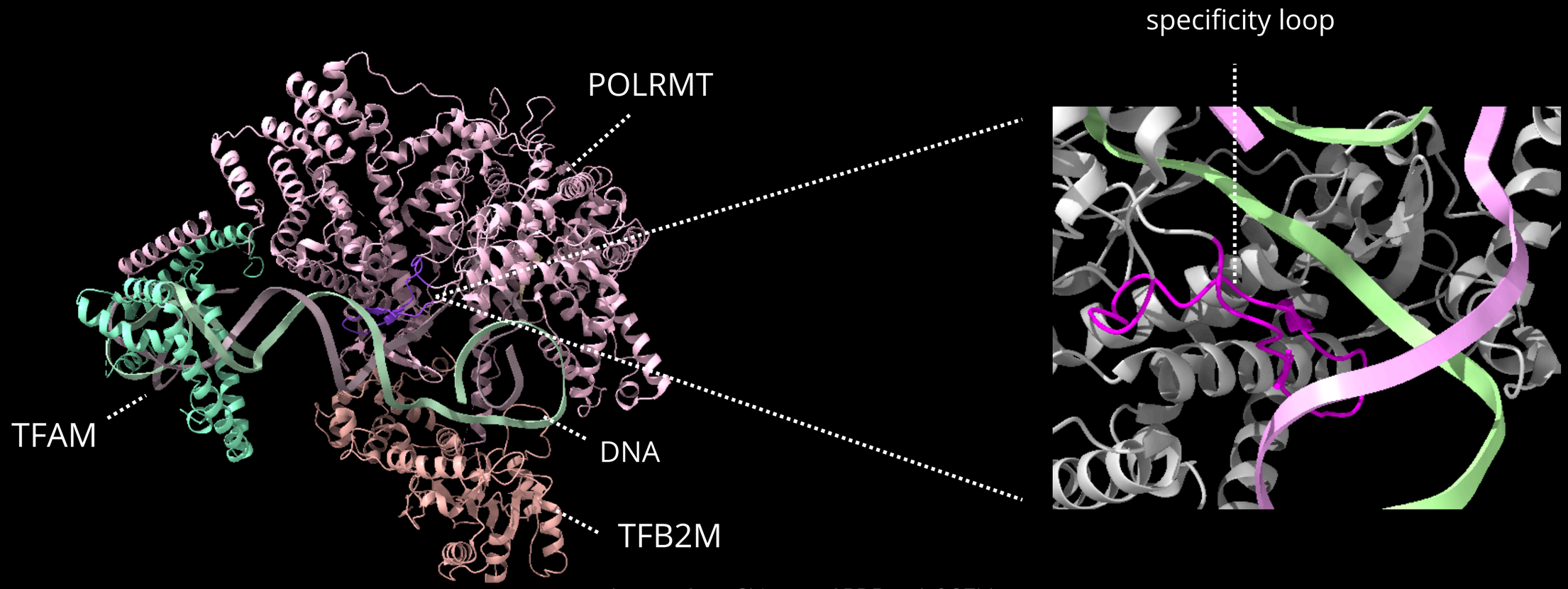


Images from Chimera of PDB code 3SPA

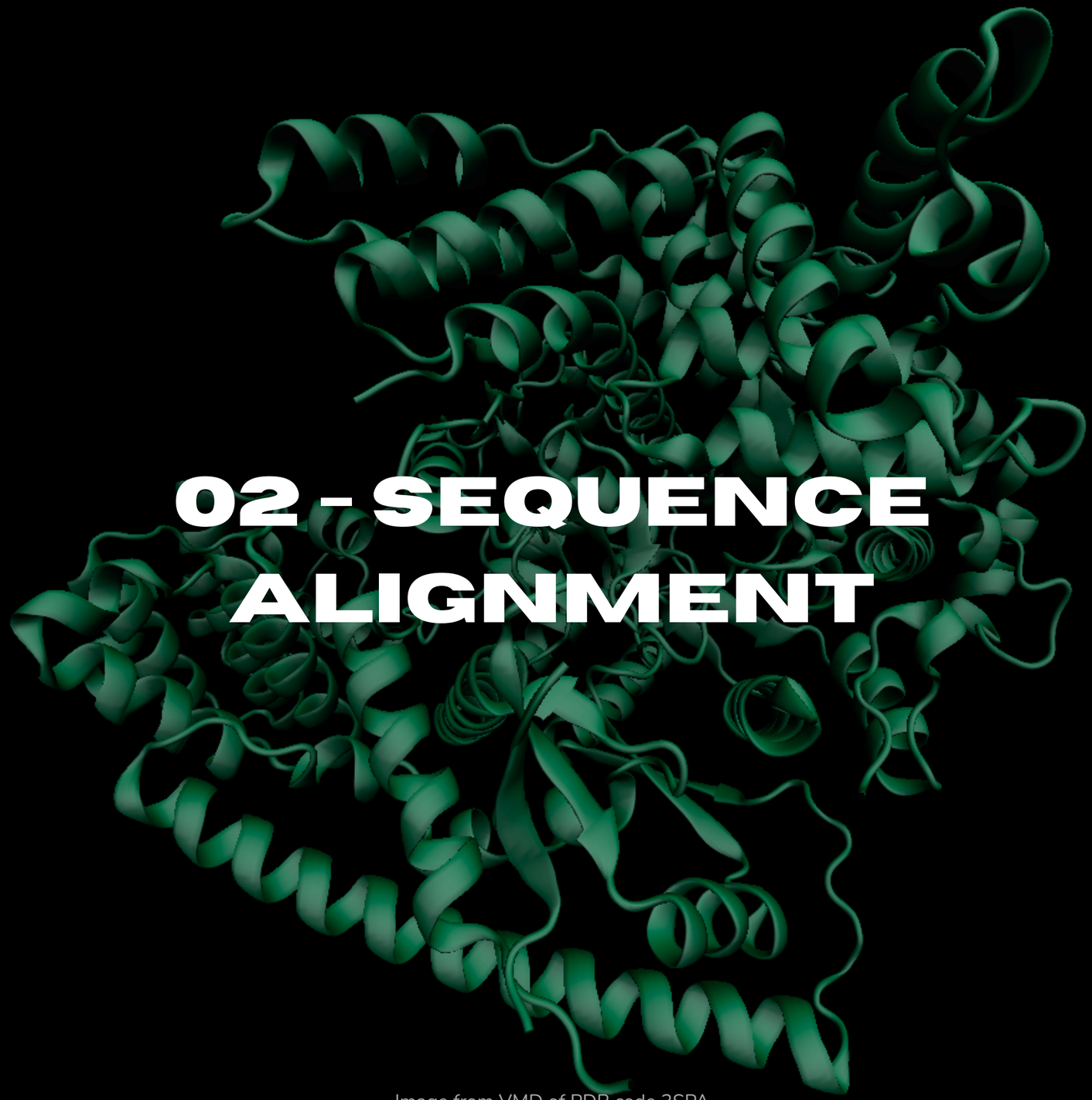
SPECIFICITY LOOP

Specificity Loop in Initiation Complex Formation:

- Stabilization of the nascent transcription bubble through interactions with the DNA major groove
- Guides the template strand toward the active site.
- Formation of an active initiation complex



Images from Chimera of PDB code9GZM



02 - SEQUENCE ALIGNMENT

Image from VMD of PDB code 3SPA

SEQUENCES SELECTION

Species aligned:

- *Homo sapiens*
- *Mus musculus*
- *Saccharomyces cerevisiae*
- *Neurospora crassa*
- *Nicotiana sylvestris*
- *Bacteriophage T7*
- *Zea mays*

UniProt codes:

- O00411
- Q8BKF1
- P13433
- P38671
- Q8VWF8
- P00573
- P10581

Sequences used in model and found again:

sp|O00411|RPOM_HUMAN DNA-directed RNA polymerase, mitochondrial O...	1211	0.0
sp|Q8BKF1|RPOM_MOUSE DNA-directed RNA polymerase, mitochondrial O...	1102	0.0
sp|Q8L6J3|RPO2B_TOBAC DNA-directed RNA polymerase 2B, chloroplast...	899	0.0
sp|Q8L6J5|RPO1B_TOBAC DNA-directed RNA polymerase 1B, mitochondri...	898	0.0
sp|Q9LFV6|RPOT2_ARATH DNA-directed RNA polymerase 2, chloroplasti...	898	0.0
sp|Q93Y94|RPOT1_NICSY DNA-directed RNA polymerase 1, mitochondria...	895	0.0
sp|Q8VWF8|RPOT2_NICSY DNA-directed RNA polymerase 2, chloroplasti...	894	0.0
sp|P92969|RPOT1_ARATH DNA-directed RNA polymerase 1, mitochondria...	894	0.0
sp|P69242|RPOT3_NICSY DNA-directed RNA polymerase 3, chloroplasti...	883	0.0
sp|P69243|RPO3A_TOBAC DNA-directed RNA polymerase 3A, chloroplast...	883	0.0
sp|O24600|RPOT3_ARATH DNA-directed RNA polymerase 3, chloroplasti...	876	0.0
sp|Q8L6J1|RPO3B_TOBAC DNA-directed RNA polymerase 3B, chloroplast...	862	0.0
sp|O13993|RPOM_SCHPO DNA-directed RNA polymerase, mitochondrial O...	803	0.0
sp|P13433|RPOM_YEAST DNA-directed RNA polymerase, mitochondrial O...	774	0.0
sp|P38671|RPOM_NEUCR DNA-directed RNA polymerase, mitochondrial O...	746	0.0
sp|P07659|RPOL_BPT3 DNA-directed RNA polymerase OS=Enterobacteria...	715	0.0
sp|P00573|RPOL_BPT7 DNA-directed RNA polymerase OS=Enterobacteria...	702	0.0
sp|P18147|RPOL_BPK11 DNA-directed RNA polymerase OS=Enterobacteri...	685	0.0
sp|P06221|RPOL_BPSP6 DNA-directed RNA polymerase OS=Enterobacteri...	604	0.0
sp|P33540|RPOP_NEUCS Probable DNA-directed RNA polymerase OS=Neur...	527	5e-169
sp|P33541|RPOP_NEUIN Probable DNA-directed RNA polymerase OS=Neur...	510	2e-163
sp|Q8L6J2|RPO2A_TOBAC DNA-directed RNA polymerase 2A (Fragment) O...	481	2e-159
sp|O03685|RPOP_GELSP Probable DNA-directed RNA polymerase OS=Gela...	500	3e-159
sp|P22372|RPOP_CLAPU Probable DNA-directed RNA polymerase OS=Clav...	477	2e-149
sp|P33539|RPOP_AGABT Probable DNA-directed RNA polymerase OS=Agar...	478	4e-148
sp|Q01521|RPOP_PODAS Probable DNA-directed RNA polymerase OS=Podo...	469	3e-146
sp|P10581|RPOP_MAIZE Probable DNA-directed RNA polymerase OS=Zea ...	407	1e-121

Image from the psi-blast we obtained

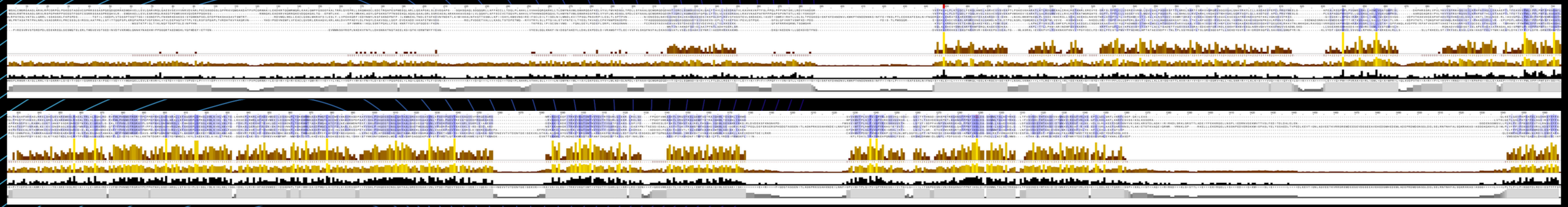


Image from Jalview of the alignment positions 1-1568

SEQUENCES SELECTION

Species aligned:

- *Homo sapiens*
- *Mus musculus*
- *Saccharomyces cerevisiae*
- *Neurospora crassa*
- *Nicotiana sylvestris*
- *Bacteriophage T7*
- *Zea mays*

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- P38671
- Q8VWF8
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- P10581

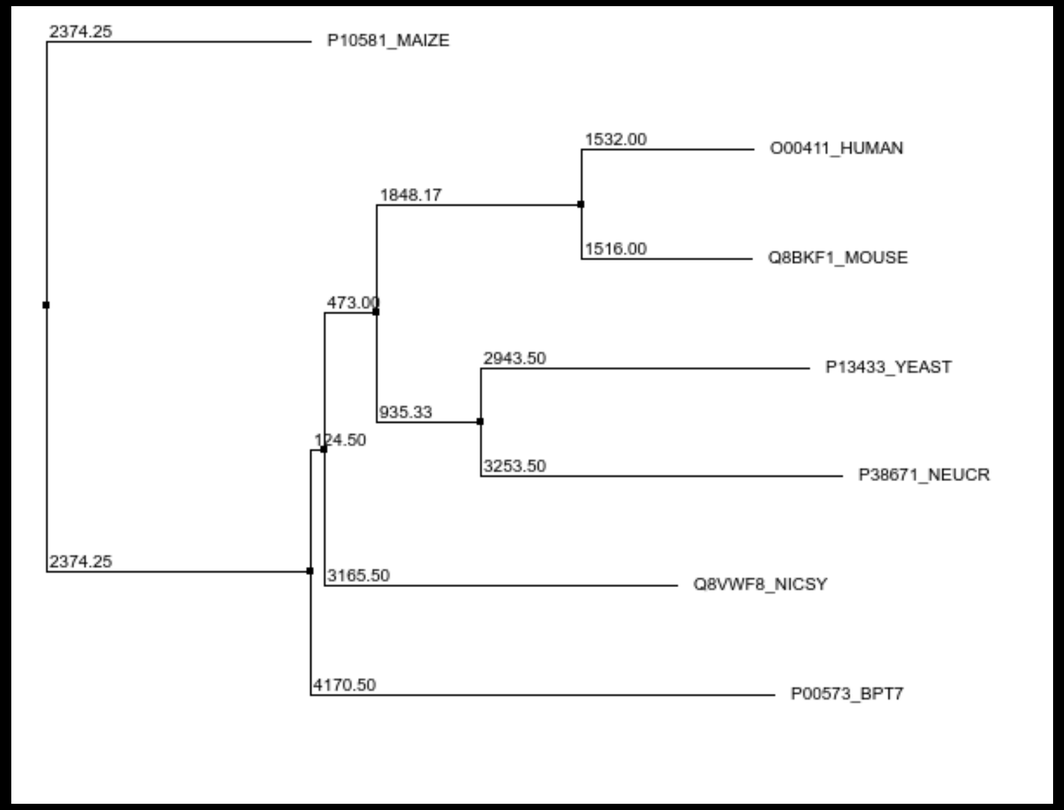


Image from Jalview

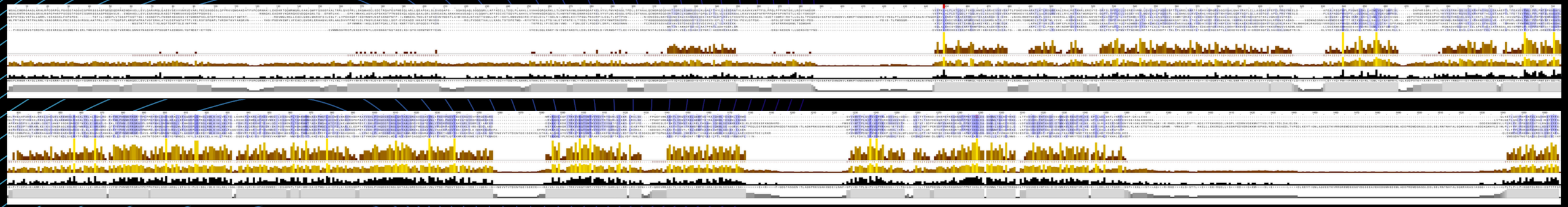


Image from Jalview of the alignment positions 1-1568

CONSERVED AMINO ACIDS

- 389 (Leu)
- 567 (Trp)
- 583 (Leu)
- 647 (Pro)
- 661 (Gly)
- 776 (Arg)
- 786 (Ala)
- 819 (Asp)
- 895 (Leu)
- 944 (Val)
- 956 (Tyr)
- 991 (Lys)
- 996 (Thr)
- 1077 (Pro)
- 1080 (Gln)
- 1126 (Ser)

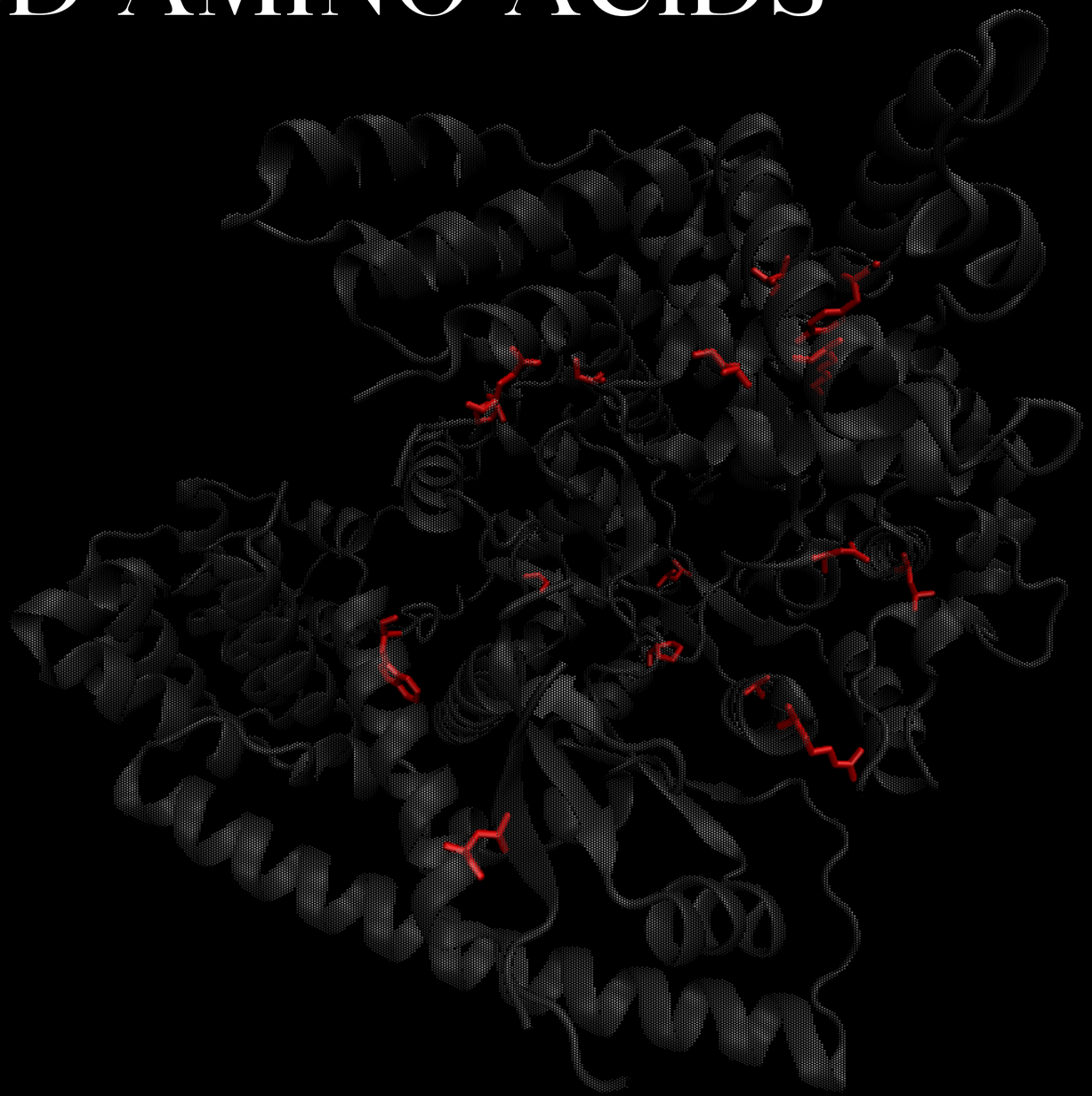


Image from VMD of PDB code 3SPA

CONSERVED AMINO ACIDS

- 389 (Leu)
- 567 (Trp)
- 583 (Leu)
- 647 (Pro)
- 661 (Gly)
- 776 (Arg)
- 786 (Ala)
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- 895 (Leu)
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- 956 (Tyr)
- 991 (Lys)
- 996 (Thr)
- 1077 (Pro)
- 1080 (Gln)
- 1126 (Ser)

