

Acknowledgements

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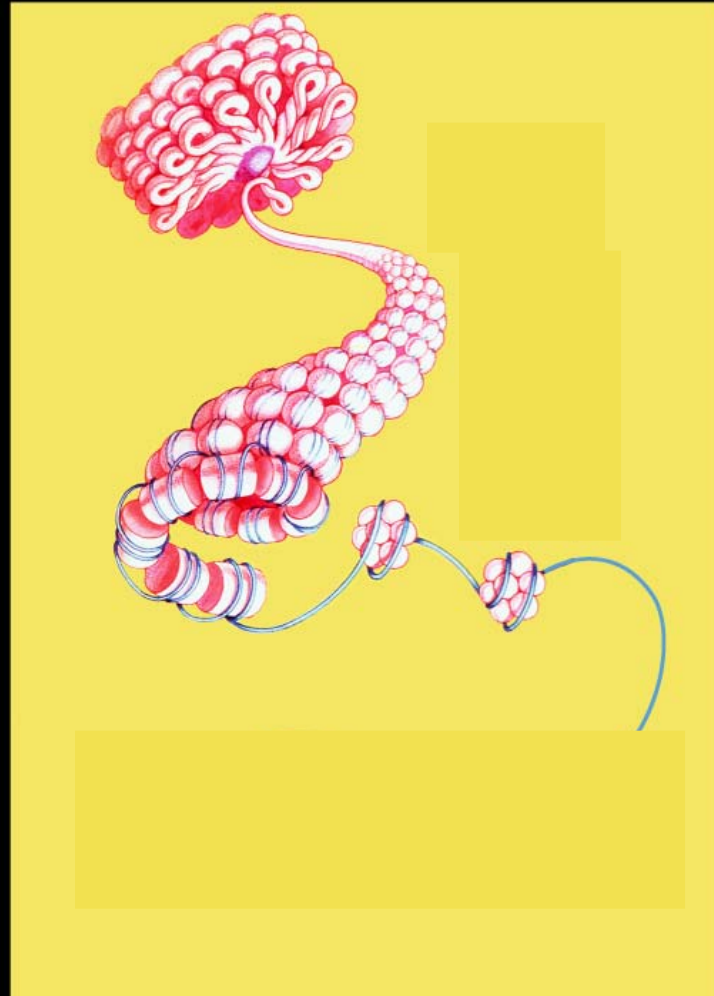
H. Tschochner

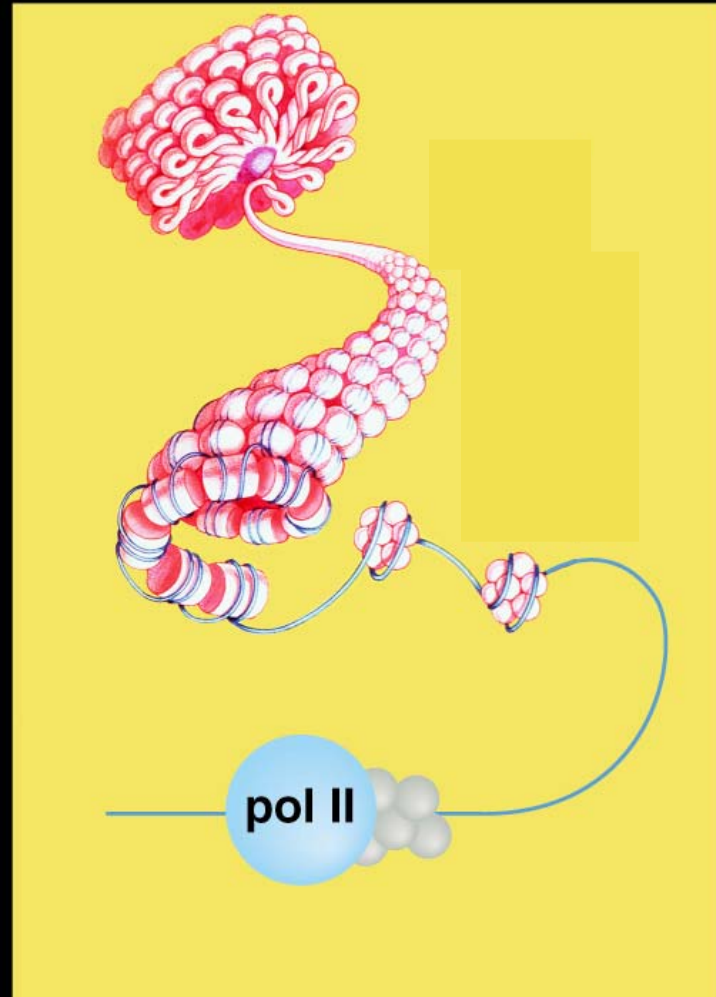
E. Uzgiris

D. Wang**K. Westover**



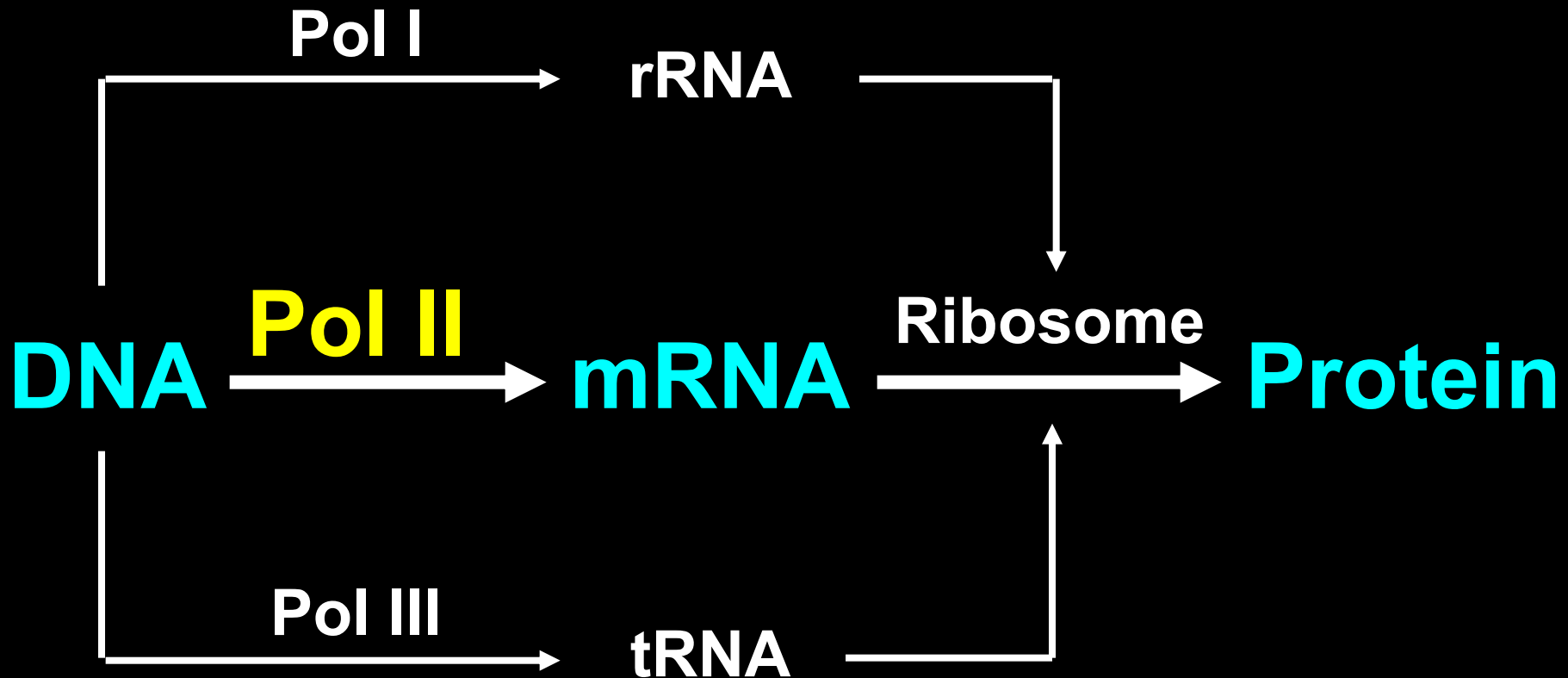
The nucleosome, fundamental particle of the chromosome







