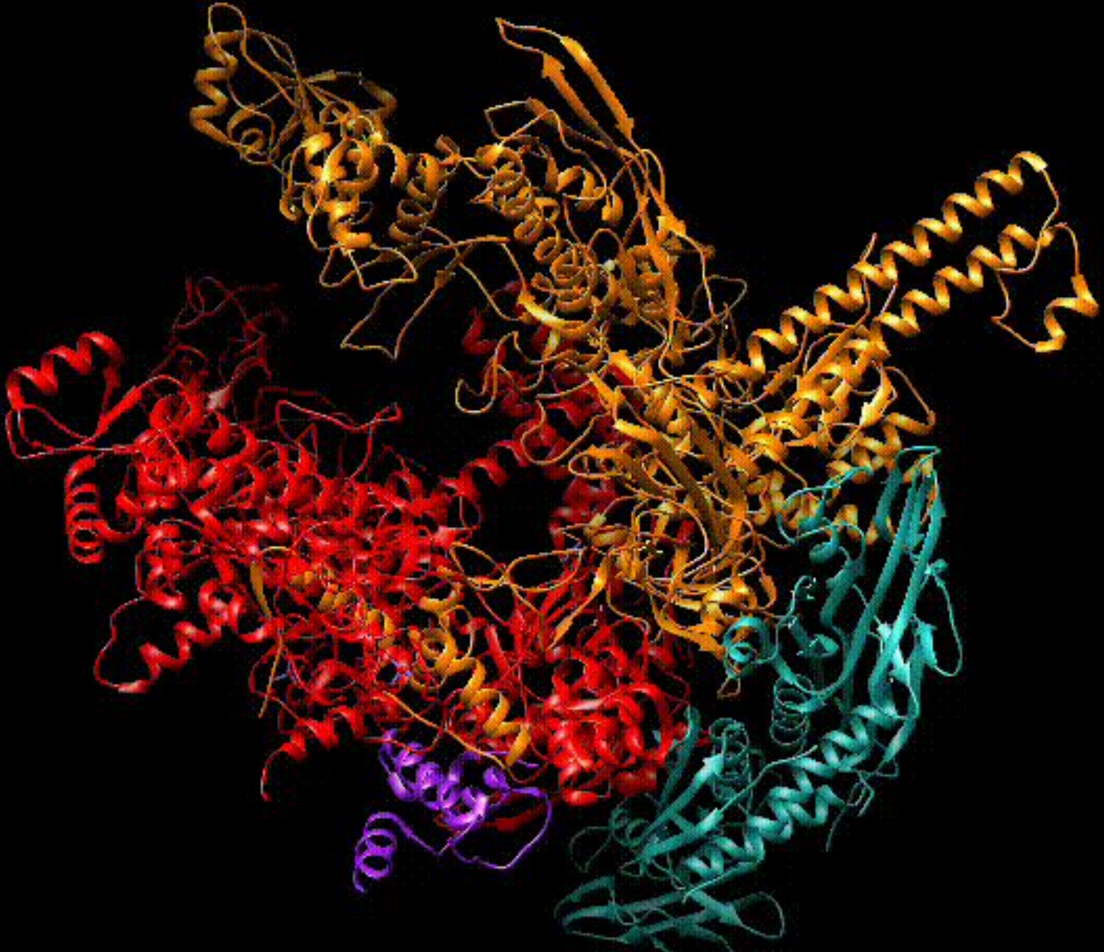
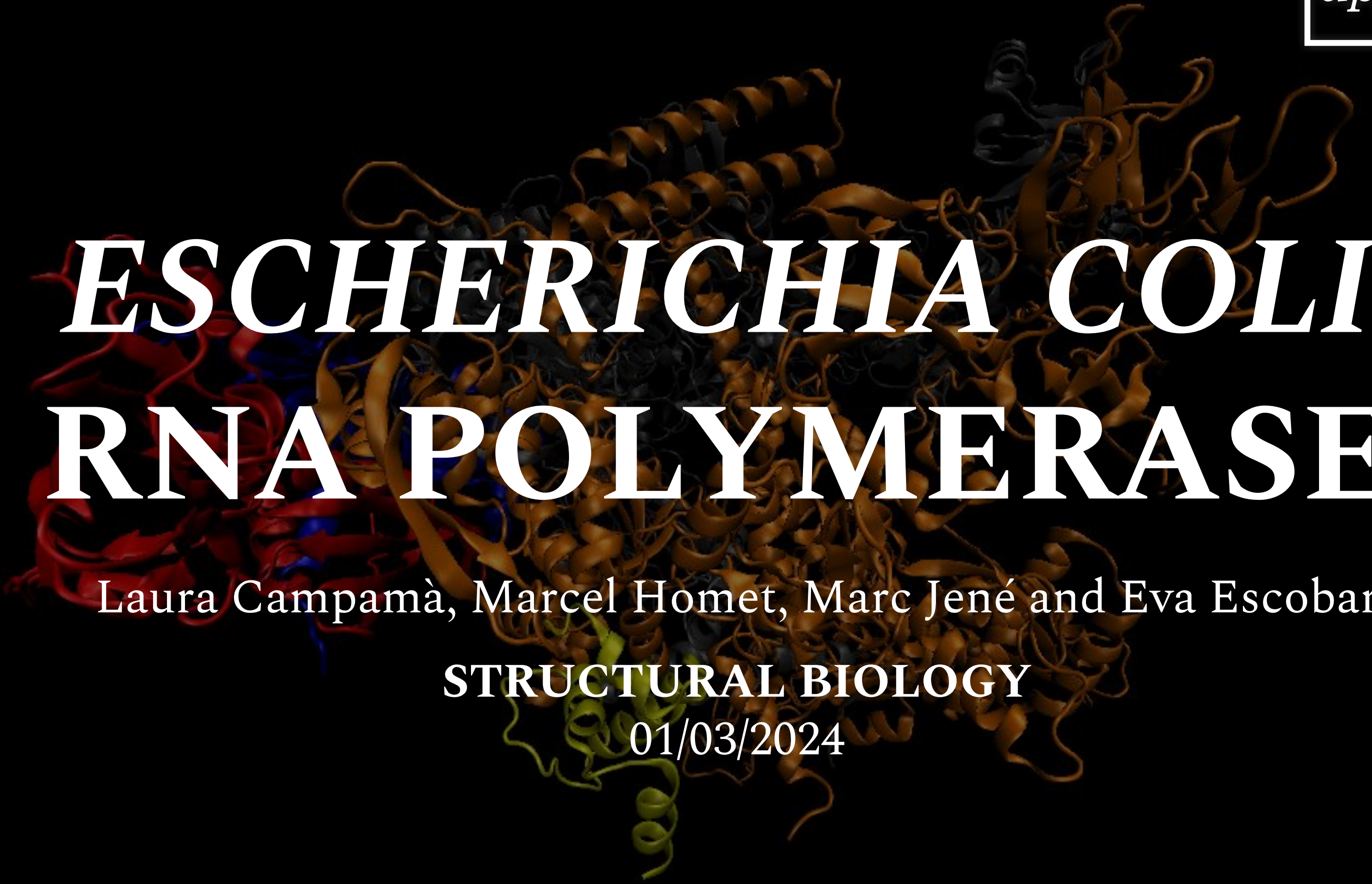


Universitat
Pompeu Fabra
Barcelona

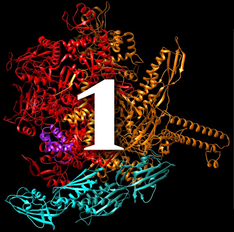
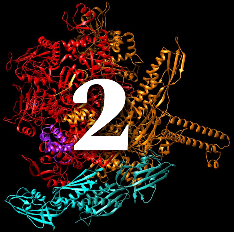


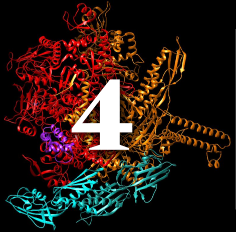
ESCHERICHIA COLI
RNA POLYMERASE

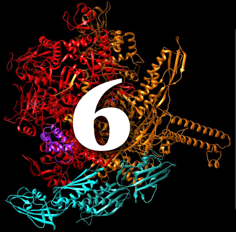
Laura Campamà, Marcel Homet, Marc Jené and Eva Escobar

STRUCTURAL BIOLOGY
01/03/2024

Index

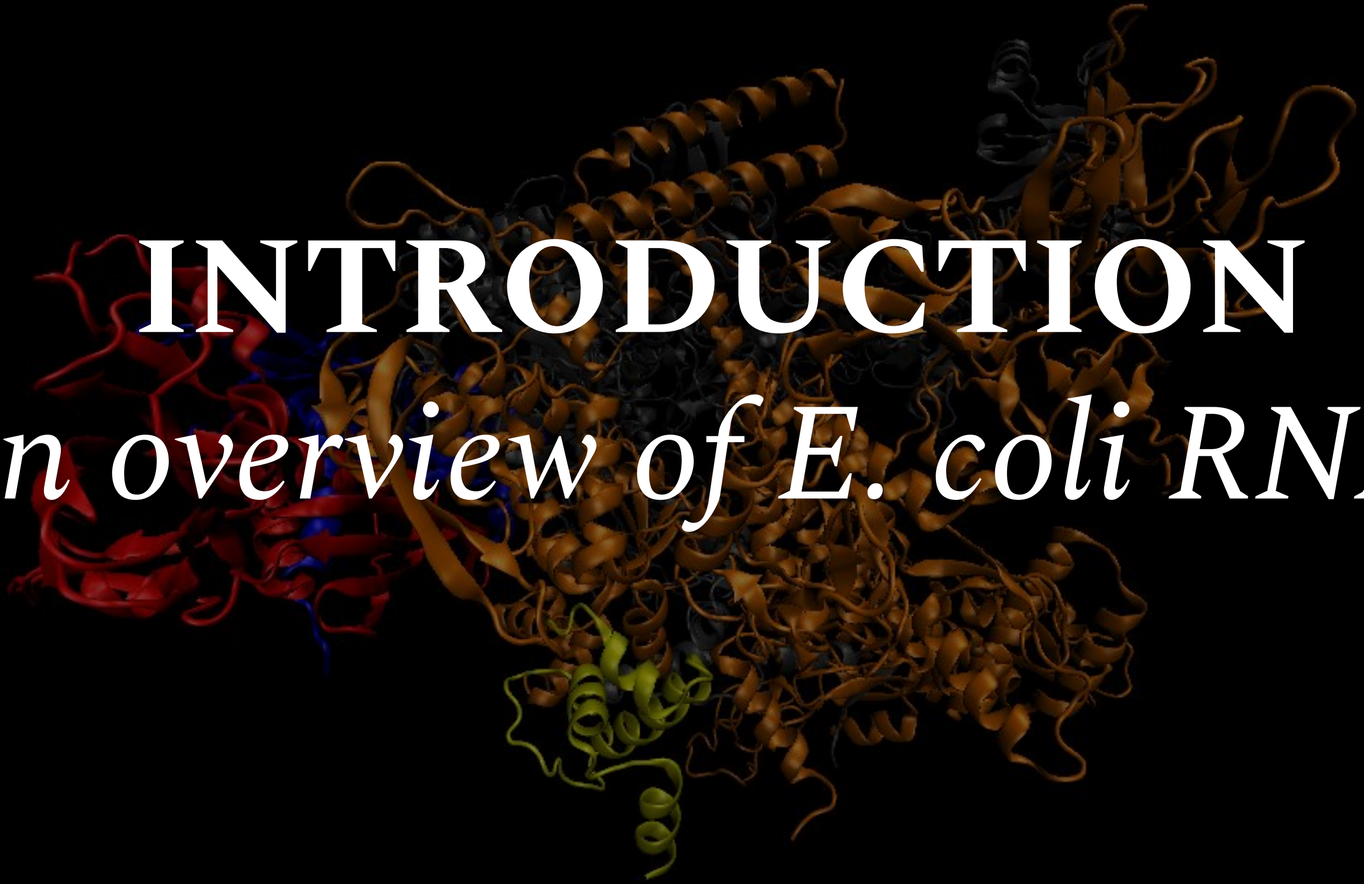





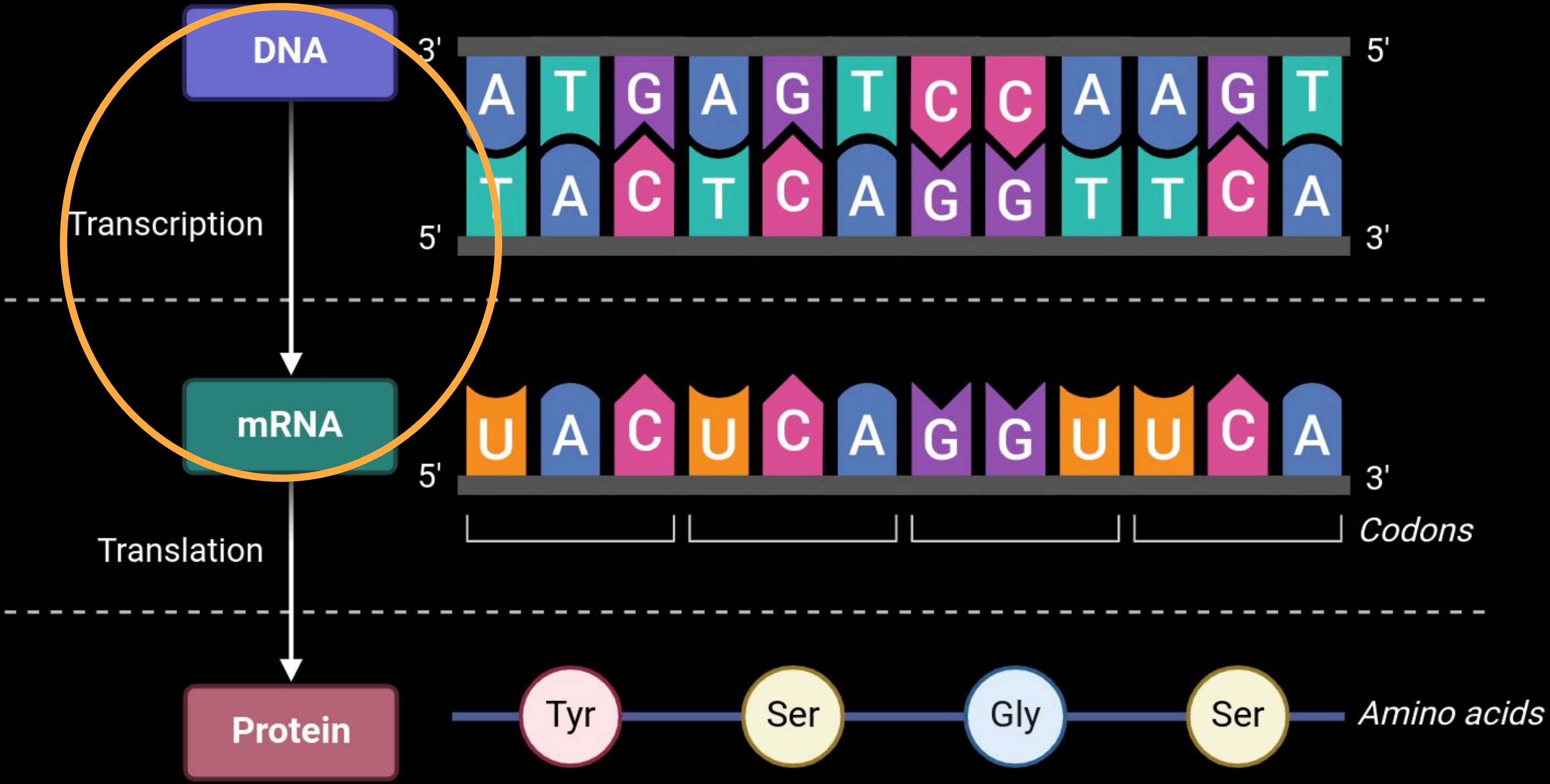


INTRODUCTION

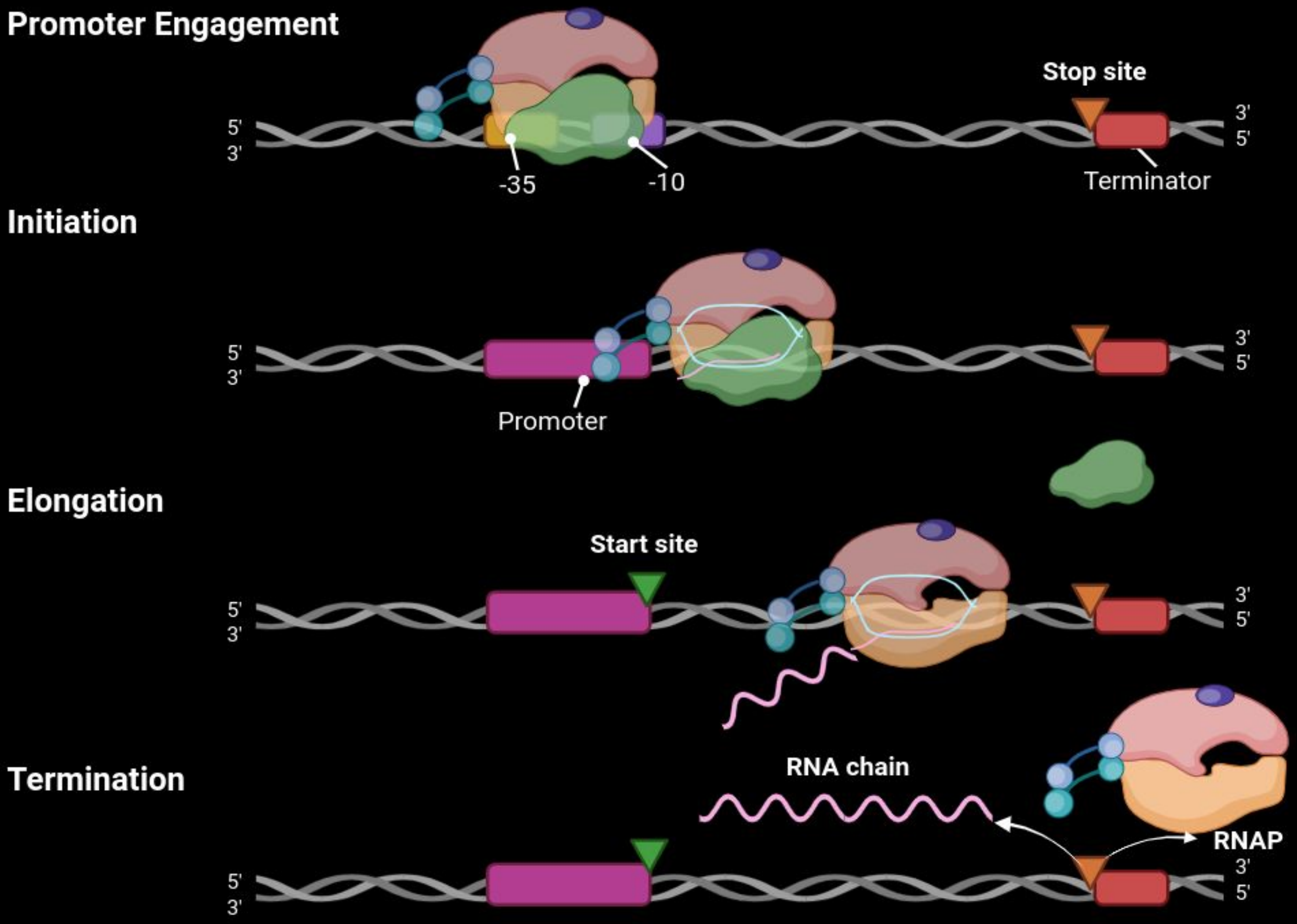
An overview of E. coli RNAP



Central Dogma of Molecular Biology



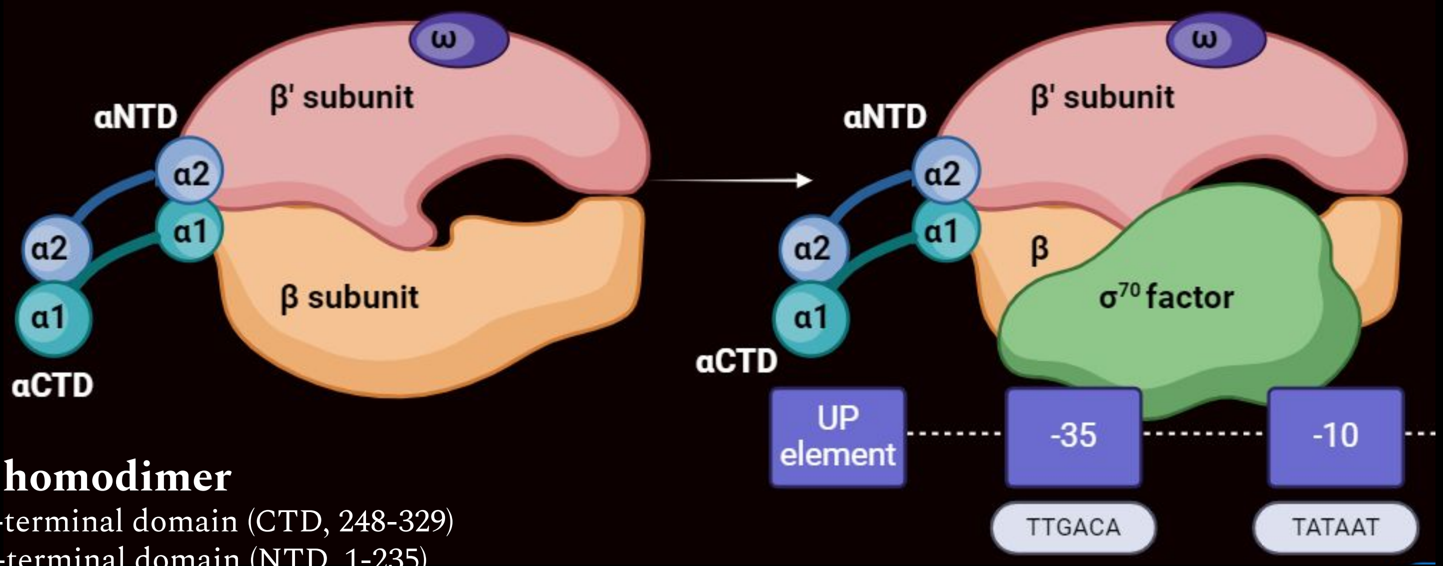
Prokaryotic Transcription



Basics of *E. coli* RNA polymerase

CORE ENZYME

HOLOENZYME



α1+α2 homodimer

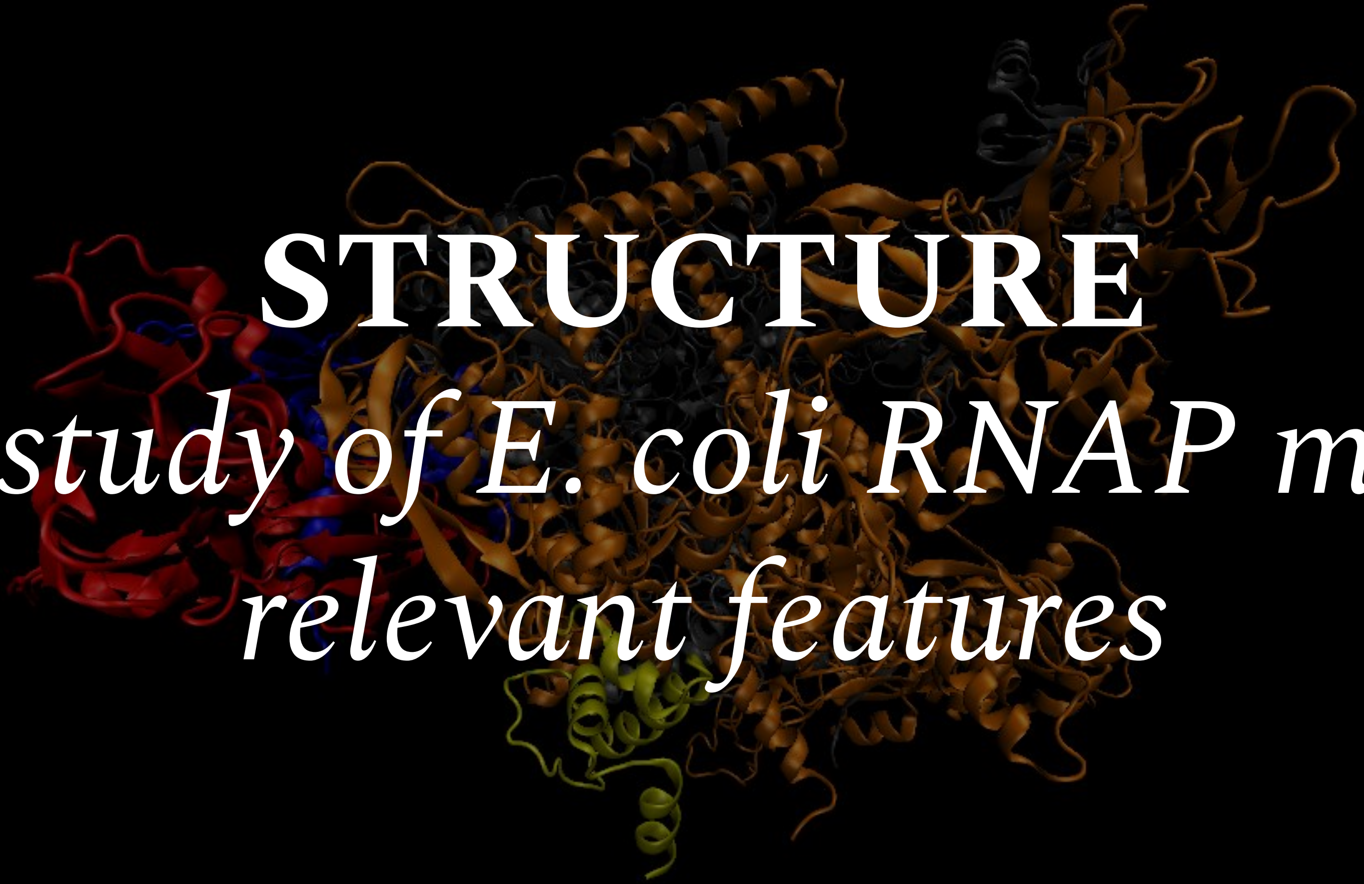
- C-terminal domain (CTD, 248-329)
- N-terminal domain (NTD, 1-235)
- Linker (236-247)