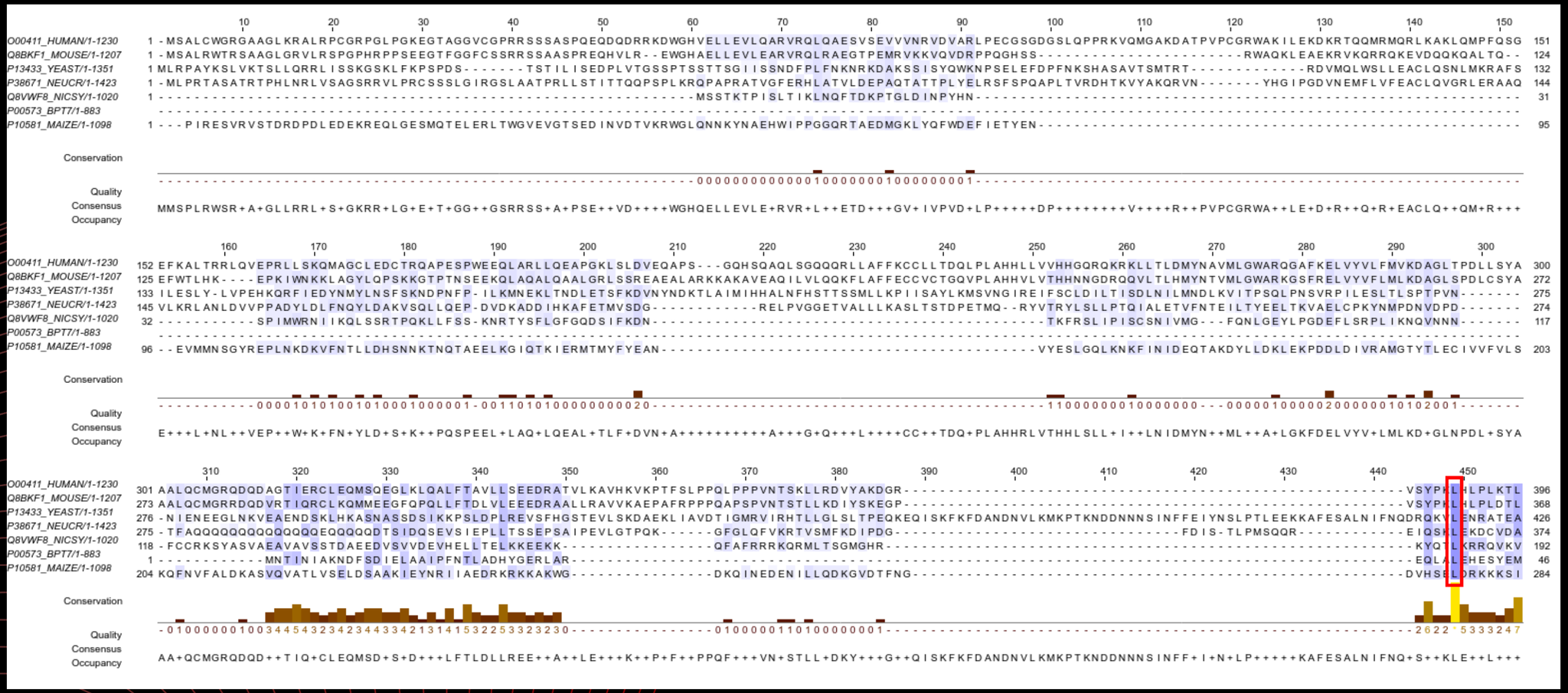


Image from Jalview of the MSA positions 1-456

CONSERVED AMINO ACIDS

- 389 (Leu)
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- 647 (Pro)
- 661 (Gly)
- 776 (Arg)
- 786 (Ala)
- 819 (Asp)
- 895 (Leu)
- 944 (Val)
- 956 (Tyr)
- 991 (Lys)
- 996 (Thr)
- 1077 (Pro)
- 1080 (Gln)
- 1126 (Ser)

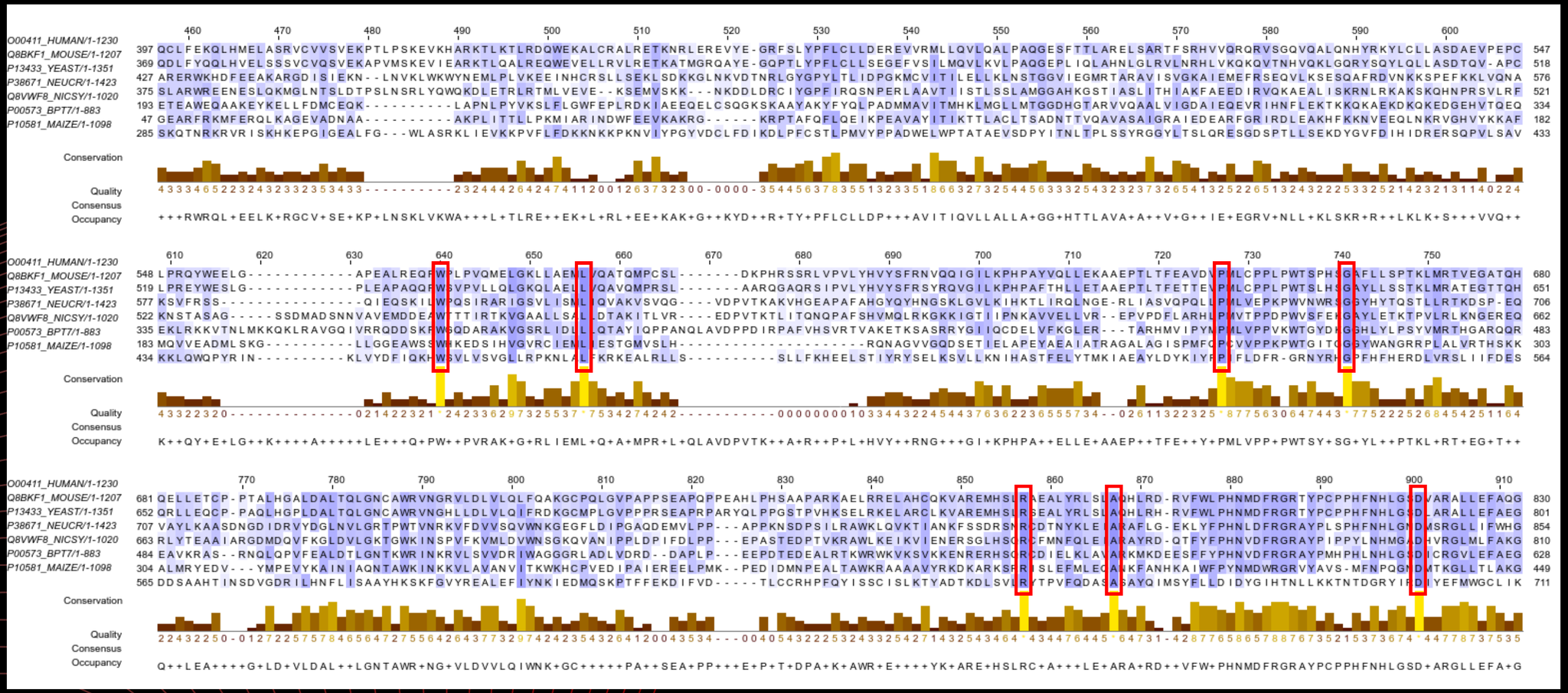


Image from Jalview of the MSA positions 457-912

CONSERVED AMINO ACIDS

- 389 (Leu)
- 567 (Trp)
- 583 (Leu)
- 647 (Pro)
- 661 (Gly)
- 776 (Arg)
- 786 (Ala)
- 819 (Asp)
- 895 (Leu)
- 944 (Val)
- 956 (Tyr)
- 991 (Lys)
- 996 (Thr)
- 1077 (Pro)
- 1080 (Gln)
- 1126 (Ser)

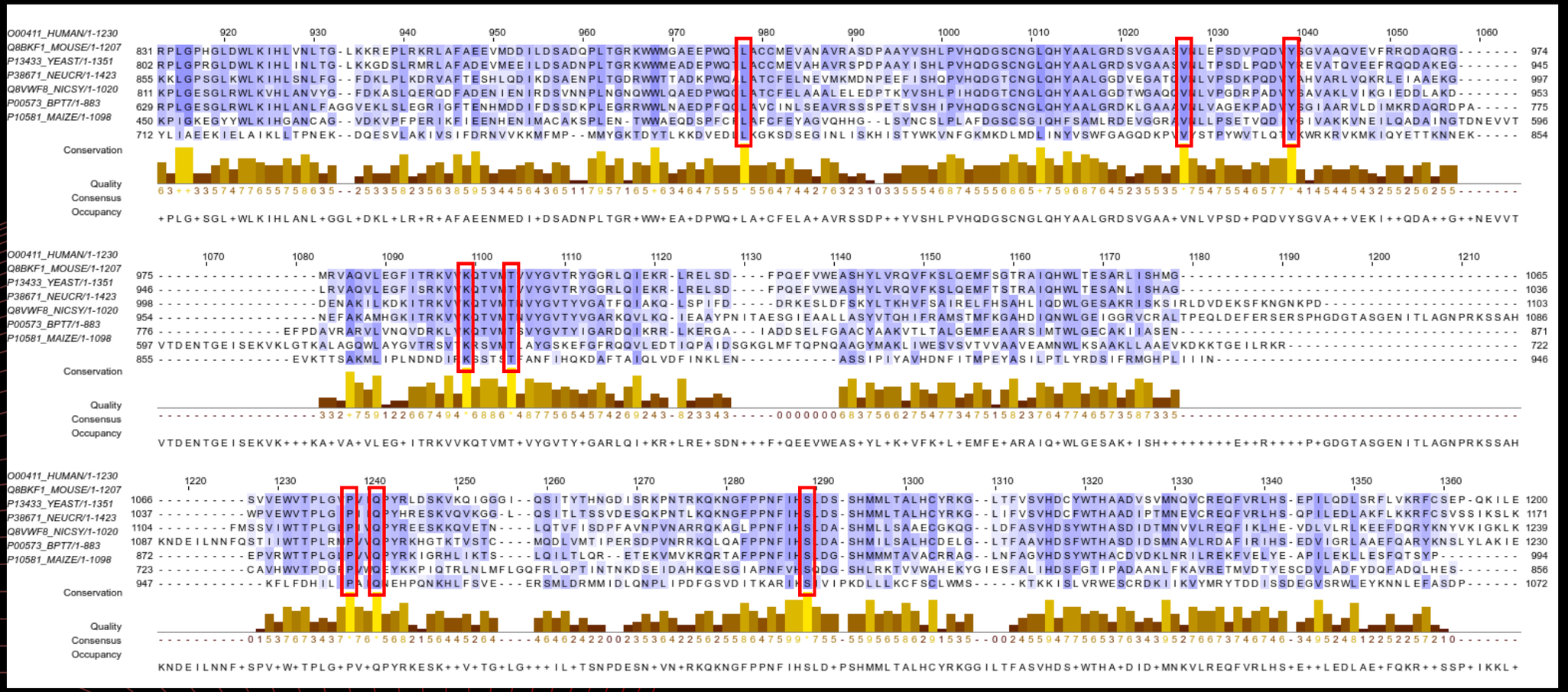


Image from Jalview of the MSA positions 913-1368

CONSERVED AMINO ACIDS

- 389 (Leu)
- 567 (Trp)
- 583 (Leu)
- 647 (Pro)
- 661 (Gly)
- 776 (Arg)
- 786 (Ala)
- 819 (Asp)
- 895 (Leu)
- 944 (Val)
- 956 (Tyr)
- 991 (Lys)
- 996 (Thr)
- 1077 (Pro)
- 1080 (Gln)
- 1126 (Ser)

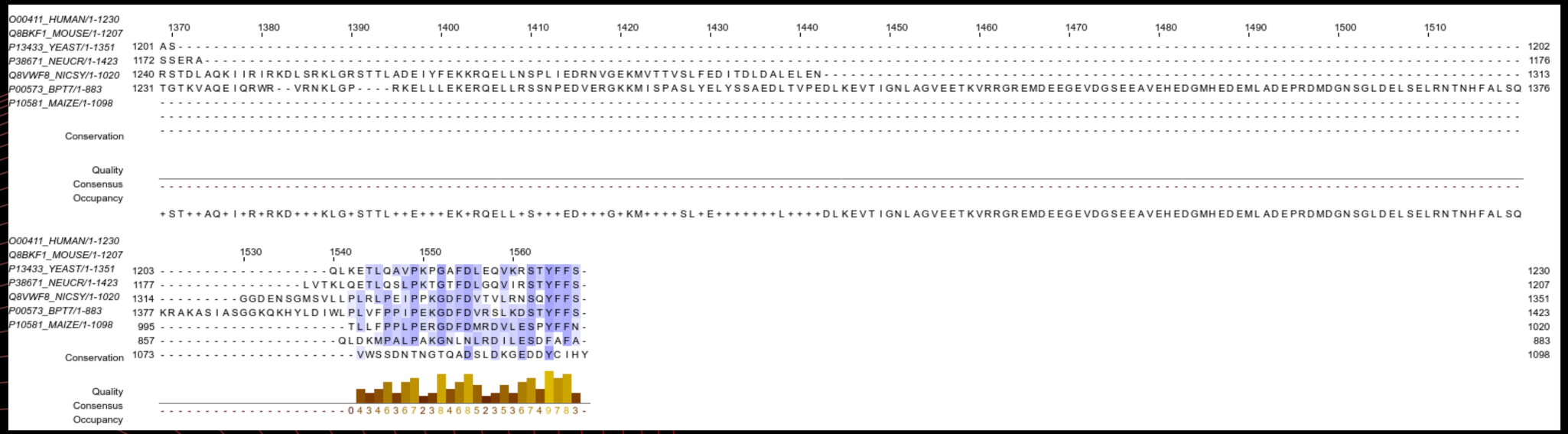


Image from Jalview of the MSA positions 1369-1568

SEQUENCES SELECTION CORRECTION

Species aligned:

- *Homo sapiens*
- *Mus musculus*
- *Saccharomyces cerevisiae*
- *Neurospora crassa*
- *Nicotiana sylvestris*
- *Bacteriophage T7*
- ~~*Zea mays*~~

UniProt codes:

- O00411
- Q8BKF1
- P13433
- P38671
- Q8VWF8
- P00573
- ~~P10581~~

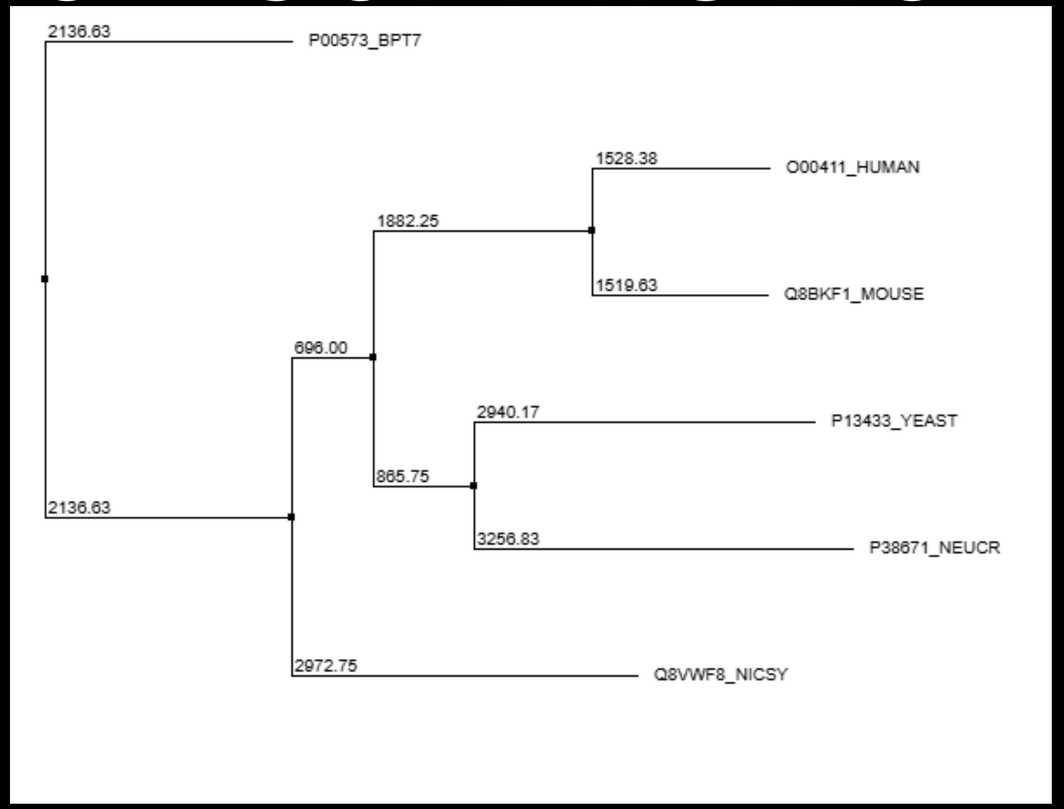
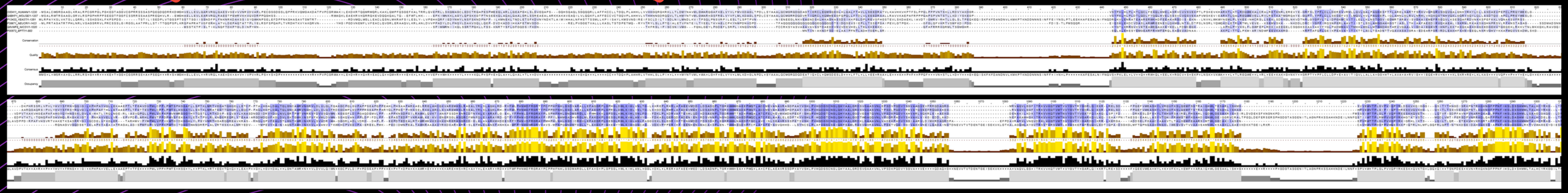