RNA polymerase II underlies the central dogma of molecular biology



RNA polymerase II transcription machinery

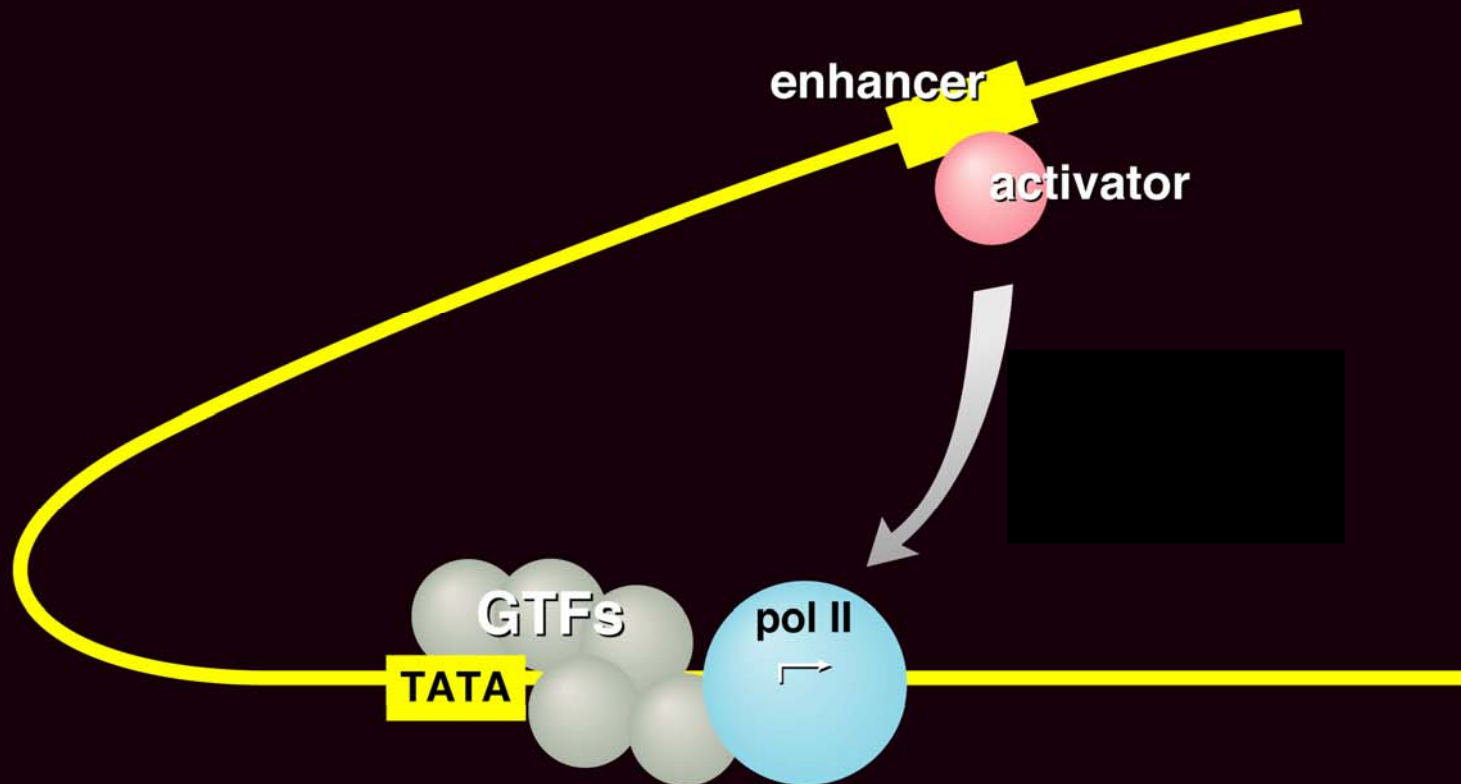
Pol II

**DNA unwinding
RNA polymerization
proofreading**

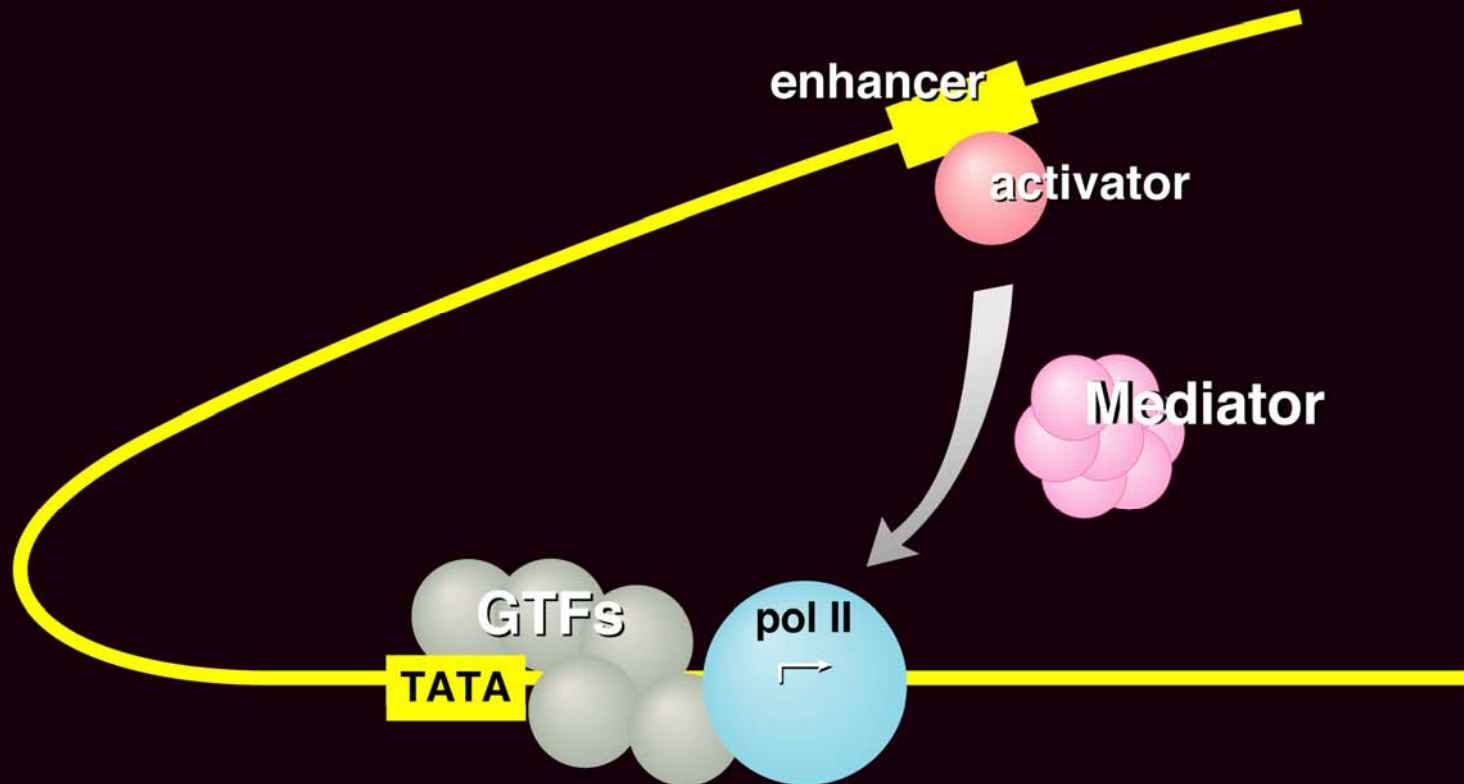
**GTFs
(TFIIB, -D, -E, -F, -H)**

promoter recognition

Regulation of RNA polymerase II transcription



Regulation of RNA polymerase II transcription



Conserved Mediator subunits

<u>yeast</u>	<u>Drosophila</u>	<u>human</u>
Med1	Trap220*	TRAP220
Med4	Trap36	TRAP36
Med6	Med6	hMed6
Med7	Med7*	hMed7
Med8	Arc32*	hNut2
Cse2/Med9	CG5134*	MED9
Nut2/Med10	Nut2*	TRAP230
Med11	Med21	MED11
Srb8	Kto*	TRAP240
Ssn2/Srb9	Skd/Pap/Bli*	TRAP170
Rgr1	Trap170	TRAP95
Gal11	Arc105*	MED15
Sin4	Trap95*	TRAP80
Srb4	Trap80	hTRFP
Srb5	p28/CG14802	MED18
Rox3	CG5546*	MED19
Srb2	Trfp	hSrb7
Srb7	Trap19	TRAP150b
Srb6	Med24	MED22
Med1	Trap220*	TRAP220
CDK8	CDK8	CDK8
cycC	cycC	cycC

Mediator of Transcriptional Regulation

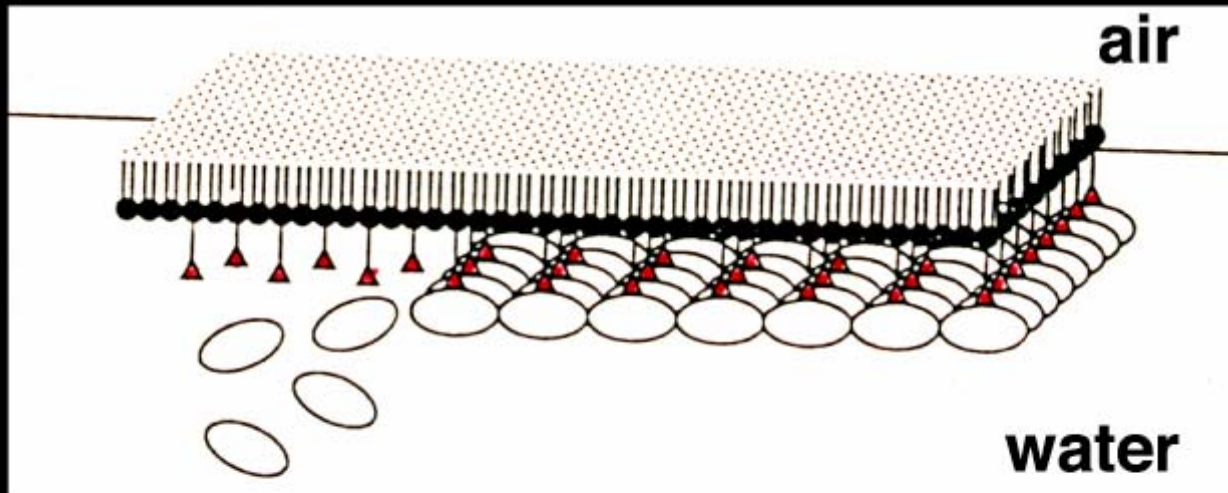
- Required for all transcription of all pol II promoters
- Essential link in the chain of communication:
enhancer → activator → Mediator → pol II → promoter
- Co-activator, co-repressor, and general transcription factor
- Processes, transduces transcriptional regulatory information in all eukaryotes

RNA polymerase II transcription machinery

	subunits	mass (kD)
pol II	12	513
GTFs	25	1558
Mediator	20	1003
	57	3074

2-D protein crystallization

- rapid, small amount of protein (μg)
- pol II without subunits 4 and 7
- 2-D crystals seed 3-D crystal growth





Obstacles to X-ray structure determination of pol II

Oxidation

Crystal polymorphism

No standard heavy atom derivatives

Structure determination of RNA polymerase II

- 1971 Lateral diffusion in lipid layers
- 1983 2-D protein crystals on lipid layers
- 1991 2-D crystals seed 3-D crystals
- 1998 Diffraction phased with heavy
atom clusters
- 2000 Structure at 2.8 Å resolution

10 years, 10,000 liters, 1 grad student





Proteins

10

Amino acids

3559

Atoms

28,378

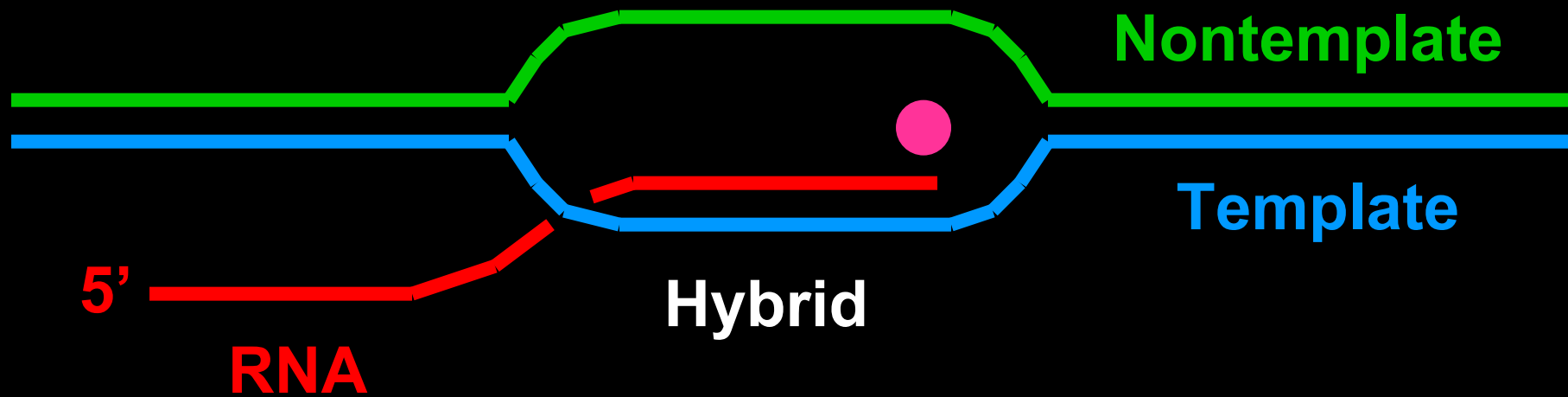
MW

0.5 MDa

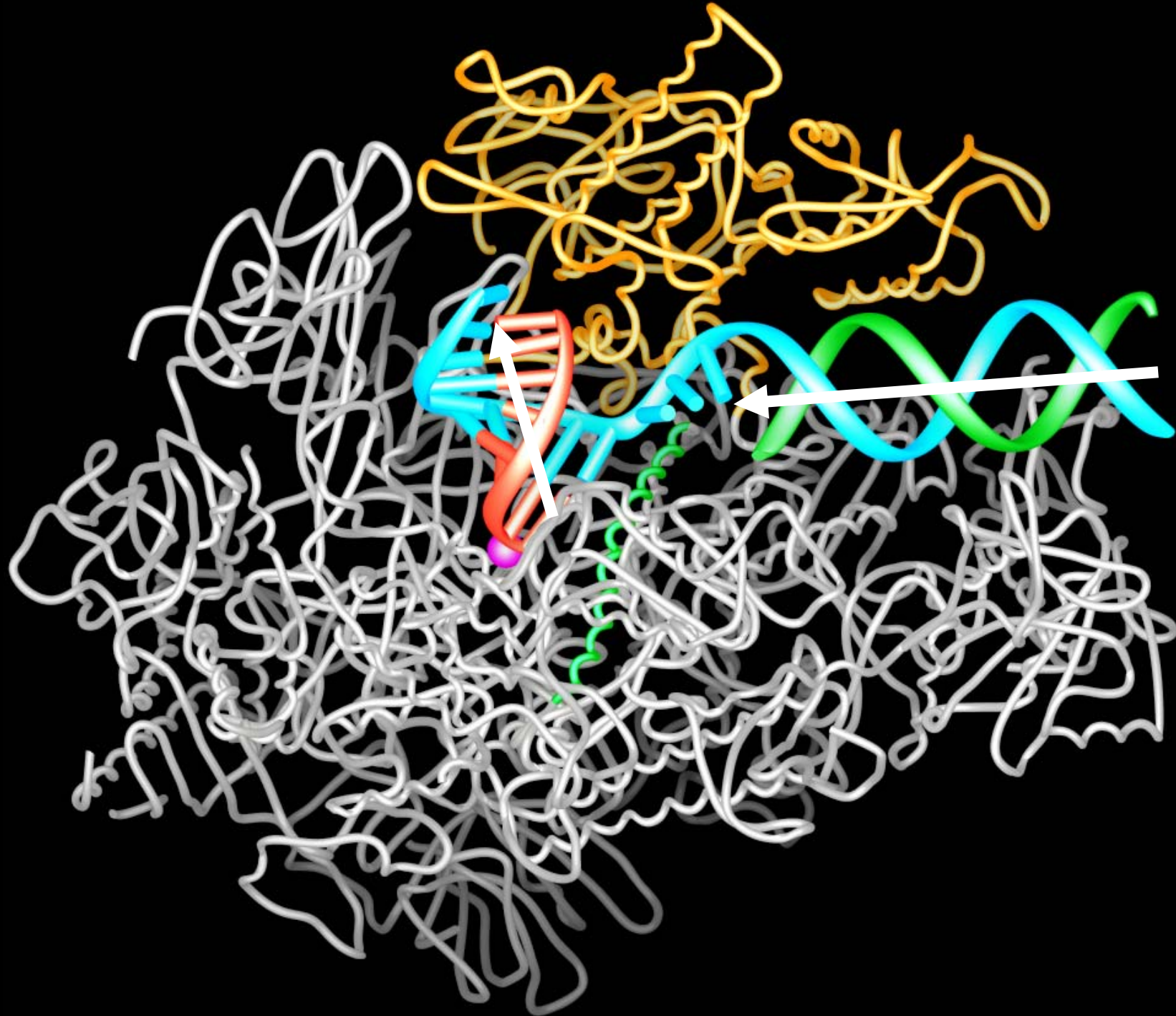
R_{free} (2.8 Å)