β + β' subunits → <u>RNApol crab claw shape</u>

ω subunit → Non-essential role

σ^{70} factor → Housekeeping genes

- Domain 4 → -35 element (dsDNA)
- Domain 2 → -10 element (ssDNA)



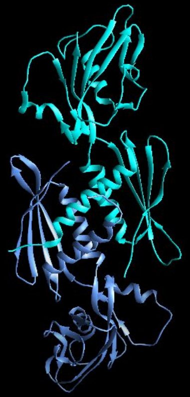
STRUCTURE

A study of E. coli RNAP most relevant features

E. coli RNAP Subunits

Alpha

α1 + α2



rpoA

329 aa (36.5 kDa)

RNAP assembly

Interactions with DNA and transcription factors for transcriptional regulation

Beta

β + β'

***rpoB* + rpoC**

1342 aa (150.4 kDa) / 1407 aa (155.0 kDa)

NTP binding

Rifampin binding site

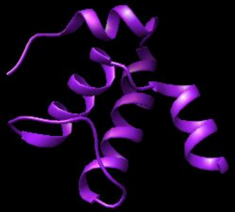
Catalytic Mg^{2+} coordination

σ factor binding

DNA and RNA binding

Omega

ω



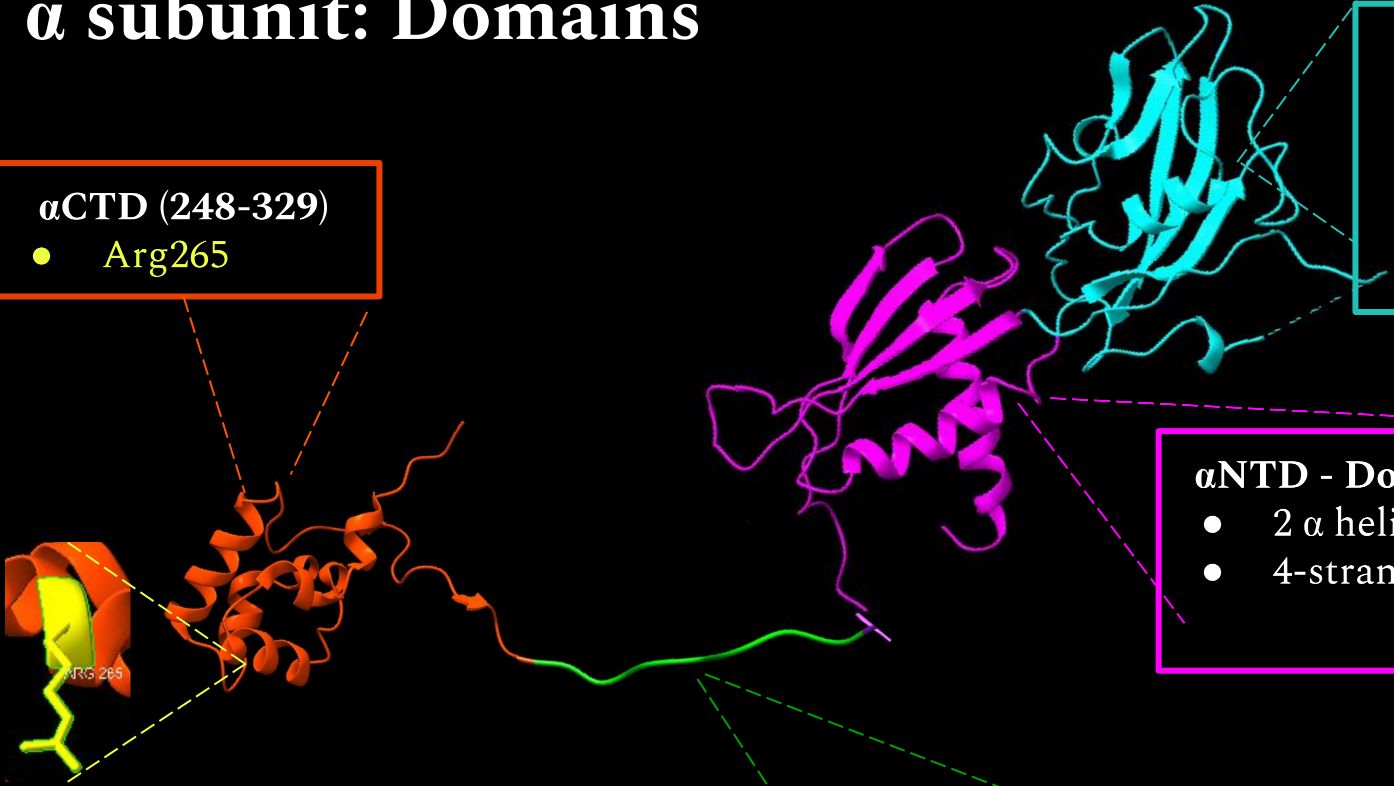
rpoZ

91 aa (10.2 kDa)

RNAP folding**?**

ppGpp binding site 1**?**

α subunit: Domains



αCTD (248-329)
- Arg265

αNTD - Domain 2
(53-179)
- 1 α helix (H3)
- 7 antiparallel β strand

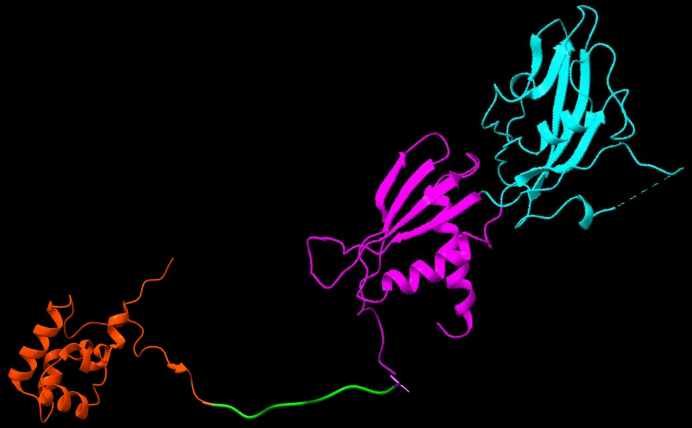
αNTD - Domain 1 (1-52; 180-235)
- 2 α helices (H1 + H3)
- 4-stranded β antiparallel sheet

α subunit CTD
3K4G 2.05Å

Linker
(236-247)

α subunit NTD
1BDF 2.50Å

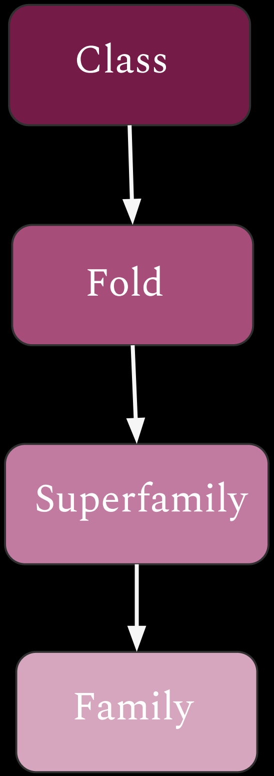
α subunit: SCOP Classification



Insert subdomain
1BDF A:53-178

Dimerisation domain
1BDF A:2-52, 179-232

CTD domain
1LB2 B:250-321



- Alpha and Beta proteins (a+b)
- Alpha and Beta proteins (a+b)
- All alpha proteins

- Insert subdomain of RNA polymerase alpha subunit
- DCoH-like
- SAM domain-like

- Insert subdomain of RNA polymerase alpha subunit
- BP11-like subunits of RNA polymerase
- C-terminal domain of RNA polymerase alpha subunit

- Insert subdomain of RNA polymerase alpha subunit
- RNA polymerase alpha subunit dimerisation domain
- C-terminal domain of RNA polymerase alpha subunit

β and β’ subunits

- Two largest subunits → **80% of the total mass of core enzyme**
- Form each **claw arm** of the **crab claw shape** of RNAP→ **cleft for DNA entry** to the enzyme active site

7MKP
3.41Å
Chimera

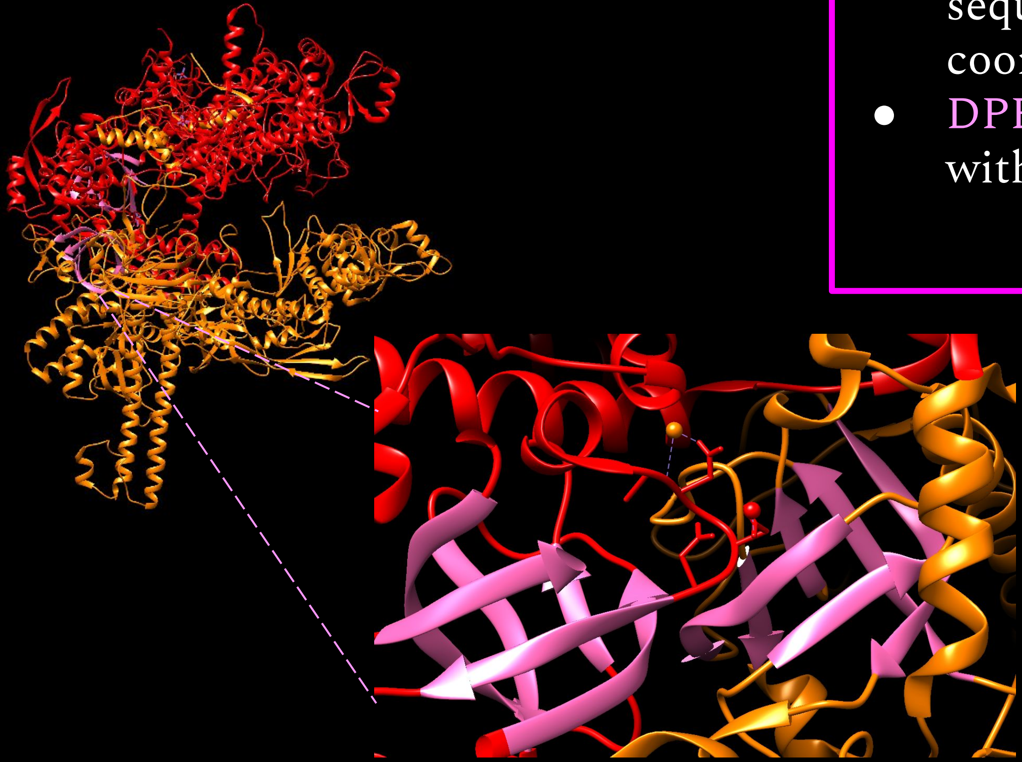
β and β’ subunits

- Two largest subunits → **80% of the total mass of core enzyme**
- Form each **claw arm** of the **crab claw shape** of RNAP→ **cleft for DNA entry** to the enzyme active site

7MKO
3.15Å
Chimera

β and β' - Active Site

Crab Claw

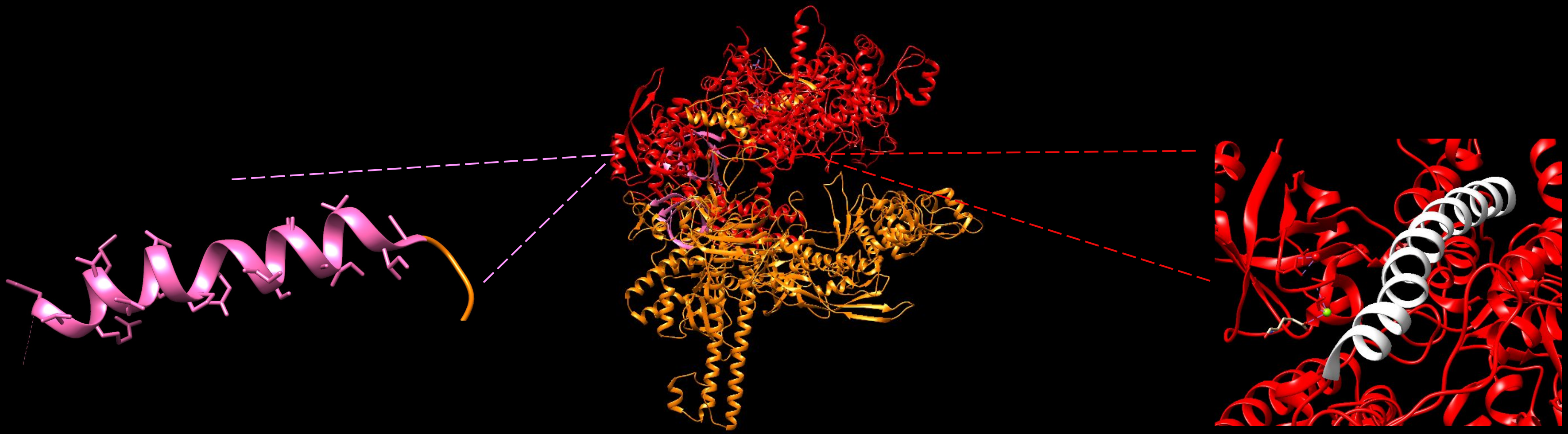


2 double-psi beta barrels (DPBB)

- DPBB (β') → **Acid Aspartic Triad**→ sequence motif - **NADFDGDQ** : coordination of catalytic Mg^{2+} ions
- DPBB (β) → Basic residues: interaction with incoming nucleotides

7MKP
3.41Å
Chimera

β' subunit - Motifs and Functions



Trigger Loop (TL) → Catalysis

- NTP bound → **TL** closes the active site → folds into **Trigger Helices (TH)** → forms a **Three-Helix Bundle (THB)** with BH → closes the active site and increases the nucleotide addition rate ~104 by positioning the NTP substrate for catalysis

Bridge Helix (BH) → Translocation

- **N-ter** → three flexible loops: β fork loop 2, β D loop II, and β' F-loop
- **C-terminal** → anchor module: flexible switch 1 and 2 elements → connects the β' clamp domain to the body of RNAP.

ω subunit

- Smallest subunit of bacterial RNAP (91 residues)

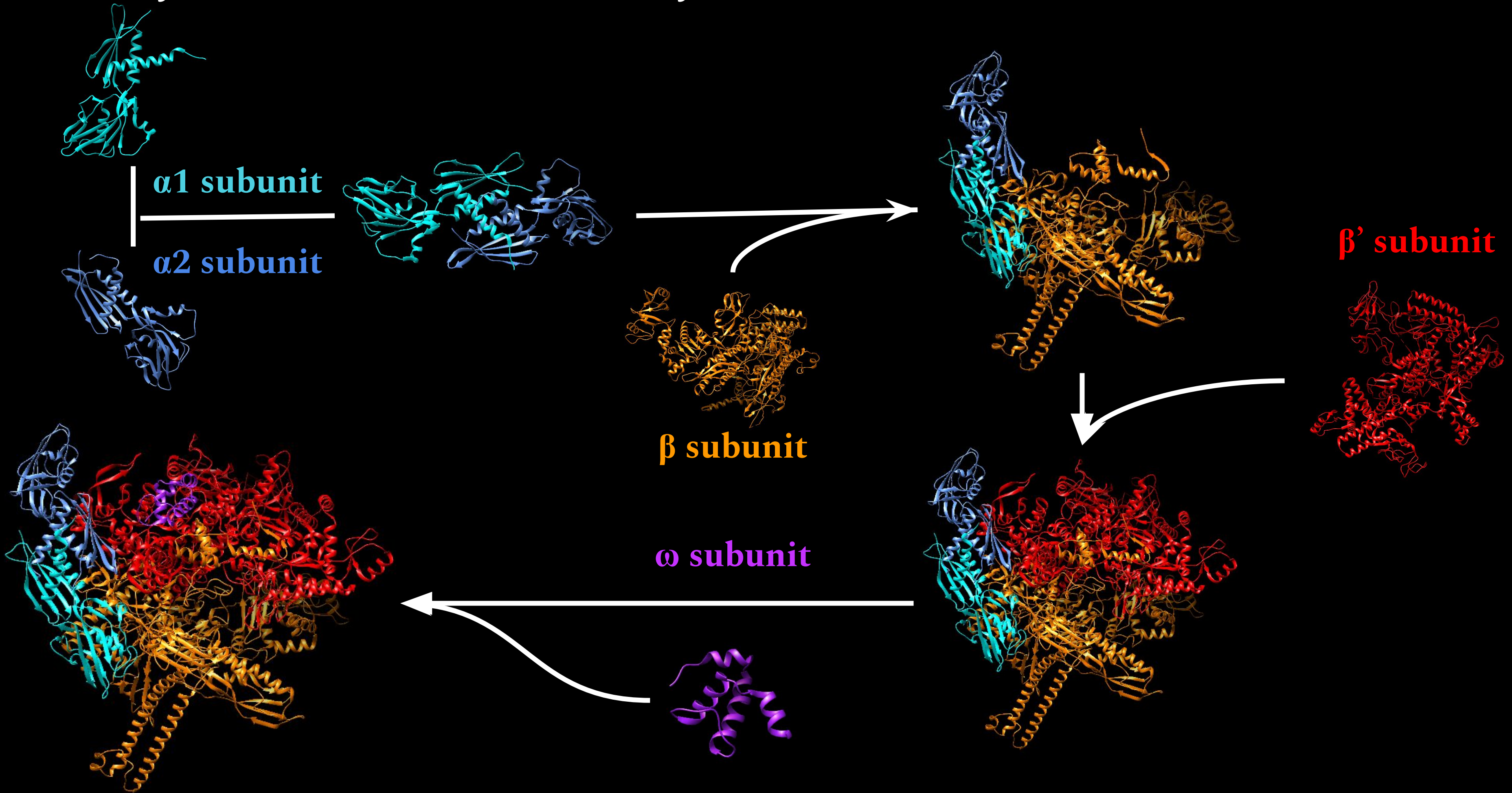
- Only one **DISPENSABLE** for cell growth and for in vitro transcription activity

- Proposed roles **?** :
    - Response to **bacterial alarmone ppGpp** during the stringent response
    - Chaperone for RNAP folding
    - Protection of the DPBB domain against various damages

- 4 α helices
- Binds mainly to β' subunit DPBB domain (contact surface: 1.348 Å)

7MKP 3.41Å
Chimera

Enzyme Core Assembly



α1 subunit

α2 subunit

β subunit

β’ subunit

ω subunit

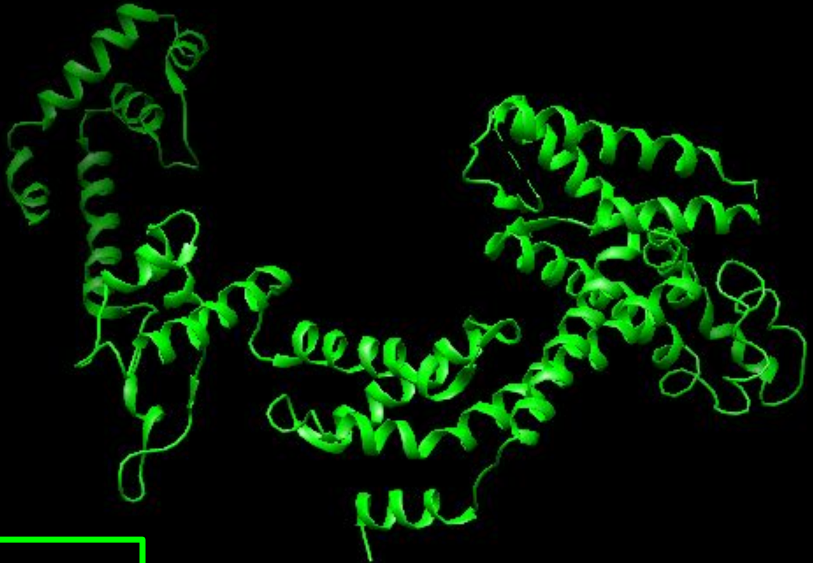
σ^{70} factor- Holoenzyme

Main functions:

- Direction of RNAP catalytic core → **transcription start sites**
- Initiation of dsDNA strand separation → **transcription bubble**

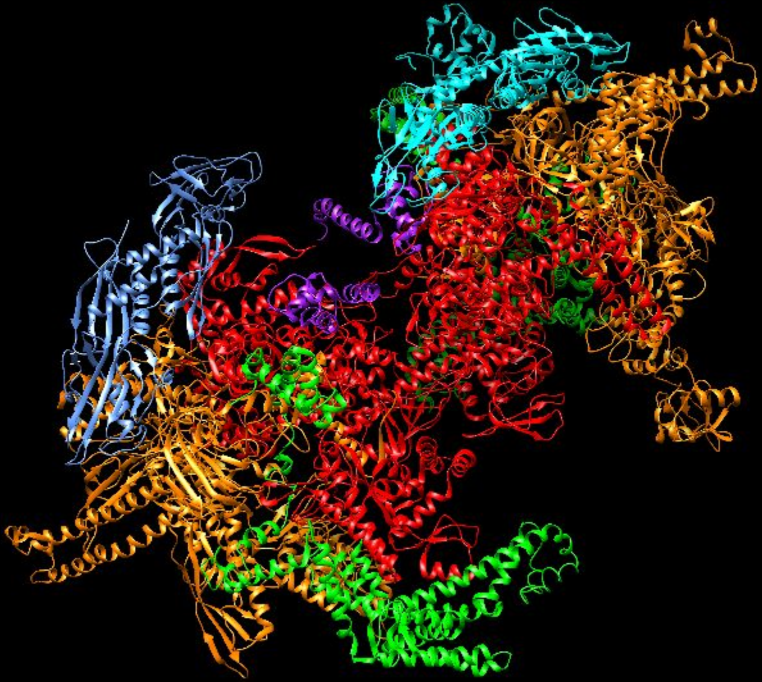
4 domains

- $\sigma_{1.1}$
- σ_2 → -10 element + discriminator motifs
- σ_3 → **α helix** → extended -10 element
- σ_4 → **helix-turn-helix DNA-binding domain** → -35 element



4YG2 3.70Å
Chimera

Holoenzyme

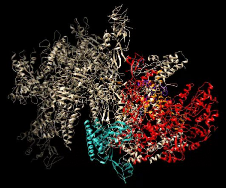


4YG2 3.70Å
Chimera

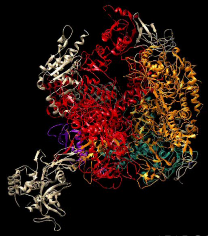
EVOLUTIONARY CONSERVATION

She knows what happened.
She was there

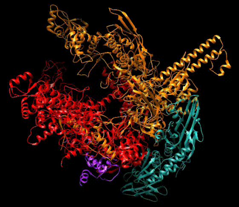
Evolutionary conservation



2PMZ



1WCM



7MKP

Hypothesized to have been one of the earliest proteins to appear.