Image from Jalview



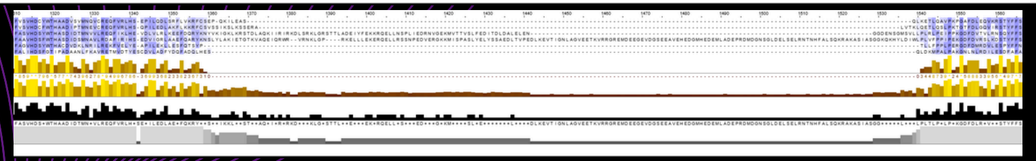


Image from Jalview of the new alignment positions 1-1566

MSA WITHOUT MAIZE

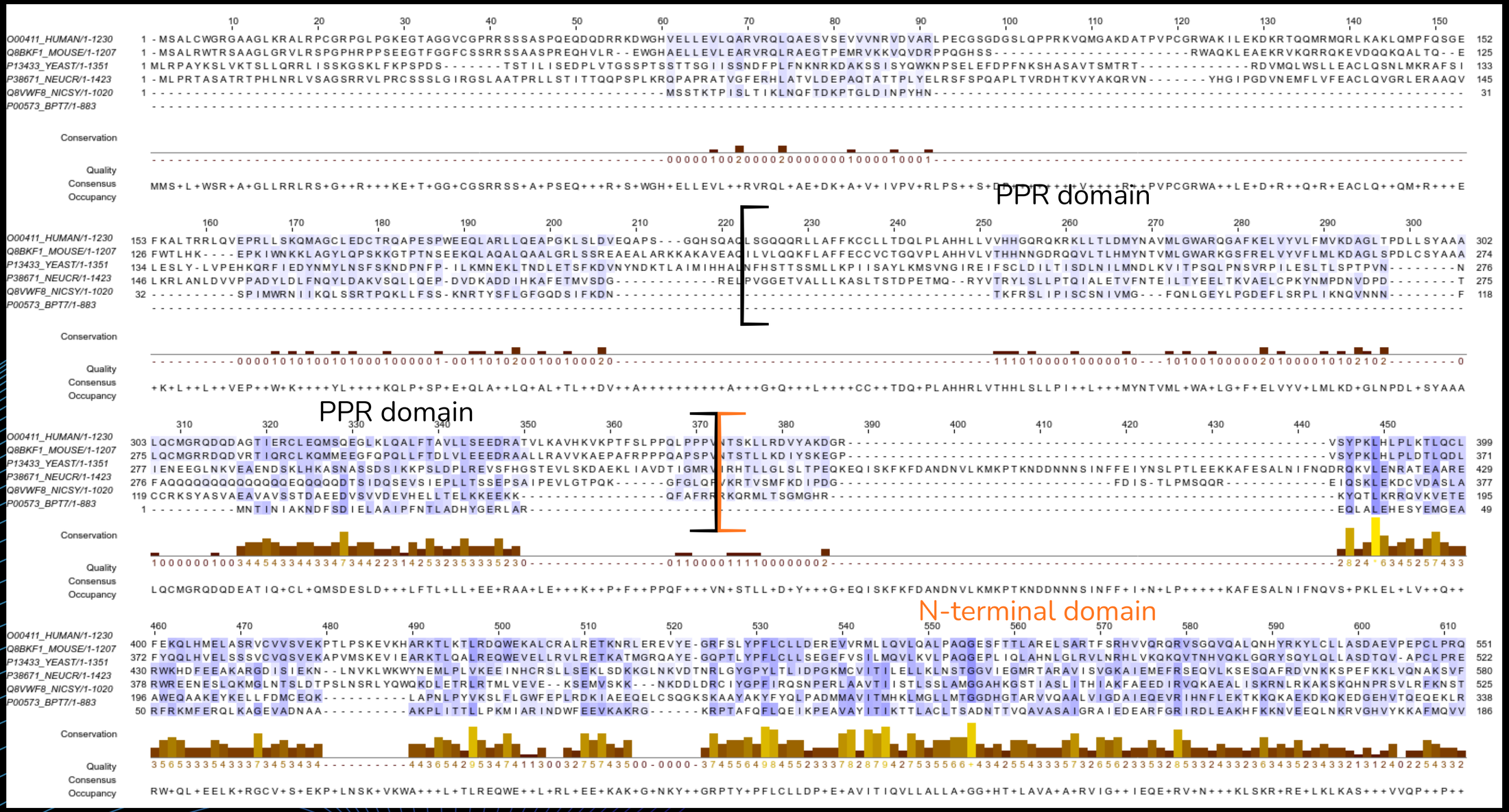


Image from Jalview of the MSA
positions 1-612

MSA WITHOUT MAIZE

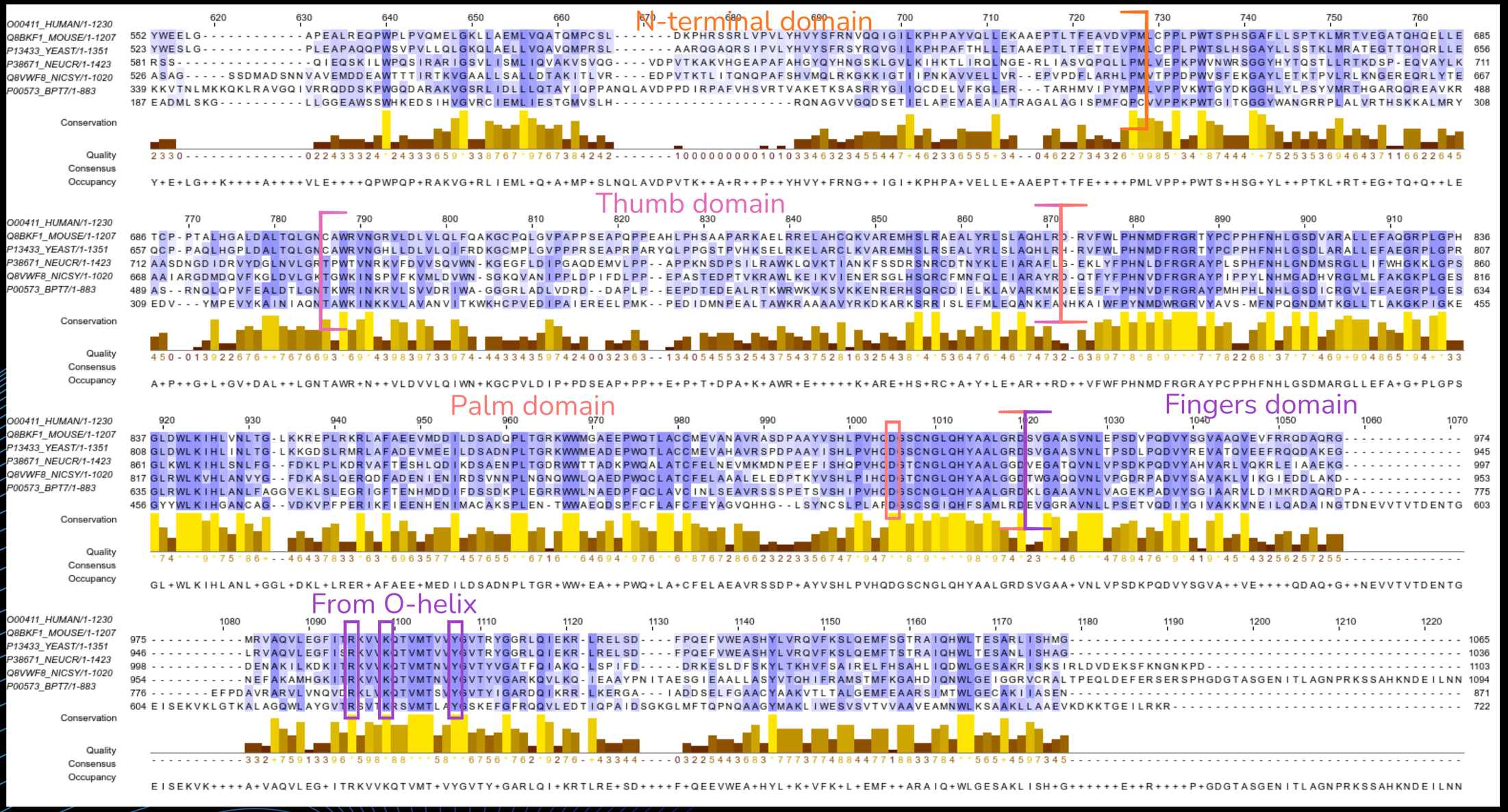


Image from Jalview of the MSA positions 613-1224

MSA WITHOUT MAIZE

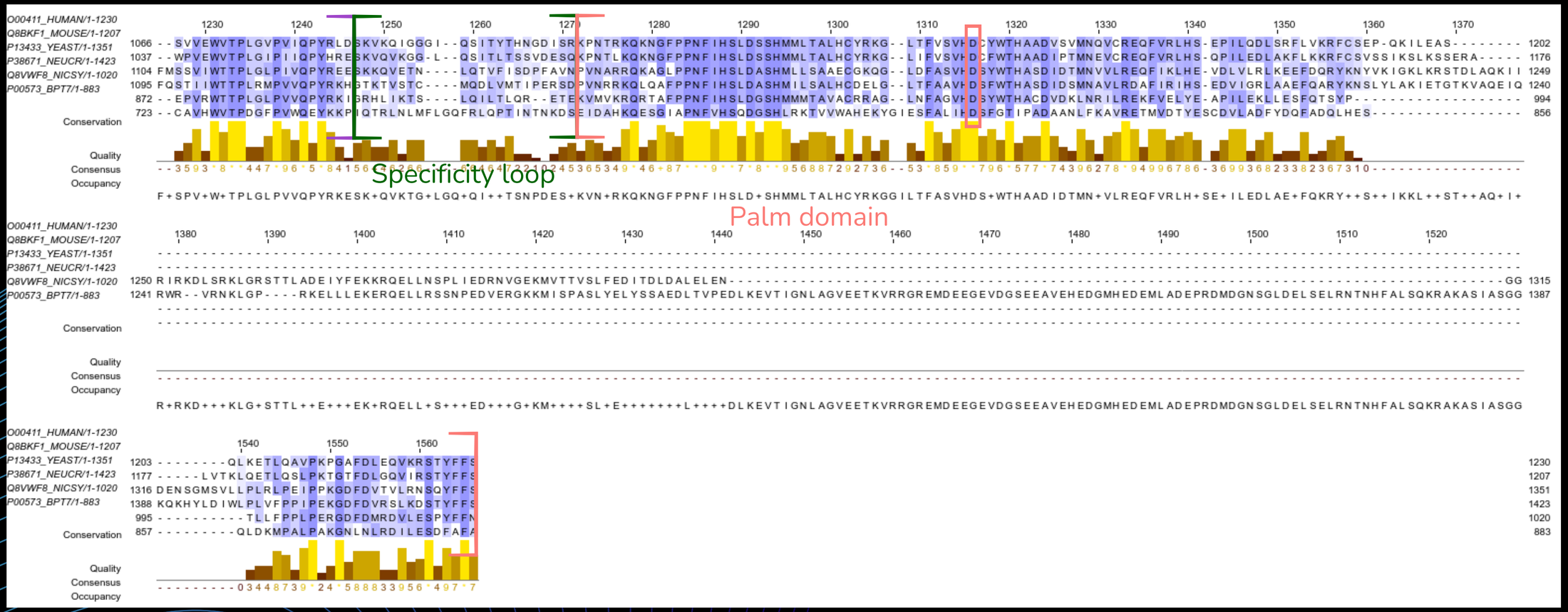
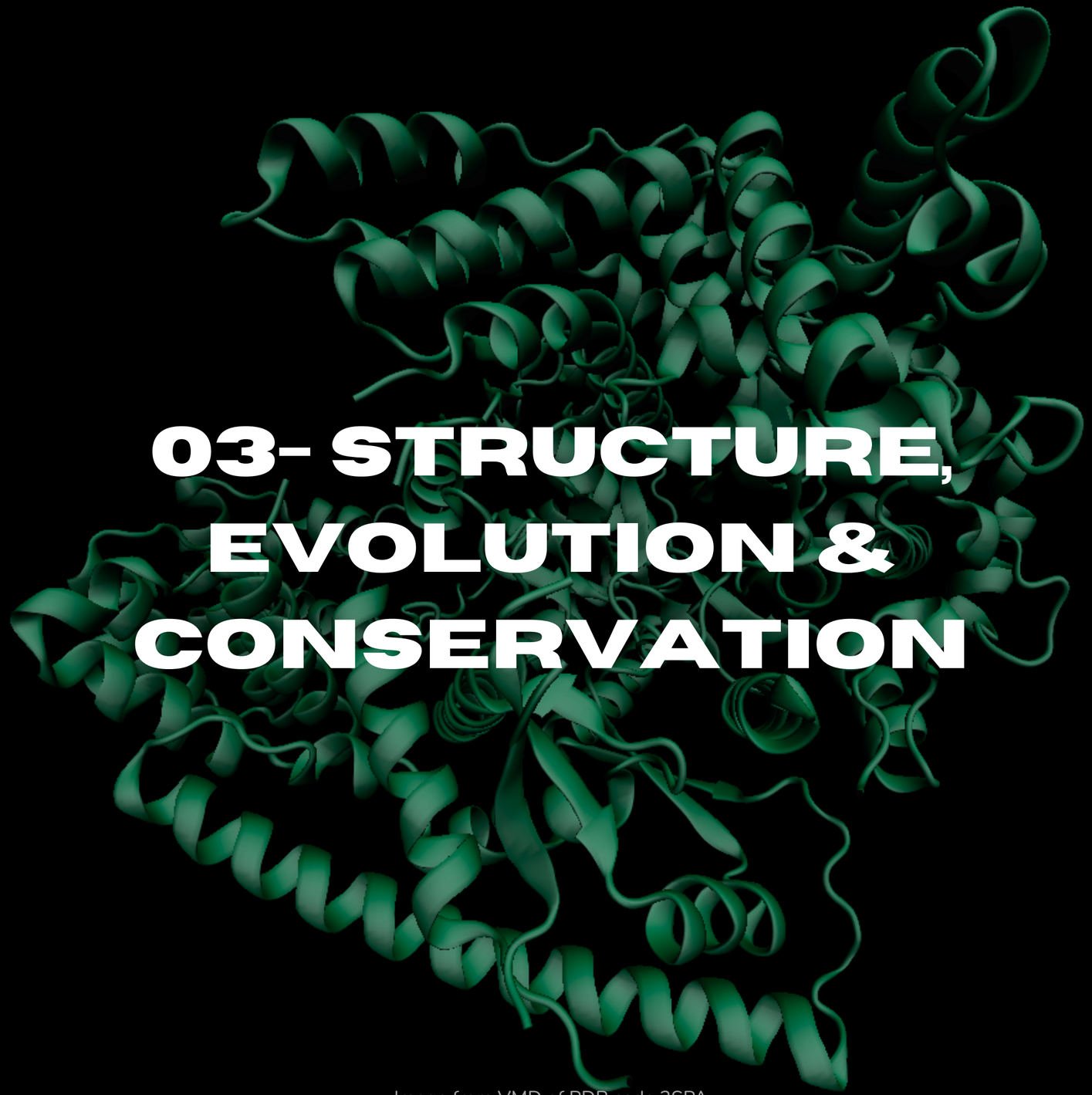


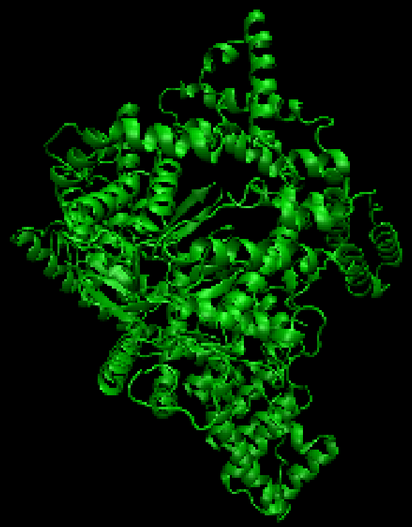
Image from Jalview of the MSA positions 1225-1566



03- STRUCTURE, EVOLUTION & CONSERVATION

Image from VMD of PDB code 3SPA

EVOLUTION & CONSERVATION



PDB: 9GZN chain A

Resolution: 3.50 Å

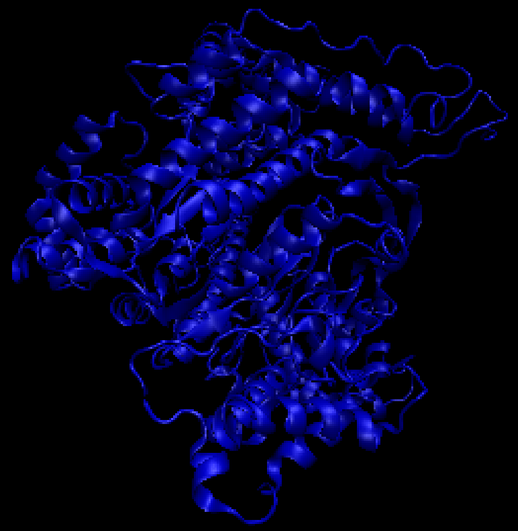
- Associated with TFB2M
- Contains a mammalian-specific PPR domain (N-terminal extension)
- Conserved “right-hand” catalytic core



PDB: 6YMW chain A

Resolution: 3.10 Å

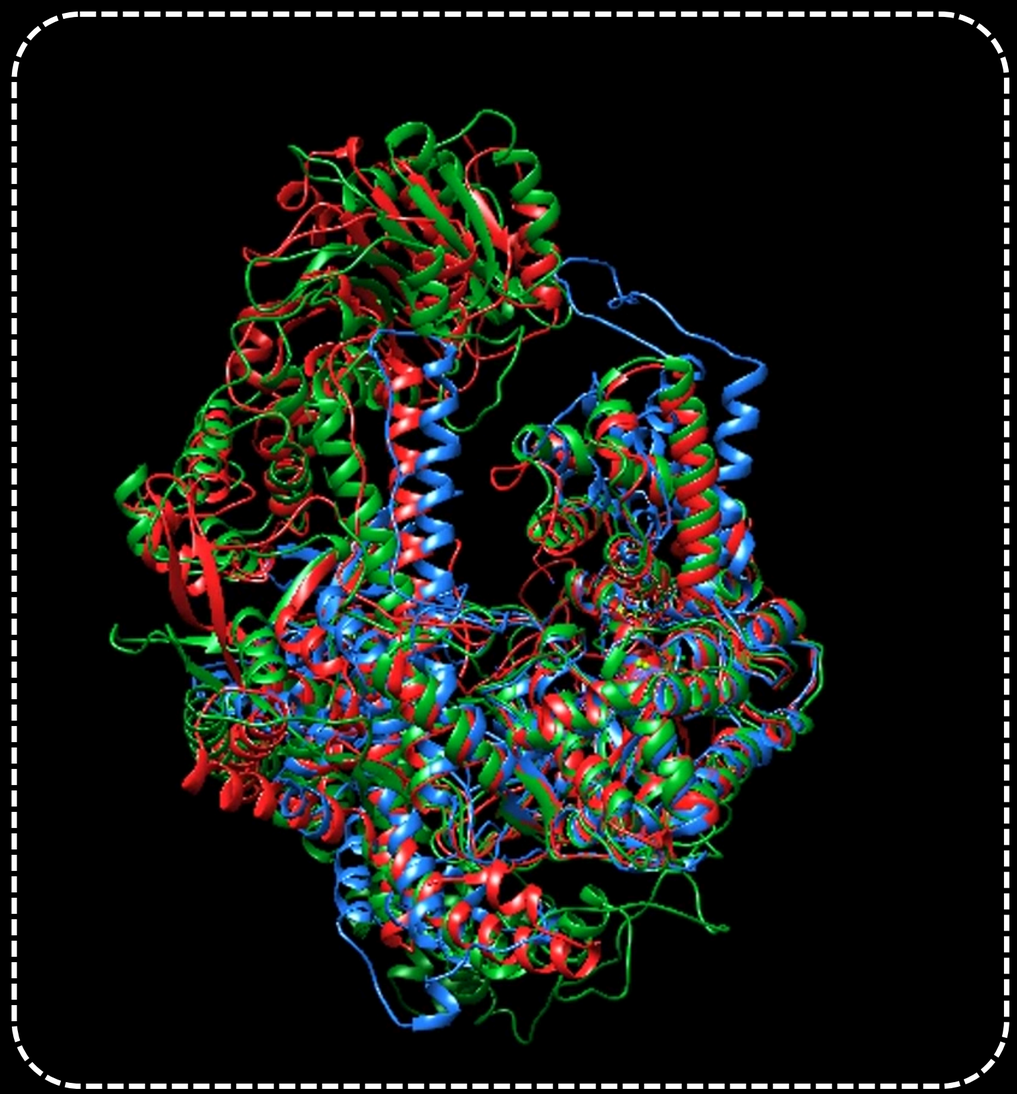
- Initiation complex with MTF1
- Conserved “right-handed” catalytic core



PDB: 1QLN chain A

Resolution: 2.40 Å

- Autonomous monomeric RNAP (no auxiliary factors)
- Direct promoter recognition
- Well-defined specificity loop
- Archetypal catalytic core



Images from Chimera

EVOLUTION & CONSERVATION

```
     No.  Domain1         Domain2         Sc     RMS    Len1 Len2  Align NFit Eq. Secs.    %I   %S    P(m)
Pair   1  9gzn           6ymv            4.81   1.54   1000  913   981  636 625    0  41.44 100.00 8.12e-94
Pair   2  9gzn           1qln            3.63   2.05   1000  862  1048  496 451    0  33.48 100.00 2.50e-41
Pair   3  6ymv           1qln            4.67   2.05    913  862  1007  567 549    0  31.69 100.00 2.05e-44
Reading in matrix file POLRMT.mat...
Doing cluster analysis...
Cluster:  1 (    9gzn  &     6ymv ) Sc  4.79 RMS   1.54 Len 984 nfit 638
 See file POLRMT.1 for the alignment and transformations
Cluster:  2 (    1qln  &     9gzn     6ymv ) Sc  6.66 RMS   2.74 Len 1147 nfit 624
 See file POLRMT.2 for the alignment and transformations
```

9GZN - 6YMW

- Very similar structures
- Strong structural conservation → same catalytic core.