28.2 %

Pol II

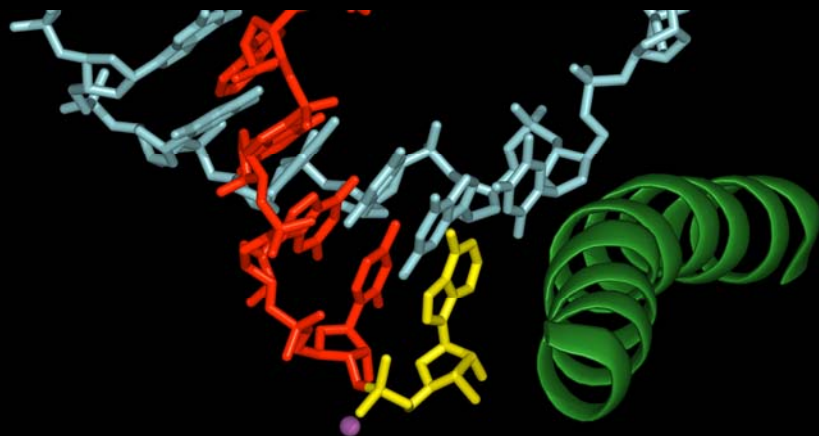




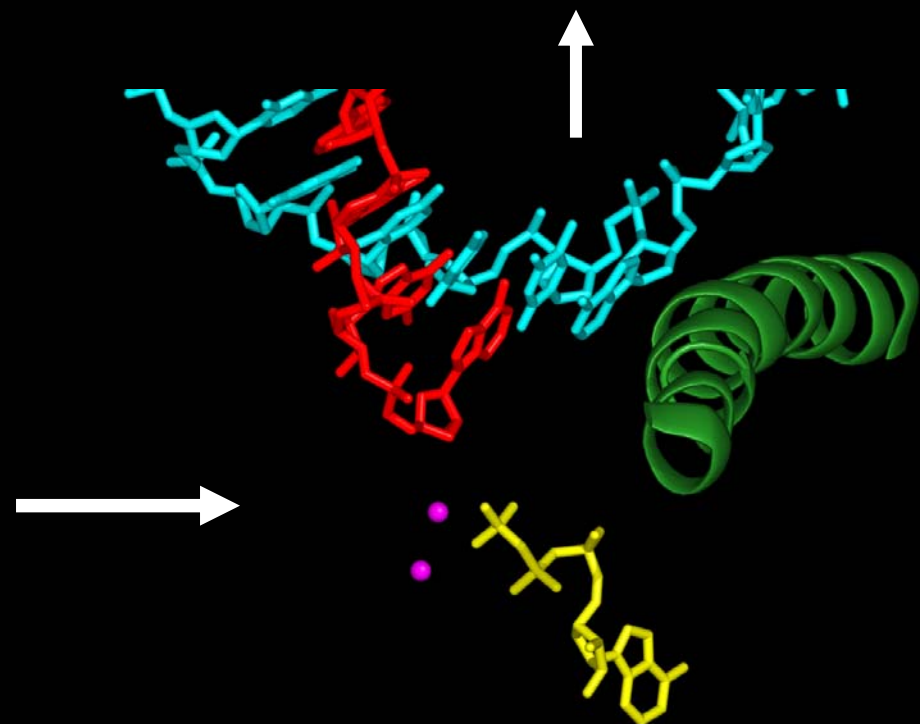
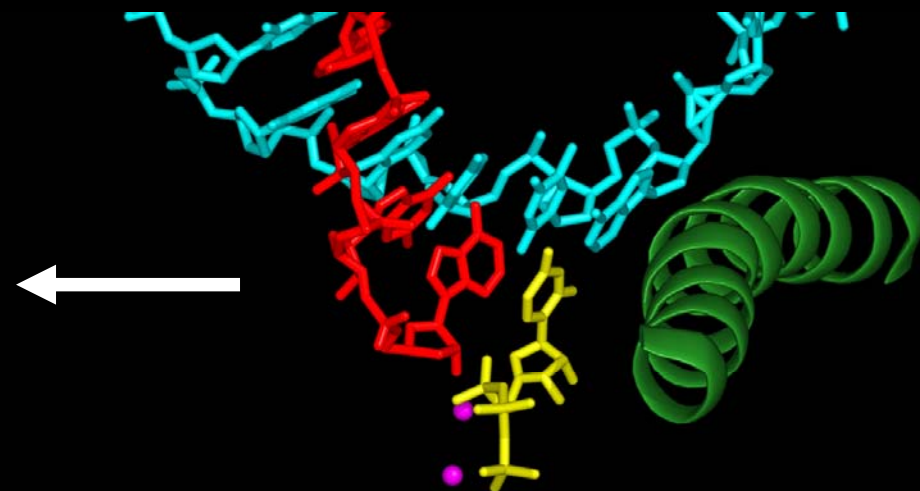