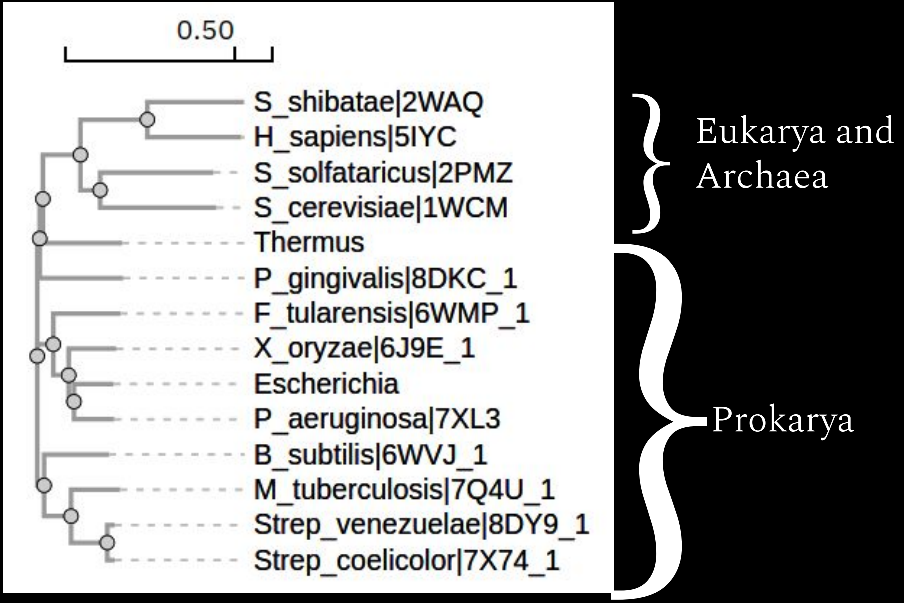
Distinction between species by complexity of RNAP

- Bacteria bind to sigma 70
- Bacteria have only 1 RNAP

Eukarya	Archaea	Prokarya
RPB 3	D	α1
RPB 11	L	α2
RPB 2	B	β
RPB 1	A”	β’
RPB 6	K	ω

Evolutionary conservation

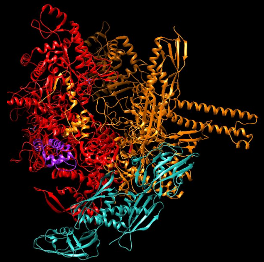
5 regions are conserved in all domains of life



Phylogenetic tree of our RNAPs

2PMZ

1WCM



7MKP

Eukarya	Archaea	Prokarya
RPB 3	D	α1
RPB 11	L	α2
RPB 2	B	β
RPB 1	A”	β’
RPB 6	K	ω

Subunit conservation RNAP

PDB ID	Species
E. coli	7MKP
P. aeruginosa	7XL3
T. aquaticus	1L9U
X. oryzae	6J9E
F. tularensis	6WMP

PDB ID	Species
P. gingivalis	8DKC
B. subtilis	6WVJ
Strep. coelicolor	7X74
Strept. venezuelae	8DY9

α_{I}

RMSD: 0.956

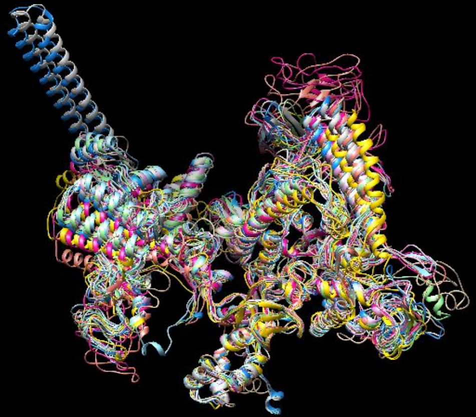
α_{II}

RMSD: 1.052

β

RMSD: 0.931

β'



RMSD: 0.902

ω

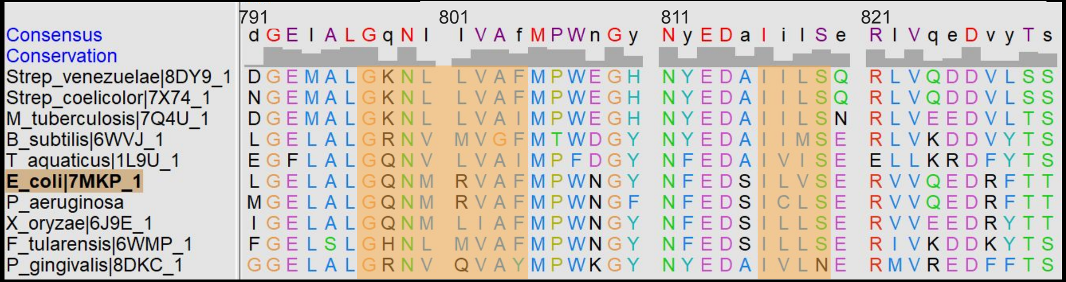
RMSD: 0.798

*Positions based on e.coli

β subunit conservation

DPBB - β strands

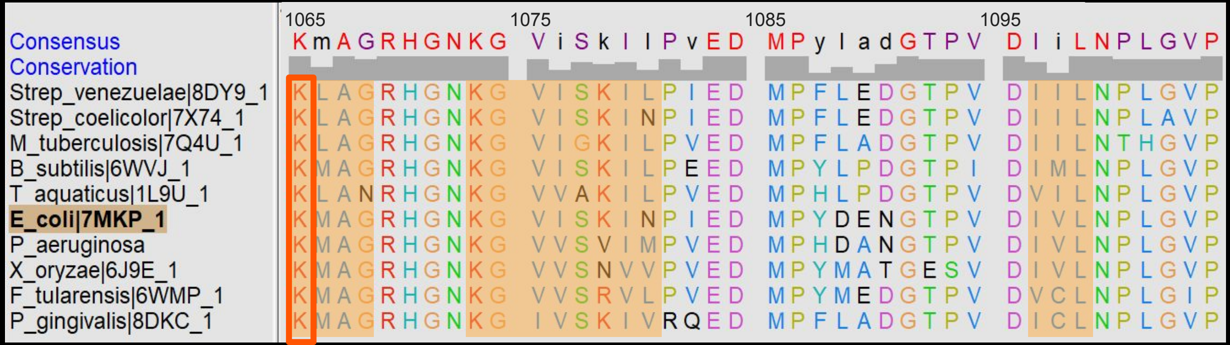
- Positively charged aa



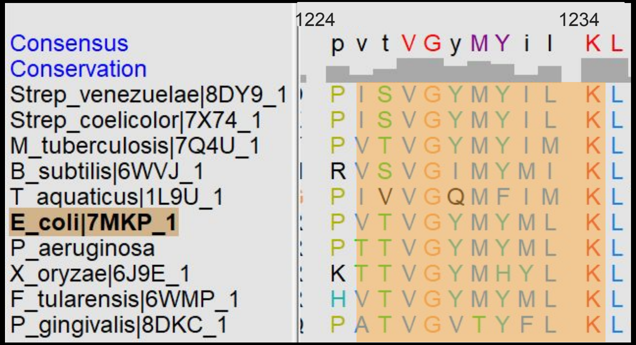
	791	801	811	821
Consensus	dGElALGqNl	lVAfMPWnGy	NyEDaIilSe	RlVqeDvyTs
Conservation				
Strep_venezuelae|8DY9_1	DGEMALGKNL	LVAFMPWEGH	NYEDAIILSQ	RLVQDDVLSS
Strep_coelicolor|7X74_1	NGEMALGKNL	LVAFMPWEGH	NYEDAIILSQ	RLVQDDVLSS
M_tuberculosis|7Q4U_1	DGEMALGKNL	LVAIMPWEGH	NYEDAIILSN	RLVEEDVLTS
B_subtilis|6WVJ_1	LGELALGRNV	MVGFMTWDGY	NYEDAIIMSE	RLVKDDVYTS
T_aquaticus|1L9U_1	EGFLALGQNV	LVAIMPFDGY	NFEDAIVISE	ELLKRDFYTS
E_coli|7MKP_1	LGELALGQNM	RVAFMPWNGY	NFEDSILVSE	RVVQEDRFTT
P_aeruginosa	MGELALGQNM	RVAFMPWNGF	NFEDSICLSE	RVVQEDRFTT
X_oryzae|6J9E_1	IGELALGQNM	LIAFMPWNGY	NFEDSILLSE	RVVEEDRYTT
F_tularensis|6WMP_1	FGELSLGHNL	MVAFMPWNGY	NFEDSILLSE	RIVKDDKYTS
P_gingivalis|8DKC_1	GGELALGRNV	QVAYMPWKGY	NYEDAIVLNE	RMVREDFFTS

RMSD: 0.931

DNA BD



	1065	1075	1085	1095
Consensus	KmAGRHGNKG	ViSkIlPvED	MPyladGTPV	DIiLNPLGVP
Conservation				
Strep_venezuelae|8DY9_1	KLAGRHGNKG	VISKILPIED	MPFLEDGTPV	DIILNPLGVP
Strep_coelicolor|7X74_1	KLAGRHGNKG	VISKINPIED	MPFLEDGTPV	DIILNPLAVP
M_tuberculosis|7Q4U_1	KLAGRHGNKG	VIGKILPVED	MPFLADGTPV	DIILNTHGVP
B_subtilis|6WVJ_1	KMAGRHGNKG	VISKILPEED	MPYLPDGTPI	DIMLNPLGVP
T_aquaticus|1L9U_1	KLANRHGNKG	VVAKILPVED	MPHLPDGTPV	DVILNPLGVP
E_coli|7MKP_1	KMAGRHGNKG	VISKINPIED	MPYDENGTPV	DIVLNPLGVP
P_aeruginosa	KMAGRHGNKG	VVSVIMPVED	MPHDANGTPV	DIVLNPLGVP
X_oryzae|6J9E_1	KMAGRHGNKG	VVSNVVPVED	MPYMATGESV	DIVLNPLGVP
F_tularensis|6WMP_1	KMAGRHGNKG	VVSRVLPVED	MPYMEDGTPV	DVCLNPLGIP
P_gingivalis|8DKC_1	KMAGRHGNKG	IVSKIVRQED	MPFLADGTPV	DICLNPLGVP



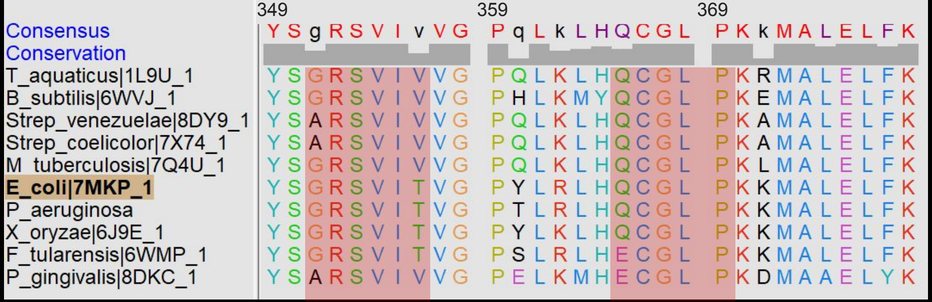
	1224	1234
Consensus	pvtVGyMYil	KL
Conservation		
Strep_venezuelae|8DY9_1	PISVGYMYIL	KL
Strep_coelicolor|7X74_1	PISVGYMYIL	KL
M_tuberculosis|7Q4U_1	PVTVGYMYIM	KL
B_subtilis|6WVJ_1	RVSVGIMYMI	KL
T_aquaticus|1L9U_1	PIVVGQMFIM	KL
E_coli|7MKP_1	PVTVGYMYML	KL
P_aeruginosa	PTTVGYMYML	KL
X_oryzae|6J9E_1	KTTVGYMHYL	KL
F_tularensis|6WMP_1	HVTVGYMYML	KL
P_gingivalis|8DKC_1	PATVGVTYFL	KL

*Positions based on e.coli

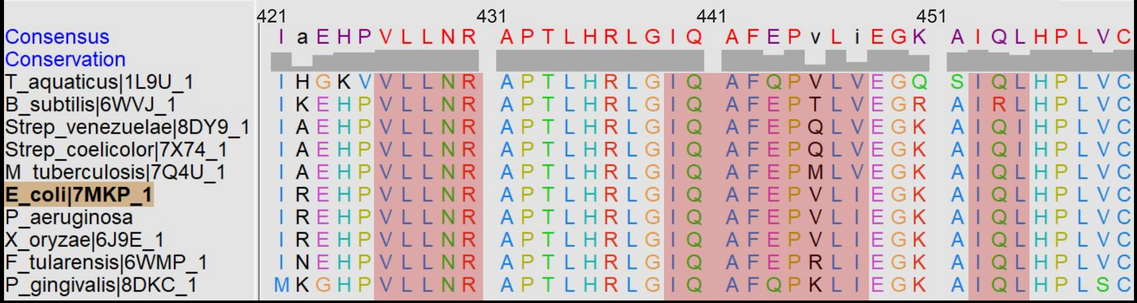
β’ subunit conservation

DPBB - β strands

RMSD: 0.902

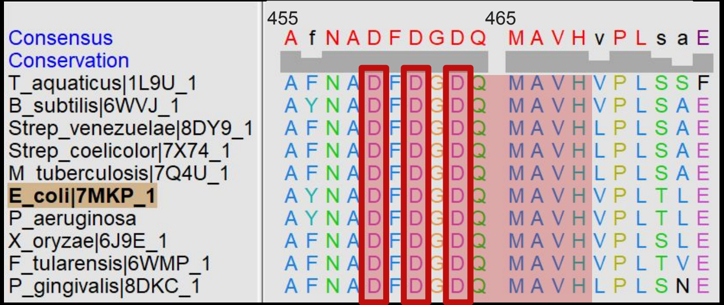


	349	359	369
Consensus	Y S g R S V I v V G	P q L k L H Q C G L	P K k M A L E L F K
Conservation			
T_aquaticus|1L9U_1	Y S G R S V I V V G	P Q L K L H Q C G L	P K R M A L E L F K
B_subtilis|6WVJ_1	Y S G R S V I V V G	P H L K M Y Q C G L	P K E M A L E L F K
Strep_venezuelae|8DY9_1	Y S A R S V I V V G	P Q L K L H Q C G L	P K A M A L E L F K
Strep_coelicolor|7X74_1	Y S A R S V I V V G	P Q L K L H Q C G L	P K A M A L E L F K
M_tuberculosis|7Q4U_1	Y S G R S V I V V G	P Q L K L H Q C G L	P K L M A L E L F K
E_coli|7MKP_1	Y S G R S V I T V G	P Y L R L H Q C G L	P K K M A L E L F K
P_aeruginosa	Y S G R S V I T V G	P T L R L H Q C G L	P K K M A L E L F K
X_oryzae|6J9E_1	Y S G R S V I T V G	P Y L K L H Q C G L	P K K M A L E L F K
F_tularensis|6WMP_1	Y S G R S V I T V G	P S L R L H E C G L	P K K M A L E L F K
P_gingivalis|8DKC_1	Y S A R S V I V V G	P E L K M H E C G L	P K D M A A E L Y K



	421	431	441	451
Consensus	I a E H P V L L N R	A P T L H R L G I Q	A F E P v L i E G K	A I Q L H P L V C
Conservation				
T_aquaticus|1L9U_1	I H G K V V L L N R	A P T L H R L G I Q	A F Q P V L V E G Q	S I Q L H P L V C
B_subtilis|6WVJ_1	I K E H P V L L N R	A P T L H R L G I Q	A F E P T L V E G R	A I R L H P L V C
Strep_venezuelae|8DY9_1	I A E H P V L L N R	A P T L H R L G I Q	A F E P Q L V E G K	A I Q I H P L V C
Strep_coelicolor|7X74_1	I A E H P V L L N R	A P T L H R L G I Q	A F E P Q L V E G K	A I Q I H P L V C
M_tuberculosis|7Q4U_1	I A E H P V L L N R	A P T L H R L G I Q	A F E P M L V E G K	A I Q L H P L V C
E_coli|7MKP_1	I R E H P V L L N R	A P T L H R L G I Q	A F E P V L I E G K	A I Q L H P L V C
P_aeruginosa	I R E H P V L L N R	A P T L H R L G I Q	A F E P V L I E G K	A I Q L H P L V C
X_oryzae|6J9E_1	I R E H P V L L N R	A P T L H R L G I Q	A F E P V L I E G K	A I Q L H P L V C
F_tularensis|6WMP_1	I N E H P V L L N R	A P T L H R L G I Q	A F E P R L I E G K	A I Q L H P L V C
P_gingivalis|8DKC_1	M K G H P V L L N R	A P T L H R L G I Q	A F Q P K L I E G K	A I Q L H P L S C

Asp Triad



	455	465
Consensus	A f N A D F D G D Q	M A V H v P L s a E
Conservation		
T_aquaticus|1L9U_1	A F N A D F D G D Q	M A V H V P L S S F
B_subtilis|6WVJ_1	A Y N A D F D G D Q	M A V H V P L S A E
Strep_venezuelae|8DY9_1	A F N A D F D G D Q	M A V H L P L S A E
Strep_coelicolor|7X74_1	A F N A D F D G D Q	M A V H L P L S A E
M_tuberculosis|7Q4U_1	A F N A D F D G D Q	M A V H L P L S A E
E_coli|7MKP_1	A Y N A D F D G D Q	M A V H V P L T L E
P_aeruginosa	A Y N A D F D G D Q	M A V H V P L T L E
X_oryzae|6J9E_1	A F N A D F D G D Q	M A V H V P L S L E
F_tularensis|6WMP_1	A F N A D F D G D Q	M A V H V P L T V E
P_gingivalis|8DKC_1	A F N A D F D G D Q	M A V H L P L S N E

σ70 conservation

Structure conservation

Alpha-helix

Spacing of hydrophobic aa (loop vary length)

PDB ID	Species
E. coli	4YG2
B. subtilis	7CKQ
T. aquaticus	4XLP
S. venezuelae	8DY9F
F. tularensis	6WMP
M. tuberculosis	7KIF

TATAAT box BD

	377 ... 387
Consensus	k h l i E A N L R L V V S i A K k Y T g
Conservation	
S_venezuelae|8DY9F	N H L L E A N L R L V V S L A K R Y T G
M_tuberculosis|7KIFF	N H L L E A N L R L V V S L A K R Y T G
T_aquaticus|4XLPF	Q H L I E A N L R L V V S I A K K Y T G
B_subtilis|7CKQF	R R L A E A N L R L V V S I A K R Y V G
E_coli|4YG2F	K E M V E A N L R L V I S I A K K Y T N
F_tularensis|6WMRZ	K E M I E A N L R L V V S E A K K Y T N

RMSD: 1.02

Subunit conservation across life

PDB ID	**Species**
E. coli	7MKP
T. aquaticus	1L9U
S. solfataricus	2PMZ
S. shibatae	2WAQ
S. cerevisiae	1WCM
H. sapiens	5IYC

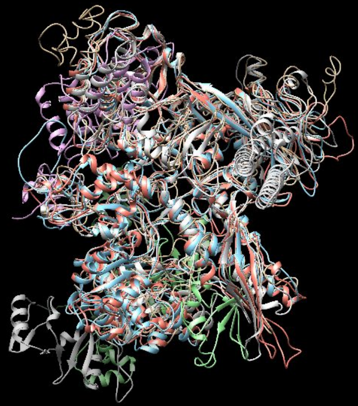
α_I

RMSD: 1.165

α_{II}

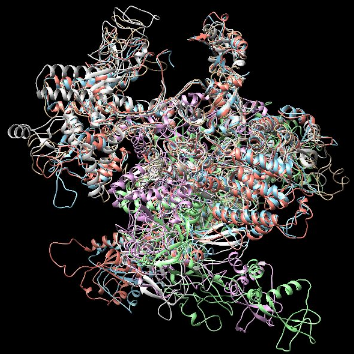
RMSD: 1.039

β



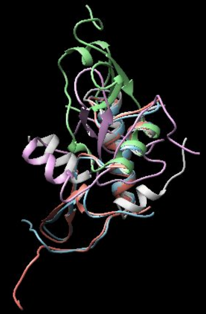
RMSD: 1.131

β'



RMSD: 1.207

ω

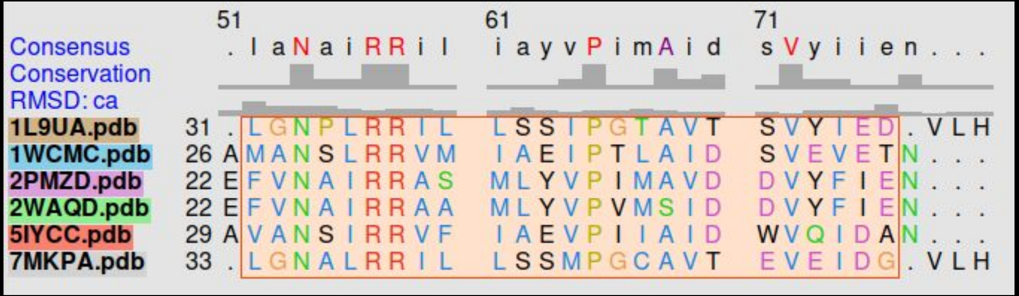


RMSD: 1.355

Subunit conservation across life

α_I structure conservation

PDB ID	Species
E. coli	7MKP
T. aquaticus	1L9U
S. solfataricus	2PMZ
S. shibatae	2WAQ
S. cerevisiae	1WCM
H. sapiens	5IYC

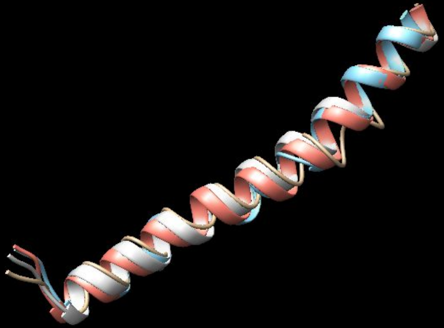


```
                 51                    61                    71
Consensus         . l a N a i R R i l   i a y v P i m A i d   s V y i i e n . . .
Conservation
RMSD: ca
1L9UA.pdb     31 . L G N P L R R I L   L S S I P G T A V T   S V Y I E D . V L H
1WCMC.pdb     26 A M A N S L R R V M   I A E I P T L A I D   S V E V E T N . . .
2PMZD.pdb     22 E F V N A I R R A S   M L Y V P I M A V D   D V Y F I E N . . .
2WAQD.pdb     22 E F V N A I R R A A   M L Y V P V M S I D   D V Y F I E N . . .
5IYCC.pdb     29 A V A N S I R R V F   I A E V P I I A I D   W V Q I D A N . . .
7MKPA.pdb     33 . L G N A L R R I L   L S S M P G C A V T   E V E I D G . V L H
```