9GZN - 1QLN

- Structural similarity, but less than that between humans and yeast.
- It retains the "right-hand" type nucleus.

6YMW -1QLN

- Catalytic core is structurally conserved.

EVOLUTION & CONSERVATION

“RIGHT-HAND” ARCHITECTURE

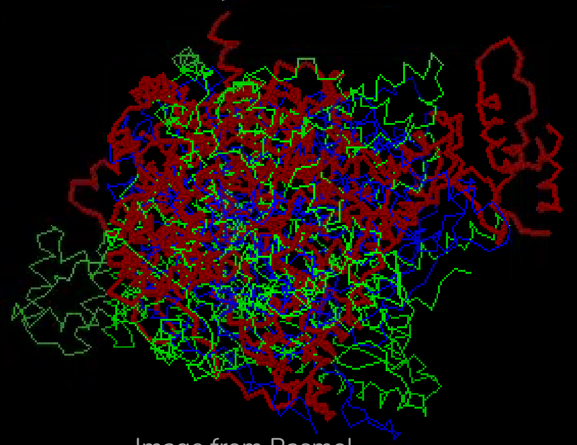
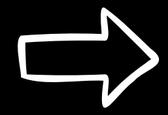


Image from Rasmol

In the central zone there are many atoms occupying almost the same space.



Image from Chimera



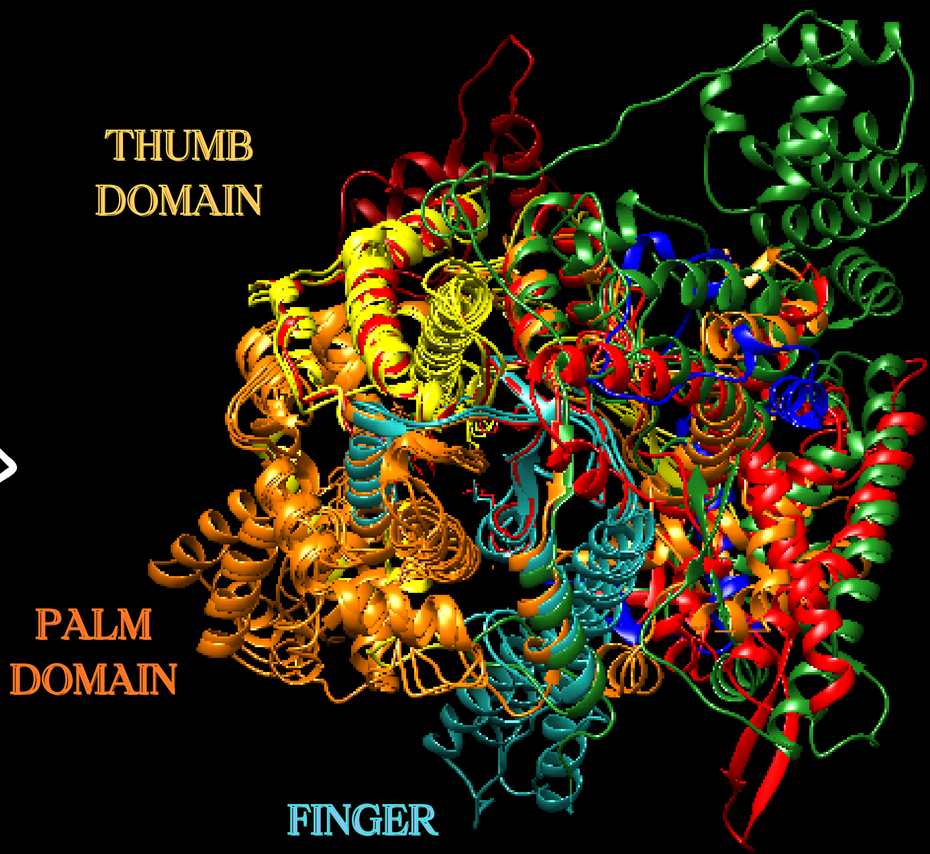


Image from Chimera

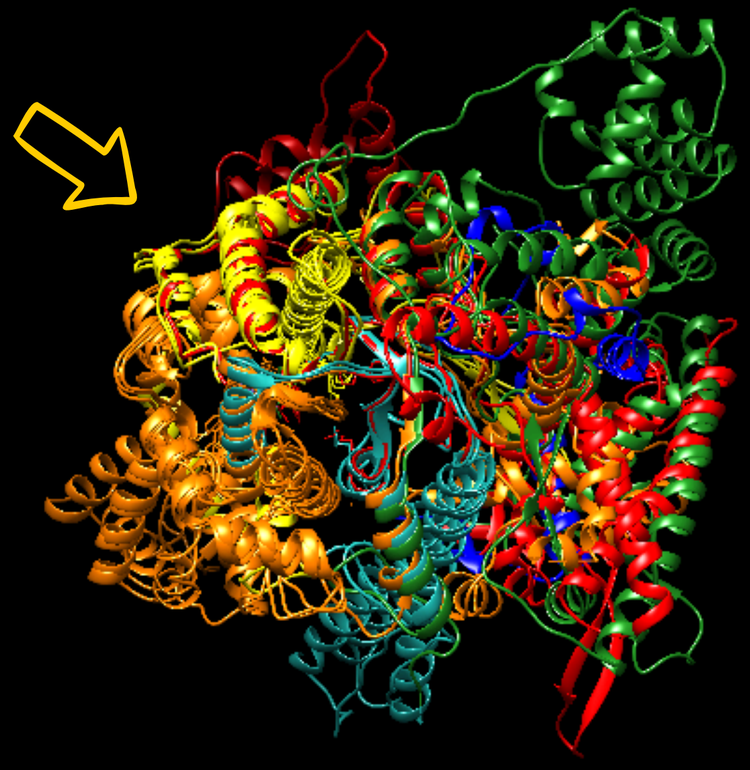
EVOLUTION & CONSERVATION

THUMB DOMAIN



Stands out for:

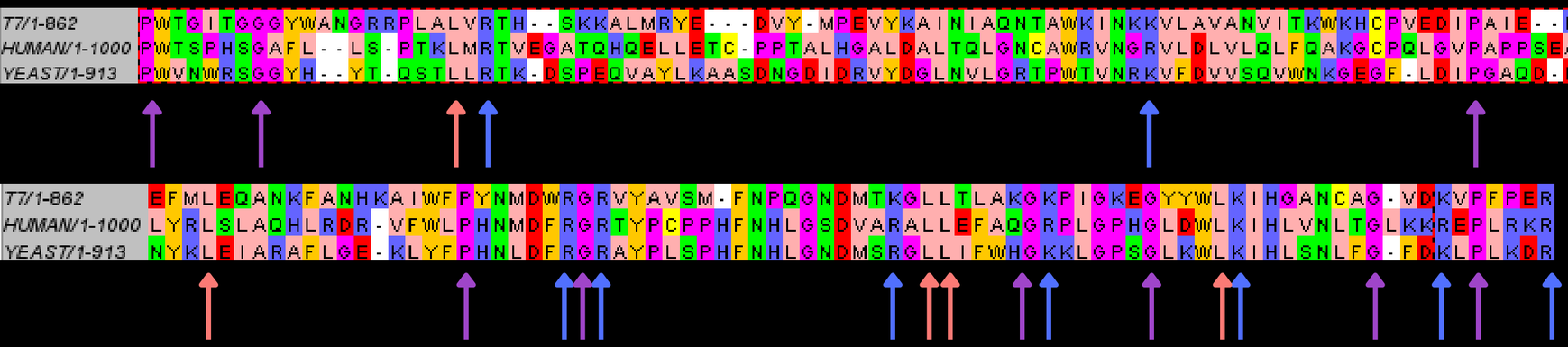
- Hydrophobic residues → maintain the helical scaffold and hydrophobic core.



In bacteriophage T7 RNA polymerase, the thumb domain exclusively stabilizes nucleic acids. In human and yeast POLRMT, although not a primary factor-binding site, it **contributes to the structural organization of the transcription initiation complex.**

EVOLUTION & CONSERVATION

FINGERS DOMAIN



Stands out for:

- **Lys / Arg** → allow interaction with the phosphates of the NTP.
- **Gly** → contribute to conformational flexibility during fingers closing.
- **Leu / Ile** / etc → maintain the helical scaffold and hydrophobic core.

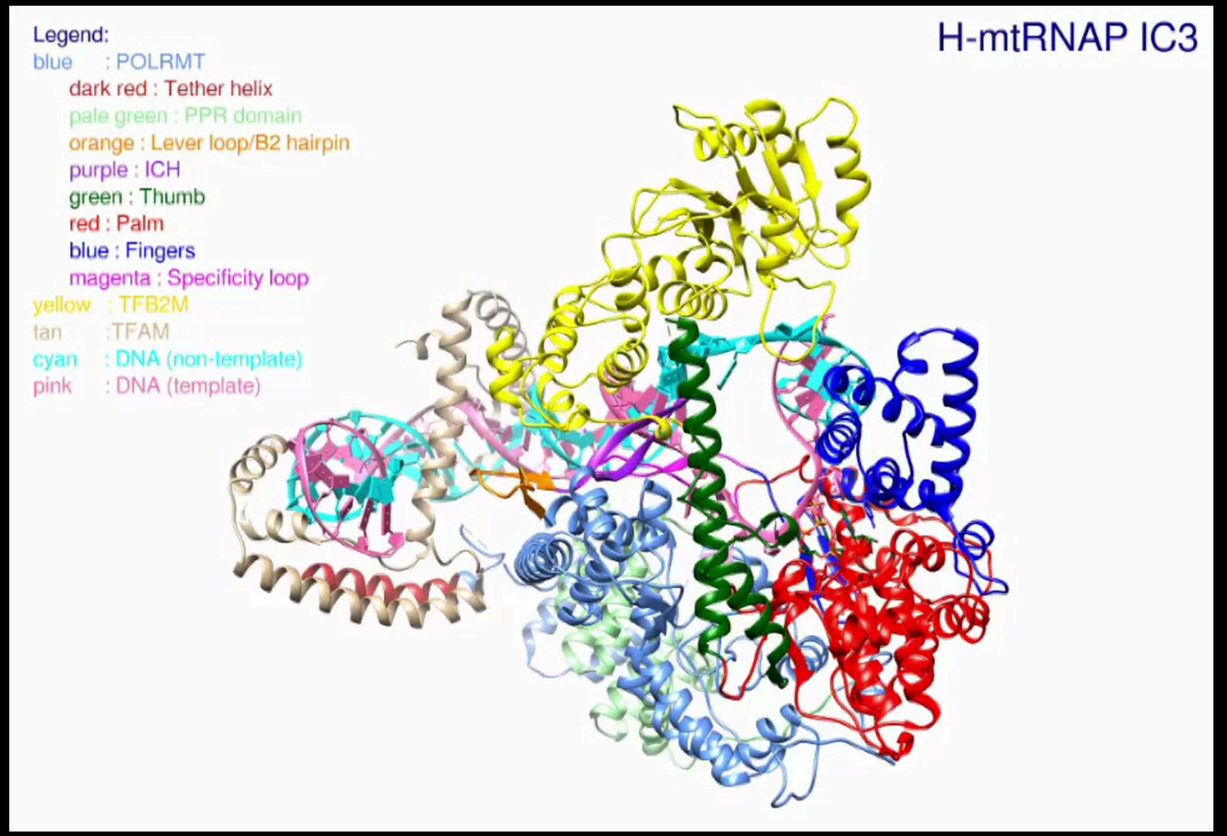
Functional conservation of physicochemical properties within the Fingers domain.



EVOLUTION & CONSERVATION

Structural reorganization associated with the opening and closing of the fingers domain





1. POLRMT bound to DNA and Finger domain is in **open conformation**
2. Nucleotide entry
3. Nucleotide recognition and **closing**
4. Catalytic alignment
5. Reopening

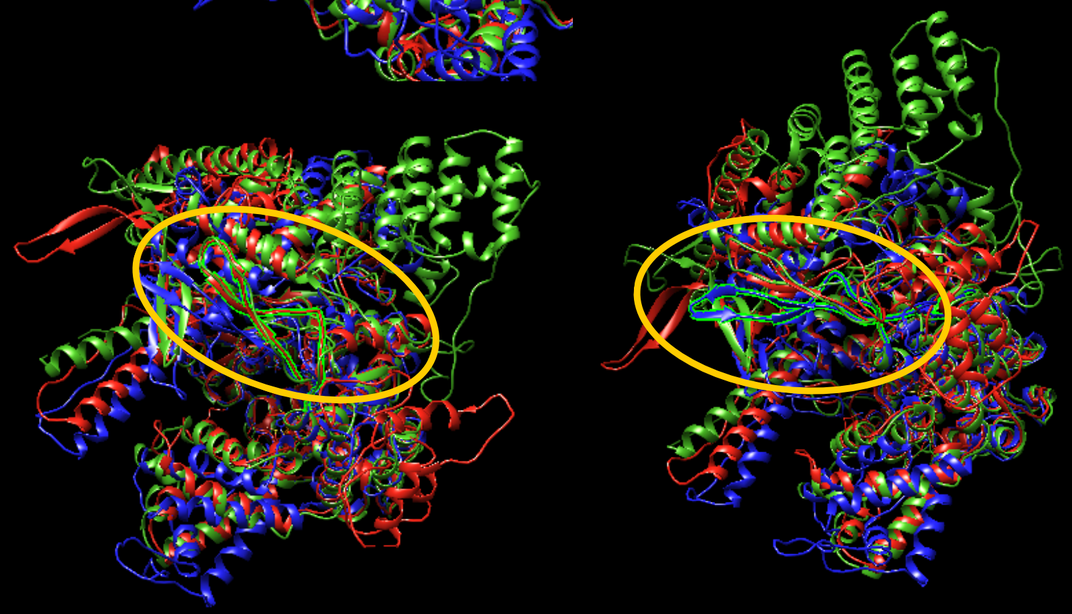
Shen et al., Mol. Cell 85, 3137–3150 (2025)

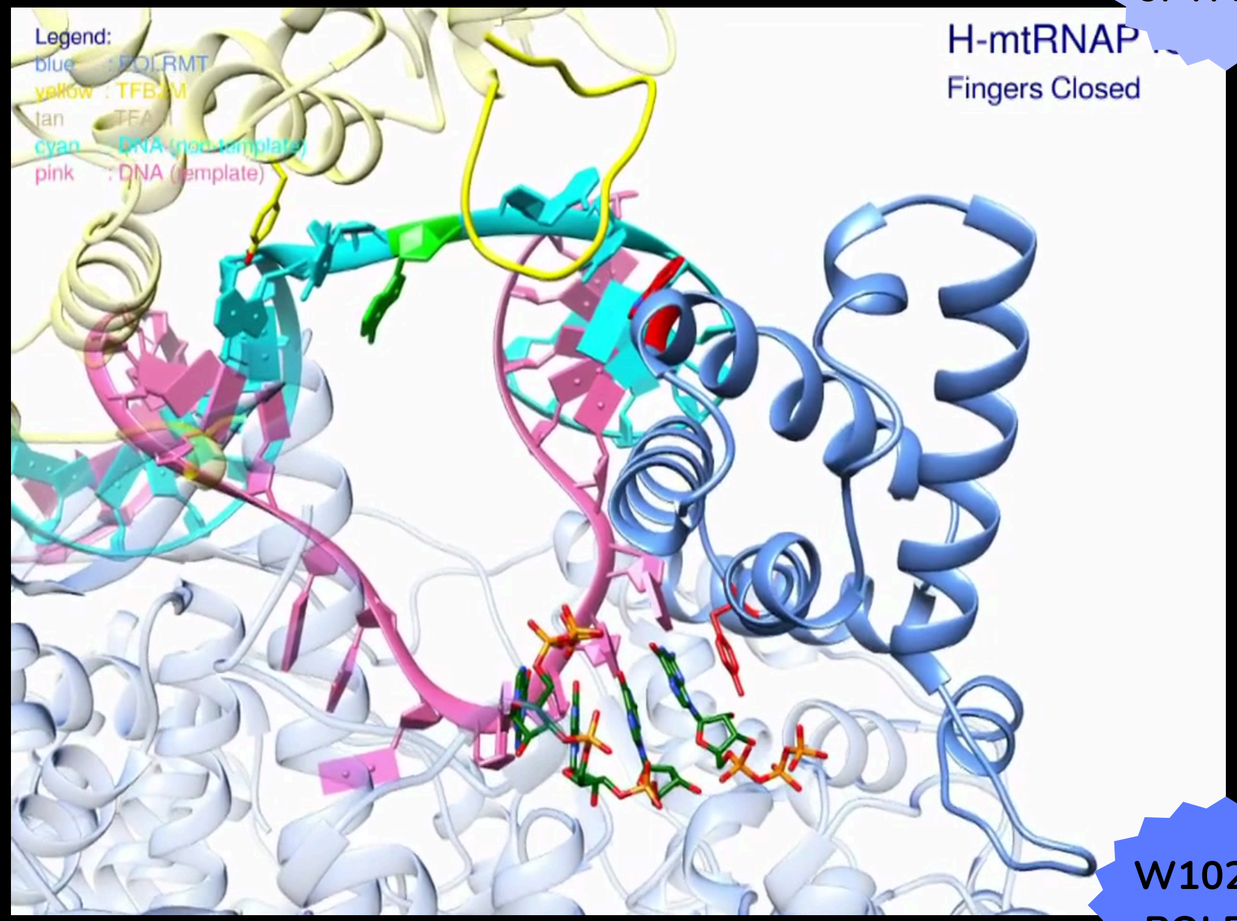
EVOLUTION & CONSERVATION

Specificity loop/ Stabilizing loop



Human (res. 1085-1108)