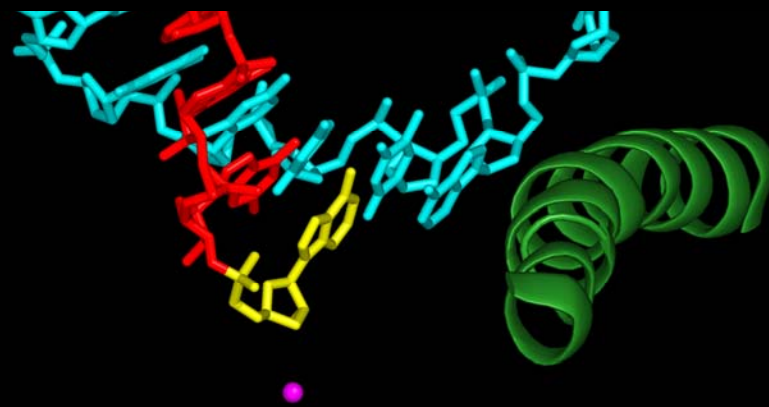
pre-translocation



NTP rotates into A site

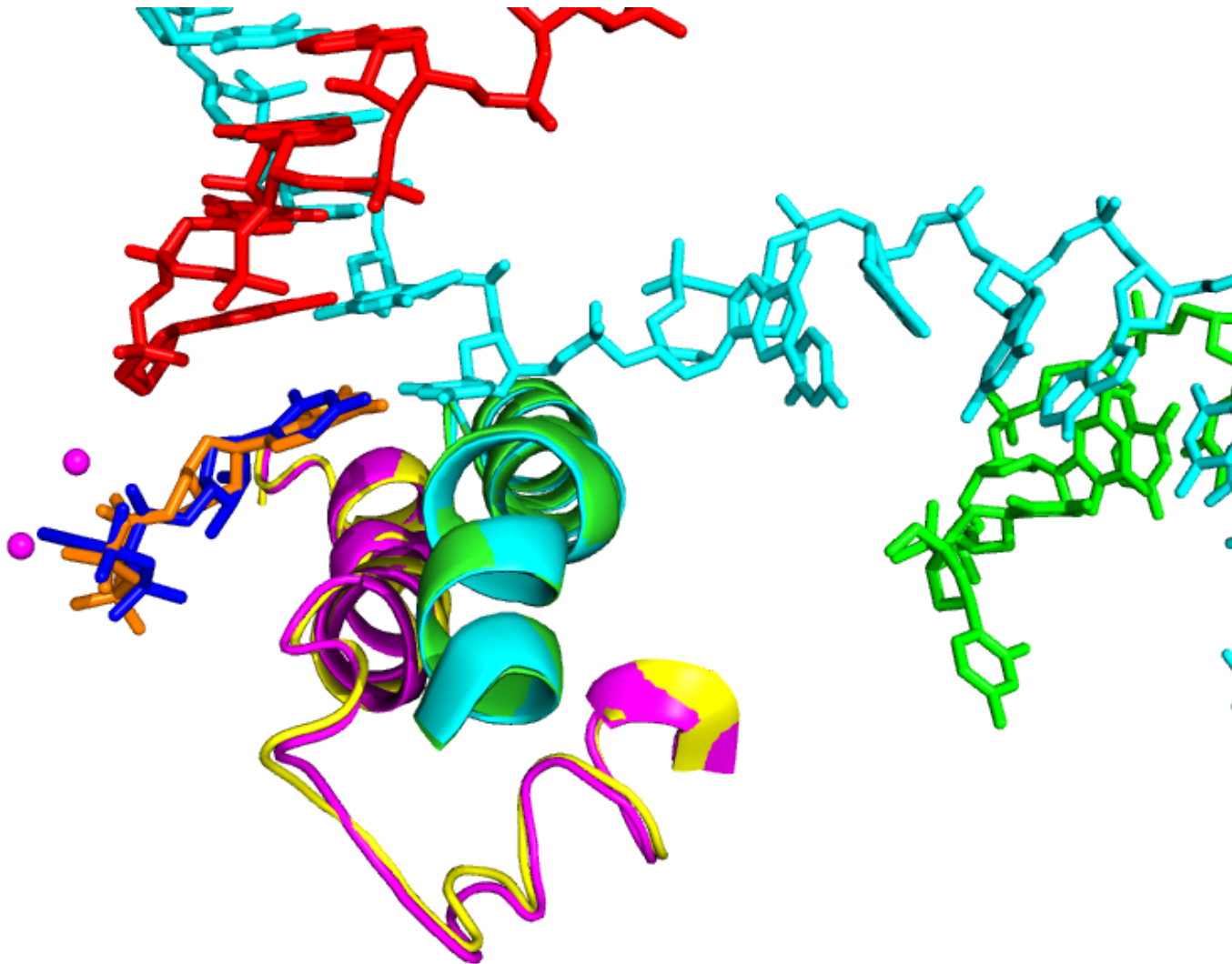


post-translocation

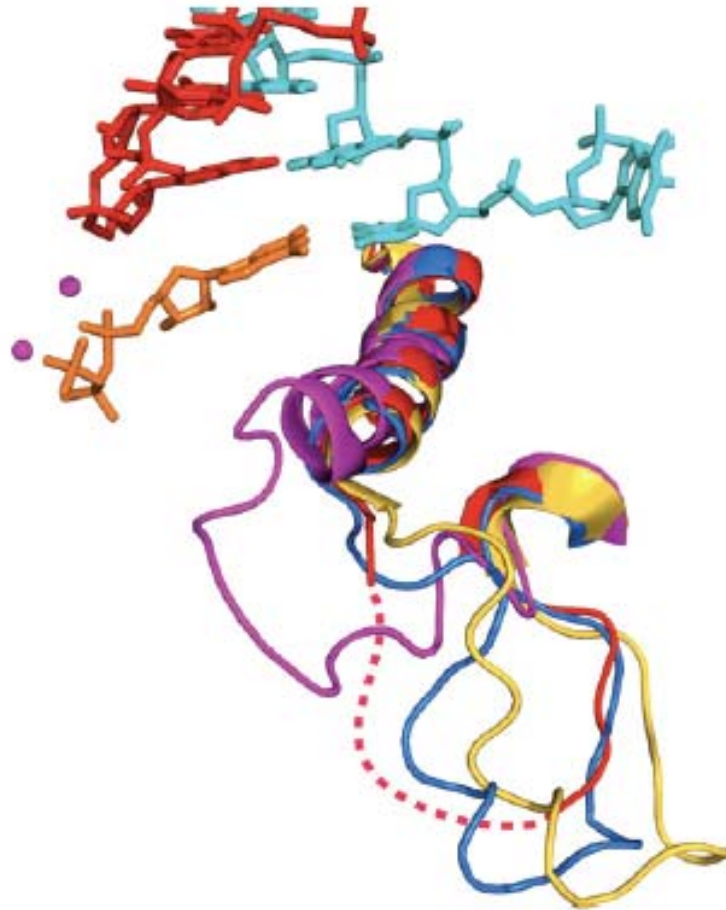


NTP enters E site

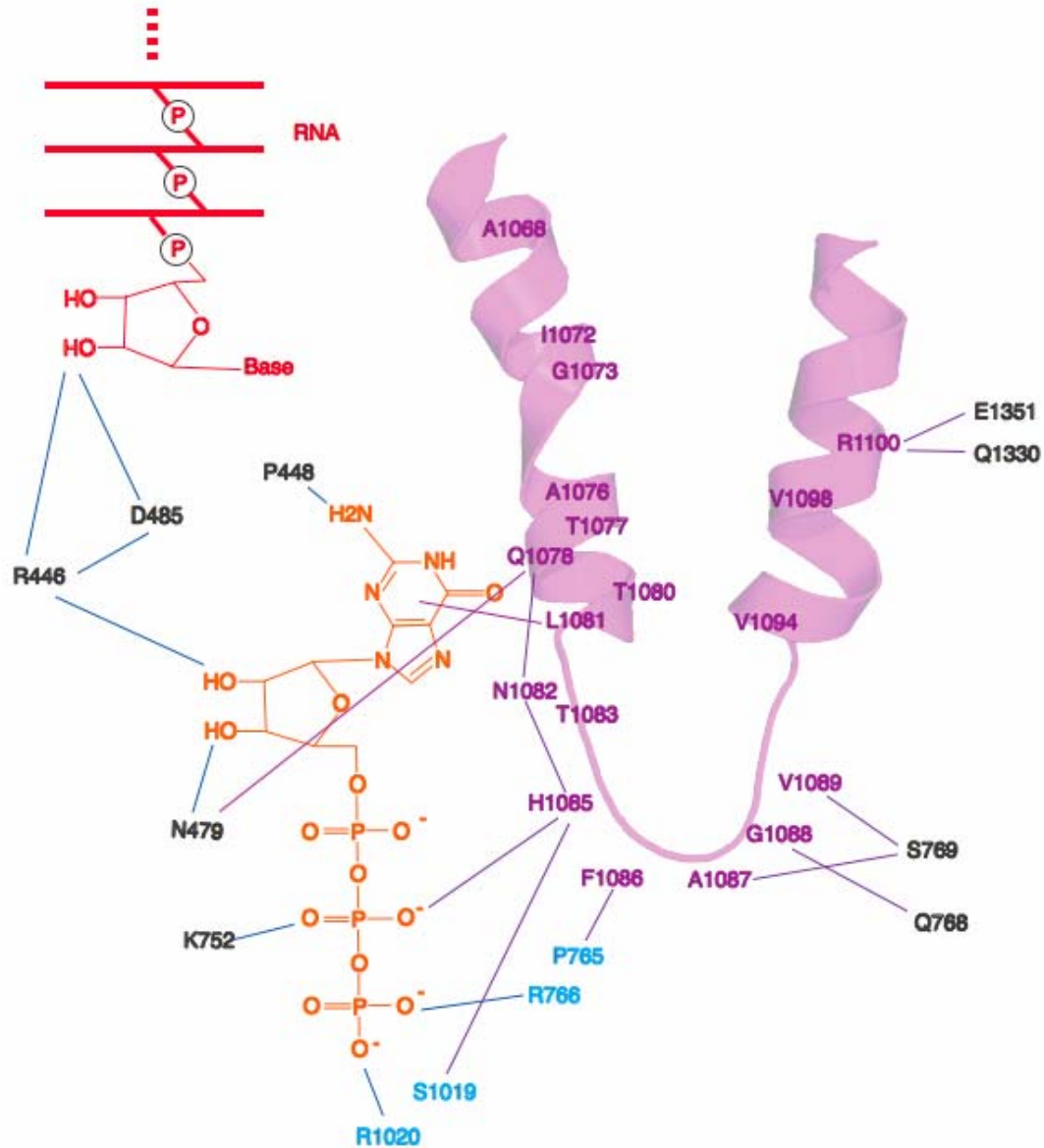
Trigger loop contacts NTP in the A site



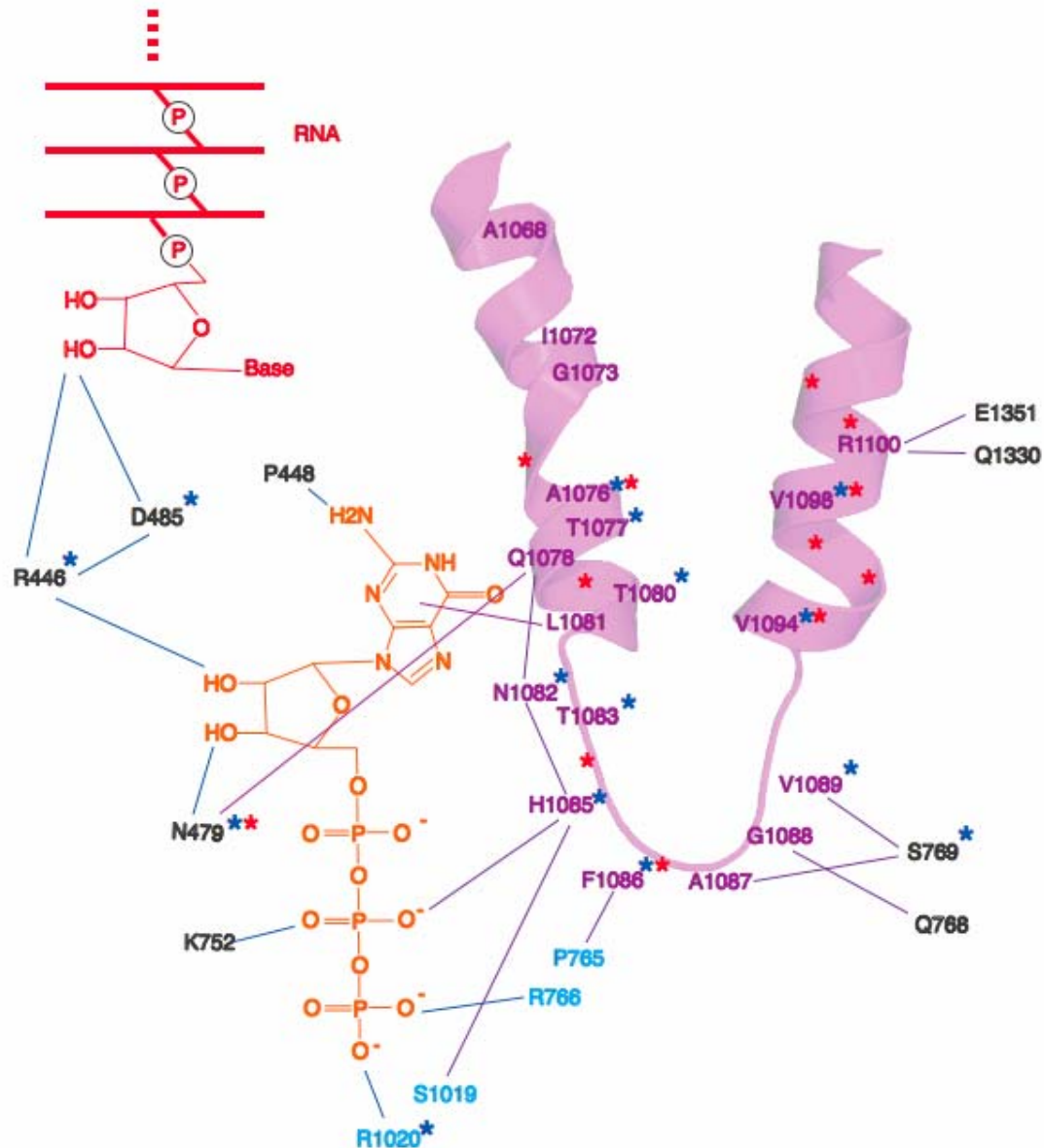
Multiple conformations of the trigger loop