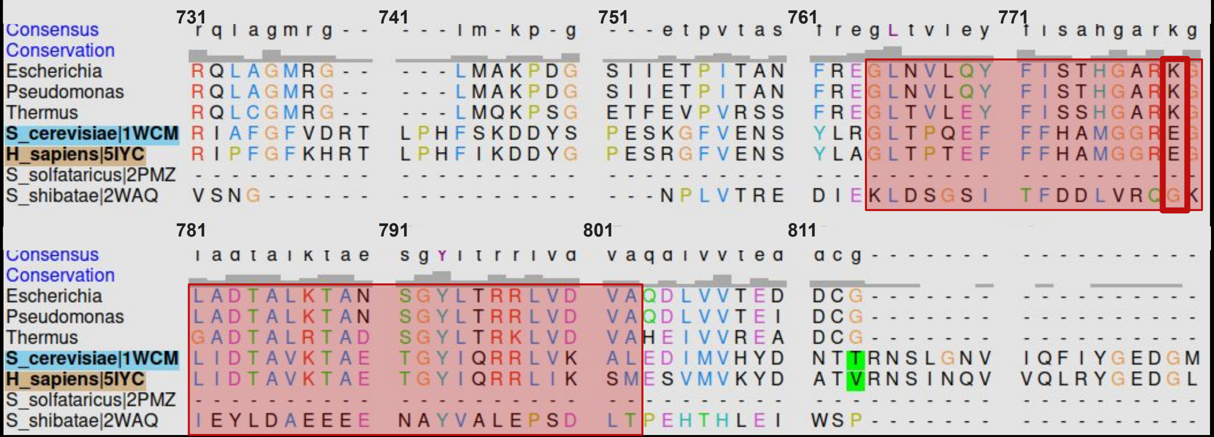
Structural alignment of RNAPs

β’ subunit differences

Bridge Helix

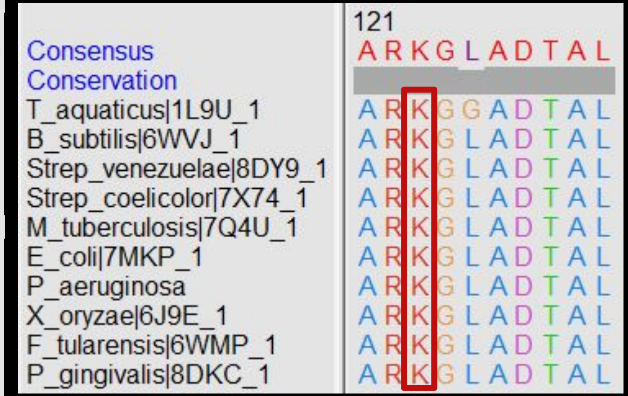


RMSD: 1.207
of β’



```
                    731         741         751         761         771
Consensus           rqlagmrg--  ---lm-kp-g  ---etpvtas  fregLtvley  fisahgarkg
Conservation
Escherichia         RQLAGMRG--  ---LMAKPDG  SIIETPITAN  FREGLNVLQY  FISTHGARKG
Pseudomonas         RQLAGMRG--  ---LMAKPDG  SIIETPITAN  FREGLNVLQY  FISTHGARKG
Thermus             RQLCGMRG--  ---LMQKPSG  ETFEVPVRSS  FREGLTVLEY  FISSHGARKG
S_cerevisiae|1WCM   RIAFGFVDRT  LPHFSKDDYS  PESKGFVENS  YLRGLTPQEF  FFHAMGGREG
H_sapiens|5IYC      RIPFGFKHRT  LPHFIKDDYG  PESRGFVENS  YLAGLTPTEF  FFHAMGGREG
S_solfataricus|2PMZ ----------  ----------  ----------  ----------  ----------
S_shibatae|2WAQ     VSNG------  ----------  ---NPLVTRE  DIEKLDSGSI  TFDDLVRQGK

                    781         791         801         811
Consensus           ladtalktae  sgYltrrlvd  vaqdlvvted  dcg-------  ----------
Conservation
Escherichia         LADTALKTAN  SGYLTRRLVD  VAQDLVVTED  DCG-------  ----------
Pseudomonas         LADTALKTAN  SGYLTRRLVD  VAQDLVVTEI  DCG-------  ----------
Thermus             GADTALRTAD  SGYLTRKLVD  VAHEIVVREA  DCG-------  ----------
S_cerevisiae|1WCM   LIDTAVKTAE  TGYIQRRLVK  ALEDIMVHYD  NTTRNSLGNV  IQFIYGEDGM
H_sapiens|5IYC      LIDTAVKTAE  TGYIQRRLIK  SMESVMVKYD  ATVRNSINQV  VQLRYGEDGL
S_solfataricus|2PMZ ----------  ----------  ----------  ----------  ----------
S_shibatae|2WAQ     IEYLDAEEEE  NAYVALEPSD  LTPEHTHLEI  WSP-------  ----------
```



```
                        121
Consensus               ARKGLADTAL
Conservation
T_aquaticus|1L9U_1      ARKGGADTAL
B_subtilis|6WVJ_1       ARKGLADTAL
Strep_venezuelae|8DY9_1 ARKGLADTAL
Strep_coelicolor|7X74_1 ARKGLADTAL
M_tuberculosis|7Q4U_1   ARKGLADTAL
E_coli|7MKP_1           ARKGLADTAL
P_aeruginosa            ARKGLADTAL
X_oryzae|6J9E_1         ARKGLADTAL
F_tularensis|6WMP_1     ARKGLADTAL
P_gingivalis|8DKC_1     ARKGLADTAL
```

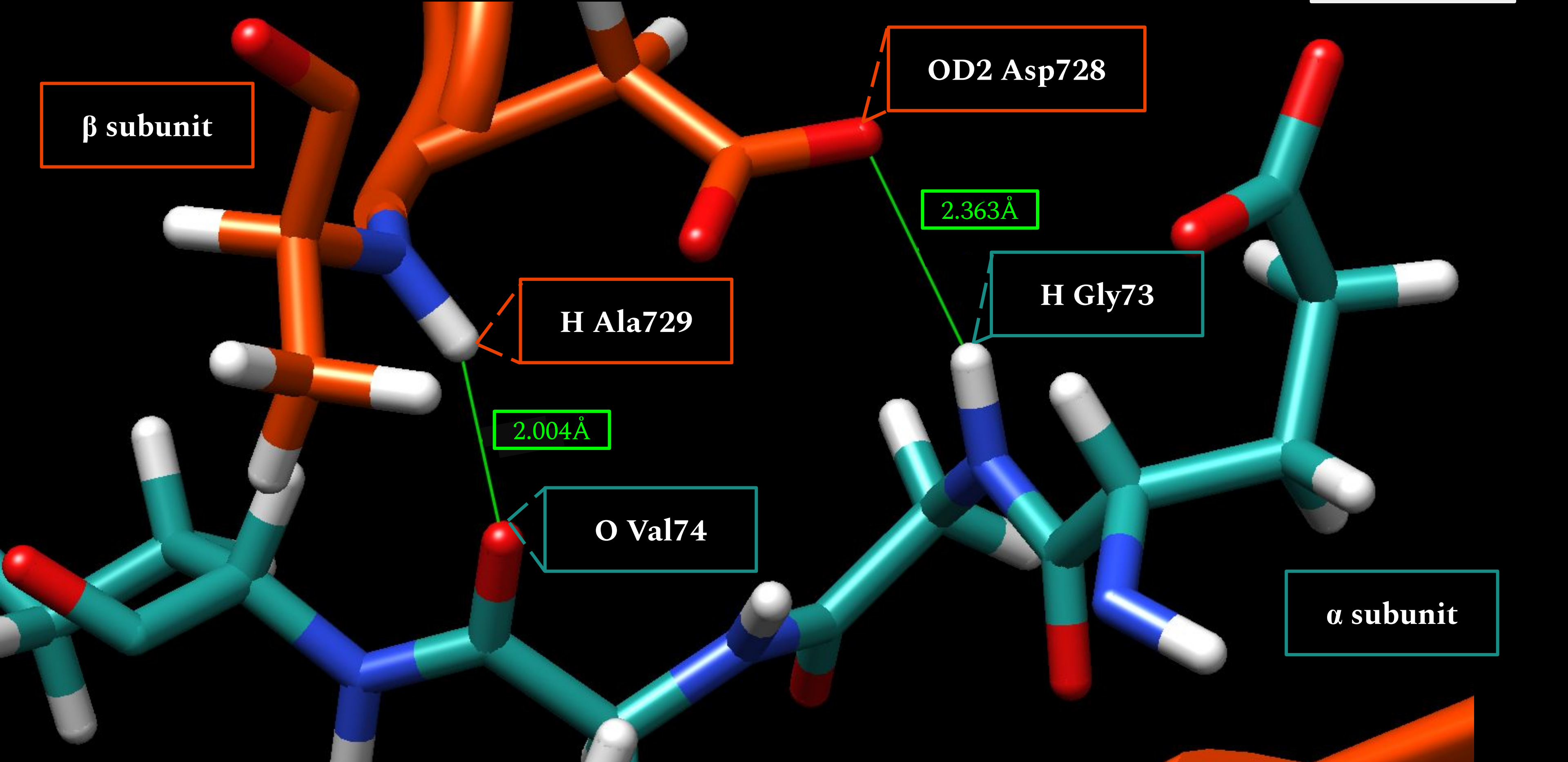
RNAP conformation

INTERACTIONS

RNAP and her friends

Between subunits - α and β (HBonds)

4YG2 3.70Å



*Positions based on e.coli

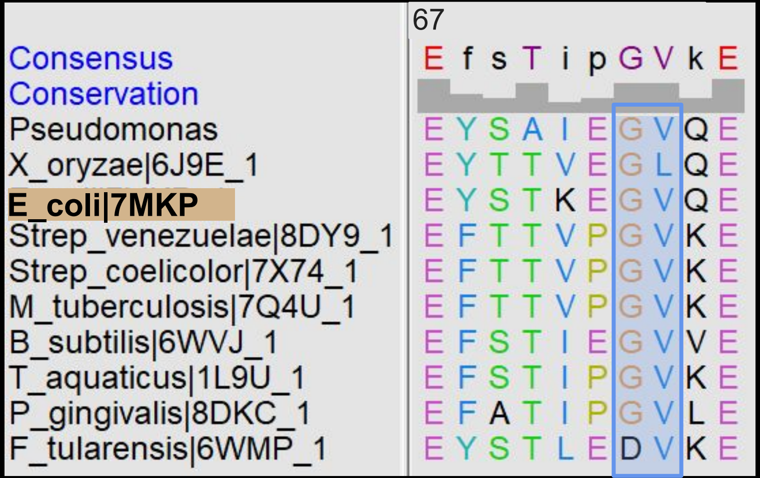
Between subunits - α and β Conservation

α subunit

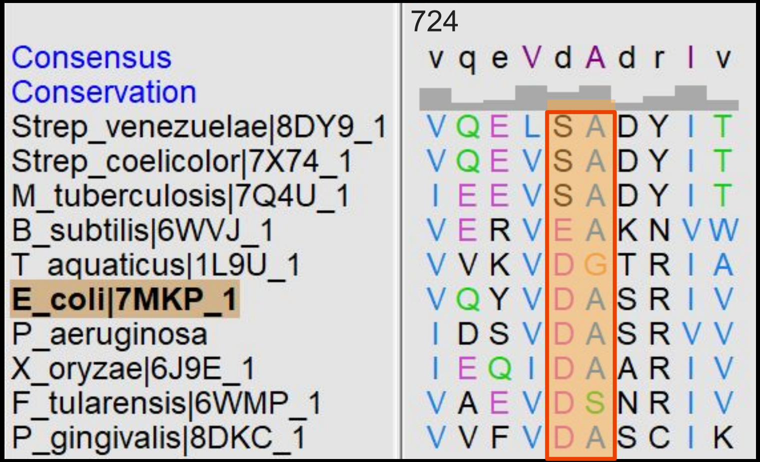
Gly73 Val74

β subunit

Ala728 Asp729



	67									
Consensus	E	f	s	T	i	p	G	V	k	E
Conservation										
Pseudomonas	E	Y	S	A	I	E	G	V	Q	E
X_oryzae|6J9E_1	E	Y	T	T	V	E	G	L	Q	E
E_coli|7MKP	E	Y	S	T	K	E	G	V	Q	E
Strep_venezuelae|8DY9_1	E	F	T	T	V	P	G	V	K	E
Strep_coelicolor|7X74_1	E	F	T	T	V	P	G	V	K	E
M_tuberculosis|7Q4U_1	E	F	T	T	V	P	G	V	K	E
B_subtilis|6WVJ_1	E	F	S	T	I	E	G	V	V	E
T_aquaticus|1L9U_1	E	F	S	T	I	P	G	V	K	E
P_gingivalis|8DKC_1	E	F	A	T	I	P	G	V	L	E
F_tularensis|6WMP_1	E	Y	S	T	L	E	D	V	K	E

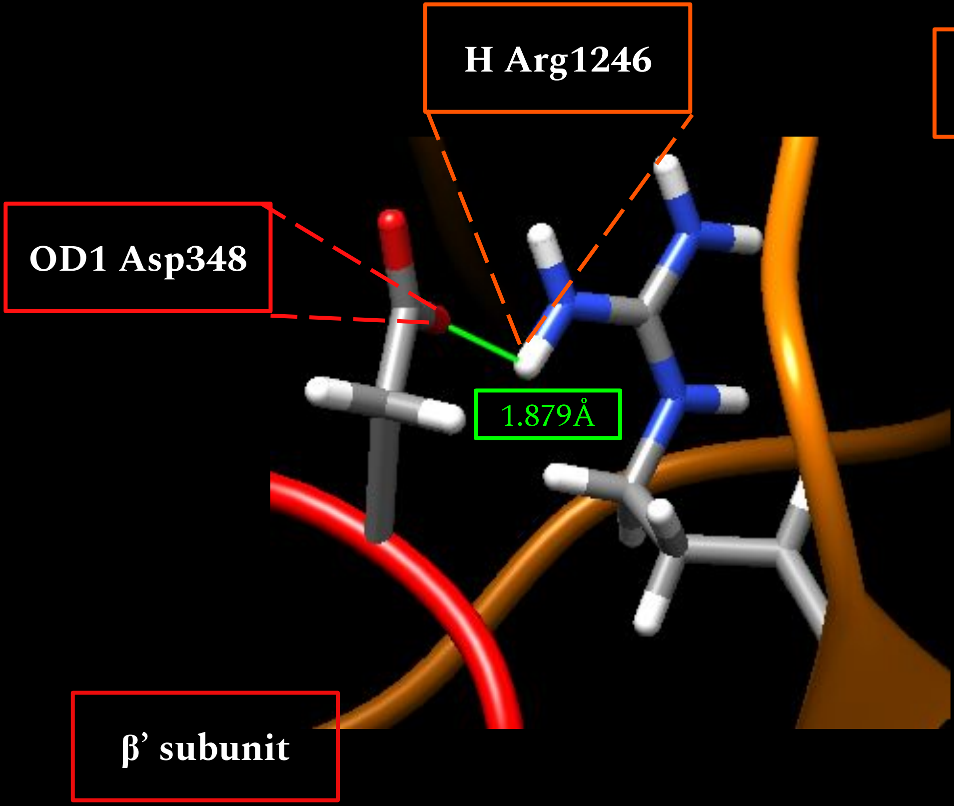


	724									
Consensus	v	q	e	V	d	A	d	r	I	v
Conservation										
Strep_venezuelae|8DY9_1	V	Q	E	L	S	A	D	Y	I	T
Strep_coelicolor|7X74_1	V	Q	E	V	S	A	D	Y	I	T
M_tuberculosis|7Q4U_1	I	E	E	V	S	A	D	Y	I	T
B_subtilis|6WVJ_1	V	E	R	V	E	A	K	N	V	W
T_aquaticus|1L9U_1	V	V	K	V	D	G	T	R	I	A
E_coli|7MKP_1	V	Q	Y	V	D	A	S	R	I	V
P_aeruginosa	I	D	S	V	D	A	S	R	V	V
X_oryzae|6J9E_1	I	E	Q	I	D	A	A	R	I	V
F_tularensis|6WMP_1	V	A	E	V	D	S	N	R	I	V
P_gingivalis|8DKC_1	V	V	F	V	D	A	S	C	I	K

Prokaryotes

Between subunits - β and β' Salt Bridges

7MKP 3.41Å


H Arg1246

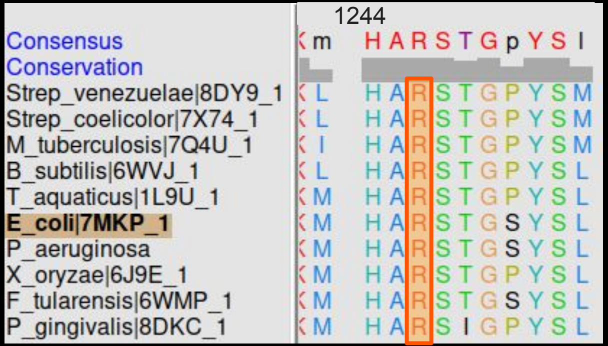
OD1 Asp348

1.879Å

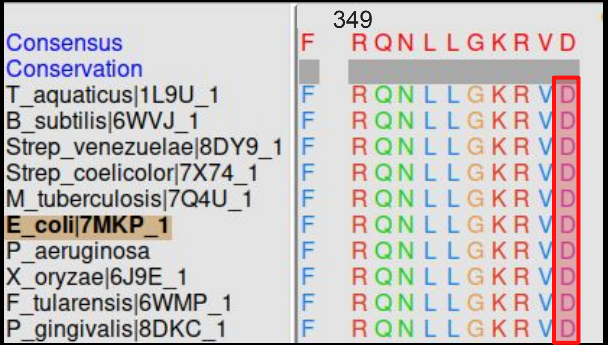
β subunit

β' subunit

Prokaryotes

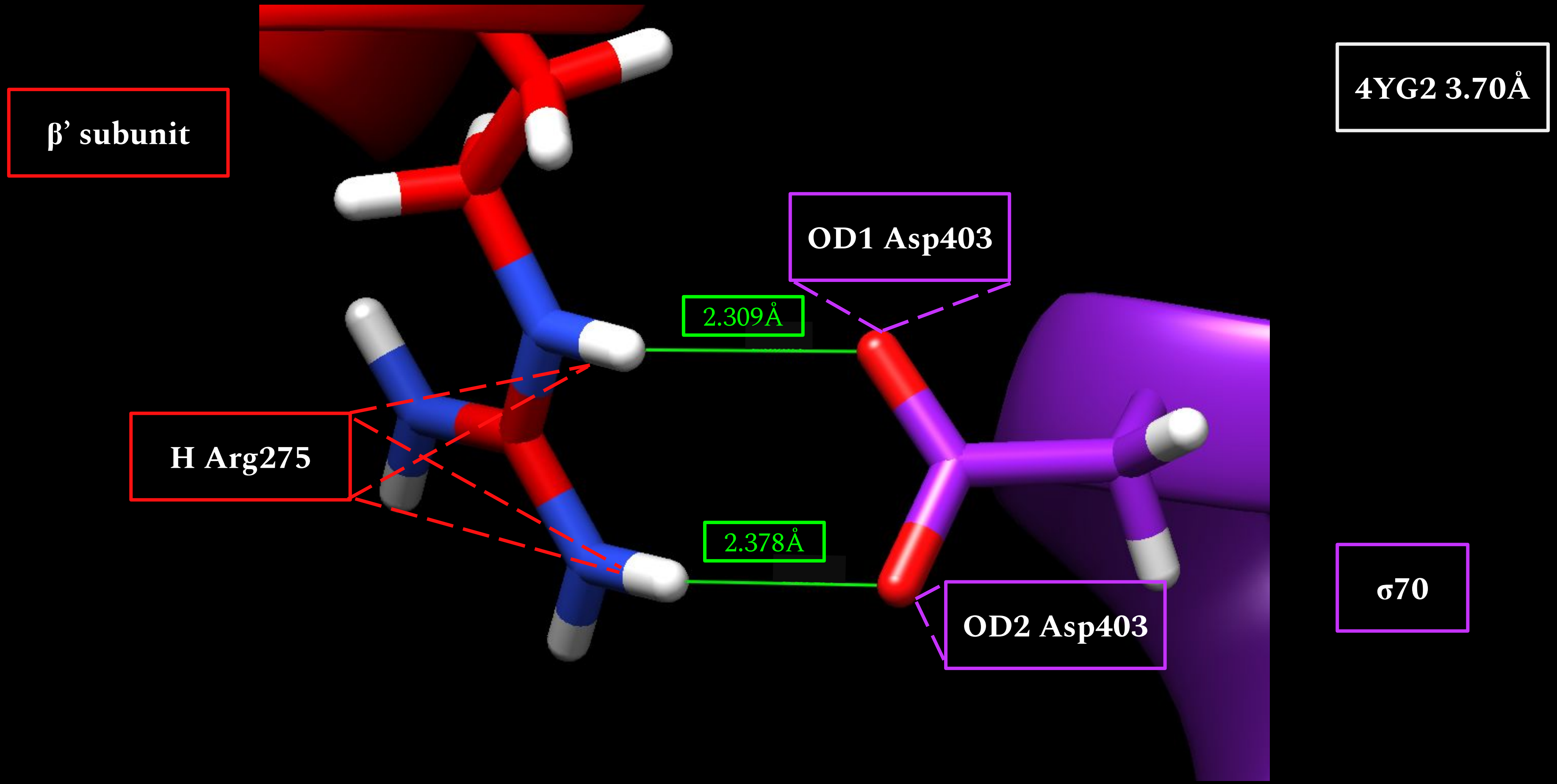


		1244
Consensus	m	H A R S T G p Y S I
Conservation		
Strep_venezuelae|8DY9_1	L	H A R S T G P Y S M
Strep_coelicolor|7X74_1	L	H A R S T G P Y S M
M_tuberculosis|7Q4U_1	I	H A R S T G P Y S M
B_subtilis|6WVJ_1	L	H A R S T G P Y S L
T_aquaticus|1L9U_1	M	H A R S T G P Y S L
E_coli|7MKP_1	M	H A R S T G S Y S L
P_aeruginosa	M	H A R S T G S Y S L
X_oryzae|6J9E_1	M	H A R S T G P Y S L
F_tularensis|6WMP_1	M	H A R S T G S Y S L
P_gingivalis|8DKC_1	M	H A R S I G P Y S L



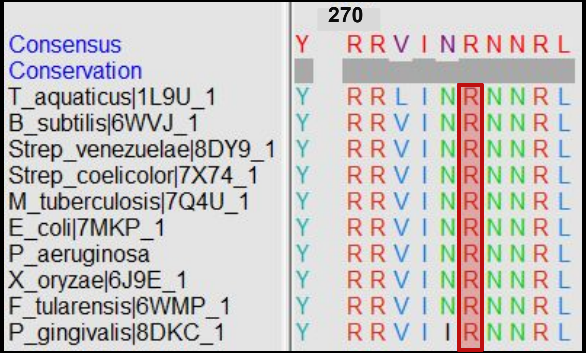
		349
Consensus	F	R Q N L L G K R V D
Conservation		
T_aquaticus|1L9U_1	F	R Q N L L G K R V D
B_subtilis|6WVJ_1	F	R Q N L L G K R V D
Strep_venezuelae|8DY9_1	F	R Q N L L G K R V D
Strep_coelicolor|7X74_1	F	R Q N L L G K R V D
M_tuberculosis|7Q4U_1	F	R Q N L L G K R V D
E_coli|7MKP_1	F	R Q N L L G K R V D
P_aeruginosa	F	R Q N L L G K R V D
X_oryzae|6J9E_1	F	R Q N L L G K R V D
F_tularensis|6WMP_1	F	R Q N L L G K R V D
P_gingivalis|8DKC_1	F	R Q N L L G K R V D

Between subunits - σ70 and RNAP Salt Bridges



Between subunits - σ70 and RNAP Salt Bridges

Prokaryotes



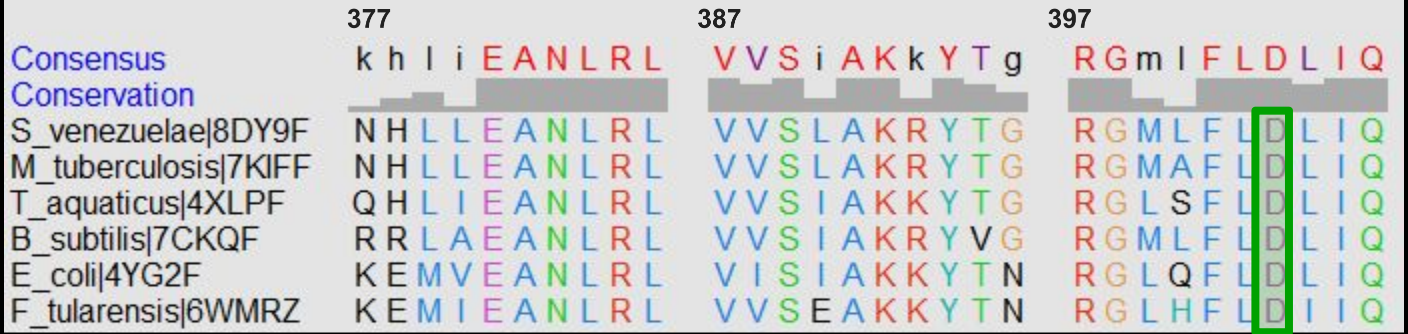
		270
Consensus	Y	RRVINRNNRL
Conservation		
T_aquaticus|1L9U_1	Y	RRLINRNNRL
B_subtilis|6WVJ_1	Y	RRVINRNNRL
Strep_venezuelae|8DY9_1	Y	RRVINRNNRL
Strep_coelicolor|7X74_1	Y	RRVINRNNRL
M_tuberculosis|7Q4U_1	Y	RRVINRNNRL
E_coli|7MKP_1	Y	RRVINRNNRL
P_aeruginosa	Y	RRVINRNNRL
X_oryzae|6J9E_1	Y	RRVINRNNRL
F_tularensis|6WMP_1	Y	RRVINRNNRL
P_gingivalis|8DKC_1	Y	RRVIIRNNRL

β’ subunit

Prokaryotes and Eukaryotes

	249	259	269
Consensus	lrpkvpfdgg	rratsdlnDl	yrriinrnnr
Conservation			
E_coli|7MKP	LRPLVPLDGG	RFATSDLNDL	YRRVINRNNR
P_aeruginosa|7XL3	LRPLVPLDGG	RFATSDLNDL	YRRVINRNNR
T_aquaticus|1L9U	LRPMVQVDGG	RFATSDLNDL	YRRLINRNNR
S_shibatae|2WAQ	VPGKIPFVIL	MRALGILTDR	DIVYAVS---
H_sapiens|5IYC	IKQEVPIIIV	FRALGFVSDR	DILEHIIYDF
S_solfataricus|2PMZ	LEEKVKQASN	ILPQKIVDDL	KNLILNK---
S_cerevisiae|1WCM	VRPSISFNES	QRGEDDLTFK	LADILKANIS