Y209 of TFB2M

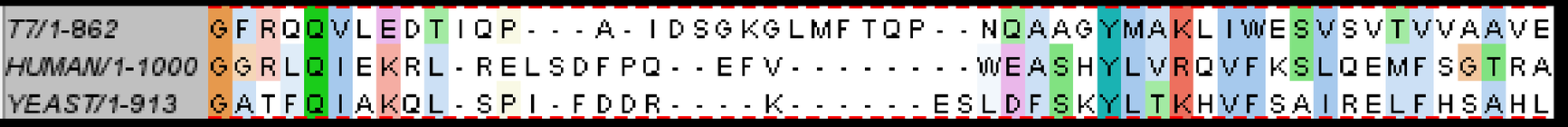
W1026 of POLRMT

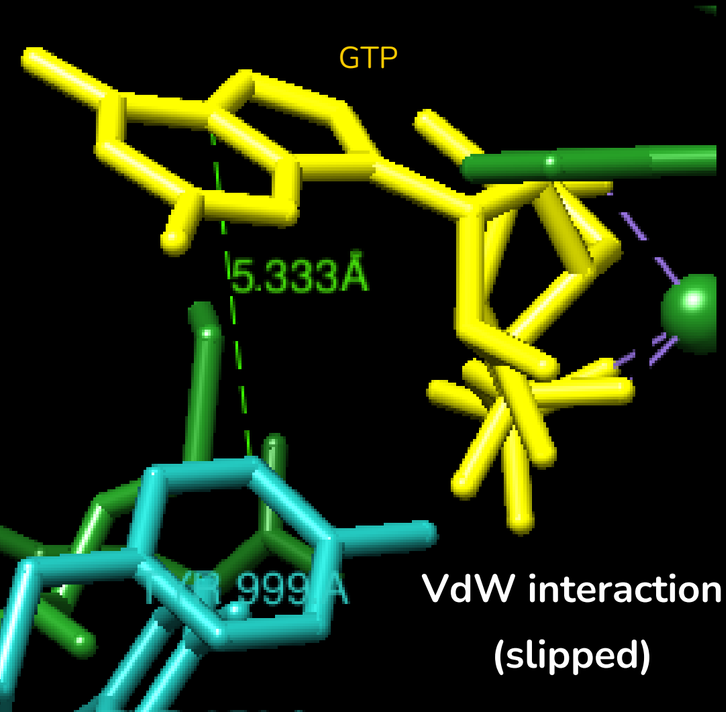
Shen et al., Mol. Cell 85, 3137–3150 (2025)

EVOLUTION & CONSERVATION

O-helix

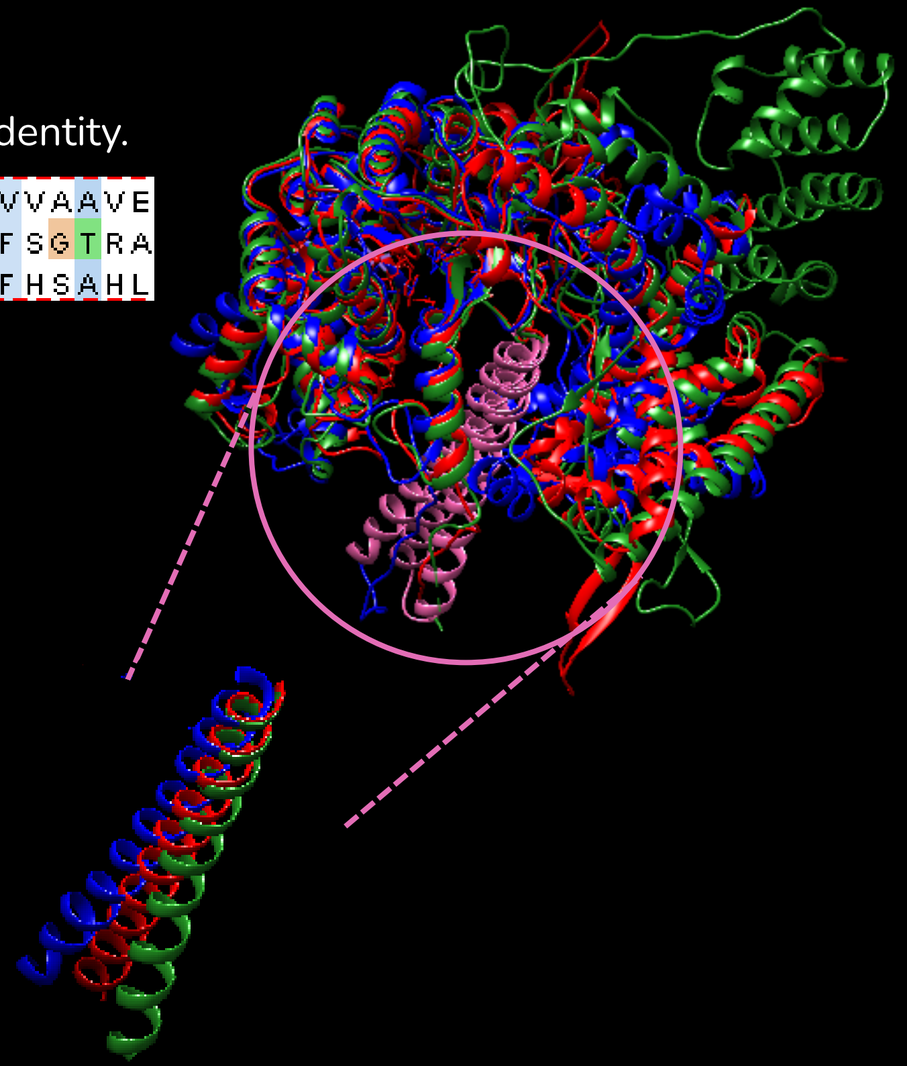
Structurally and functionally preserved, but they do not have the same sequence identity.





VdW interaction

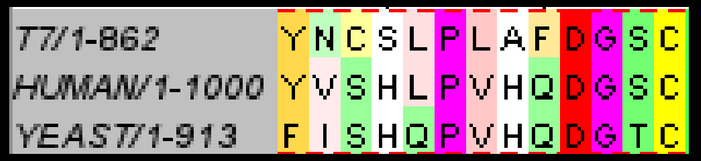
(slipped)



EVOLUTION & CONSERVATION

CATALYTIC CORE

PALM DOMAIN

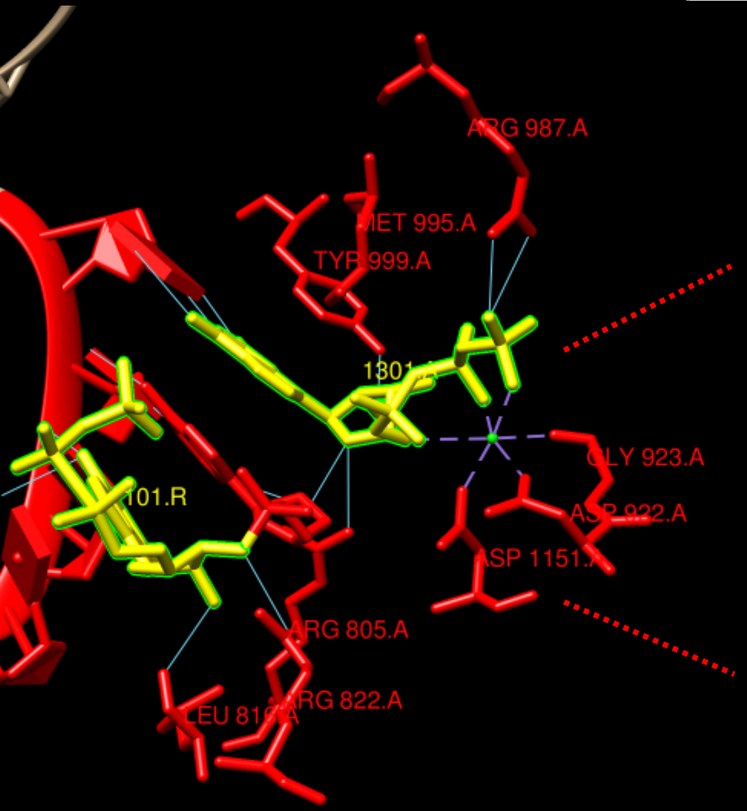


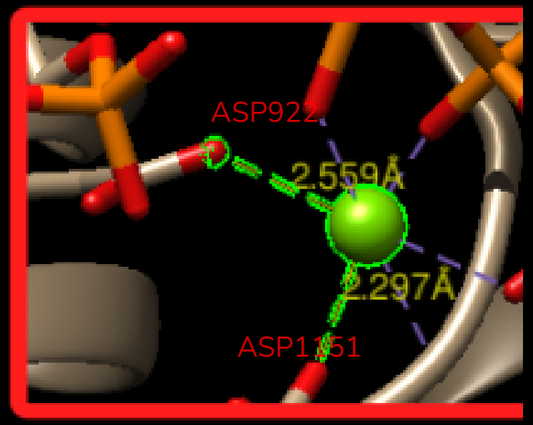
Asp 922

...



Asp 1151



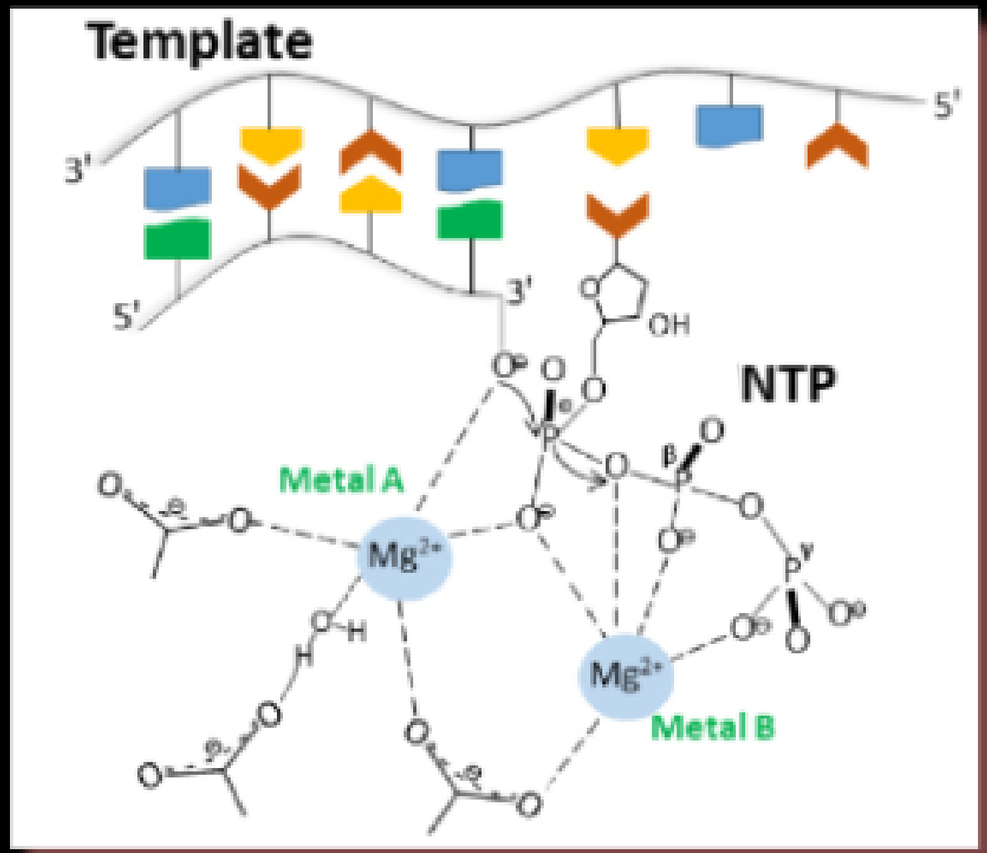


Electrostatic interaction

- Catalytic Asp residues are conserved across species
- Mg^2 coordination is maintained
- Active-site architecture is structurally preserved
- Peripheral regions show higher variability

EVOLUTION & CONSERVATION

Two-metal ion mechanism in the catalytic Palm domain of POLRMT



The conserved residues **Asp922 and Asp1151** coordinate two **Mg^{2+} ions** within the active site.

Metal A

↓

Activation of the 3'-OH of the nascent RNA strand, facilitating nucleophilic attack on the α-phosphate of the incoming NTP.

Metal B

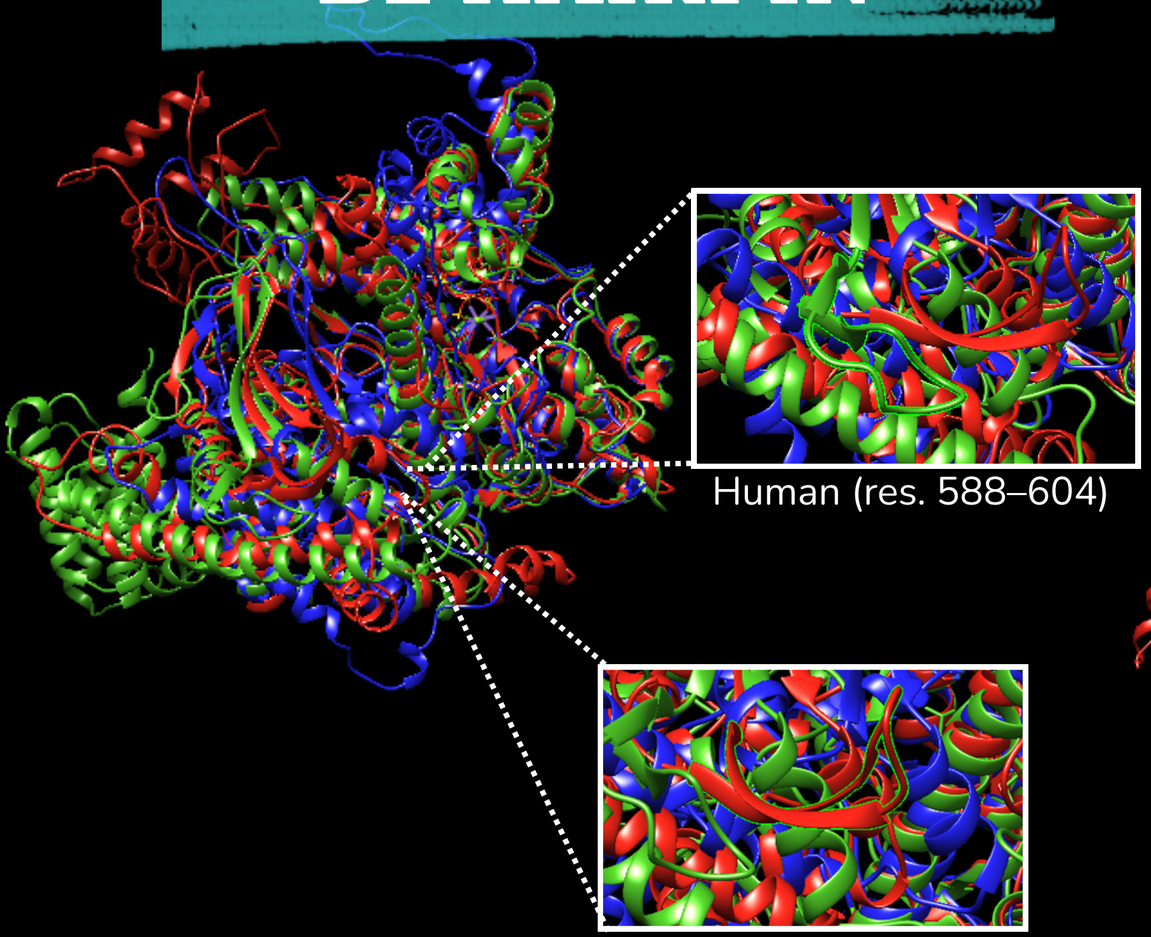
↓

Stabilizes the negatively charged during phosphodiester bond formation.

Modified from: Kuznetsova et al., 2022. Life Science Alliance 5(12):e202201428. https://doi.org/10.26508/lsa.202201428

EVOLUTION & CONSERVATION

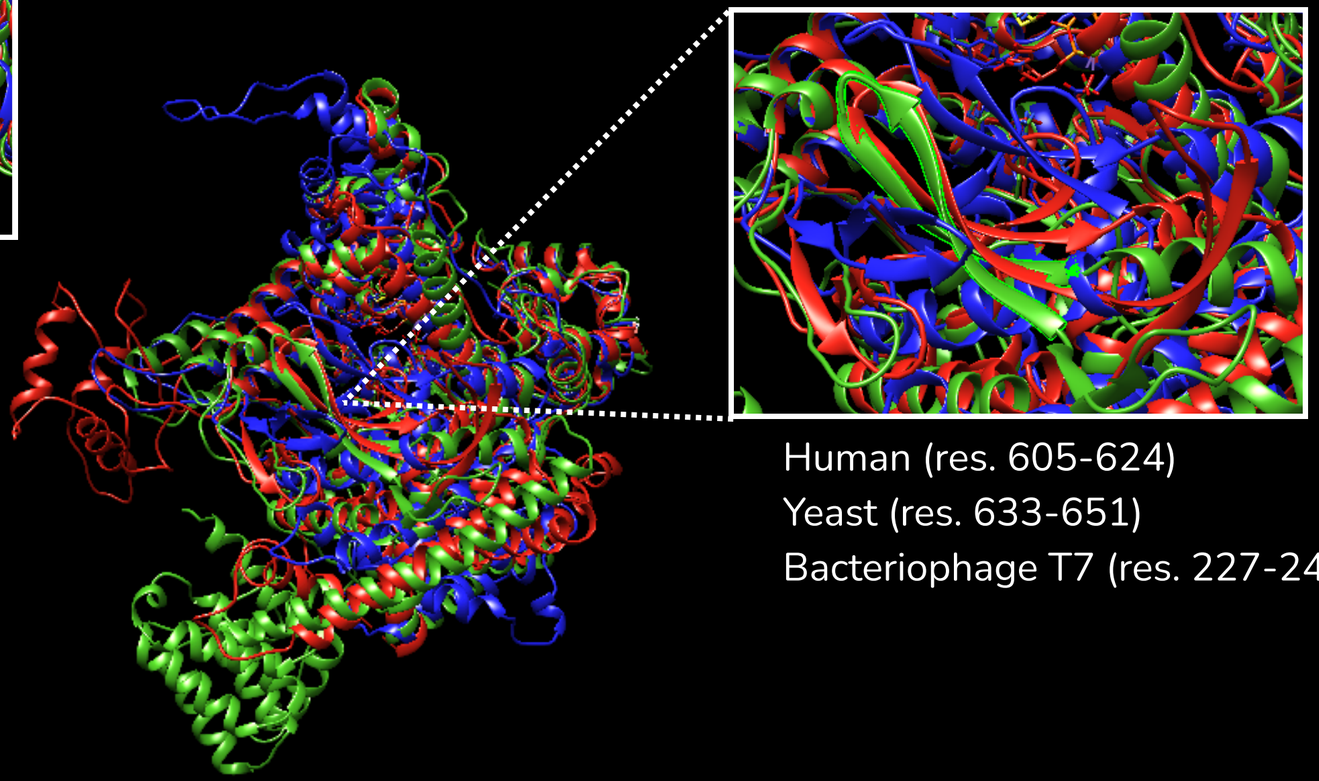
LEVER LOOP/ B2 HAIRPIN



Human (res. 588–604)