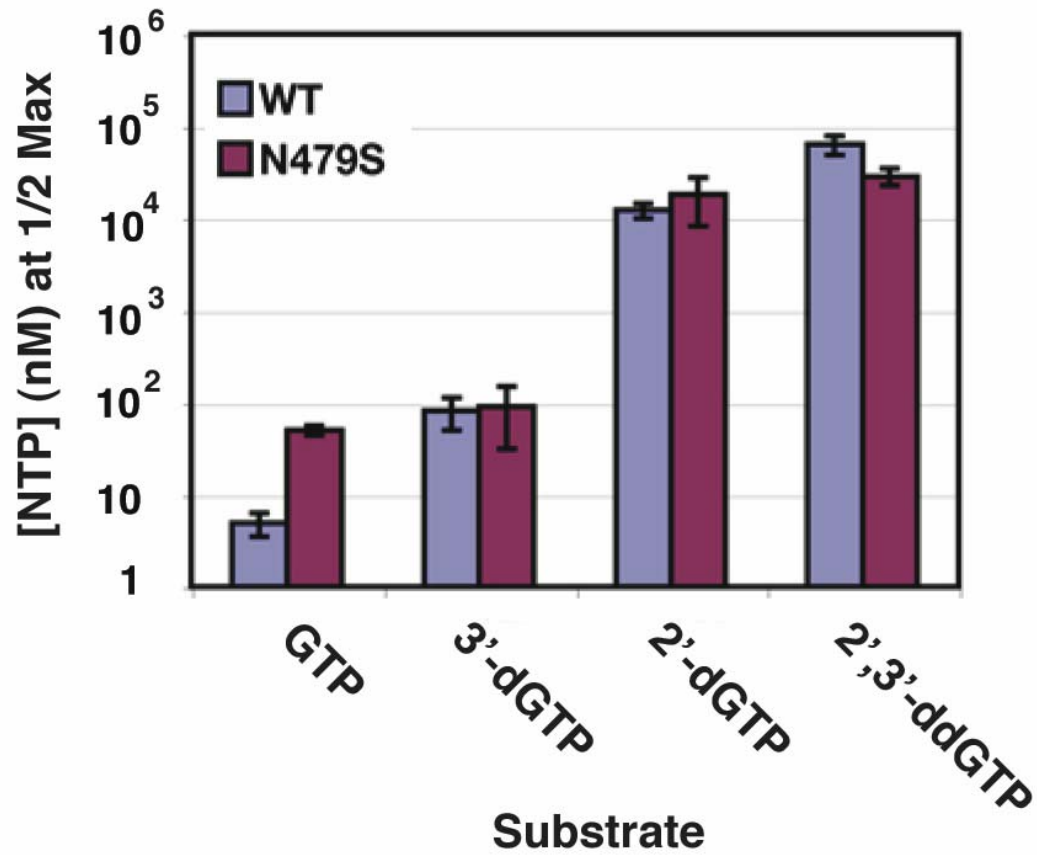
Trigger Loop Network



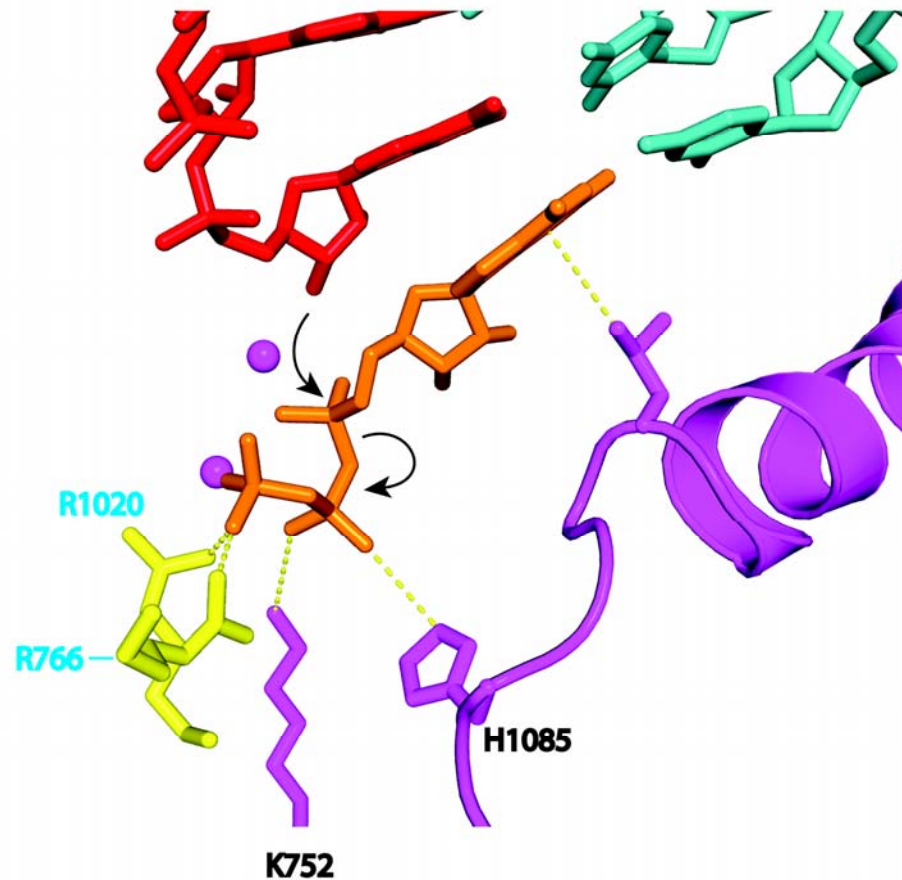
Trigger Loop Network



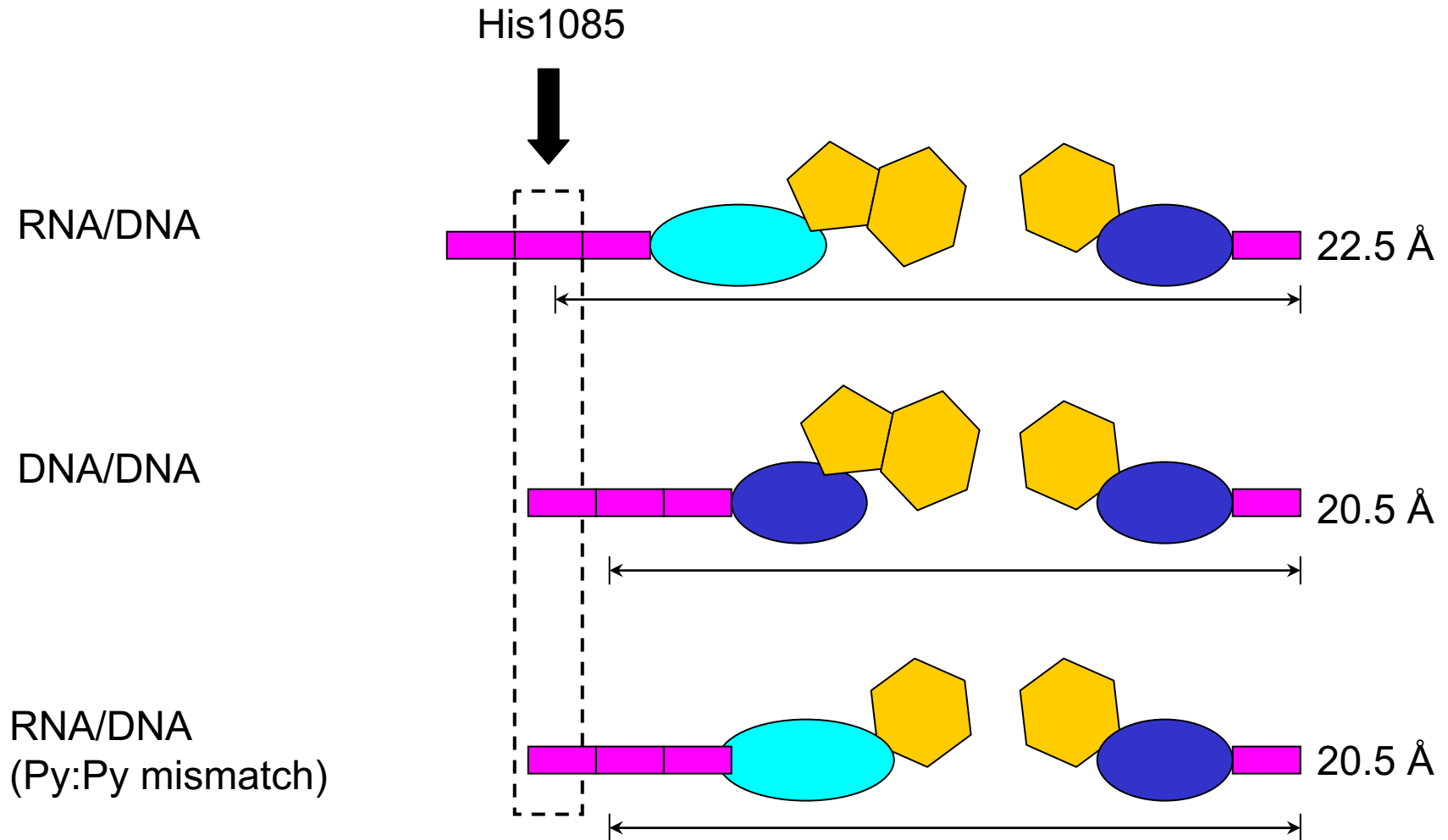
Nucleotide selection in RNA chain extension from 10 to 11 residues



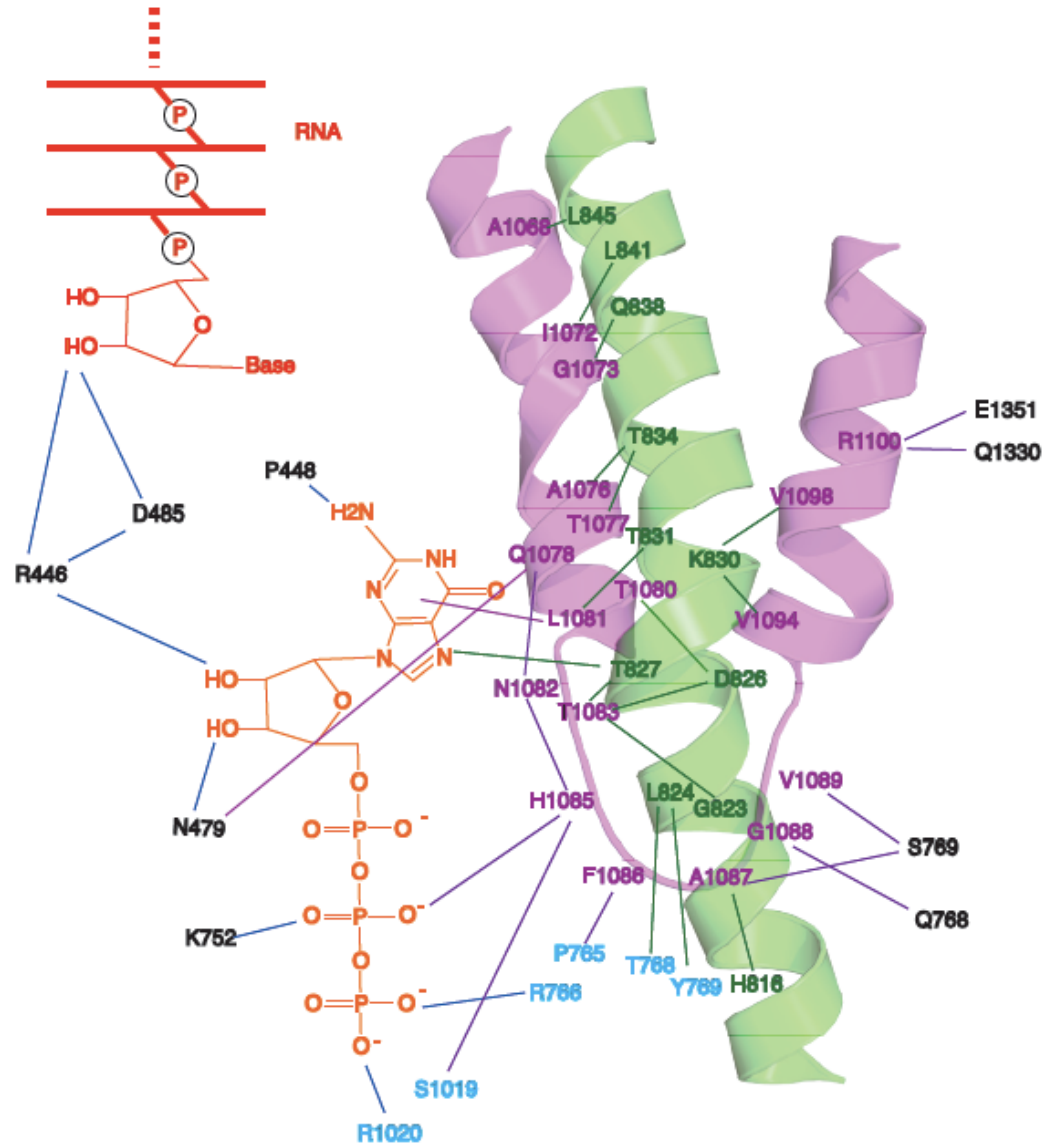
Trigger loop couples nucleotide selection to catalysis