Prokaryotes

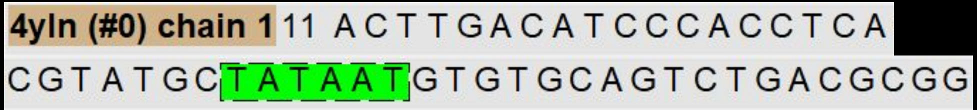


	377	387	397
Consensus	khliEANLRL	VVSiAKkYTg	RGmlFLDLIQ
Conservation			
S_venezuelae|8DY9F	NHLLEANLRL	VVSLAKRYTG	RGMLFLDLIQ
M_tuberculosis|7KIFF	NHLLEANLRL	VVSLAKRYTG	RGMAFLDLIQ
T_aquaticus|4XLPF	QHLIEANLRL	VVSIAKKYTG	RGLSFLDLIQ
B_subtilis|7CKQF	RRLAEANLRL	VVSIAKRYVG	RGMLFLDLIQ
E_coli|4YG2F	KEMVEANLRL	VISIAKKYTN	RGLQFLDLIQ
F_tularensis|6WMRZ	KEMIEANLRL	VVSEAKKYTN	RGLHFLDIIQ

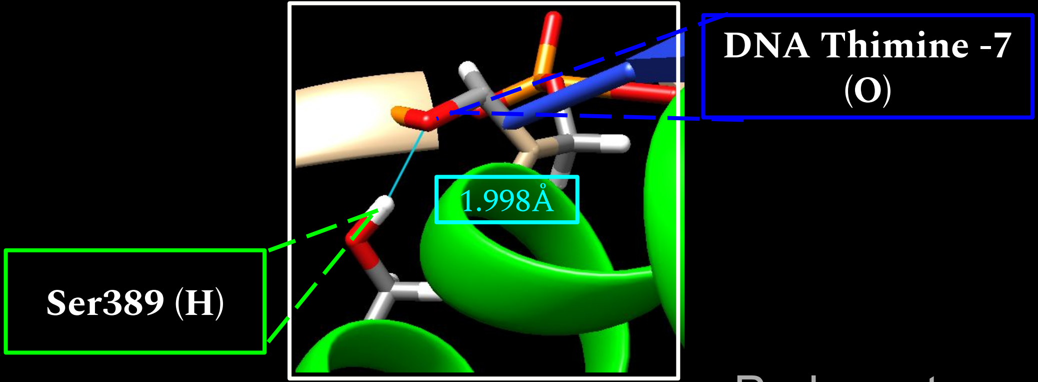
σ70

σ70 - DNA negative element

4YLN 5.5Å



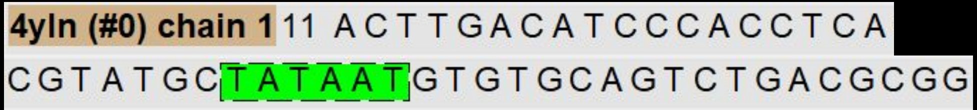
4yln (#0) chain 1 11 ACTTGACATCCCACCTCA
CGTATGCTATAATGTGTGCAGTCTGACGCGG



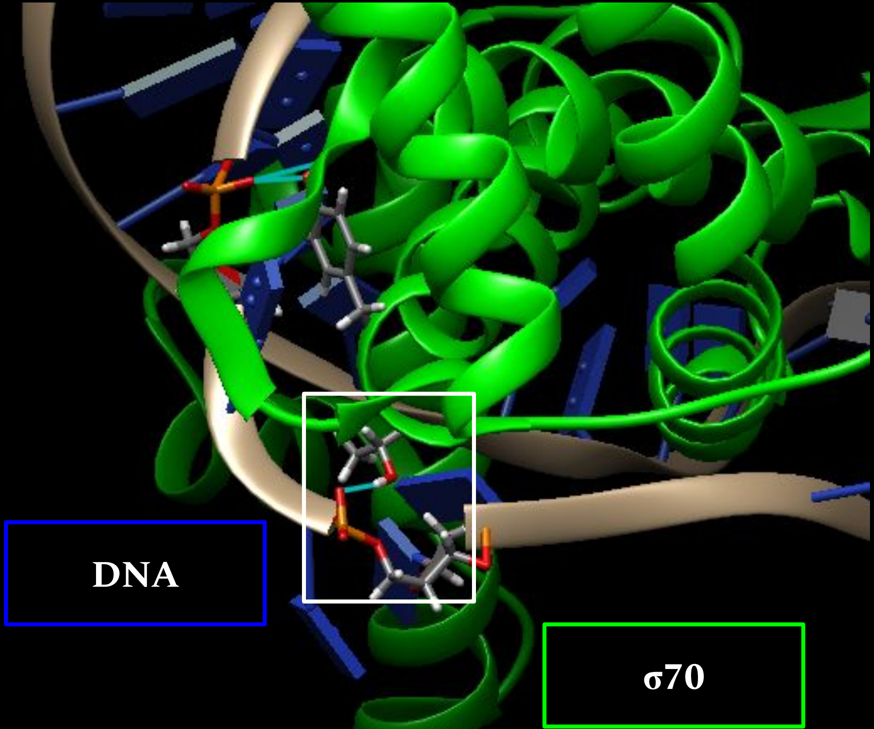
Prokaryotes

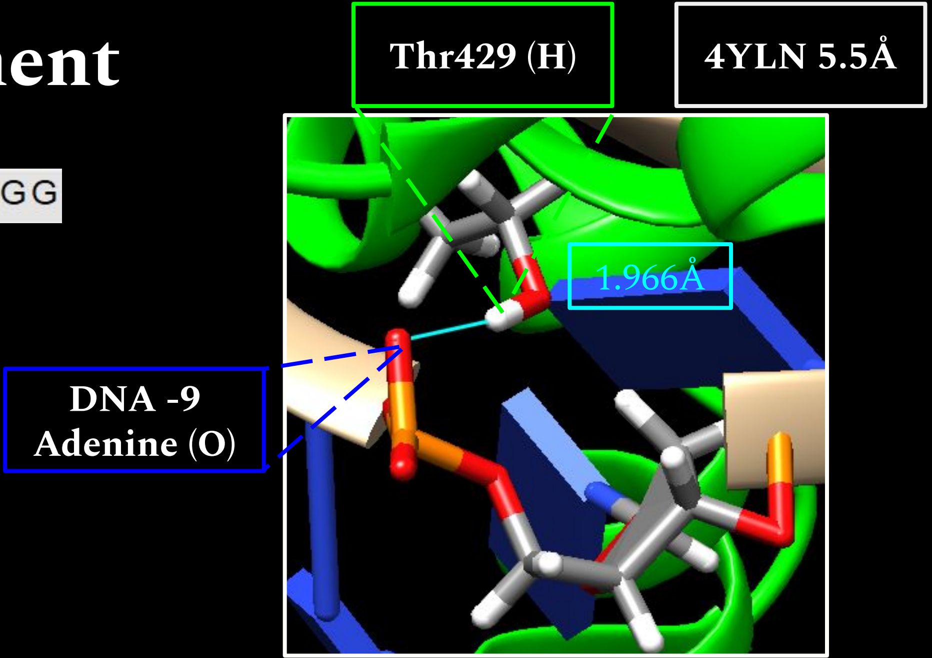
	377	387
Consensus	k h l i E A N L R L	V V S i A K k Y T g
Conservation		
S_venezuelae|8DY9F	N H L L E A N L R L	V V S L A K R Y T G
M_tuberculosis|7KIFF	N H L L E A N L R L	V V S L A K R Y T G
T_aquaticus|4XLPF	Q H L I E A N L R L	V V S I A K K Y T G
B_subtilis|7CKQF	R R L A E A N L R L	V V S I A K R Y V G
E_coli|4YG2F	K E M V E A N L R L	V I S I A K K Y T N
F_tularensis|6WMRZ	K E M I E A N L R L	V V S E A K K Y T N

σ70 - DNA negative element

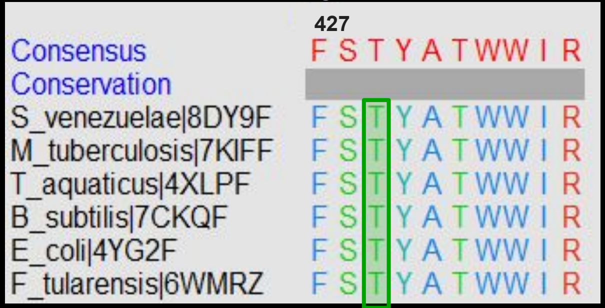


4yln (#0) chain 1 11 ACTTGACATCCCACCTCA
CGTATGCTATAATGTGTGCAGTCTGACGCGG



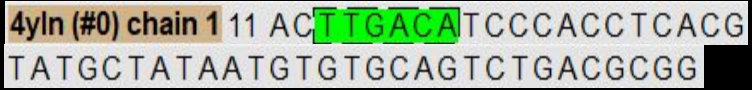


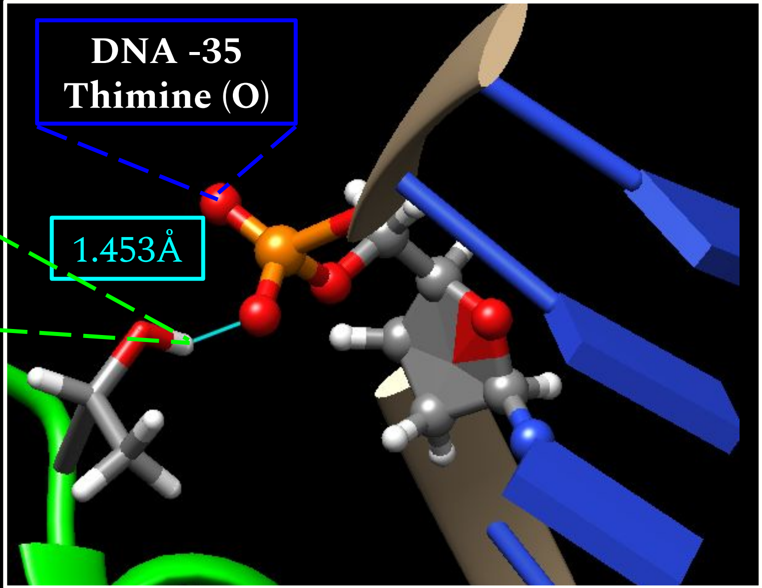
Prokaryotes



	427
Consensus	FSTYATWWIR
Conservation	
S_venezuelae|8DY9F	FSTYATWWIR
M_tuberculosis|7KIFF	FSTYATWWIR
T_aquaticus|4XLPF	FSTYATWWIR
B_subtilis|7CKQF	FSTYATWWIR
E_coli|4YG2F	FSTYATWWIR
F_tularensis|6WMRZ	FSTYATWWIR

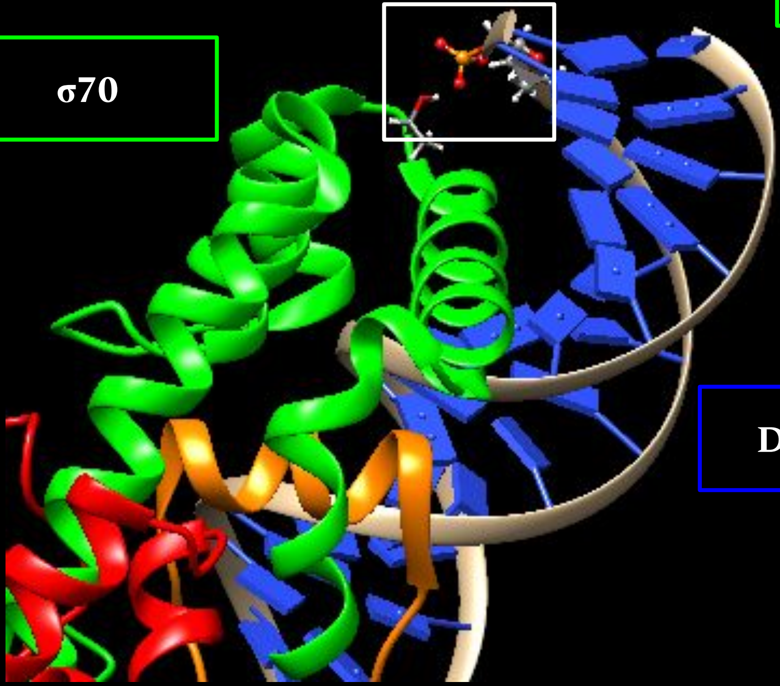
σ70 - DNA negative element


4yln (#0) chain 1 11 ACTTGACATCCCACCTCACG
TATGCTATAATGTGTGCAGTCTGACGCGG



Thr583 (H)

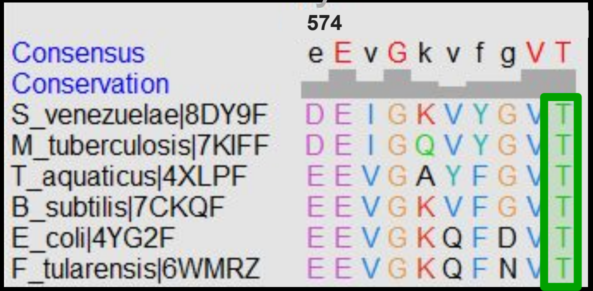
σ70



DNA

4YLN 5.5Å

Prokaryotes



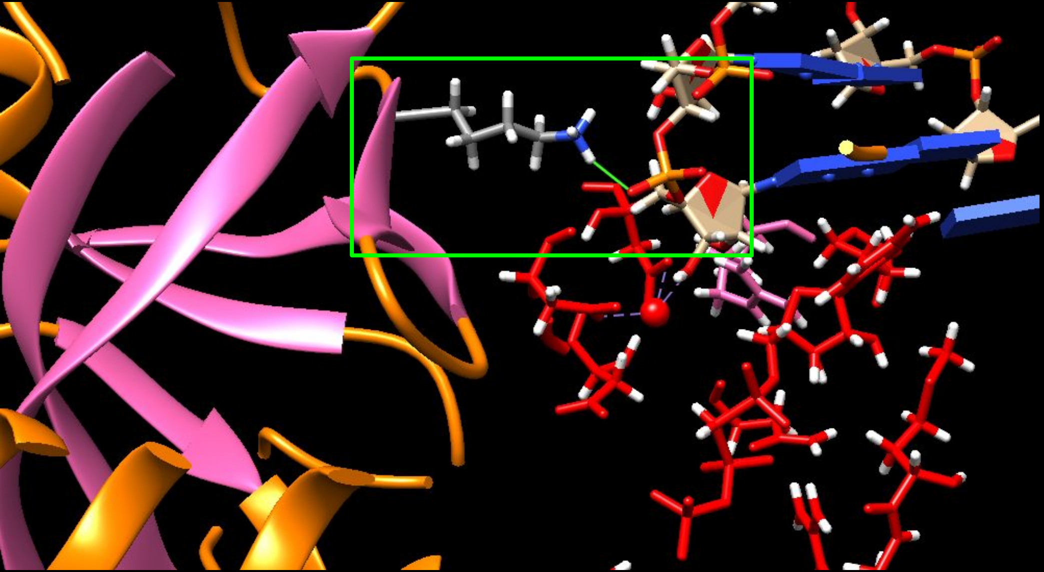
	574									
Consensus	e	E	v	G	k	v	f	g	V	T
Conservation										
S_venezuelae|8DY9F	D	E	I	G	K	V	Y	G	V	T
M_tuberculosis|7KIFF	D	E	I	G	Q	V	Y	G	V	T
T_aquaticus|4XLPF	E	E	V	G	A	Y	F	G	V	T
B_subtilis|7CKQF	E	E	V	G	K	V	F	G	V	T
E_coli|4YG2F	E	E	V	G	K	Q	F	D	V	T
F_tularensis|6WMRZ	E	E	V	G	K	Q	F	N	V	T

DPBB - DNA

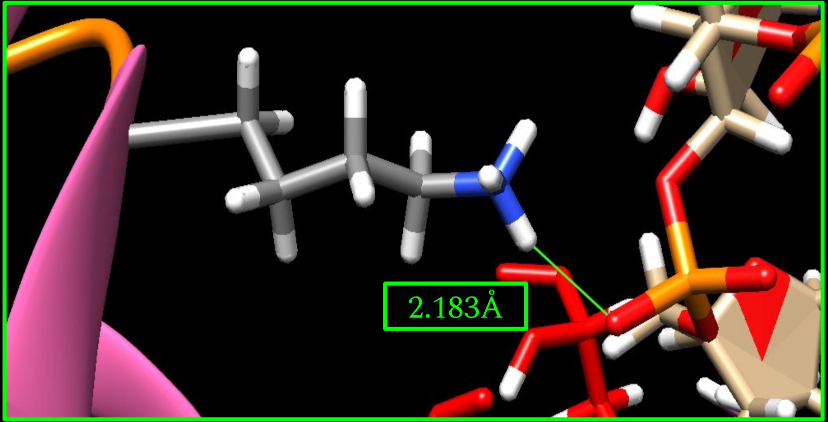
7MKO 3.15Å

β subunit

DNA

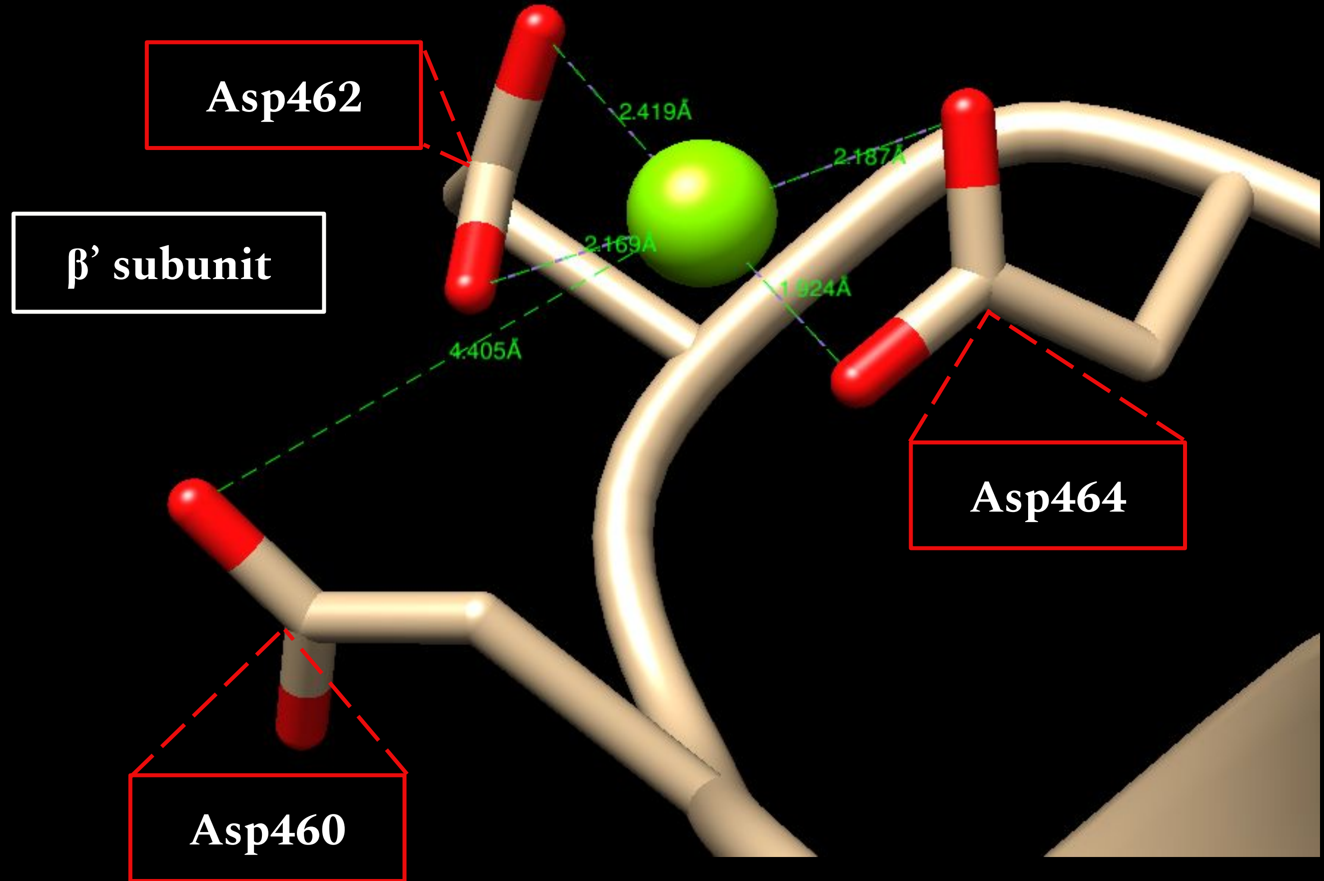


Lys1065 (H)



DNA Adenine 20 (O)

Coenzyme binding - Mg^{2+}



Asp462

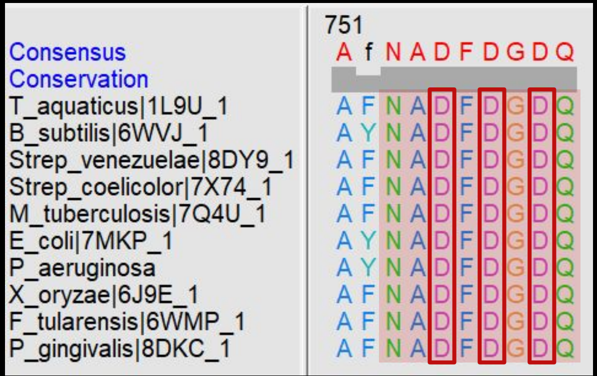
β’ subunit

Asp464

Asp460

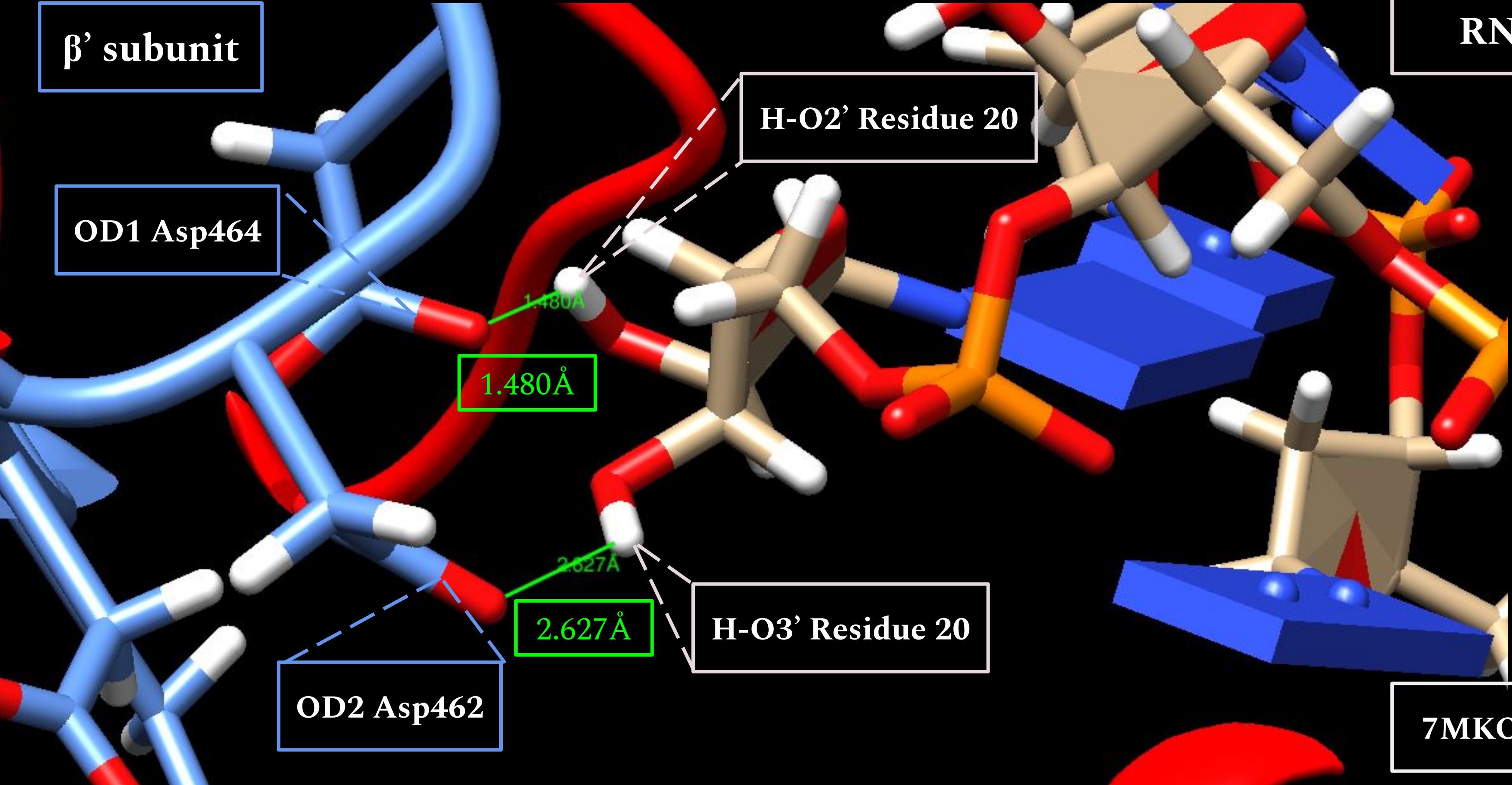
7MKP 3.41Å

Prokaryotes



	751									
Consensus	A	f	N	A	D	F	D	G	D	Q
Conservation										
T_aquaticus|1L9U_1	A	F	N	A	D	F	D	G	D	Q
B_subtilis|6WVJ_1	A	Y	N	A	D	F	D	G	D	Q
Strep_venezuelae|8DY9_1	A	F	N	A	D	F	D	G	D	Q
Strep_coelicolor|7X74_1	A	F	N	A	D	F	D	G	D	Q
M_tuberculosis|7Q4U_1	A	F	N	A	D	F	D	G	D	Q
E_coli|7MKP_1	A	Y	N	A	D	F	D	G	D	Q
P_aeruginosa	A	Y	N	A	D	F	D	G	D	Q
X_oryzae|6J9E_1	A	F	N	A	D	F	D	G	D	Q
F_tularensis|6WMP_1	A	F	N	A	D	F	D	G	D	Q
P_gingivalis|8DKC_1	A	F	N	A	D	F	D	G	D	Q