Yeast (res. 613-632)

INTERCALATING HAIRPIN

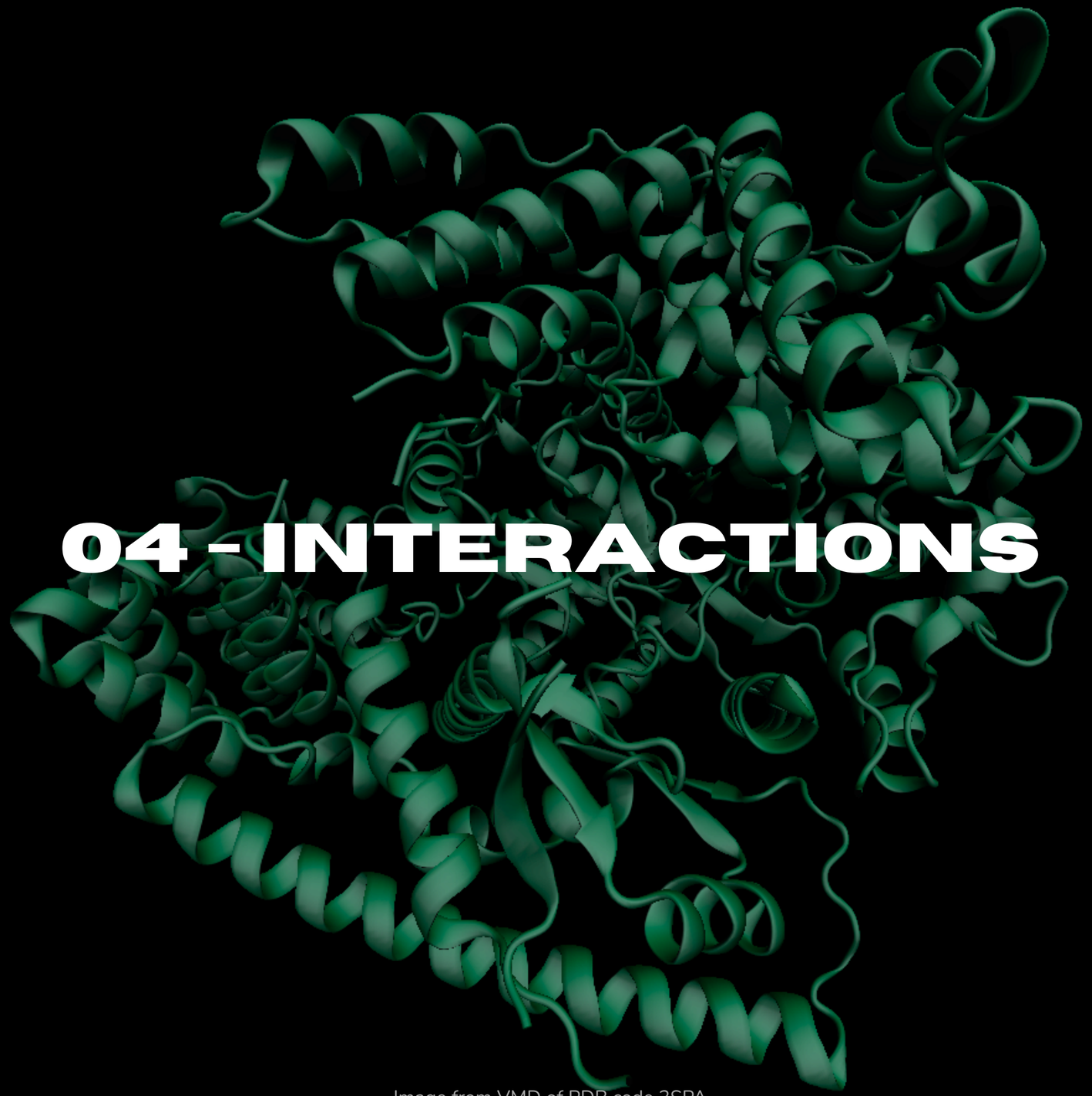


Human (res. 605-624)
Yeast (res. 633-651)
Bacteriophage T7 (res. 227-246)

EVOLUTION & CONSERVATION

N-TERMINAL EXTENSION

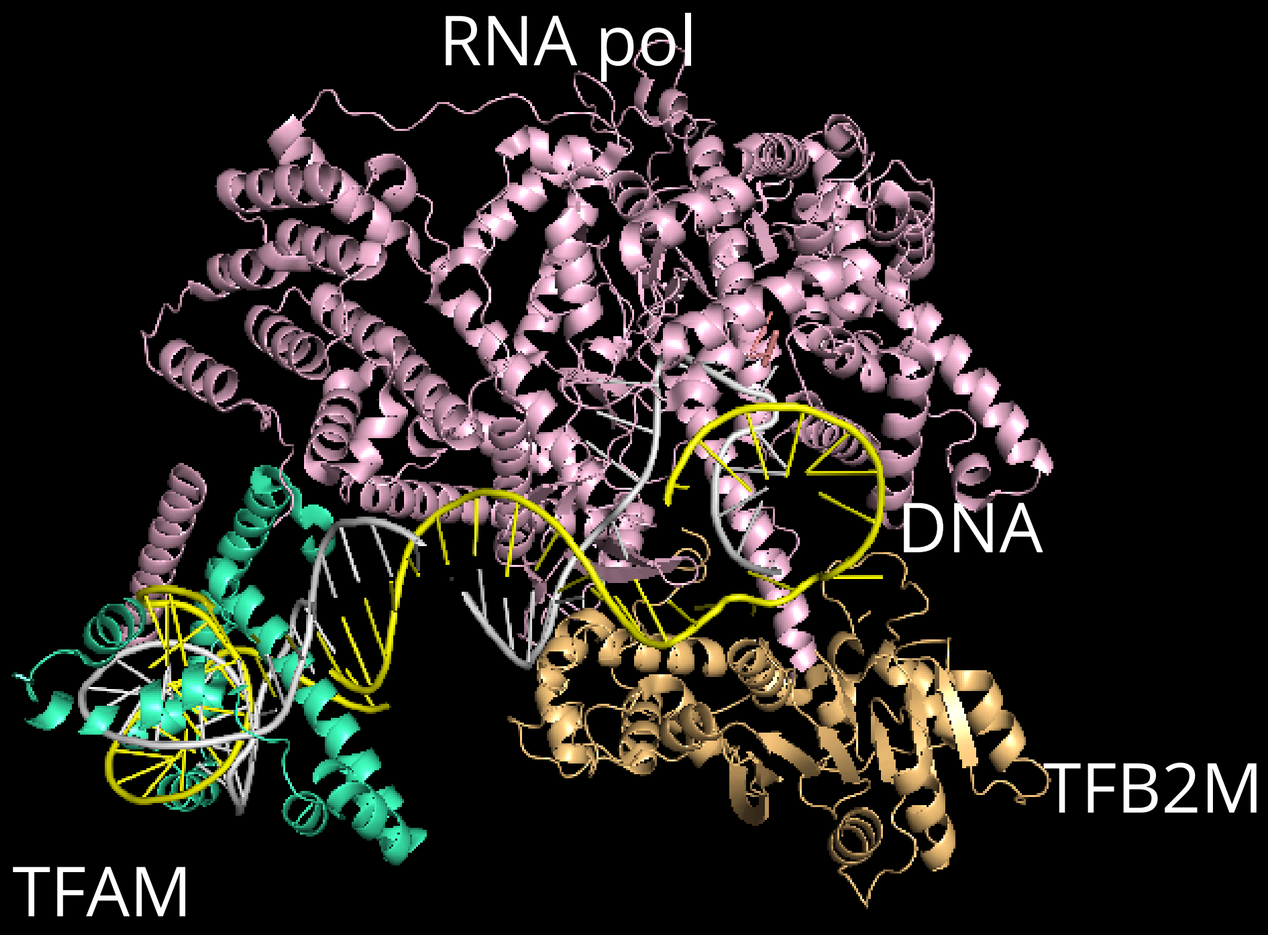
```
T7/1-862      - - -
```



04 - INTERACTIONS

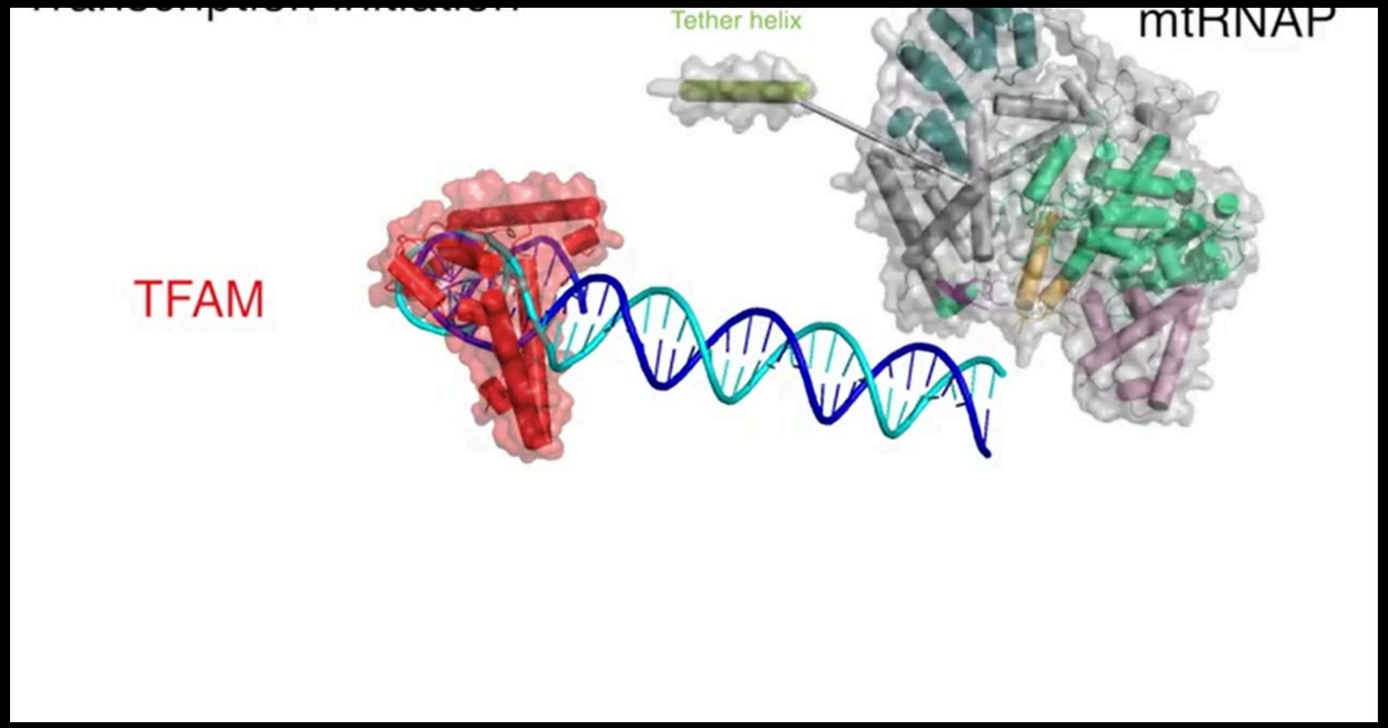
Image from VMD of PDB code 3SPA

PROMOTER MELTING BY TFAM & TFB2M

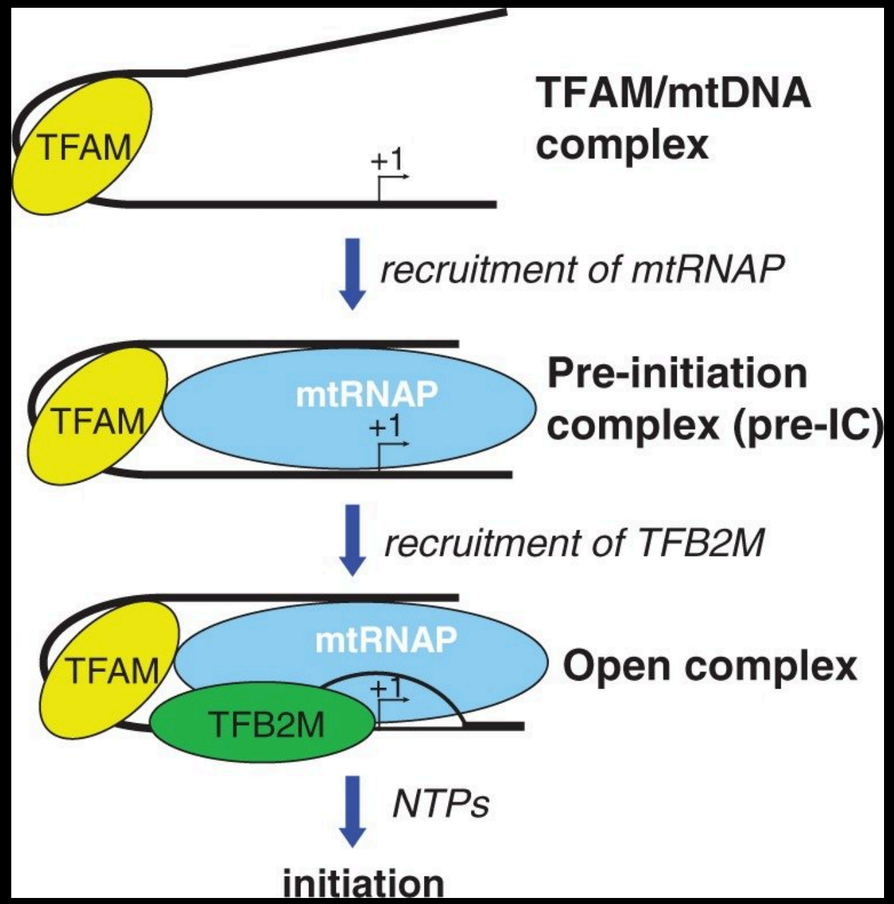


Images from Pymol using PDB code 9GZM

TFAM + TFB2M



https://www.youtube.com/watch?v=QmjCvqfSdXk

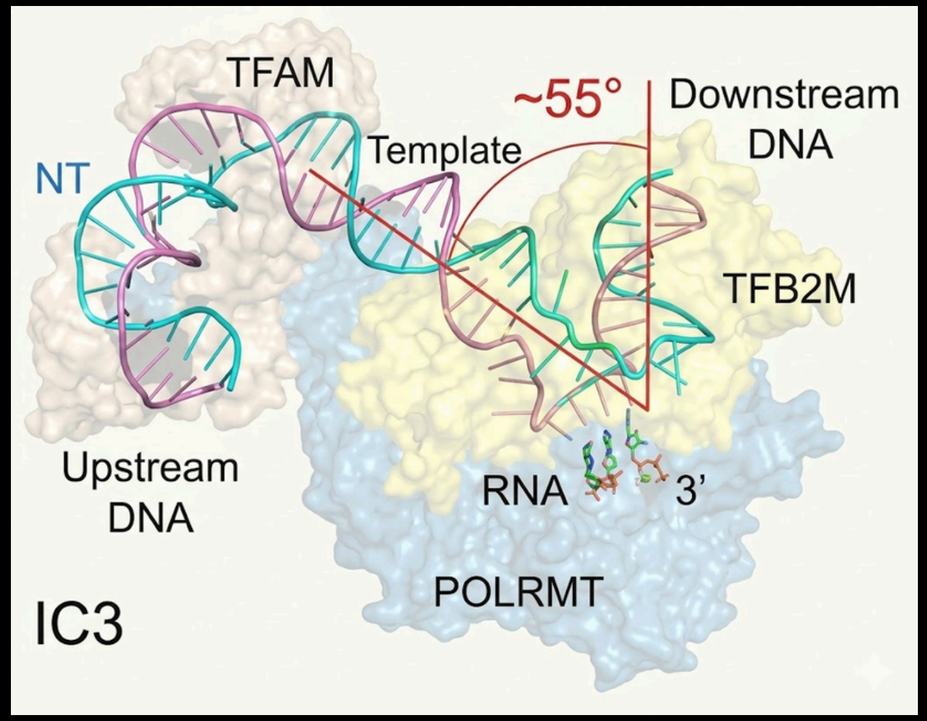


- POLRMT (core polymerase) → catalytic RNA polymerase

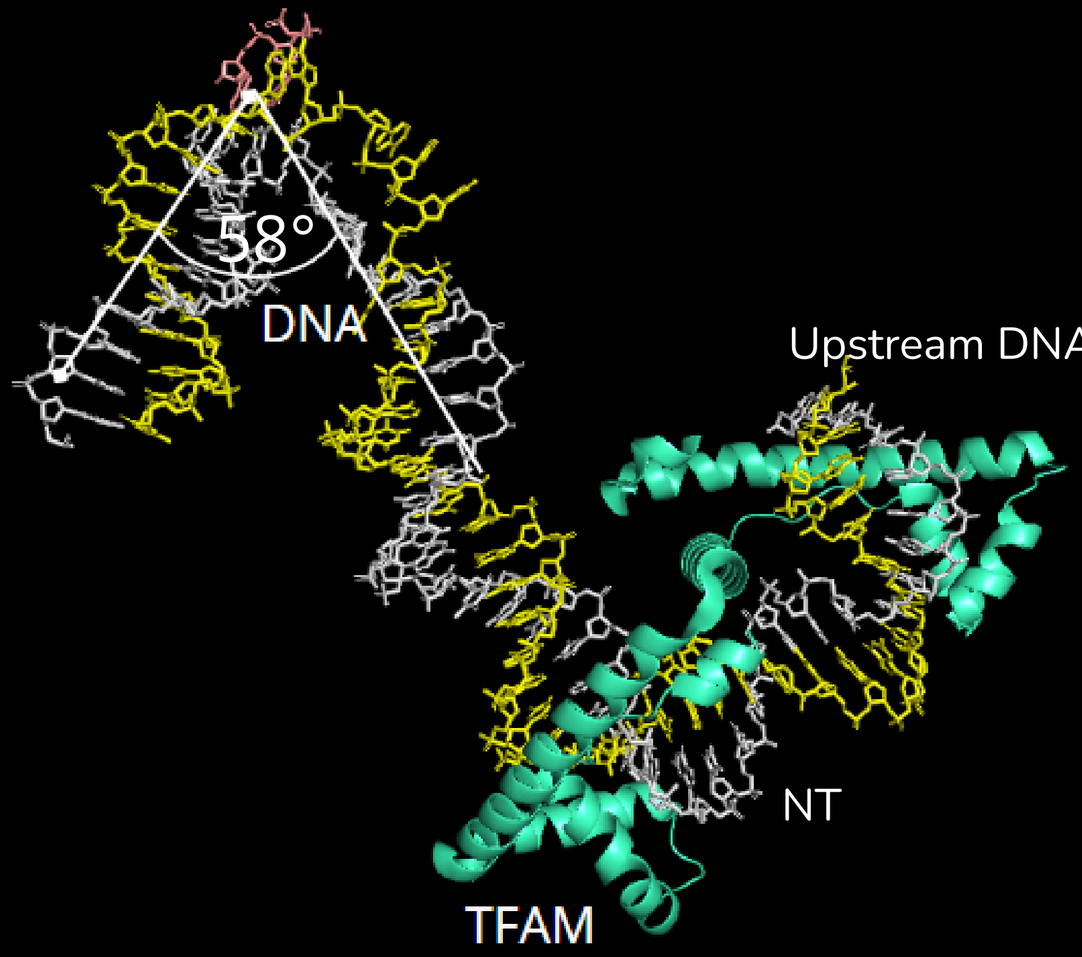
- TFAM : Binds upstream and bends the DNA template
  - → creates necessary torsional strain.
- TFB2M → Inserts near the TSS
  - Stabilizes the melted non-template strand.
  - Locks the -1A base to ensure initiation at +1