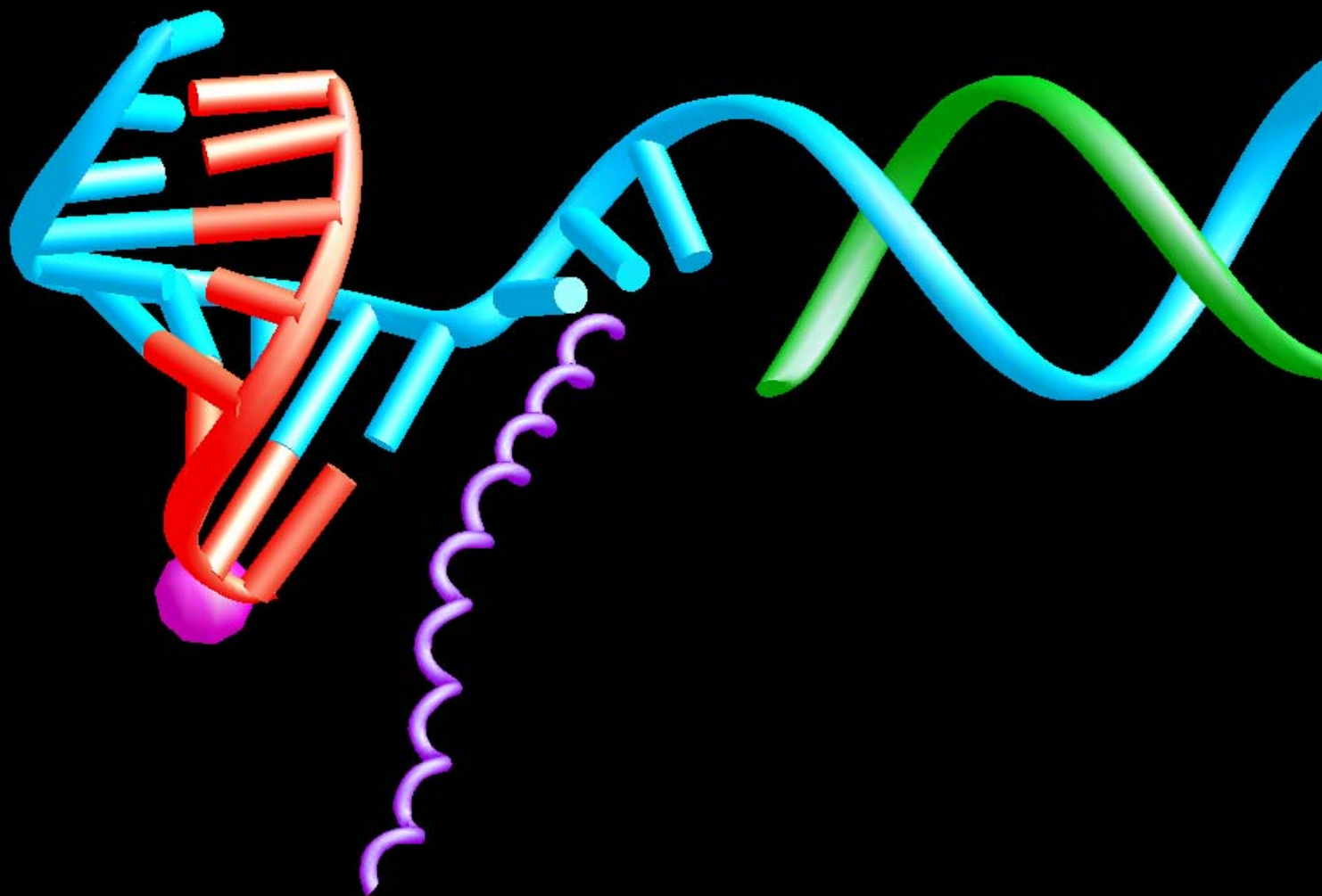


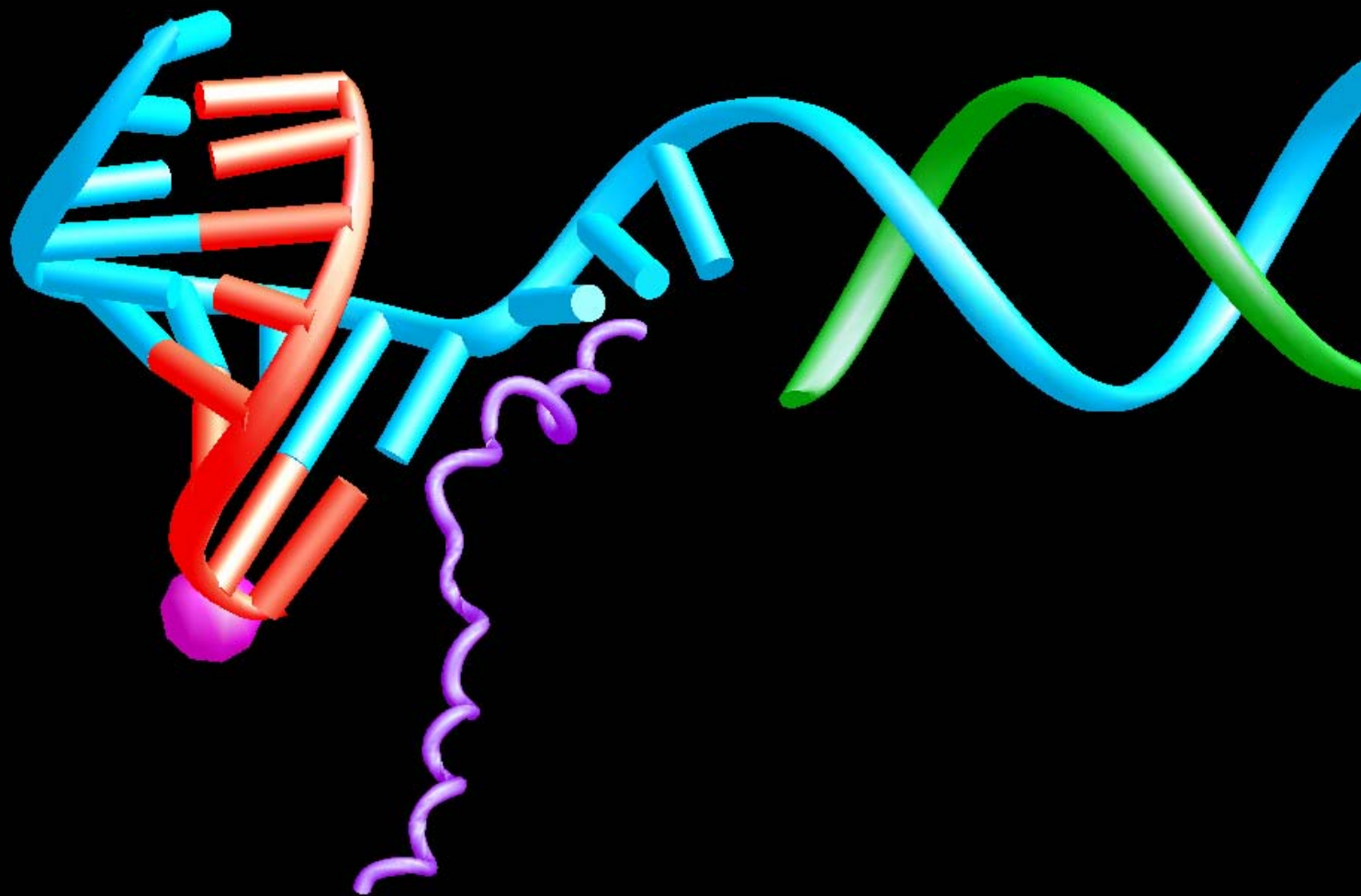
Nucleotide selection by alignment with the trigger loop, coupling recognition to catalysis