Active Site - Interaction between RNA and Asp Triad



β' subunit

RNA

H-O2' Residue 20

OD1 Asp464

1.480Å

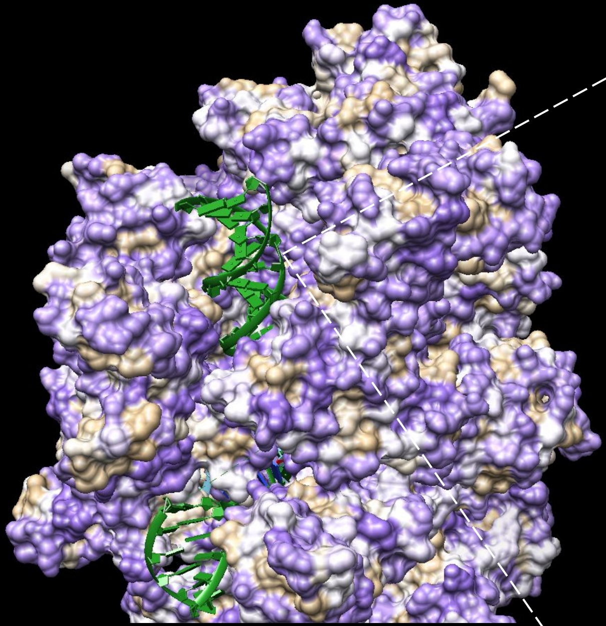
2.627Å

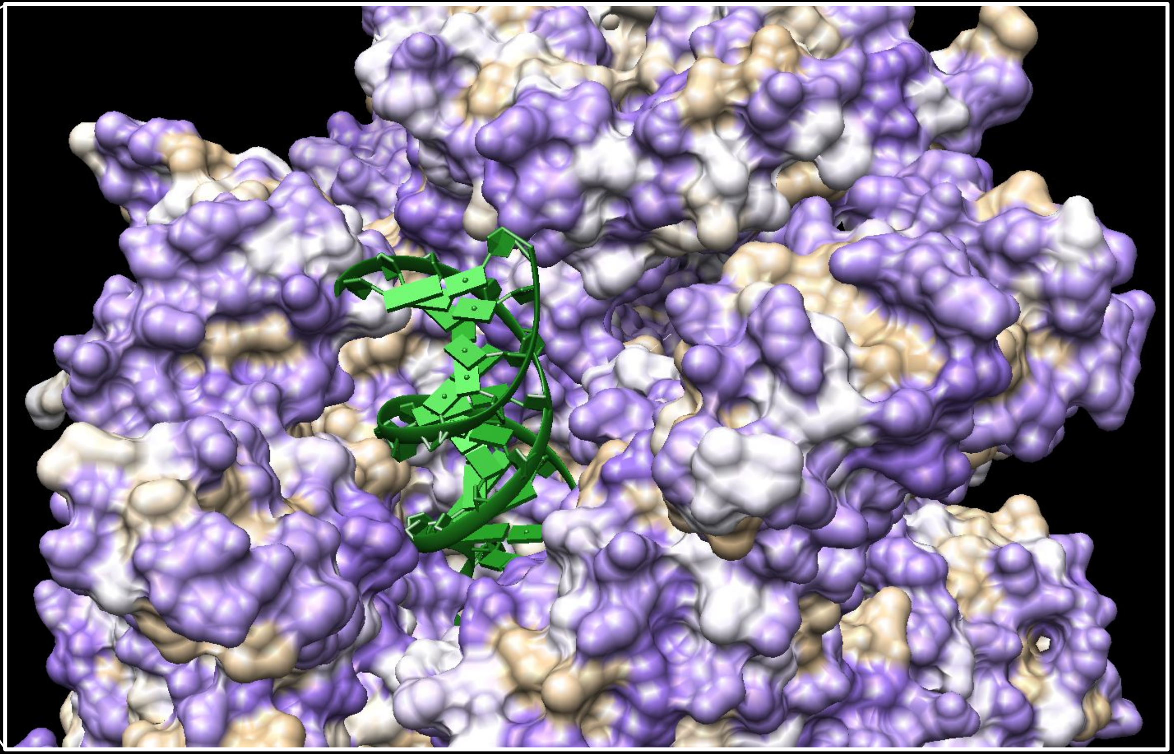
H-O3' Residue 20

OD2 Asp462

7MKO 3.15Å

Active Site - Hydrophobic Interactions

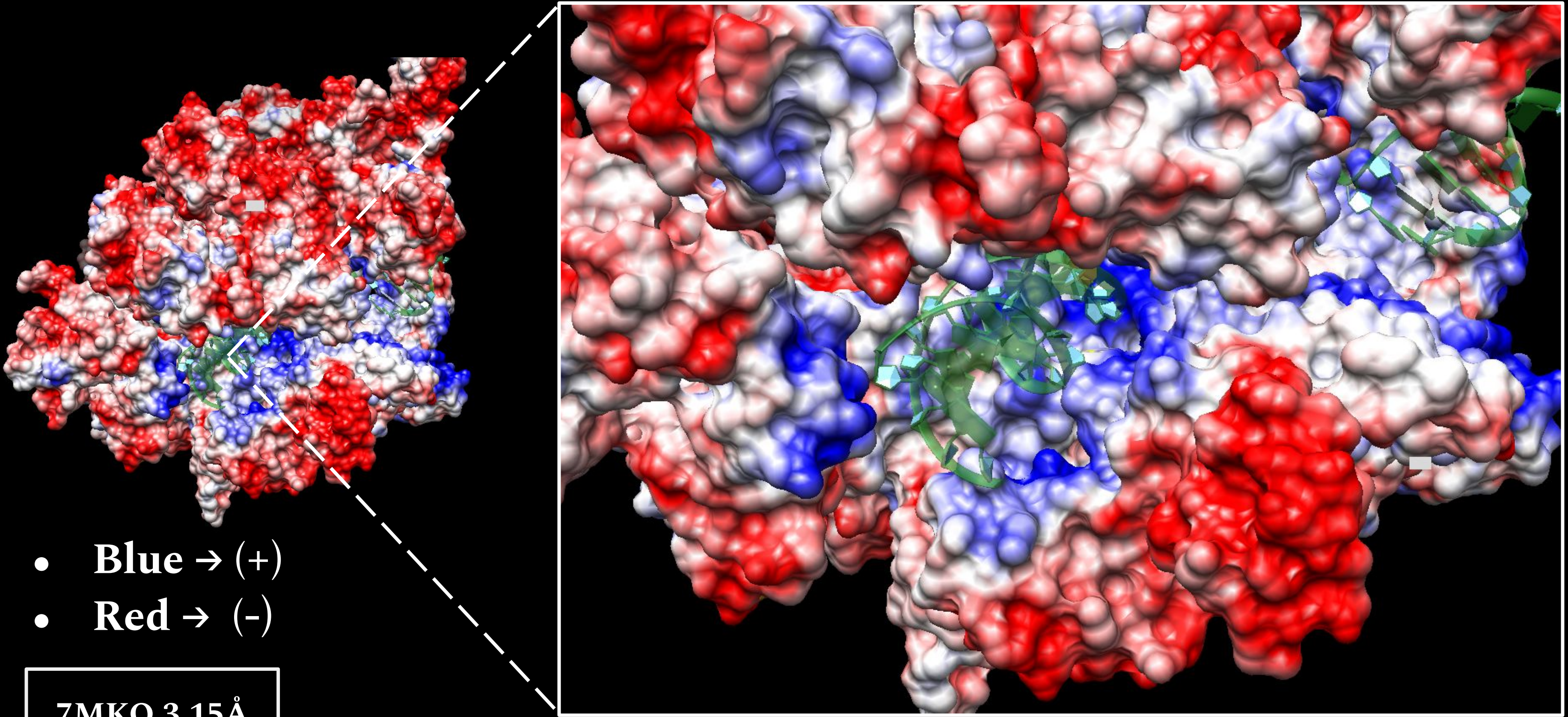




- Purple → Hydrophilic
- Brown → Hydrophobic

7MKO 3.15Å

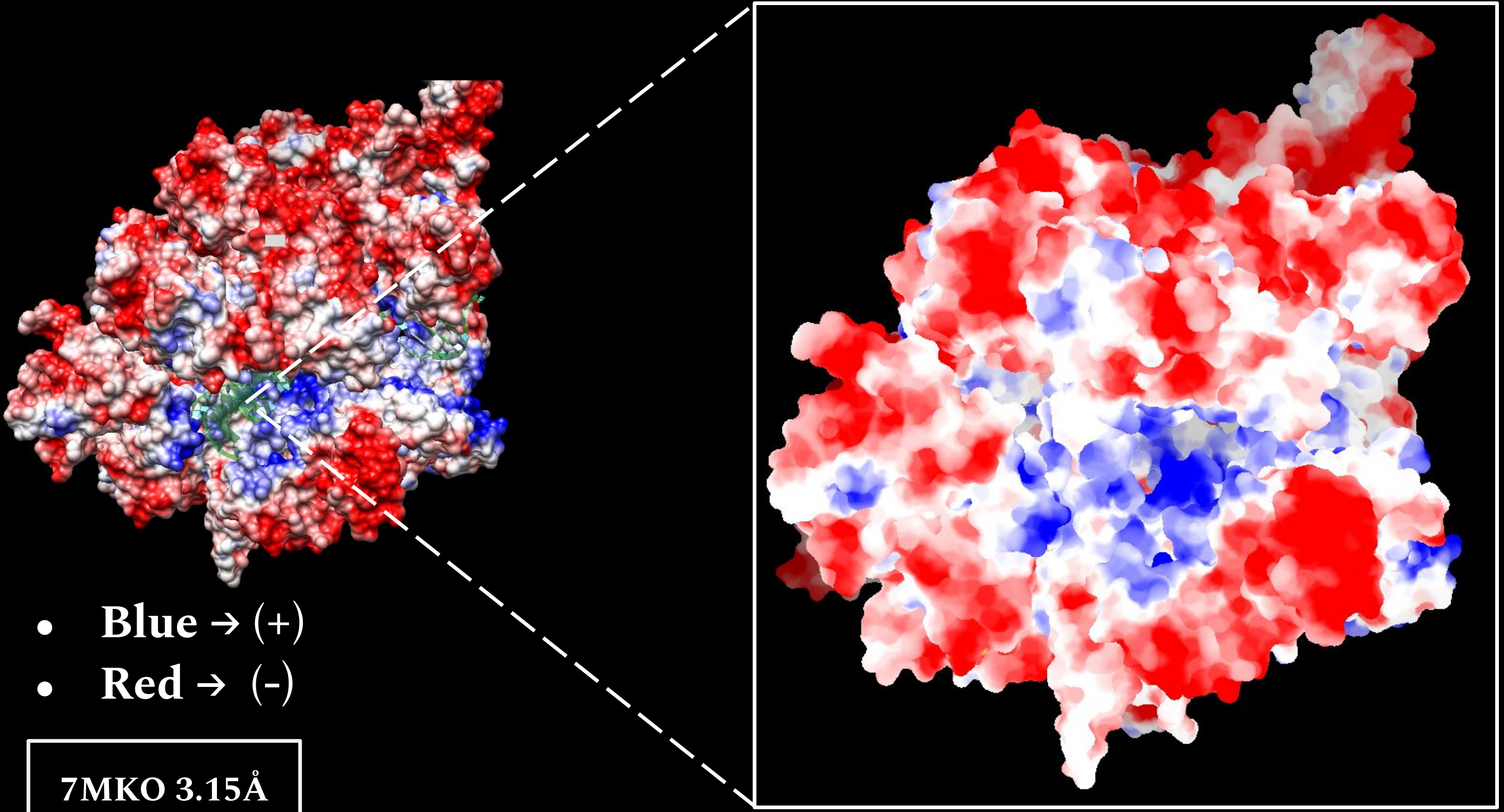
Active Site - Electrostatic Interactions



- Blue → (+)
- Red → (-)

7MKO 3.15Å

Active Site - Electrostatic Interactions

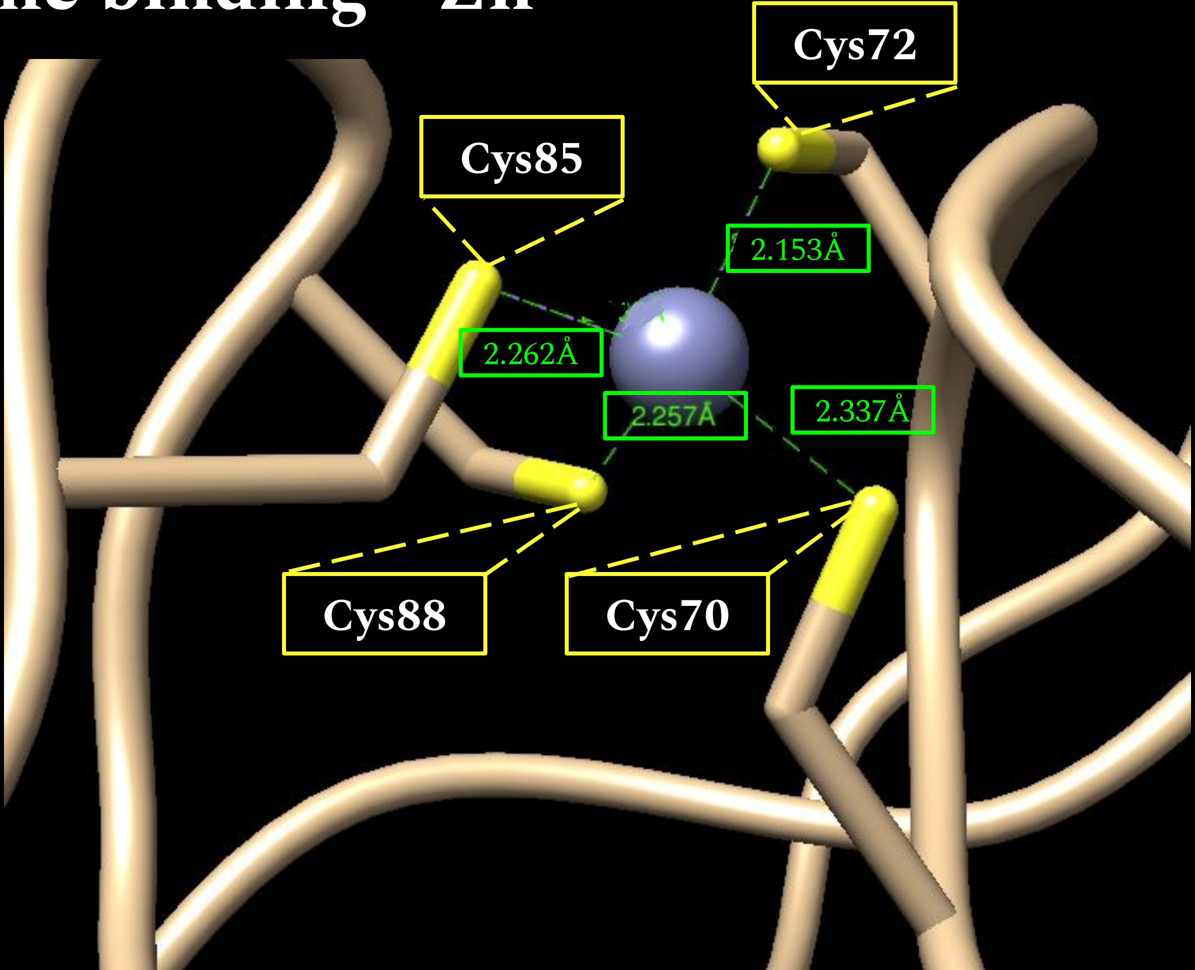


- **Blue** → (+)
- **Red** → (-)

7MKO 3.15Å

Coenzyme binding - Zn^{2+}

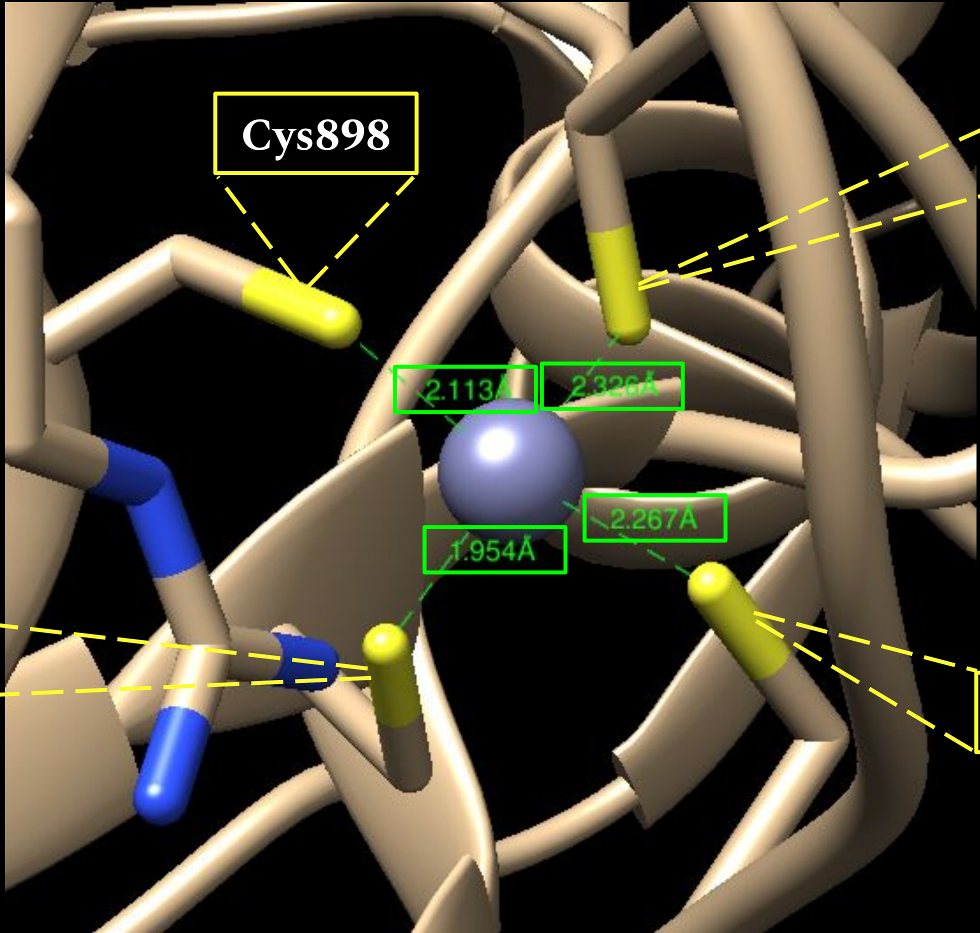
β' subunit



7MKP 3.41Å

Coenzyme binding - Zn^{2+}

β' subunit



Cys898

Cys888

7MKP 3.41Å

Cys895

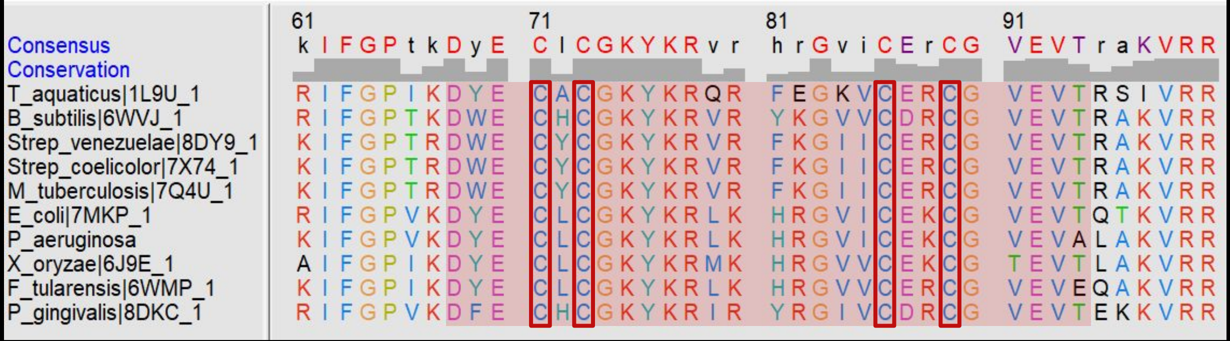
Cys814

β’ subunit

Zinc Binding Domain

Prokaryotes

*Positions based on e.coli



	61	71	81	91
Consensus	k I F G P t k D y E	C I C G K Y K R v r	h r G v i C E r C G	V E V T r a K V R R
Conservation				
T_aquaticus|1L9U_1	R I F G P I K D Y E	C A C G K Y K R Q R	F E G K V C E R C G	V E V T R S I V R R
B_subtilis|6WVJ_1	R I F G P T K D W E	C H C G K Y K R V R	Y K G V V C D R C G	V E V T R A K V R R
Strep_venezuelae|8DY9_1	K I F G P T R D W E	C Y C G K Y K R V R	F K G I I C E R C G	V E V T R A K V R R
Strep_coelicolor|7X74_1	K I F G P T R D W E	C Y C G K Y K R V R	F K G I I C E R C G	V E V T R A K V R R
M_tuberculosis|7Q4U_1	K I F G P T R D W E	C Y C G K Y K R V R	F K G I I C E R C G	V E V T R A K V R R
E_coli|7MKP_1	R I F G P V K D Y E	C L C G K Y K R L K	H R G V I C E K C G	V E V T Q T K V R R
P_aeruginosa	K I F G P V K D Y E	C L C G K Y K R L K	H R G V I C E K C G	V E V A L A K V R R
X_oryzae|6J9E_1	A I F G P I K D Y E	C L C G K Y K R M K	H R G V V C E K C G	T E V T L A K V R R
F_tularensis|6WMP_1	K I F G P I K D Y E	C L C G K Y K R L K	H R G V V C E R C G	V E V E Q A K V R R
P_gingivalis|8DKC_1	R I F G P V K D F E	C H C G K Y K R I R	Y R G I V C D R C G	V E V T E K K V R R