Morozov, Y. I., Agaronyan, K., Cheung, A. C., & Temiakov, D. (2014)

TFAM BENDS DNA (~55°)



https://pmc.ncbi.nlm.nih.gov/articles/PMC11642874/

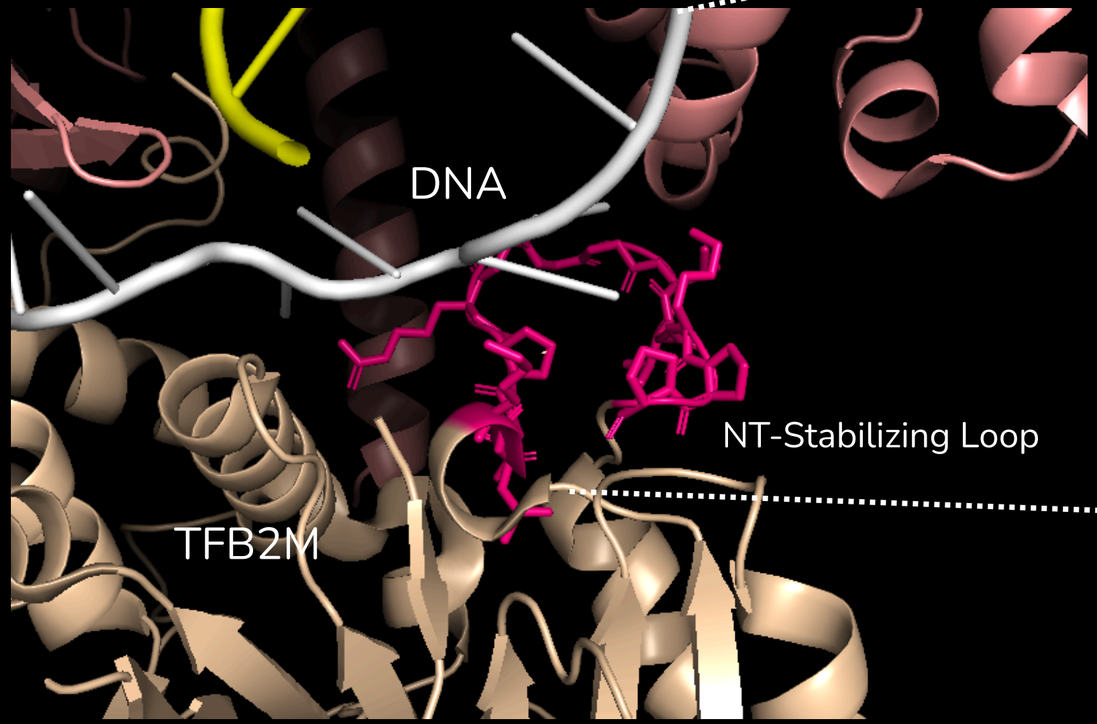


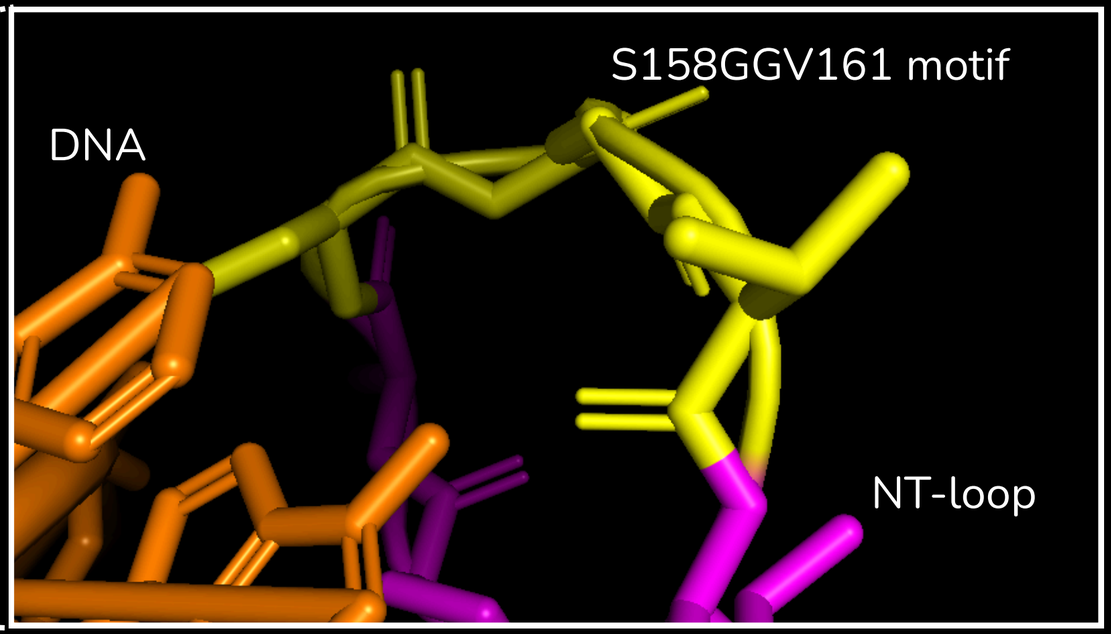
Images from Pymol using PDB code 9GZM

- Induces a ~55° bend via HMG-box binding to organize promoter geometry
- Reduces base-stacking energy to lower the barrier for DNA strand separation → prepares for "melting"
- Does NOT directly melt DNA

TFB2M STABILIZING LOOP

Once the promoter melts → non-template strand must be held open without collapsing back into duplex DNA → S158GG motif

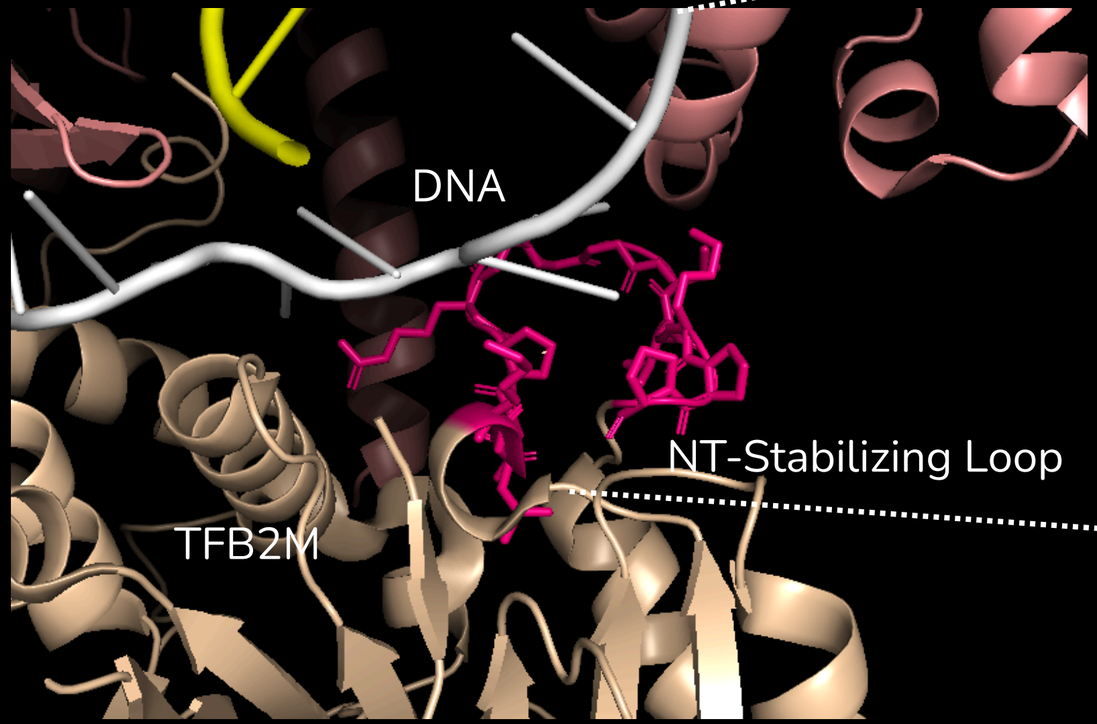


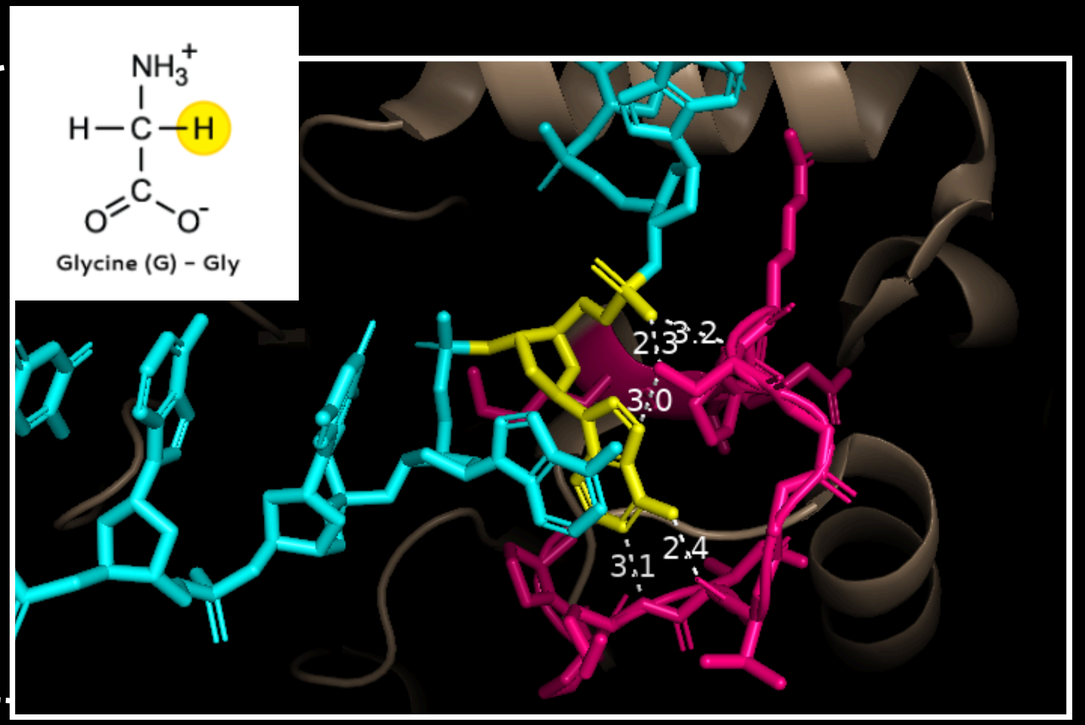


Images from Pymol using PDB code 9GZM

TFB2M STABILIZING LOOP

TFB2M stabilizes the non-template strand via backbone-mediated hydrogen bonds





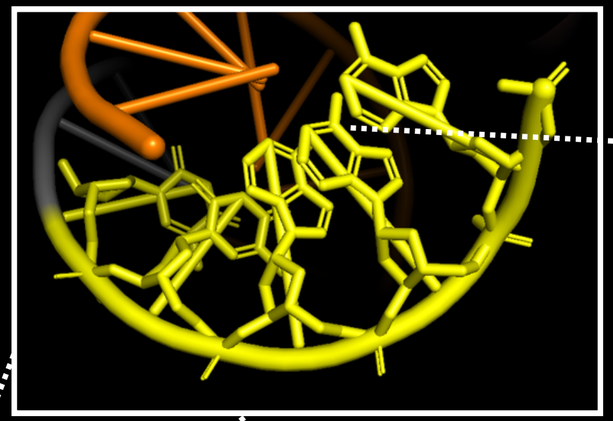
-1A

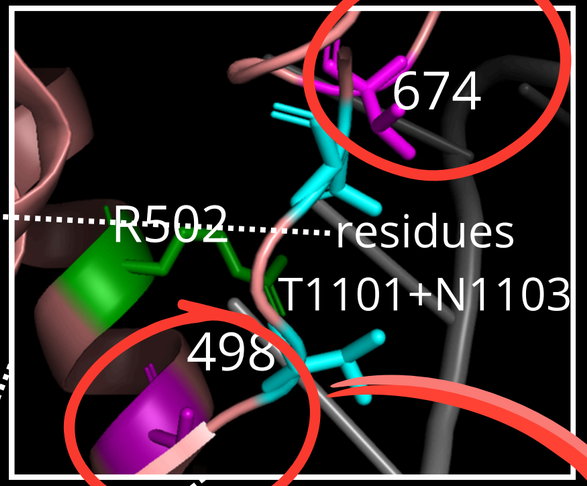
Hydrogen Bonds (H-bond Distances)