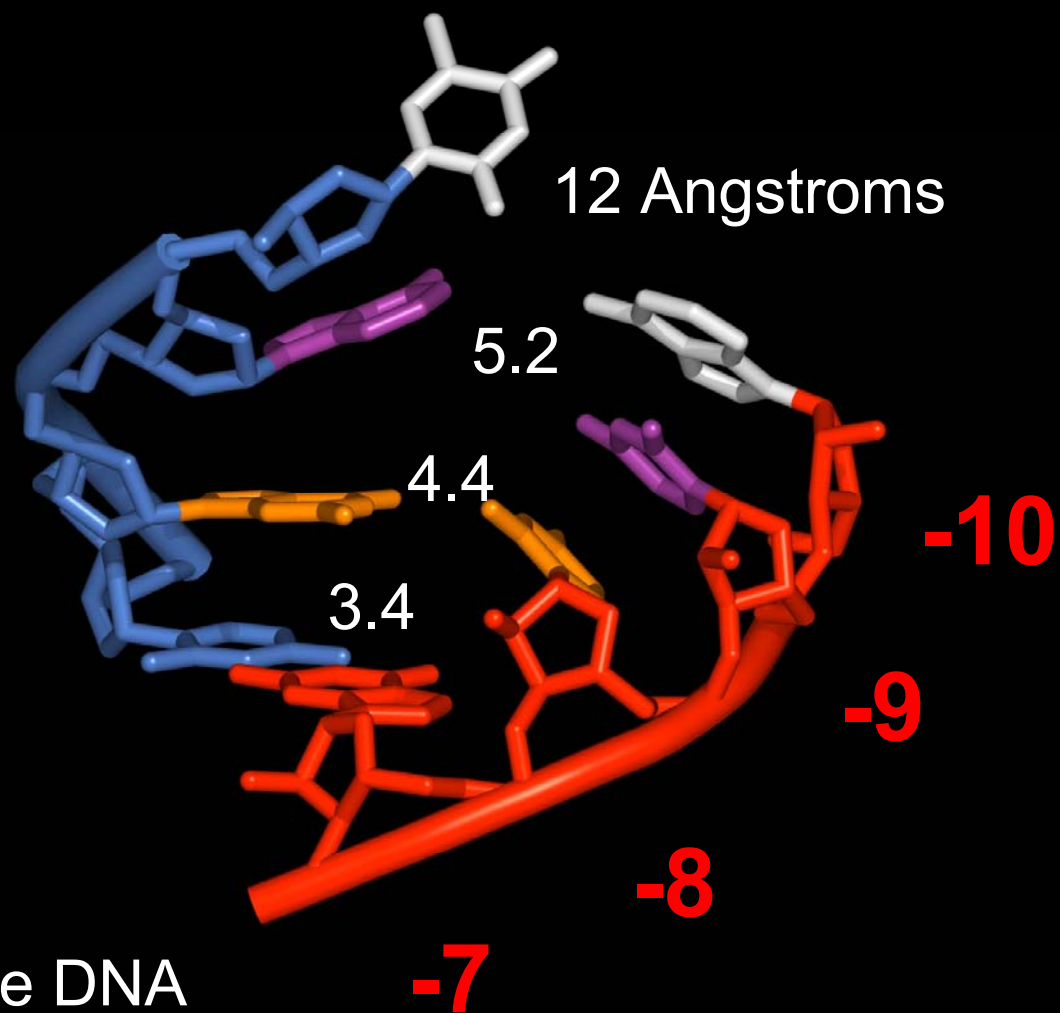


Trigger Loop Network



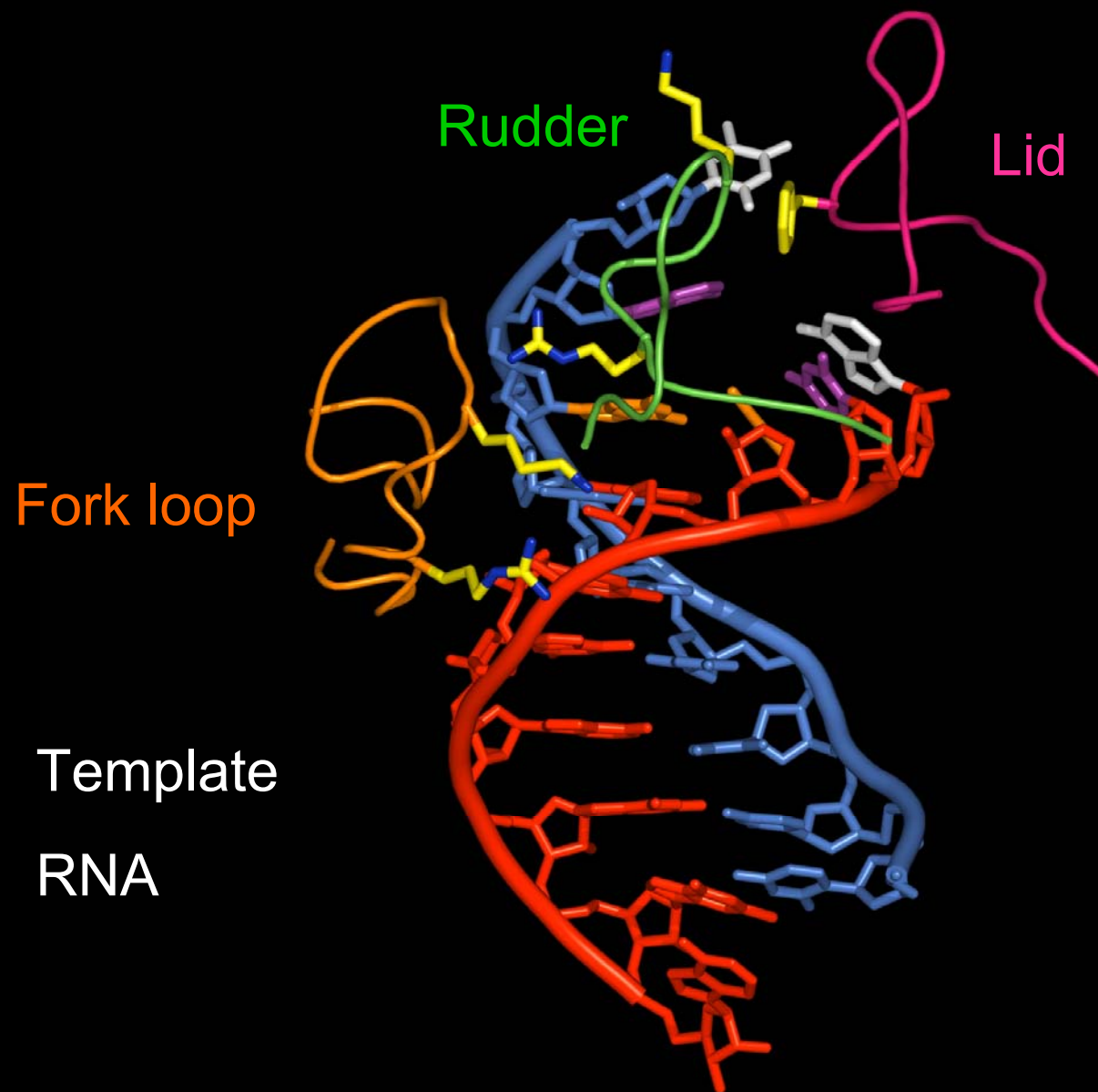




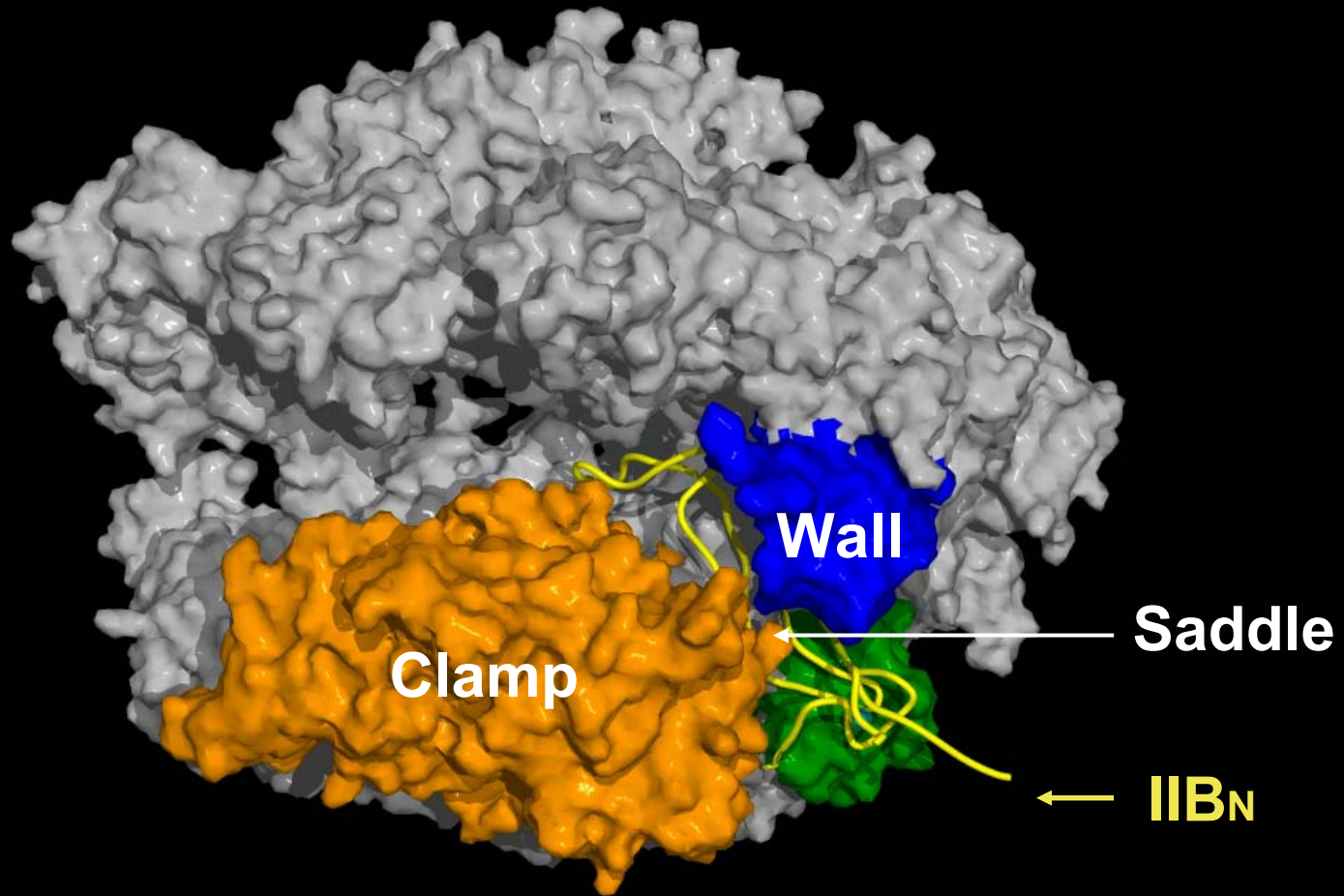


Template DNA

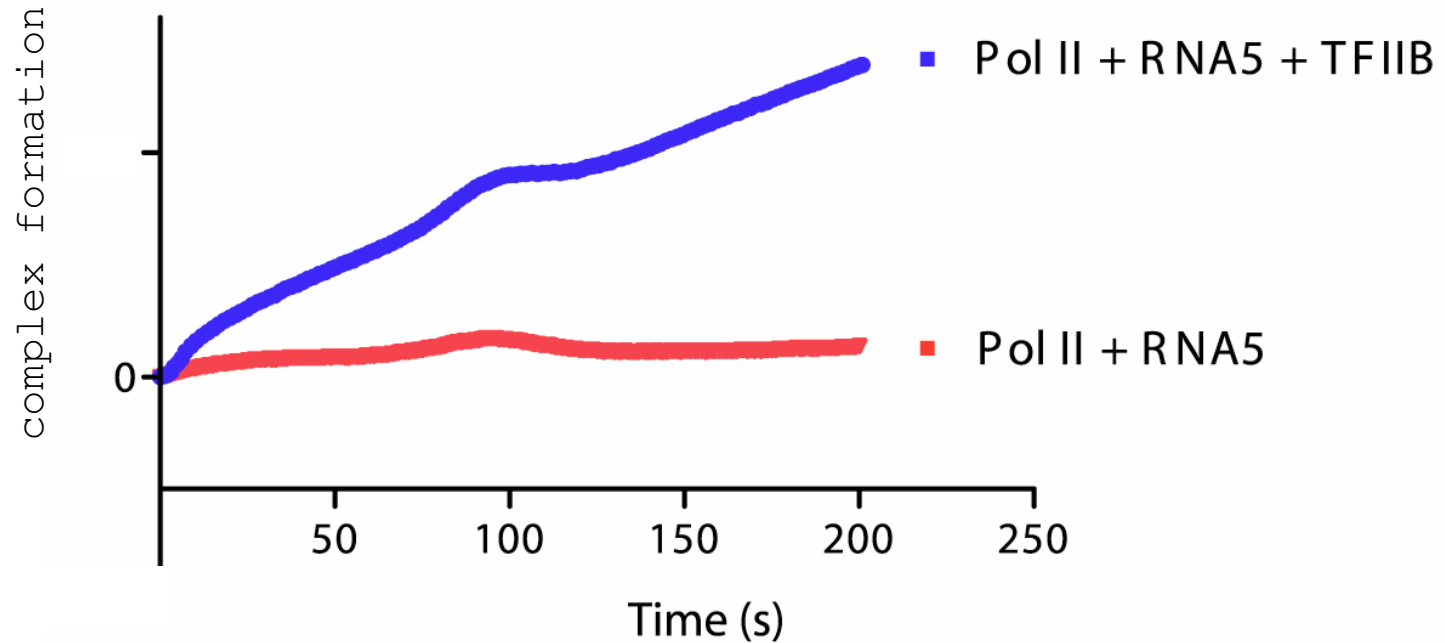
RNA

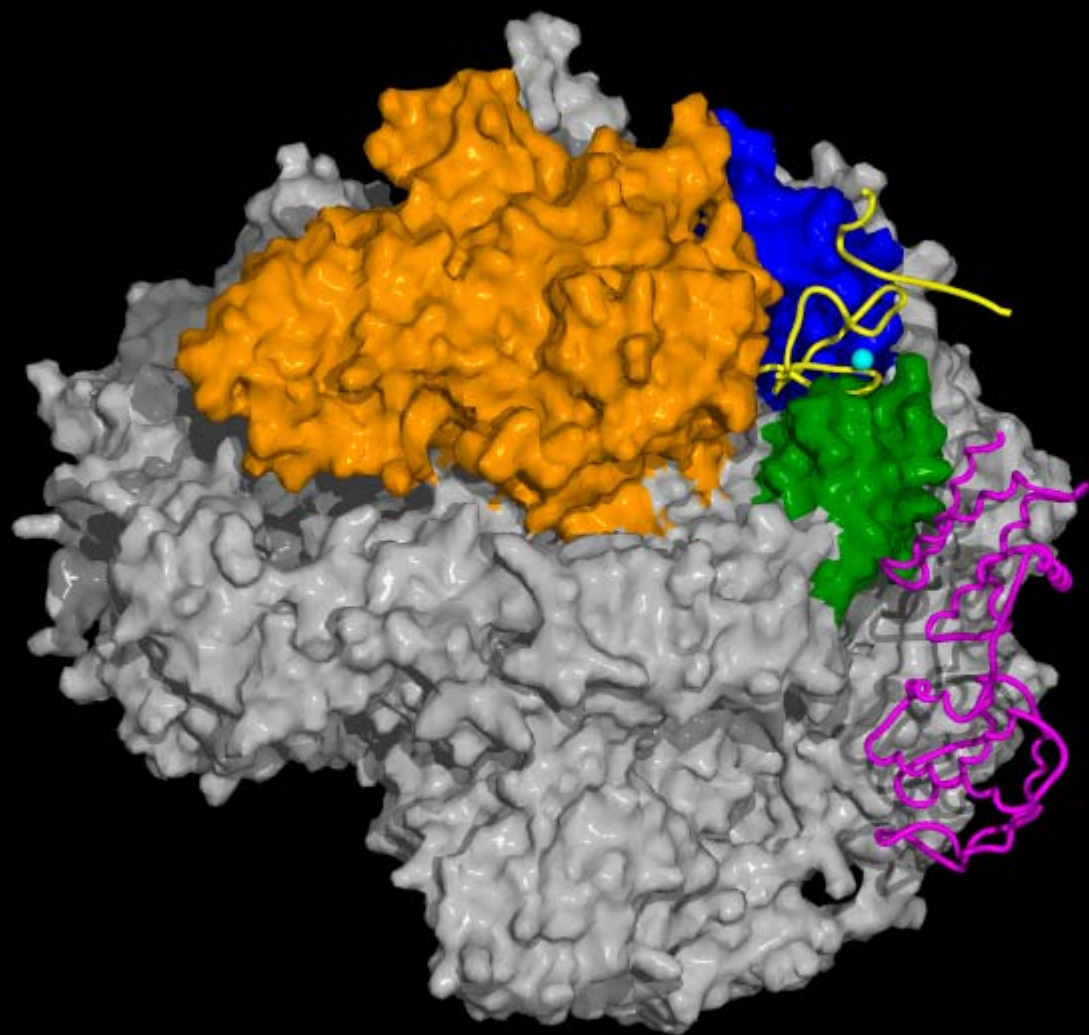


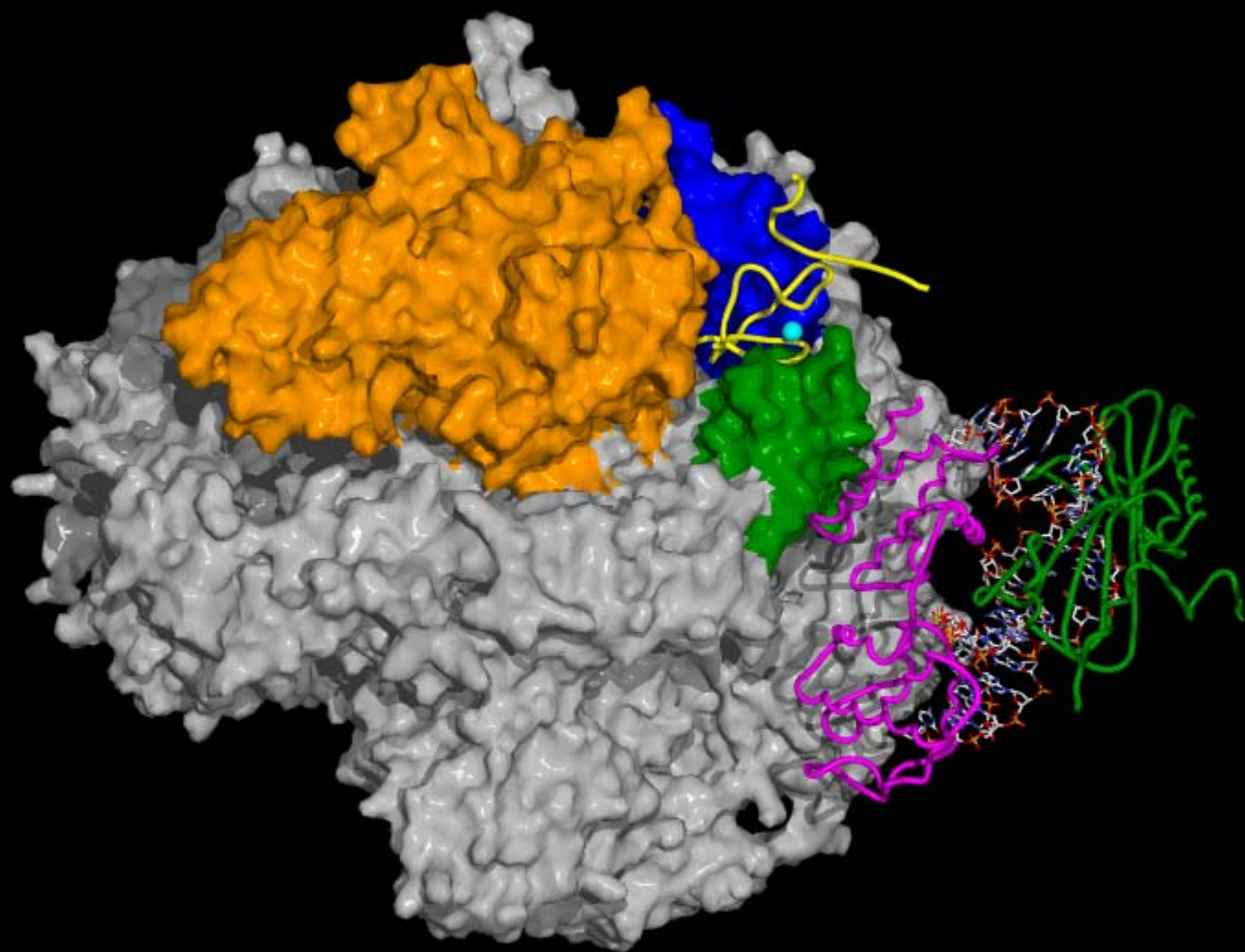
Structure of pol II - TFIIB complex: N-terminal region of TFIIB forms Zn ribbon and B finger