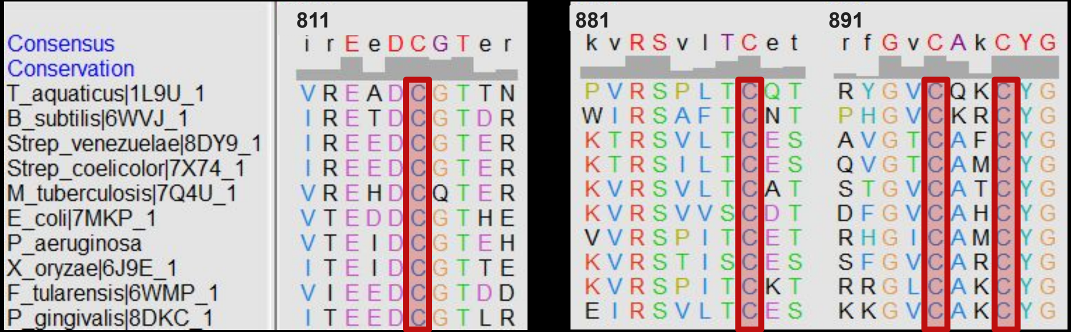
Conservation of residues 65-94

- 4 Cys residues for Zn^{2+} binding
- Positively charged aa

Conservation of residues 814 - 898

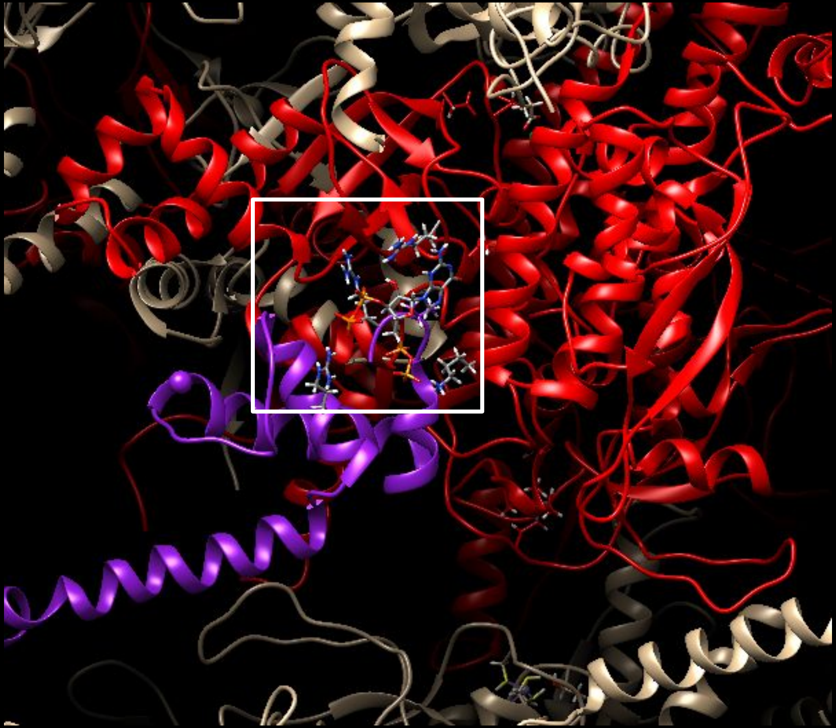
- 4 Cys residues for Zn^{2+} binding

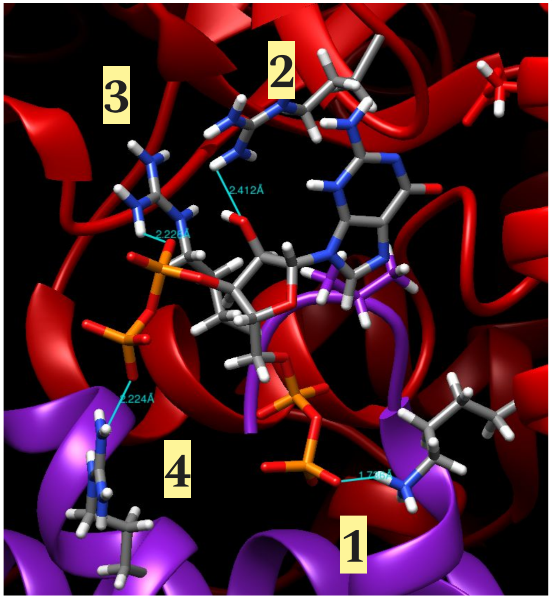
Prokaryotes



	811	881	891
Consensus	i r E e D C G T e r	k v R S v l T C e t	r f G v C A k C Y G
Conservation			
T_aquaticus|1L9U_1	V R E A D C G T T N	P V R S P L T C Q T	R Y G V C Q K C Y G
B_subtilis|6WVJ_1	I R E T D C G T D R	W I R S A F T C N T	P H G V C K R C Y G
Strep_venezuelae|8DY9_1	I R E E D C G T E R	K T R S V L T C E S	A V G T C A F C Y G
Strep_coelicolor|7X74_1	I R E E D C G T E R	K T R S I L T C E S	Q V G T C A M C Y G
M_tuberculosis|7Q4U_1	V R E H D C Q T E R	K V R S V L T C A T	S T G V C A T C Y G
E_coli|7MKP_1	V T E D D C G T H E	K V R S V V S C D T	D F G V C A H C Y G
P_aeruginosa	V T E I D C G T E H	V V R S P I T C E T	R H G I C A M C Y G
X_oryzae|6J9E_1	I T E I D C G T T E	K V R S T I S C E S	S F G V C A R C Y G
F_tularensis|6WMP_1	V I E E D C G T D D	K V R S P I T C K T	R R G L C A K C Y G
P_gingivalis|8DKC_1	I T E E D C G T L R	E I R S V L T C E S	K K G V C A K C Y G

Inhibitor: ppGpp alarmone





β’	ppGpp	Distance
1. Lys615 H	O2B	2.736Å
2. Arg362 H	O2’	2.412Å

ω	ppGpp	Distance
3. Arg3 H	O1C	2.26Å
4. Arg52 H	O2D	2.224Å

4JKR 4.20Å

LIMITATIONS

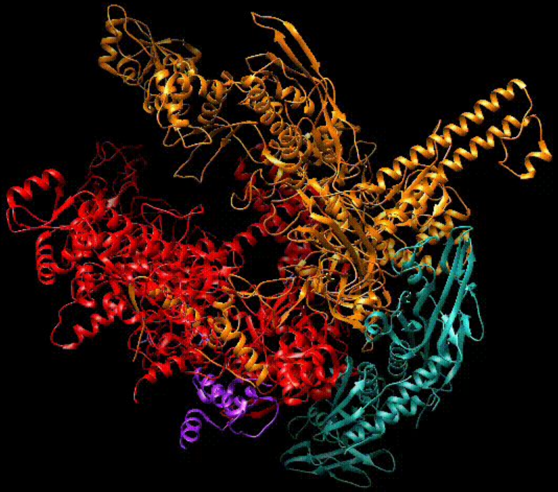
We are not perfect...

Resolution of the structure
(both EM and X-ray)

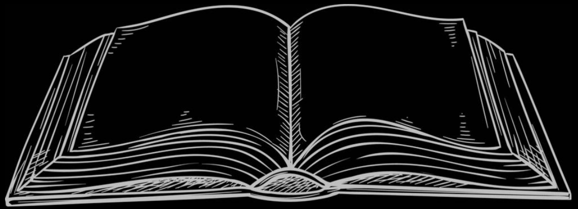
>3Å

😔

Size of the molecule



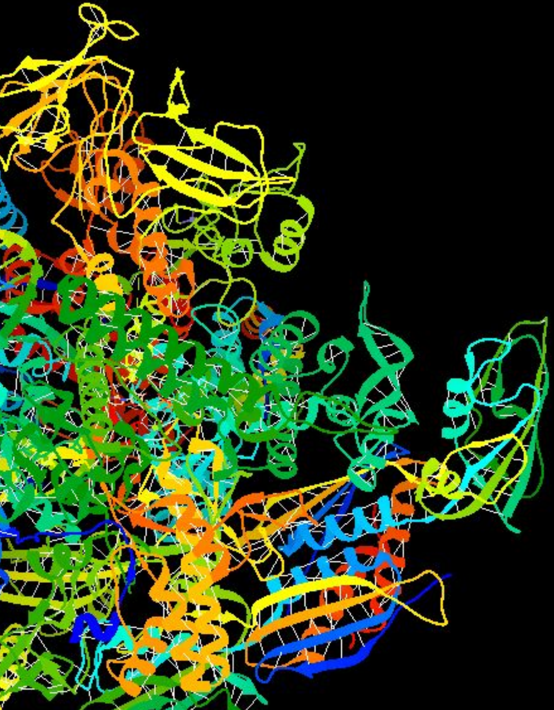
Conflicting literature
and missing information



CONCLUSIONS

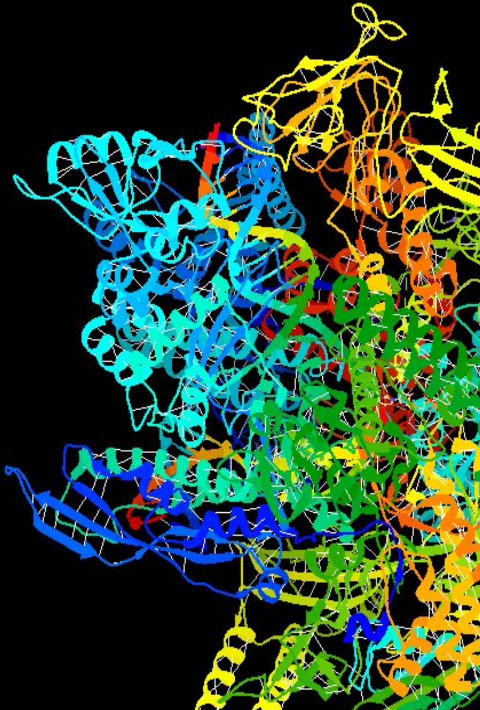
Take-home messages

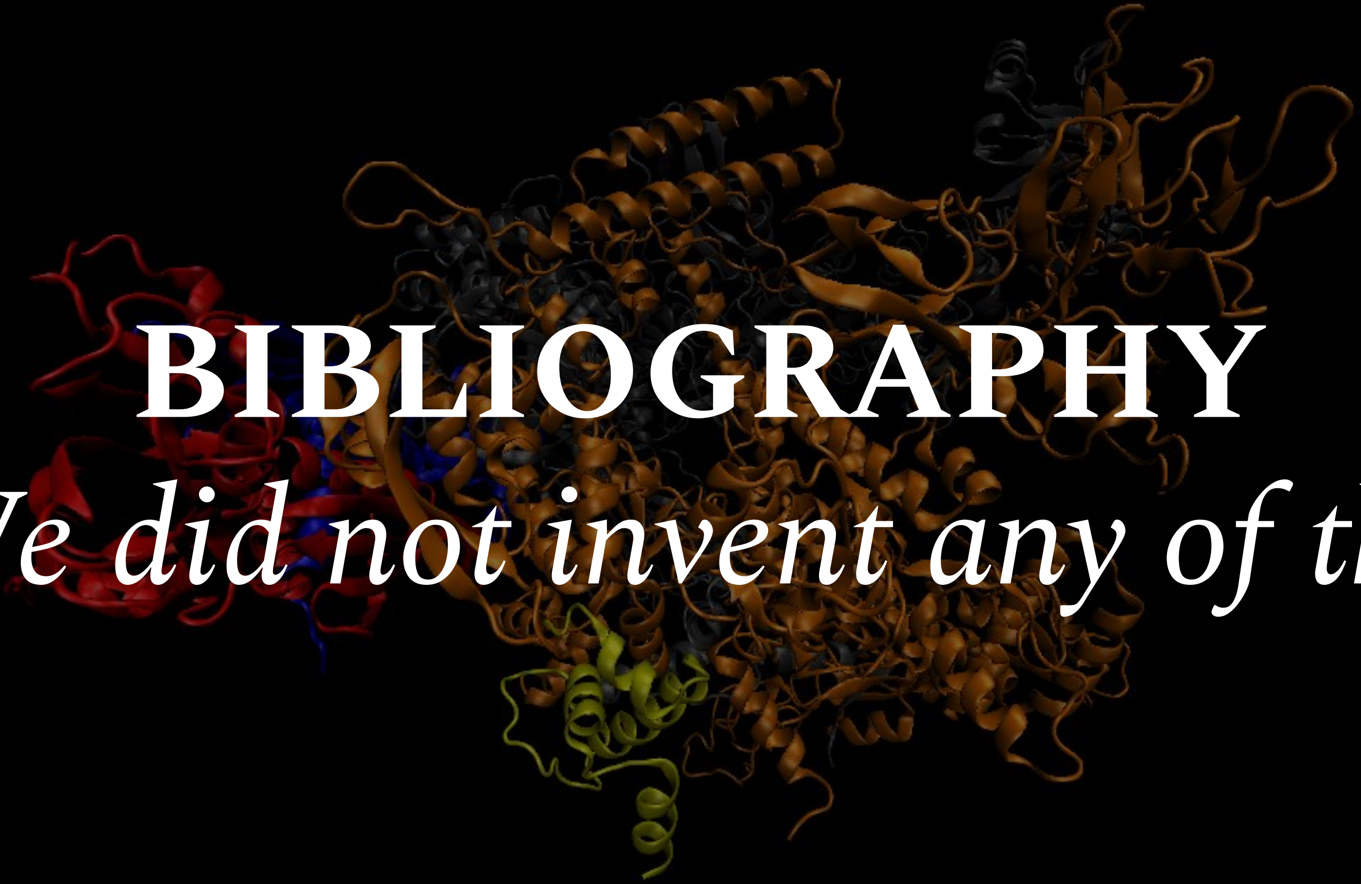
Complex structure, multifunctional enzyme



The most functional residues are evolutionarily conserved

Many relevant interactions between subunits, ligands and inhibitors





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We did not invent any of this

The following PDB IDs were referenced in this work: 7MKP, 7MKO, 7Q4U, 1L9U, 6J9E, 6WMP, 8DKC, 6WVJ, 8DY9, 5NSR, 5NSS, 2PMZ, 2WAQ, 1WCM, 8HYJ, 7AST, 4YG2, 4XLP, 8DY9, 7X74, 7KIF, 6WMR, 7CKQ, 5UAC, . PDB: Protein Data Bank. Retrieved from https://www.rcsb.org/

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MULTIPLE CHOICE?

Easy, we all want to pass BE

1. **What is true about σ70?**
    a. It’s part of the region that binds DNA
    b. Is conserved among prokaryotes
    c. Also binds to subunit β’
    d. Is separated from the core enzyme after DNA binding
    e. All of the above
2. **RNA polymerase …**
    a. Is only conserved in Eukaryotes
    b. Consists of three enzymes in Humans RNA-pol I / II / III
    c. It has low conservation among species
    d. Has a Gly triad in the alpha subunit
    e. Is made of one subunit
3. **RNAP function is to …**
    a. Transcribe DNA into RNA
    b. Replicate DNA
    c. Translate RNA into proteins
    d. Act as a cell cycle control
    e. It does not have a determined function yet
4. **RNAP is a …**
    a. Ribosomal nucleus activating protein
    b. A transcription factor
    c. DNA-dependant RNA polymerase
    d. A cell cycle inhibitor
    e. A cancer drug
5. **Which statement(s) is true?**
    a. The β and β’ subunit form two double psi beta barrels (DPBB)
    b. The alpha subunit is a homodimer
    c. a and b are correct
    d. The β’ subunit interacts with DNA
    e. All of the above

6. **What is the importance of Aspartic acid triad in RNAP?**
    a. Is just a conserved sequence recently discovered
    b. Helps bind Mg2+ as well as RNA and DNA
    c. There is no such sequence in the RNAP enzyme
    d. It has an inhibitor purpose
    e. Generate a charge free zone in the surface of the RNAP
7. **Which statement is correct?**
    a. The binding sites between subunits are not conserved
    b. RNAP is a small molecule easily crystallized
    c. RNAP‘s core enzyme consist only of the beta claw domain
    d. All of them are false
    e. RNAP is a good example to prove that sequence is more important than structure
8. **RNAP interacts with …**
    a. RNA, DNA, co-factors, promoters, …
    b. Ribosomes
    c. The cell membrane
    d. Only DNA
    e. Other structures to form a functional tertiary structure
9. **What is true about the β’ subunit of *E.coli* RNA polymerase?**
    a. It is a small polypeptide chain made by approximately 50 amino acids
    b. It is considered as a non-essential subunit
    c. Together with the α subunit they create the crab claw where DNA will remain to be transcripted
    d. It plays an important role in the nucleotide addition cycle
    e. All of the above
10. **What is true about *E.coli* RNA polymerase?**
    a. It can change its double-psi beta barrel (DPBB) conformation according to multiple interactions
    b. It is considered as a non-essential enzyme
    c. With only the α subunit it can create the crab claw where DNA will remain to be transcripted
    d. It plays an important role in proliferation
    e. All of the above

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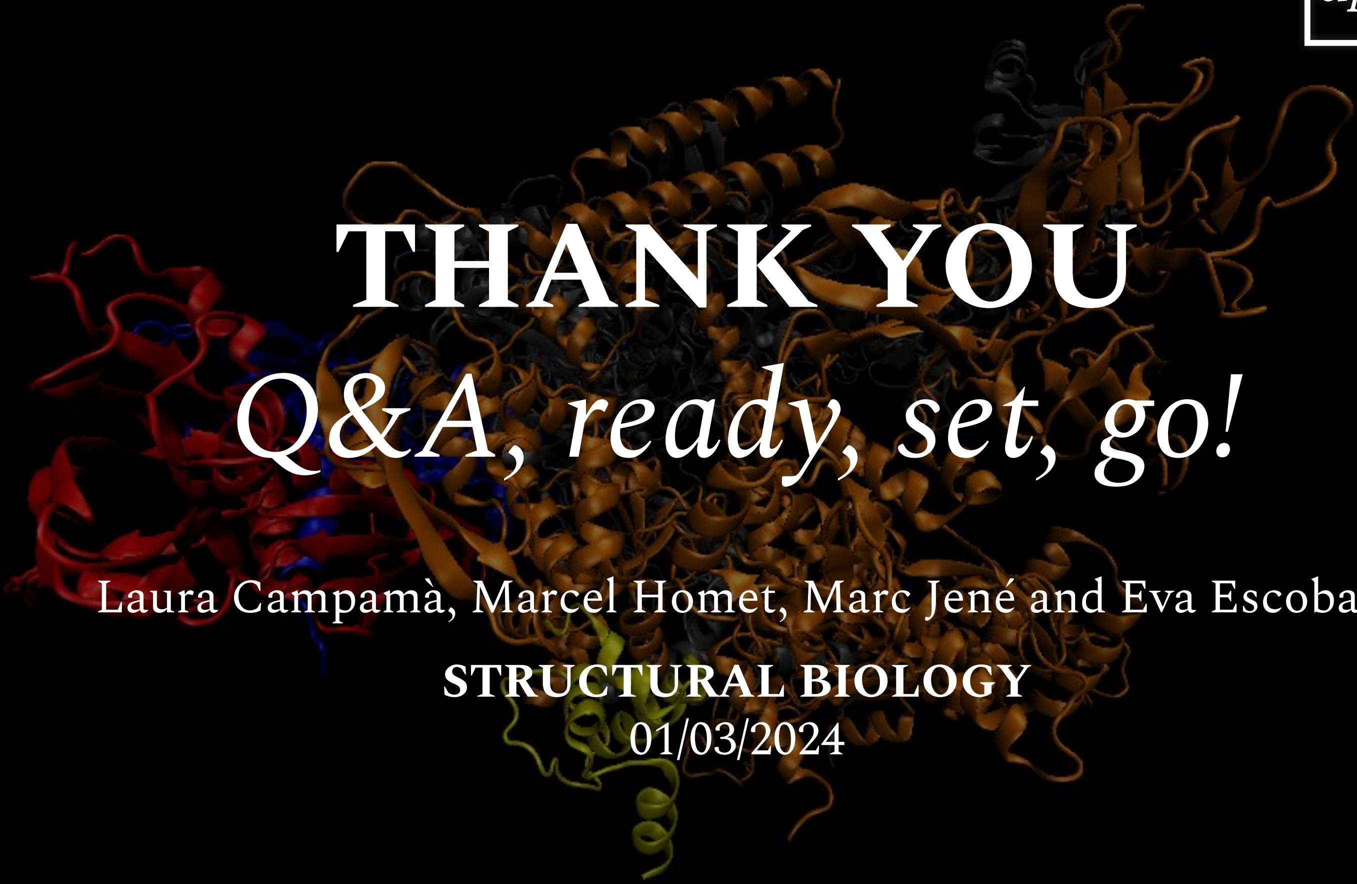


THANK YOU

Q&A, ready, set, go!

Laura Campamà, Marcel Homet, Marc Jené and Eva Escobar

STRUCTURAL BIOLOGY

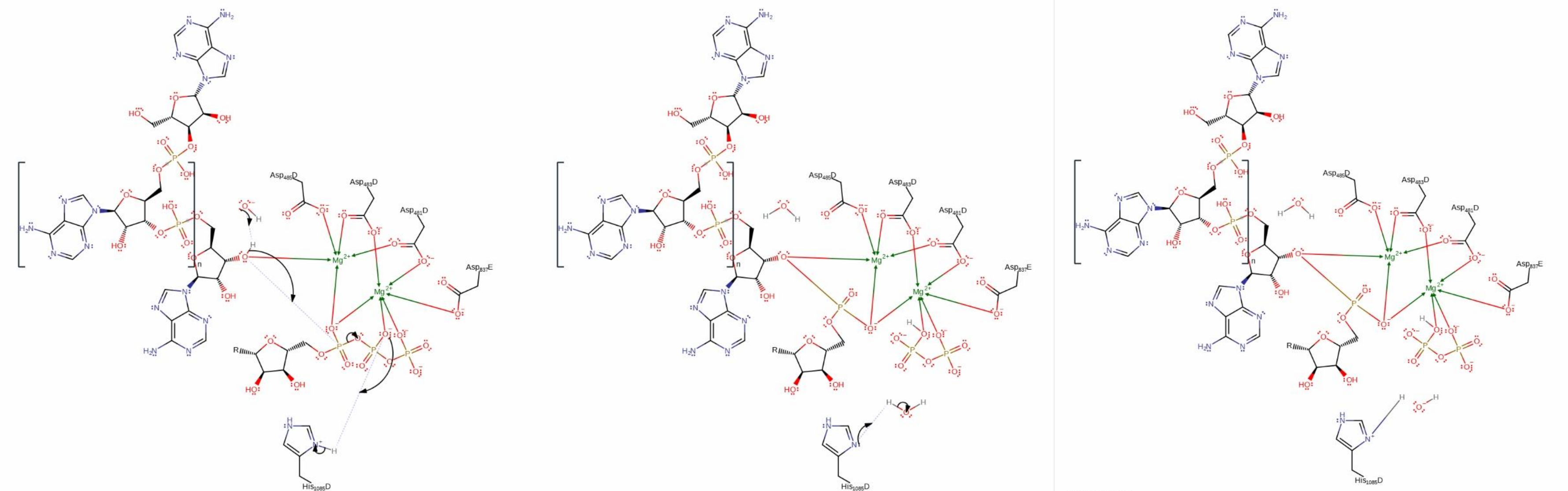
01/03/2024

EXTRA INFORMATION

But wait… there's more!