Images from Pymol using PDB code 9GZM

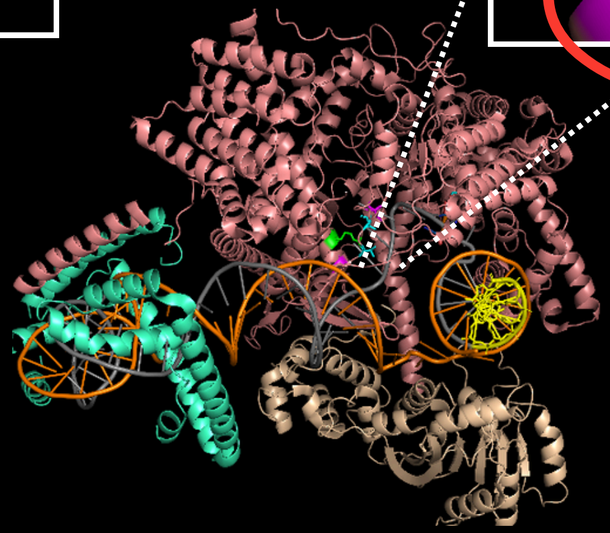
TEMPLATE STRAND RECOGNITION

DNA Promoter





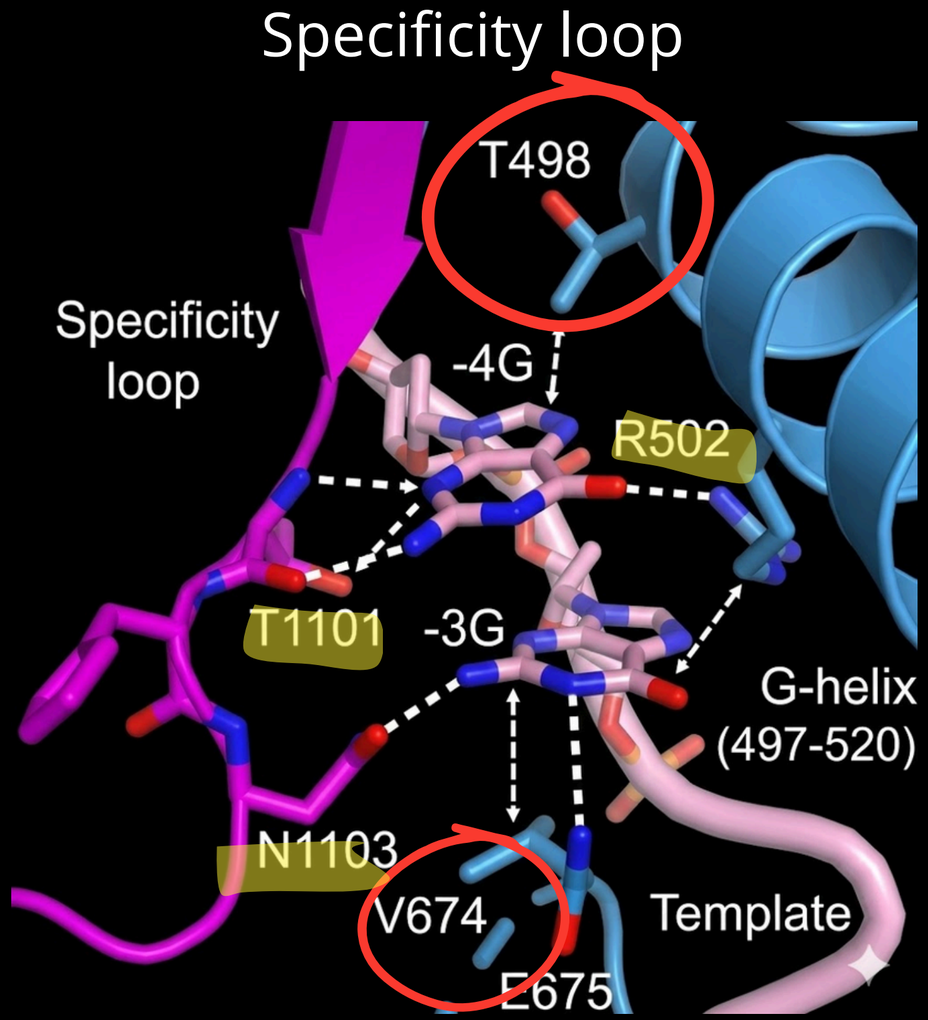




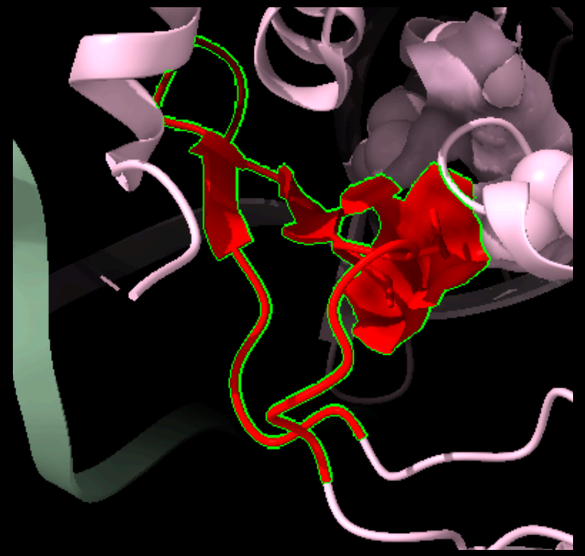
HYDROPHOBIC SANDWICH

Images from Pymol using PDB code 9GZM

TEMPLATE STRAND RECOGNITION

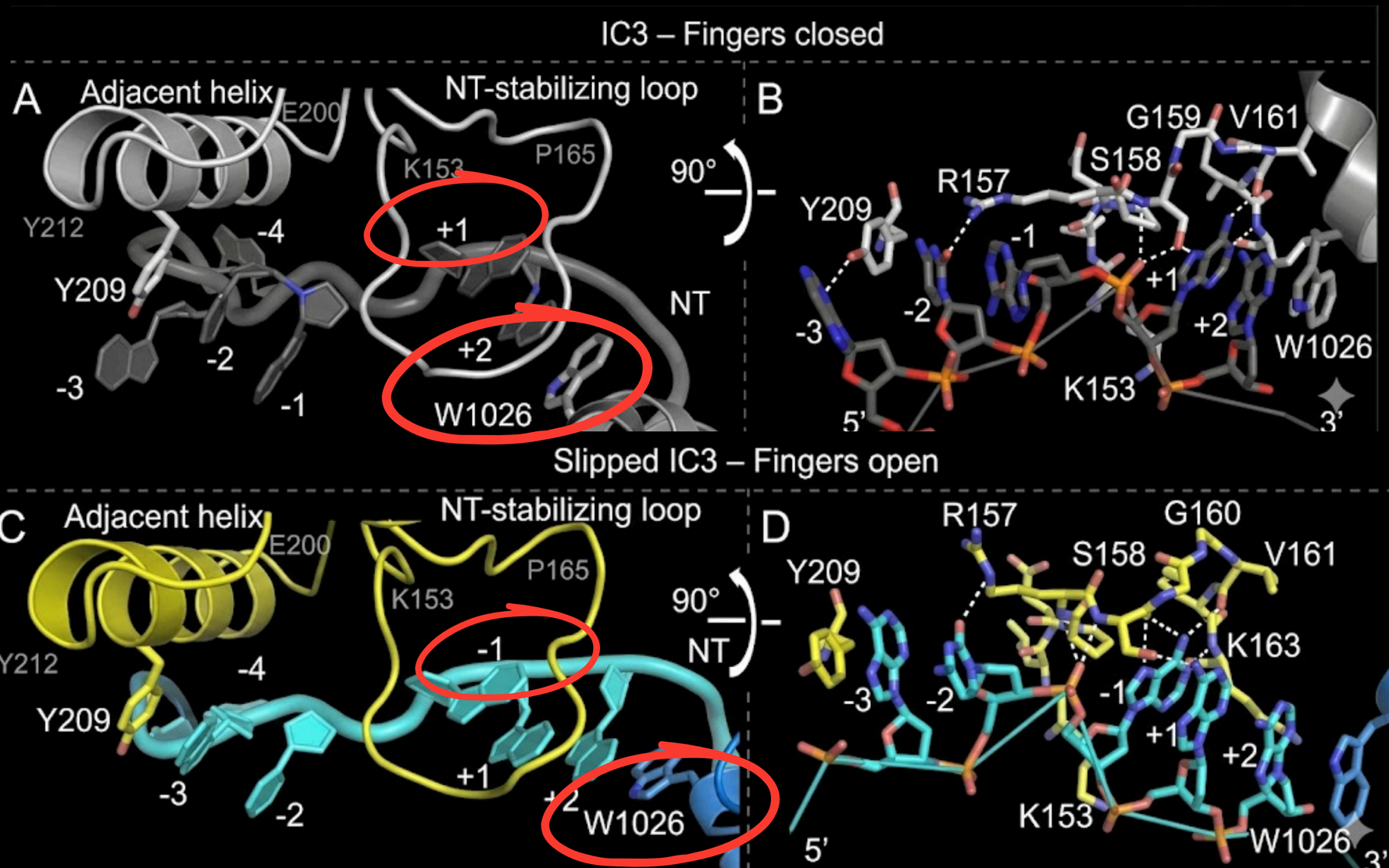


- POLRMT reads promoter bases –4G and –3G on template strand
- H-bonds: T1101 (–4G), N1103 (–3G)
- R502 double-checks both bases
- Hydrophobic “sandwich”: T498 + V674 stabilizes DNA environment
- Specificity loop contributes to promoter sequence verification



Images from Pymol using PDB code 9GZM

DYNAMIC NT STRAND REPOSITIONING (W1026 + Fingers Domain)



Images from Shen, J., Goovaerts, Q., Ajjugal, Y., et al. (2024). Human mitochondrial RNA polymerase structures reveal transcription start-site and slippage mechanism. PMC11642874, Figure 2. Licensed under CC BY-NC-ND 4.0.

05 - CONCLUSIONS



Image from VMD of PDB code 3SPA

TAKE HOME MESSAGE

- POLRMT retains the ancestral **T7-like right-hand architecture**, confirming its evolutionary origin from bacteriophage-type polymerases.
- Catalytic regions are highly conserved driven by **two-metal ion mechanism** that cooridinates Mg^{2+} and enables phosphodiester bond formation.
- The **N-terminal region** has diversified, display greater variability, reflecting their regulatory specialization to **adapt better to the mitochondrial promoter environment.**
- Human mithocondrial transcription is **factor-dependent**, requiring **TFAM** for promoter architecture and **TFB2M** for DNA melting and dynamic stabilization of initiation complex.

Image from VMD of PDB code 3SPA



THANK YOU!
ANY QUESTIONS?

Image from VMD of PDB code 3SPA

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Ringel, R., Sologub, M., Morozov, Y. I., Litonin, D., Cramer, P., & Temiakov, D. (2011). Structure of human mitochondrial RNA polymerase. Nature, 478(7368), 269–273. https://doi.org/10.1038/nature10435

Shen, J., Goovaerts, Q., Ajjugal, Y., De Wijngaert, B., Das, K., & Patel, S. S. (2025). Human mitochondrial RNA polymerase structures reveal transcription start site and slippage mechanism. Molecular Cell, 85(16), 3137–3150. https://doi.org/10.1016/j.molcel.2025.07.XXX

Image from VMD of PDB code 3SPA

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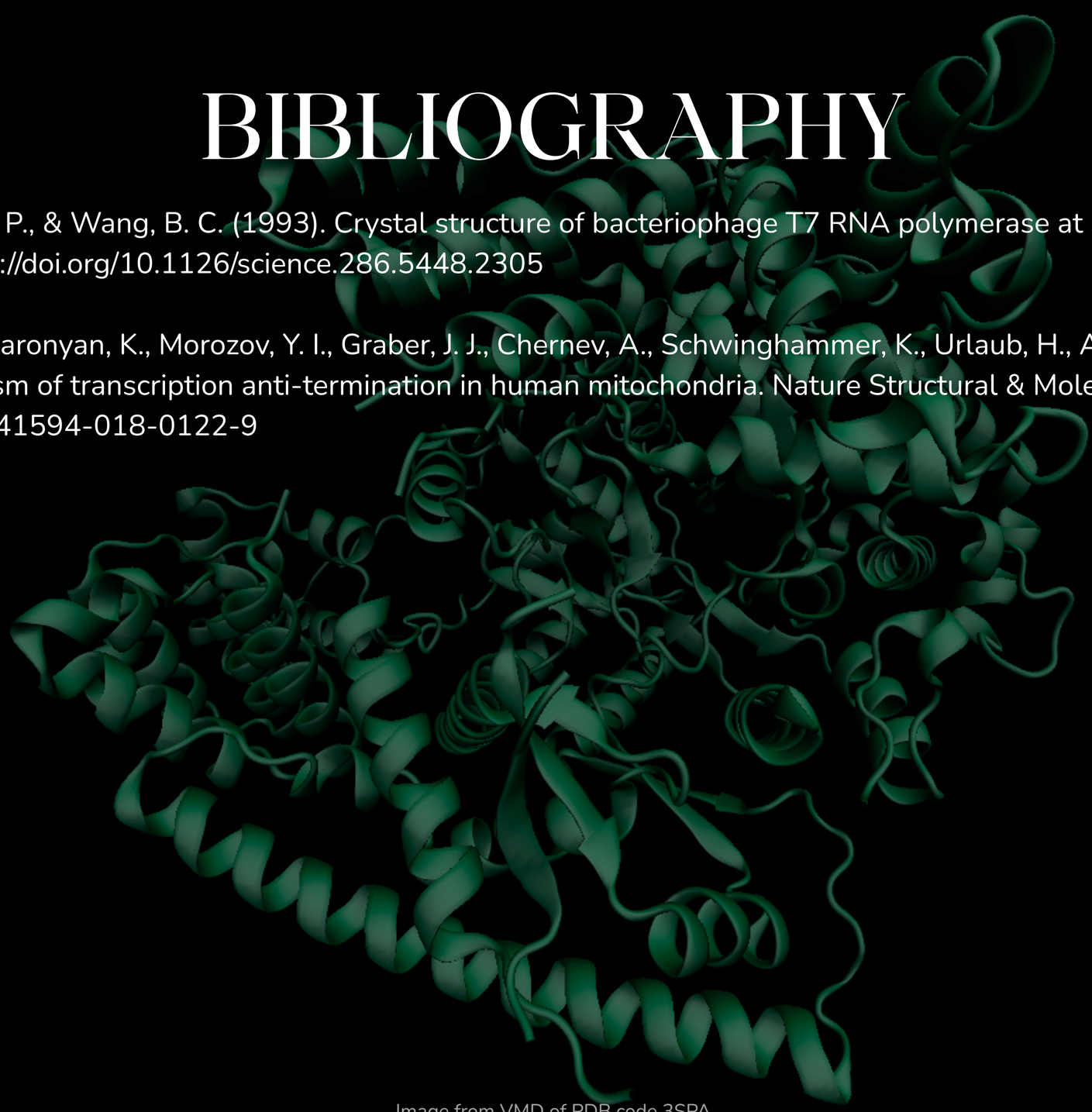


Image from VMD of PDB code 3SPA

PEM

1. Which of the following choices are correct in relationship to the catalytic core of POLRMT?

a) It adopts a right-hand architecture organized into thumb, palm, and fingers subdomains.
b) It belongs to the nucleotide cyclase/DNA polymerase palm domain-like superfamily.
c) Both above are correct.
d) It is structurally unrelated to the bacteriophage T7 RNA polymerase family.
e) All are correct.

2. In which CTD subdomain is the catalytic active site located?

a) Thumb
b) Fingers
c) Palm
d)PPR
e) N-terminal extensio

3. What structural feature supports the bacteriophage-type evolutionary origin of POLRMT?

a) The presence of multiple subunits
b) **The “right-hand” architecture**
c) Dependence on nuclear factors
d) The presence of PPR domains
e) Mitochondrial localization

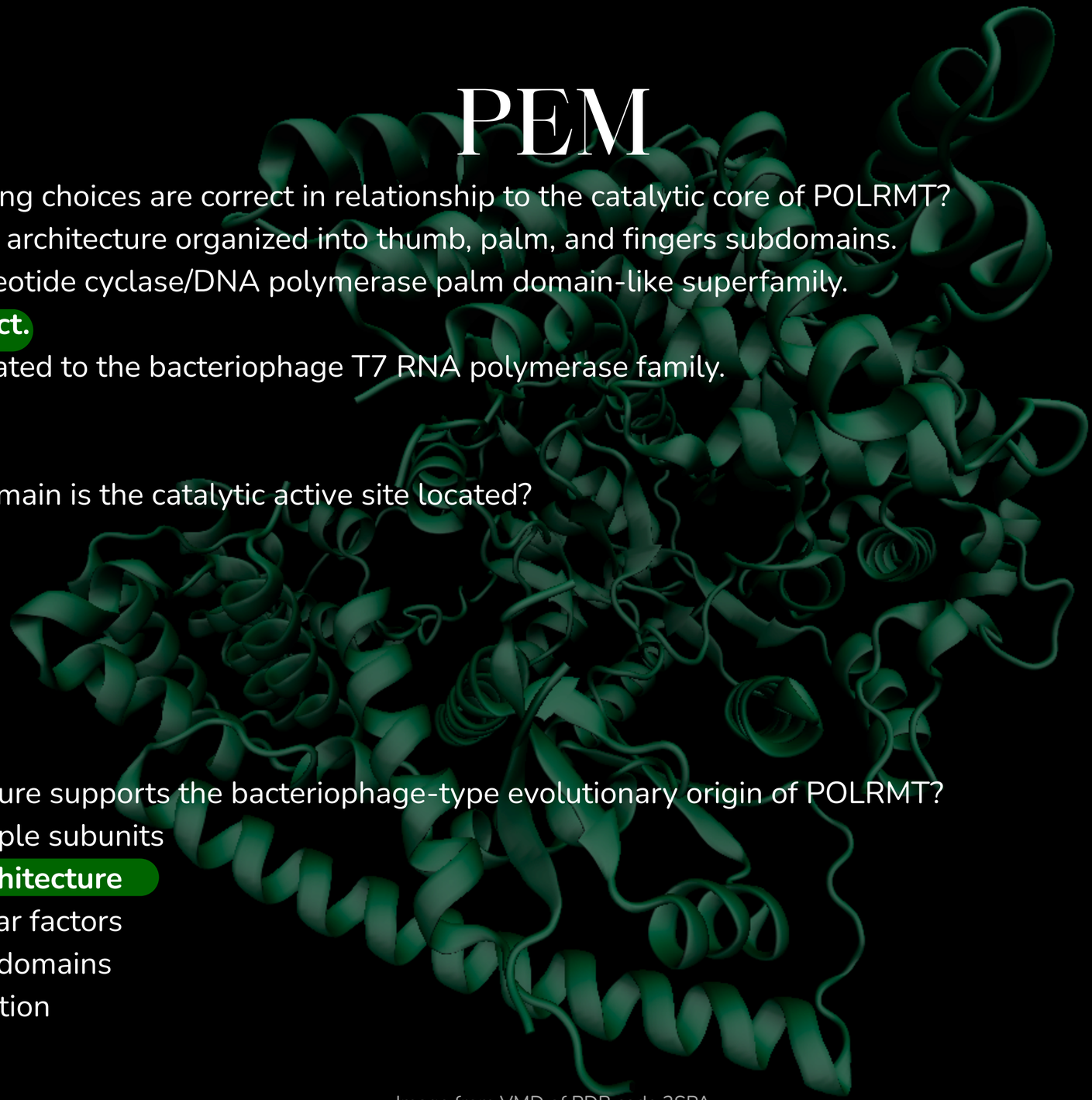


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4. How does the "intercalating hairpin" contribute to mitochondrial transcription initiation?

a) It sequesters nascent RNA to prevent rehybridization with the template.

b) It acts as a physical wedge to separate DNA strands and stabilize the transcription bubble.

c) It recruits the TEFM factor to the elongation complex.

d) It provides the Mg2+ ions necessary for polymerization.

e) It links the N-terminal extension to the PBD.

5. According to the sequence alignment analysis, which region of POLRMT is the least conserved across species?

a) The palm subdomain of the catalytic core.

b) The C-terminal catalytic domain (CTD).

c) The O-helix and Y-helix regions.

d) The N-terminal end, particularly the regulatory regions.

e) The residues Asp922 and Asp1151.

6. **How does TFB2M stabilize the open state of the transcription initiation complex?**

a) By forming rigid aromatic stacking interactions with the +2 base.

b) **By using its NT-stabilizing loop to form backbone-mediated hydrogen bonds with the non-template -1 adenine**.

c) By enzymatically cleaving the DNA template strand.

d) By acting as a chaperone that folds the N-terminal extension (NTE).

e) By phosphorylating the thumb domain of POLRMT.

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7. What is the primary structural role of TFAM during mitochondrial transcription initiation?

a) Catalyzing phosphodiester bond formation
b) Coordinating Mg^{2+} ions
c) Bending promoter DNA to reduce stacking energy
d) Stabilizing the RNA–DNA hybrid
e)Acting as a helicase

8. During the transition from open to closed conformation of the fingers domain:

a) The thumb domain dissociates
b) The non-template strand shifts by one nucleotide
c) Mg^{2+} ions are released
d) TFAM dissociates
e) The PPR motif unfolds

9. Lys and Arg, in the fingers domain contribute to NTP stabilization primarily through which type of interaction?

a) Hydrophobic interactions with the ribose
b) Covalent bonding with the α-phosphate
c) Electrostatic interactions with the negatively charged phosphate groups
d) Disulfide bridge formation
e) Van der Waals interactions with the DNA template

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10. The helical hydrophobic scaffold of the fingers domain is enriched in Leu and Ile residues. What is the primary structural consequence of this enrichment?

a) It directly coordinates Mg^{2+} ions during catalysis

b) **It stabilizes the tertiary structure through hydrophobic packing interactions**

c) It forms hydrogen bonds with the NTP triphosphate

d) It promotes DNA melting by intercalation

e) It creates π–π stacking interactions with guanine bases

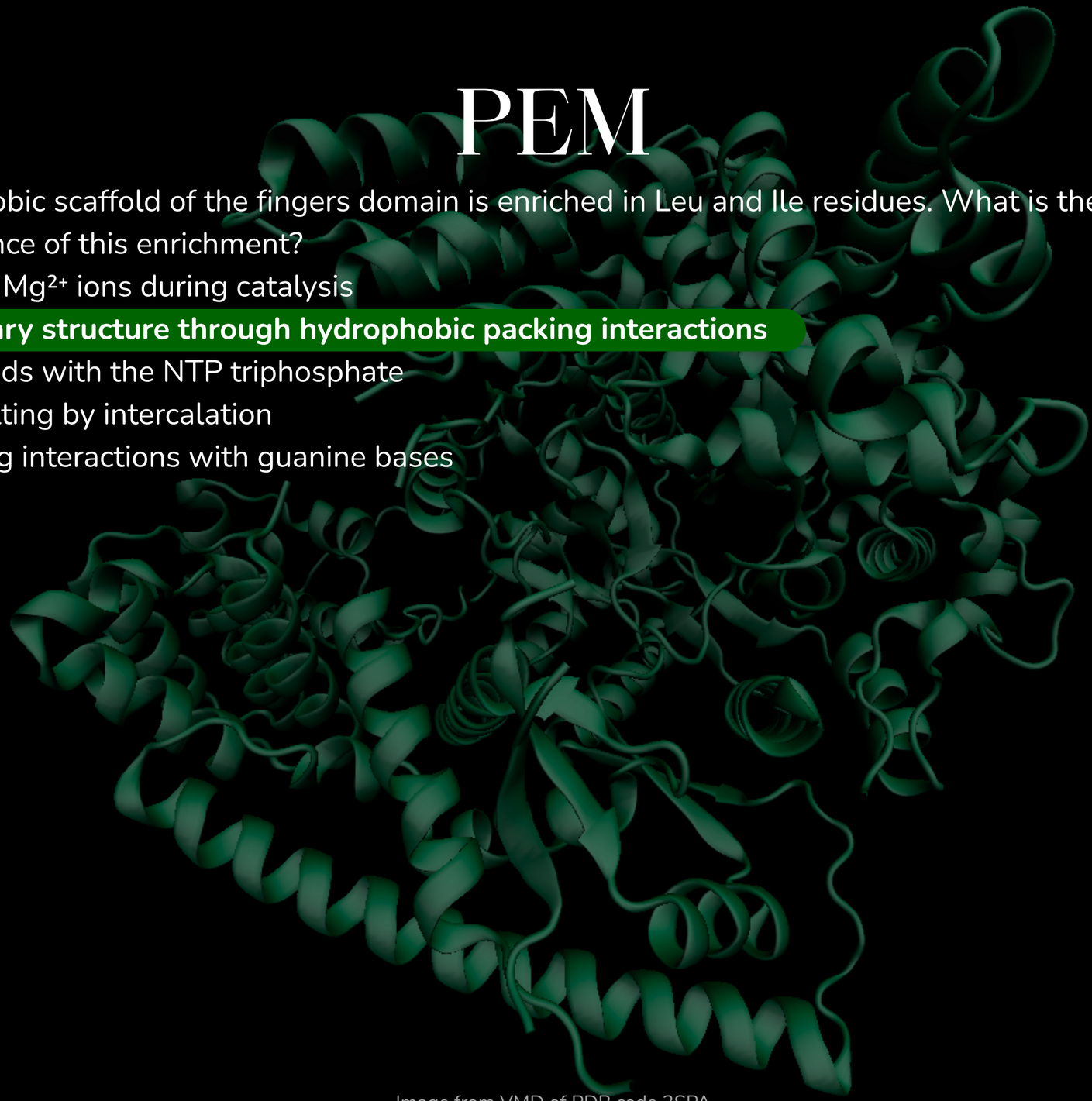


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