RNA elongation: NTP addition (catalysis)

Nucleophilic attack of a bound nucleoside 5'-triphosphate by the 3'-hydroxyl of an RNA primer = incorporation of a nucleoside monophosphate into RNA and the release of pyrophosphate



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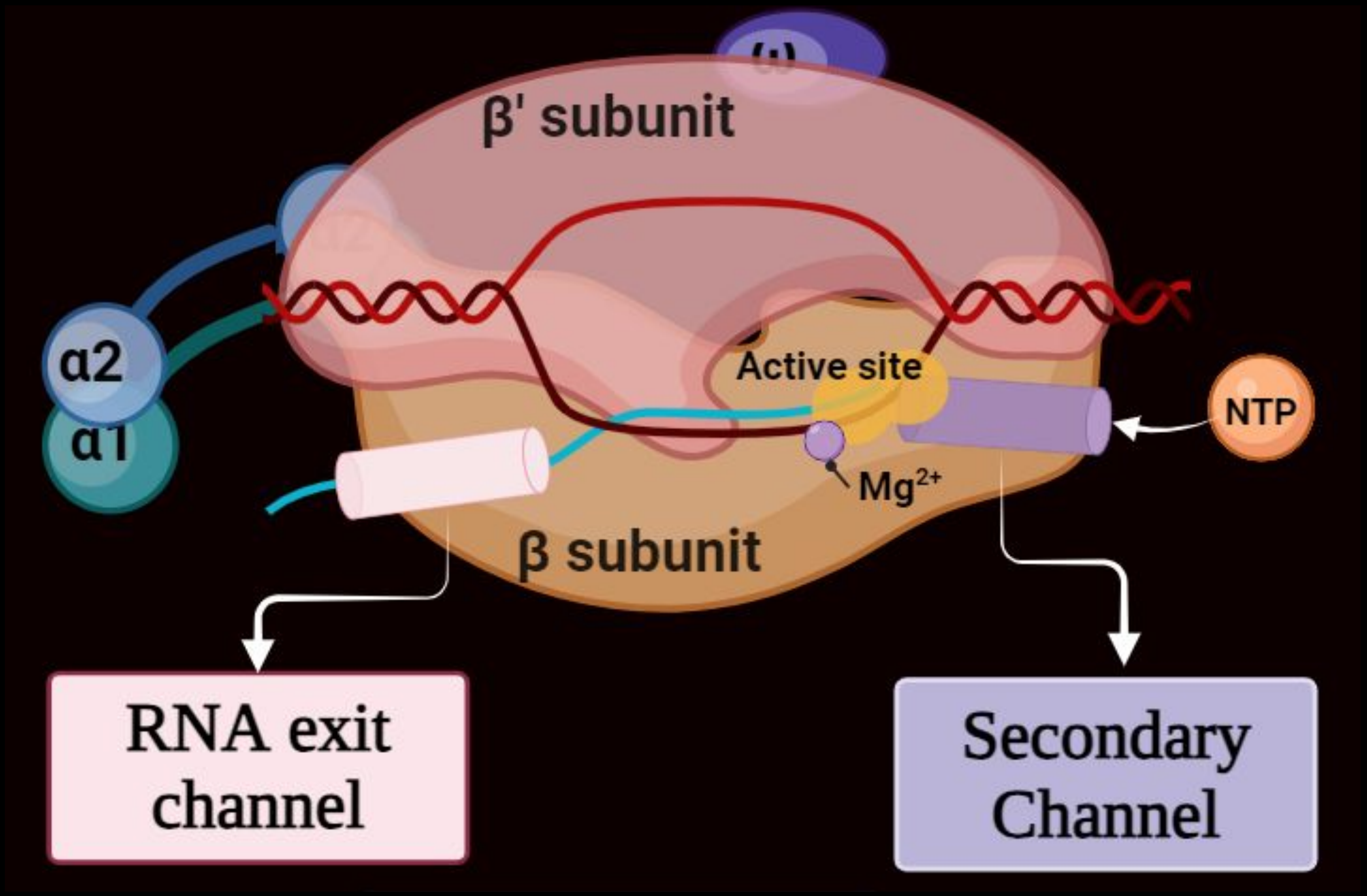
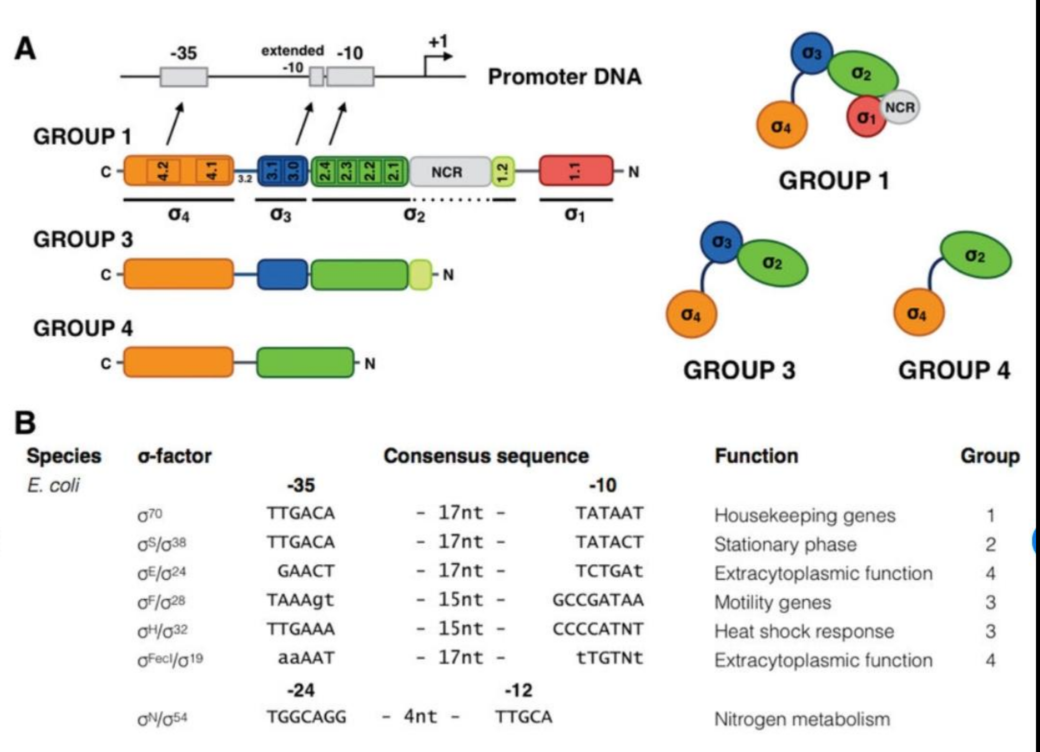
RNAP Channels



Bacterial σ factors



Species	σ-factor	-35		-10	Function	Group
E. coli						
	σ^{70}	TTGACA	- 17nt -	TATAAT	Housekeeping genes	1
	σ^{S}/σ^{38}	TTGACA	- 17nt -	TATACT	Stationary phase	2
	σ^{E}/σ^{24}	GAACT	- 17nt -	TCTGAt	Extracytoplasmic function	4
	σ^{F}/σ^{28}	TAAAgt	- 15nt -	GCCGATAA	Motility genes	3
	σ^{H}/σ^{32}	TTGAAA	- 15nt -	CCCCATNT	Heat shock response	3
	$\sigma^{FecI}/\sigma^{19}$	aaAAT	- 17nt -	tTGTNt	Extracytoplasmic function	4
		-24		-12		
	σ^{N}/σ^{54}	TGGCAGG	- 4nt -	TTGCA	Nitrogen metabolism	

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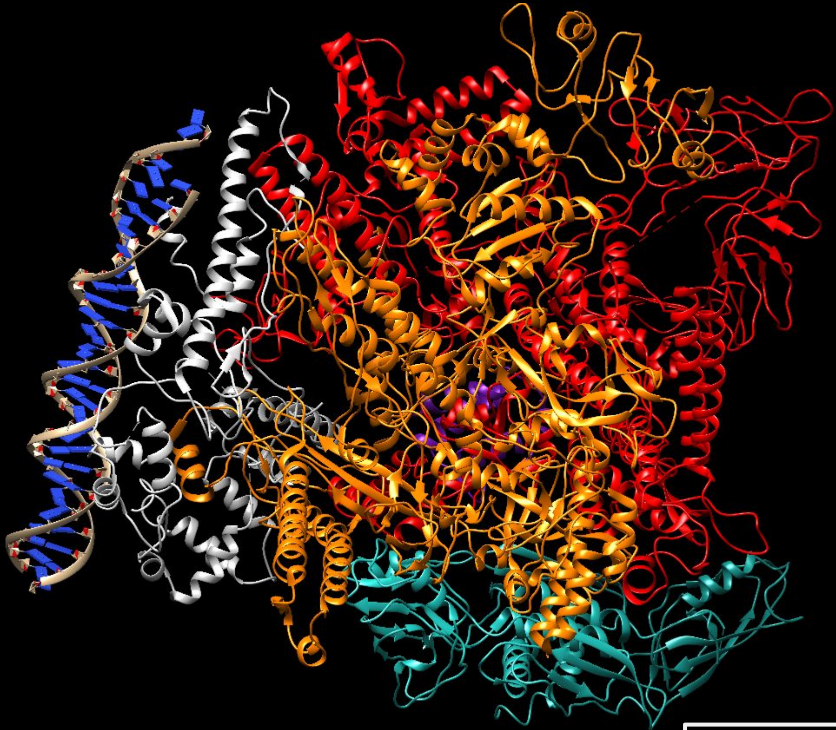
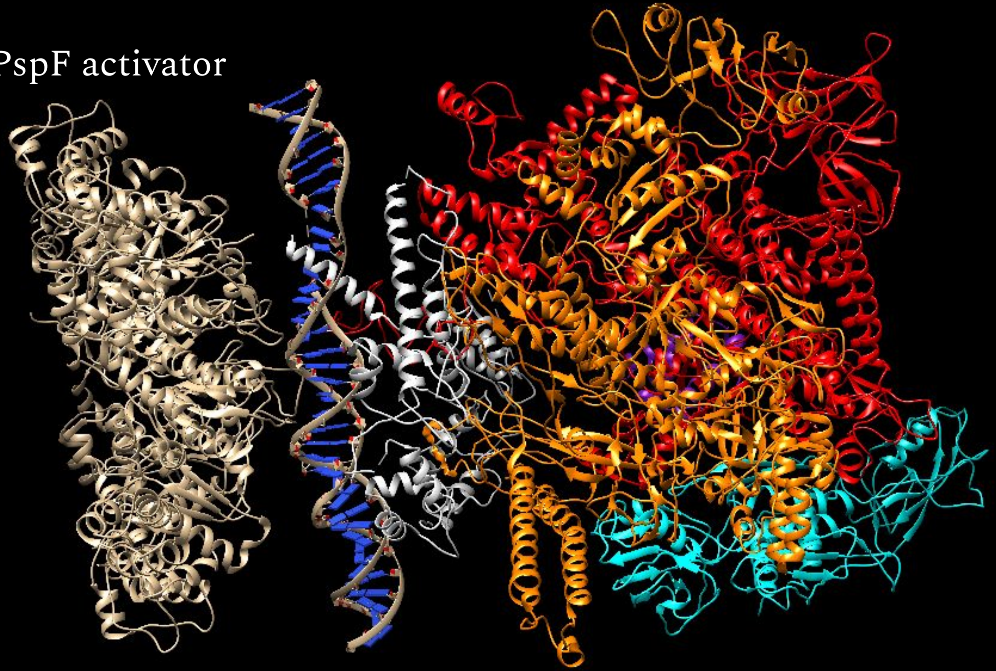
Active and inactive conformations

σ54 RNAP closed



5NSR 3.8Å

σ54 RNAP intermediate



5NSS 5.8Å

Active and inactive conformations

σ54 RNAP closed

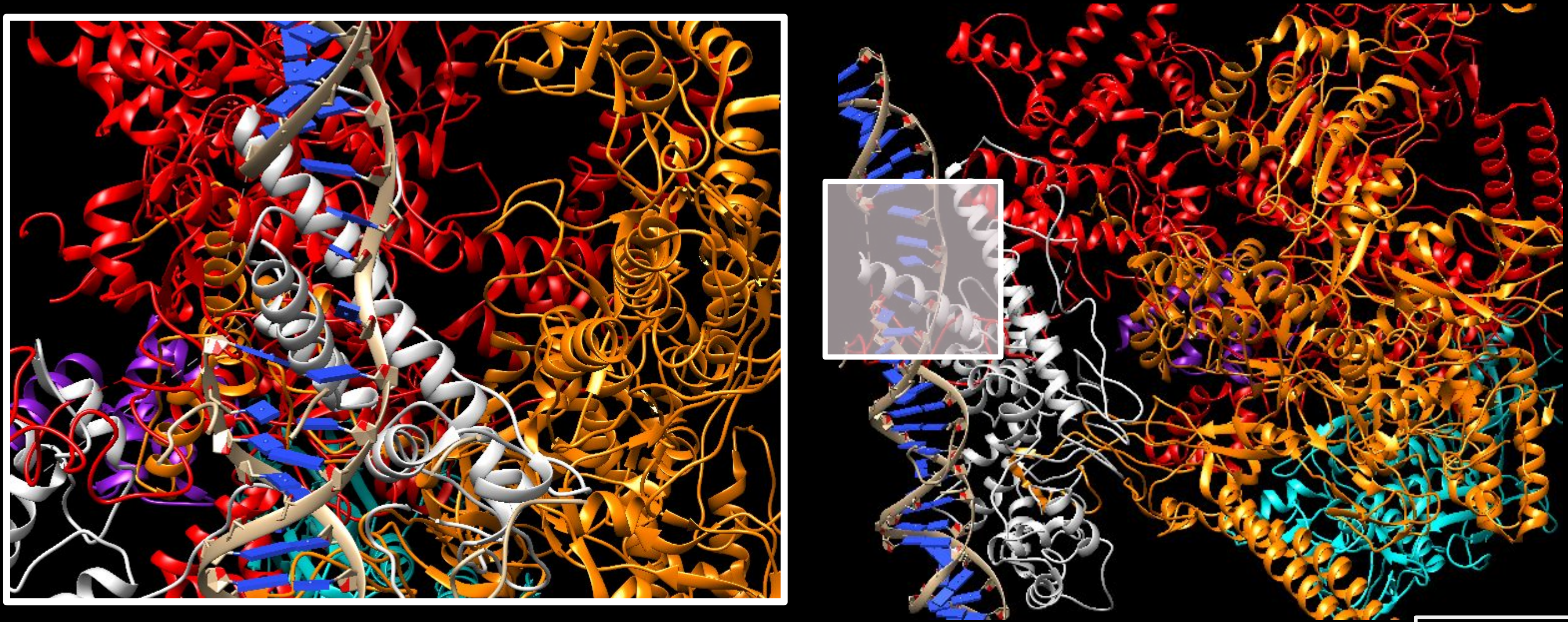
5NSR 3.8Å

σ54 RNAP intermediate

5NSS 5.8Å

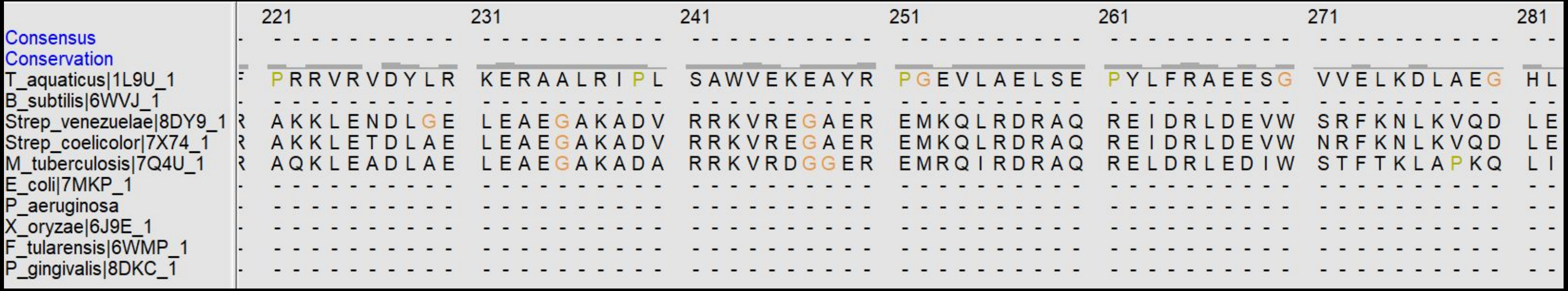
Active and inactive conformations

σ54 RNAP intermediate



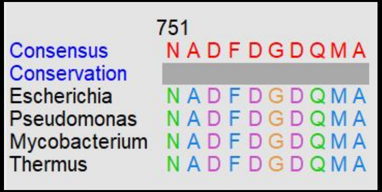
5NSS 5.8Å

Triad Acid binding site not on position 460 in the alignment.

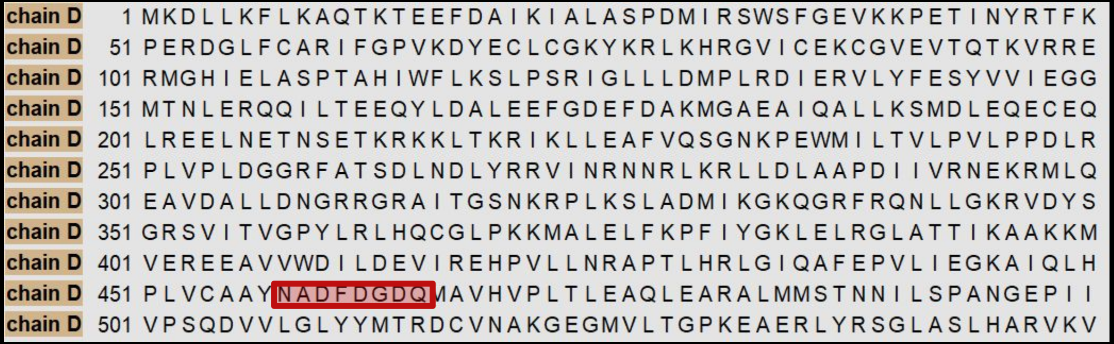


```
                          221        231        241        251        261        271        281
Consensus                 - ---------- ---------- ---------- ---------- ---------- ---------- --
Conservation
T_aquaticus|1L9U_1        F PRRVRVDYLR KERAALRIPL SAWVEKEAYR PGEVLAELSE PYLFRAEESG VVELKDLAEG HL
B_subtilis|6WVJ_1         - ---------- ---------- ---------- ---------- ---------- ---------- --
Strep_venezuelae|8DY9_1   R AKKLENDLGE LEAEGAKADV RRKVREGAER EMKQLRDRAQ REIDRLDEVW SRFKNLKVQD LE
Strep_coelicolor|7X74_1   R AKKLETDLAE LEAEGAKADV RRKVREGAER EMKQLRDRAQ REIDRLDEVW NRFKNLKVQD LE
M_tuberculosis|7Q4U_1     R AQKLEADLAE LEAEGAKADA RRKVRDGGER EMRQIRDRAQ RELDRLEDIW STFTKLAPKQ LI
E_coli|7MKP_1             - ---------- ---------- ---------- ---------- ---------- ---------- --
P_aeruginosa              - ---------- ---------- ---------- ---------- ---------- ---------- --
X_oryzae|6J9E_1           - ---------- ---------- ---------- ---------- ---------- ---------- --
F_tularensis|6WMP_1       - ---------- ---------- ---------- ---------- ---------- ---------- --
P_gingivalis|8DKC_1       - ---------- ---------- ---------- ---------- ---------- ---------- --
```

T. aquaticus has insertions in the β‘ sequence.

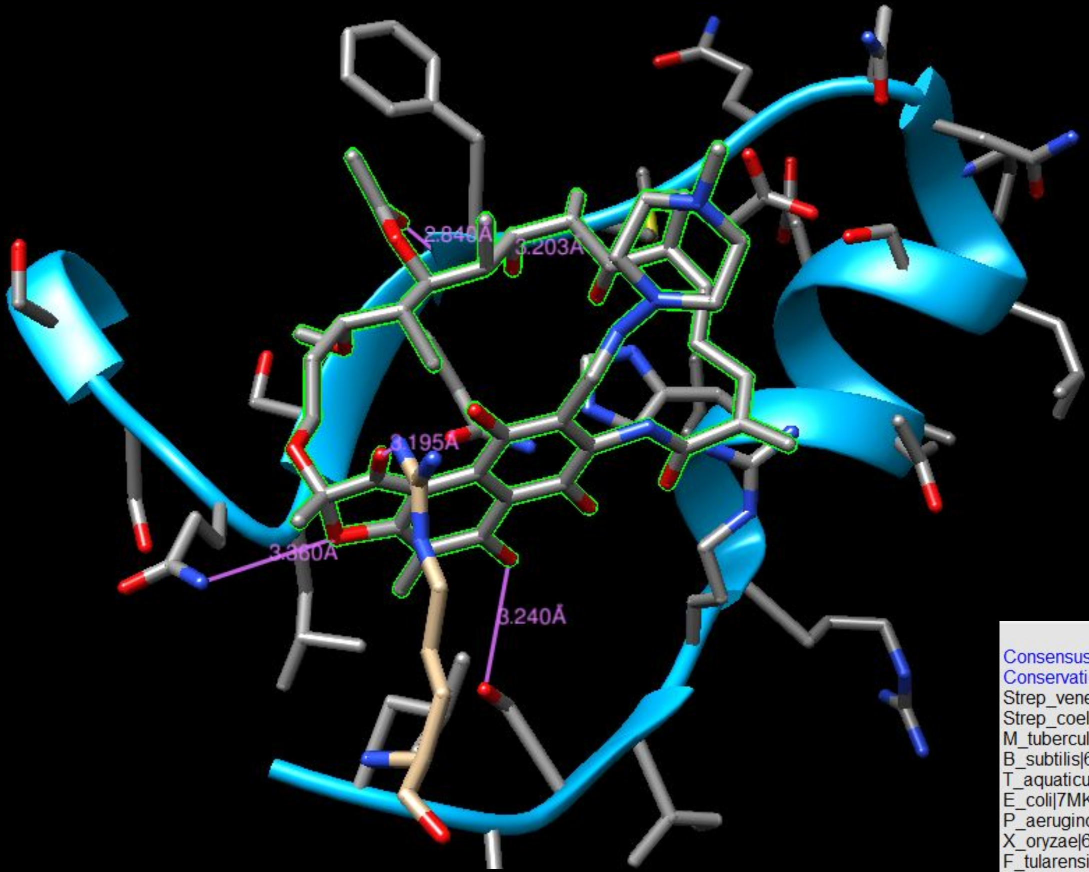


```
                751
Consensus       NADFDGDQMA
Conservation
Escherichia     NADFDGDQMA
Pseudomonas     NADFDGDQMA
Mycobacterium   NADFDGDQMA
Thermus         NADFDGDQMA
```

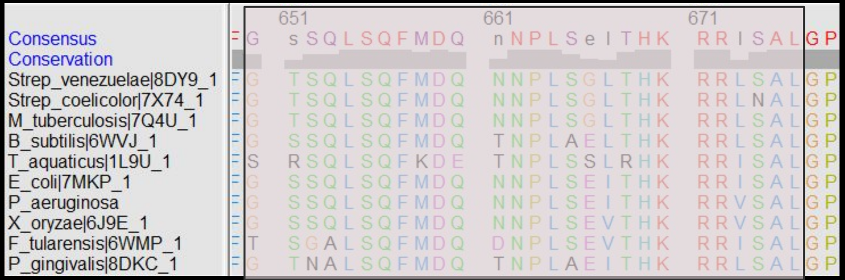


```
chain D    1 MKDLLKFLKAQTKTEEFDAIKIALASPDMIRSWSFGEVKKPETINYRTFK
chain D   51 PERDGLFCARIFGPVKDYECLCGKYKRLKHRGVICEKCGVEVTQTKVRRE
chain D  101 RMGHIELASPTAHIWFLKSLPSRIGLLLDMPLRDIERVLYFESYVVIEGG
chain D  151 MTNLERQQILTEEQYLDALEEFGDEFDAKMGAEAIQALLKSMDLEQECEQ
chain D  201 LREELNETNSETKRKKLTKRIKLLEAFVQSGNKPEWMILTVLPVLPPDLR
chain D  251 PLVPLDGGRFATSDLNDLYRRVINRNNRLKRLLDLAAPDIIVRNEKRMLQ
chain D  301 EAVDALLDNGRRGRAITGSNKRPLKSLADMIKGKQGRFRQNLLGKRVDYS
chain D  351 GRSVITVGPYLRLHQCGLPKKMALELFKPFIYGKLELRGLATTIKAAKKM
chain D  401 VEREEAVVWDILDEVIREHPVLLNRAPTLHRLGIQAFEPVLIEGKAIQLH
chain D  451 PLVCAAYNADFDGDQMAVHVPLTLEAQLEARALMMSTNNILSPANGEPII
chain D  501 VPSQDVVLGLYYMTRDCVNAKGEGMVLTGPKEAERLYRSGLASLHARVKV
```

Inhibitor: Rifampin (Antibiotic)



5UAC 3.80Å



		651	661	671	
Consensus	G	s S Q L S Q F M D Q	n N P L S e I T H K	R R I S A L	G P
Conservation					
Strep_venezuelae|8DY9_1	G	T S Q L S Q F M D Q	N N P L S G L T H K	R R L S A L	G P
Strep_coelicolor|7X74_1	G	T S Q L S Q F M D Q	N N P L S G L T H K	R R L N A L	G P
M_tuberculosis|7Q4U_1	G	T S Q L S Q F M D Q	N N P L S G L T H K	R R L S A L	G P
B_subtilis|6WVJ_1	G	S S Q L S Q F M D Q	T N P L A E L T H K	R R L S A L	G P
T_aquaticus|1L9U_1	S	R S Q L S Q F K D E	T N P L S S L R H K	R R I S A L	G P
E_coli|7MKP_1	G	S S Q L S Q F M D Q	N N P L S E I T H K	R R I S A L	G P
P_aeruginosa	G	S S Q L S Q F M D Q	N N P L S E I T H K	R R V S A L	G P
X_oryzae|6J9E_1	G	S S Q L S Q F M D Q	N N P L S E V T H K	R R V S A L	G P
F_tularensis|6WMP_1	T	S G A L S Q F M D Q	D N P L S E V T H K	R R I S A L	G P
P_gingivalis|8DKC_1	G	T N A L S Q F M D Q	T N P L A E I T H K	R R L S A L	G P