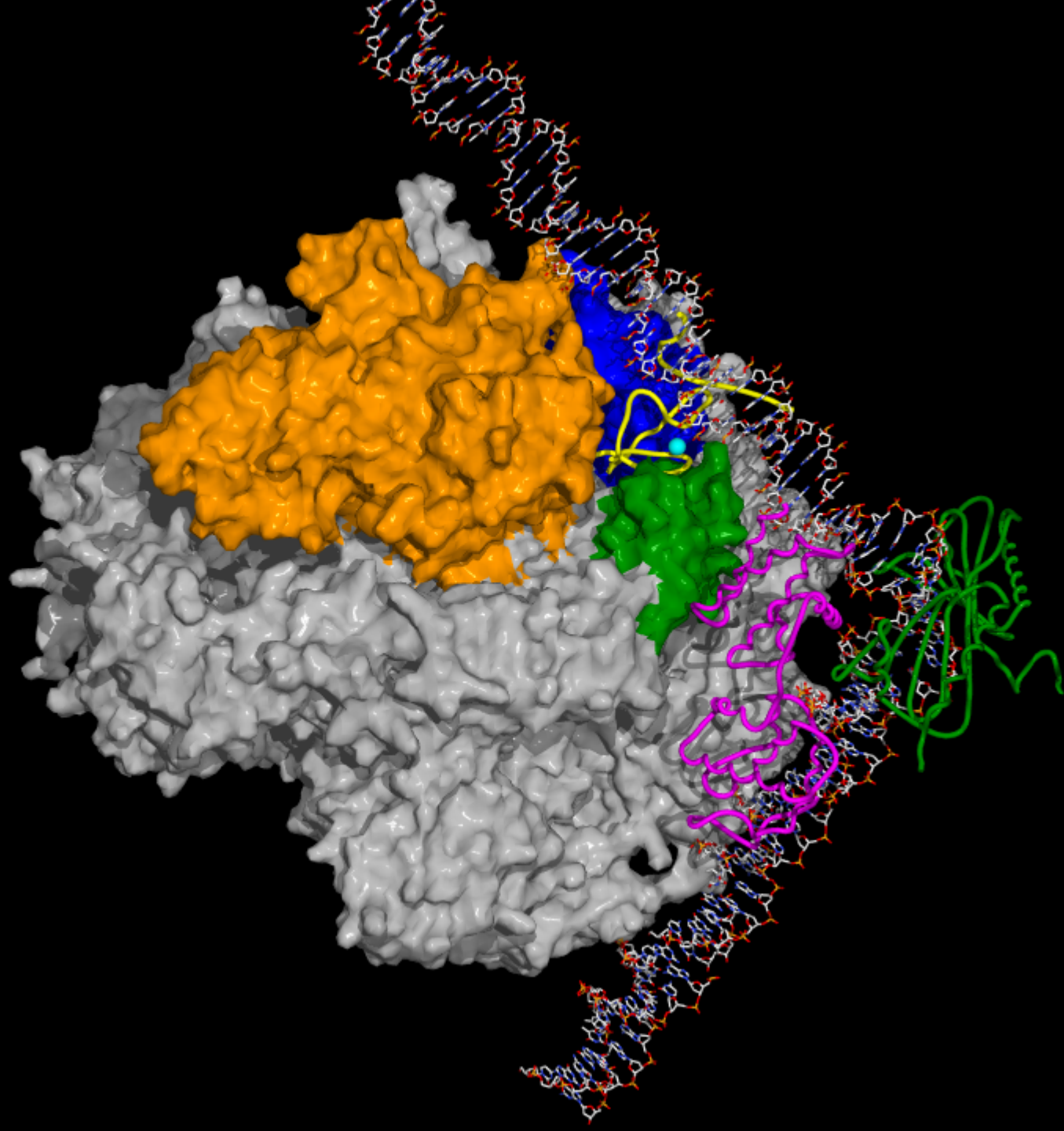


TFIIB stabilizes an initial transcribing complex

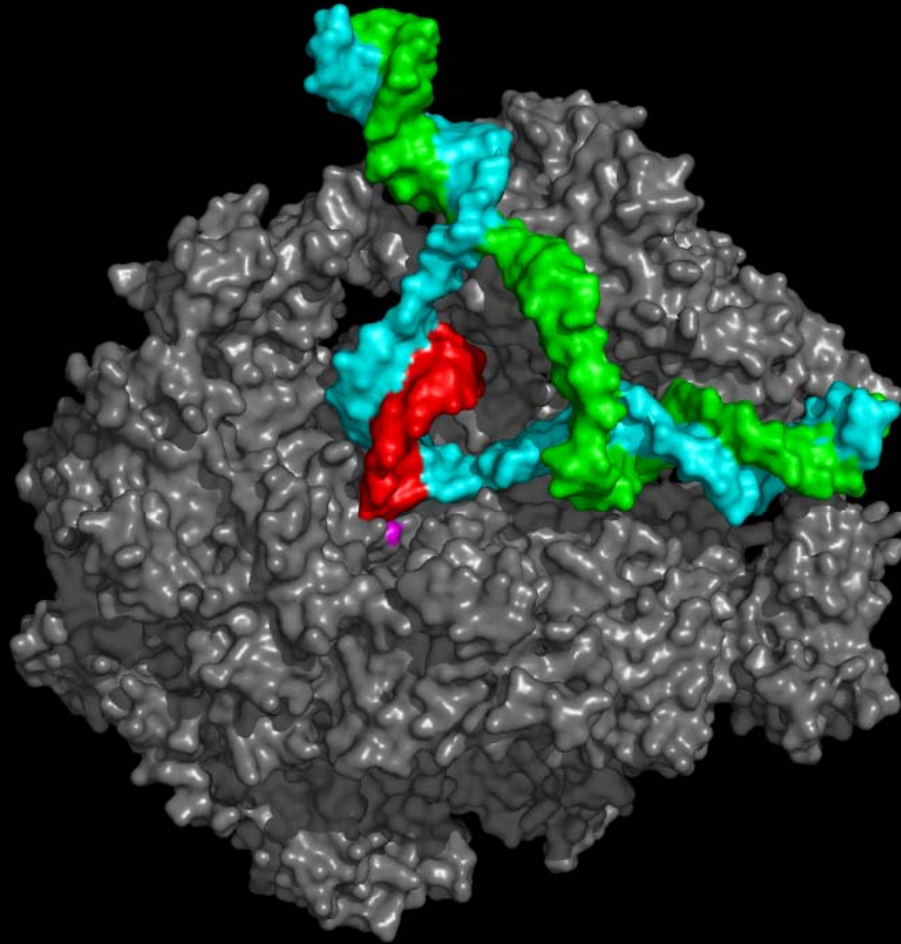


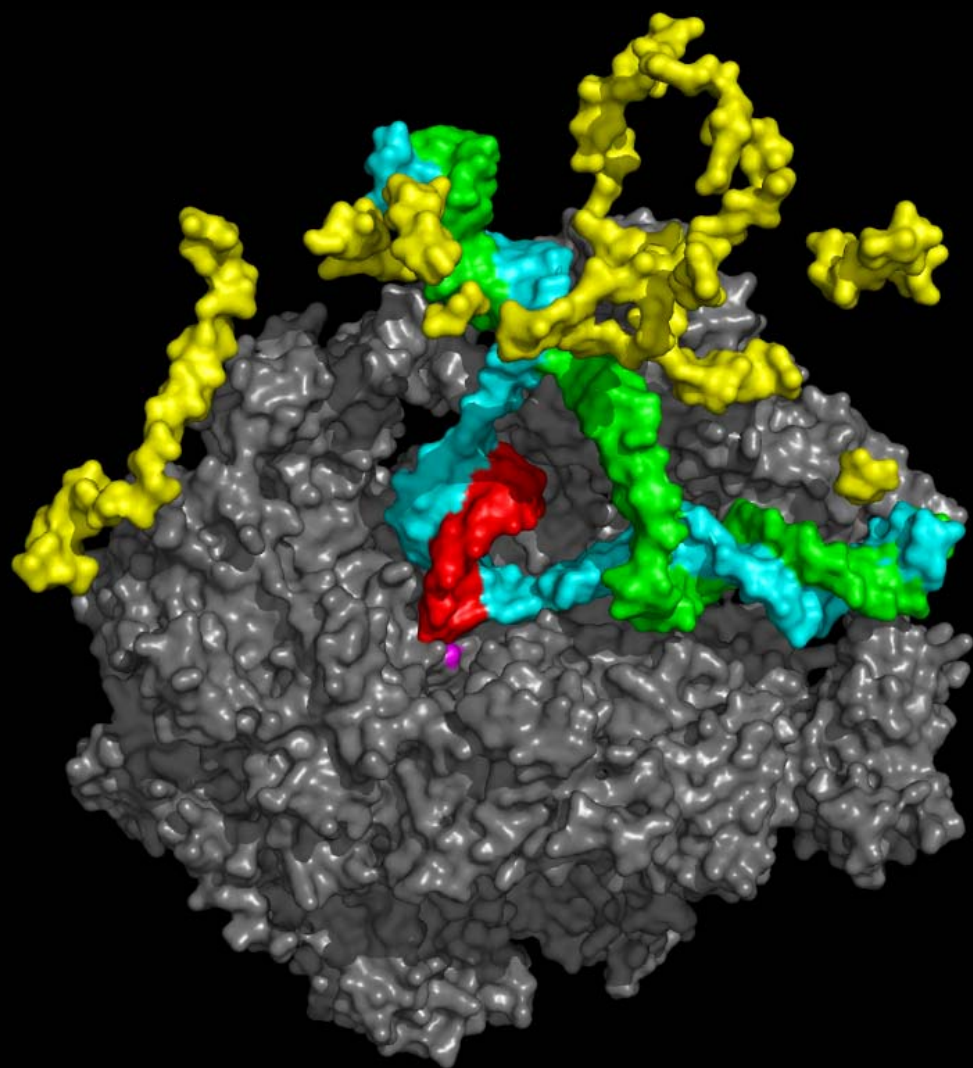


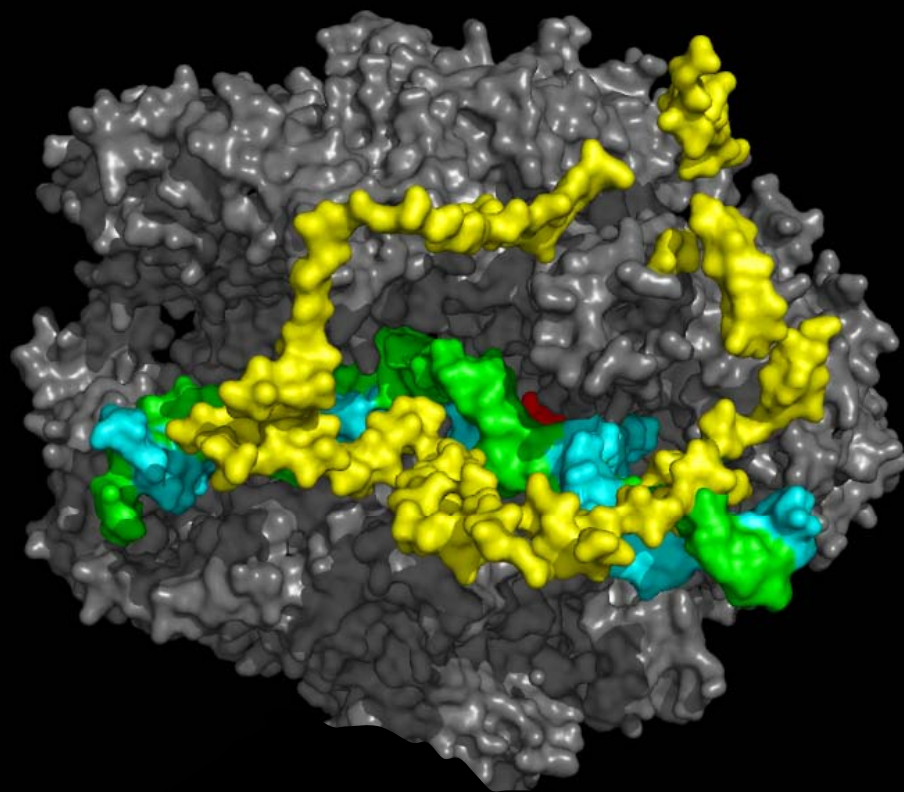


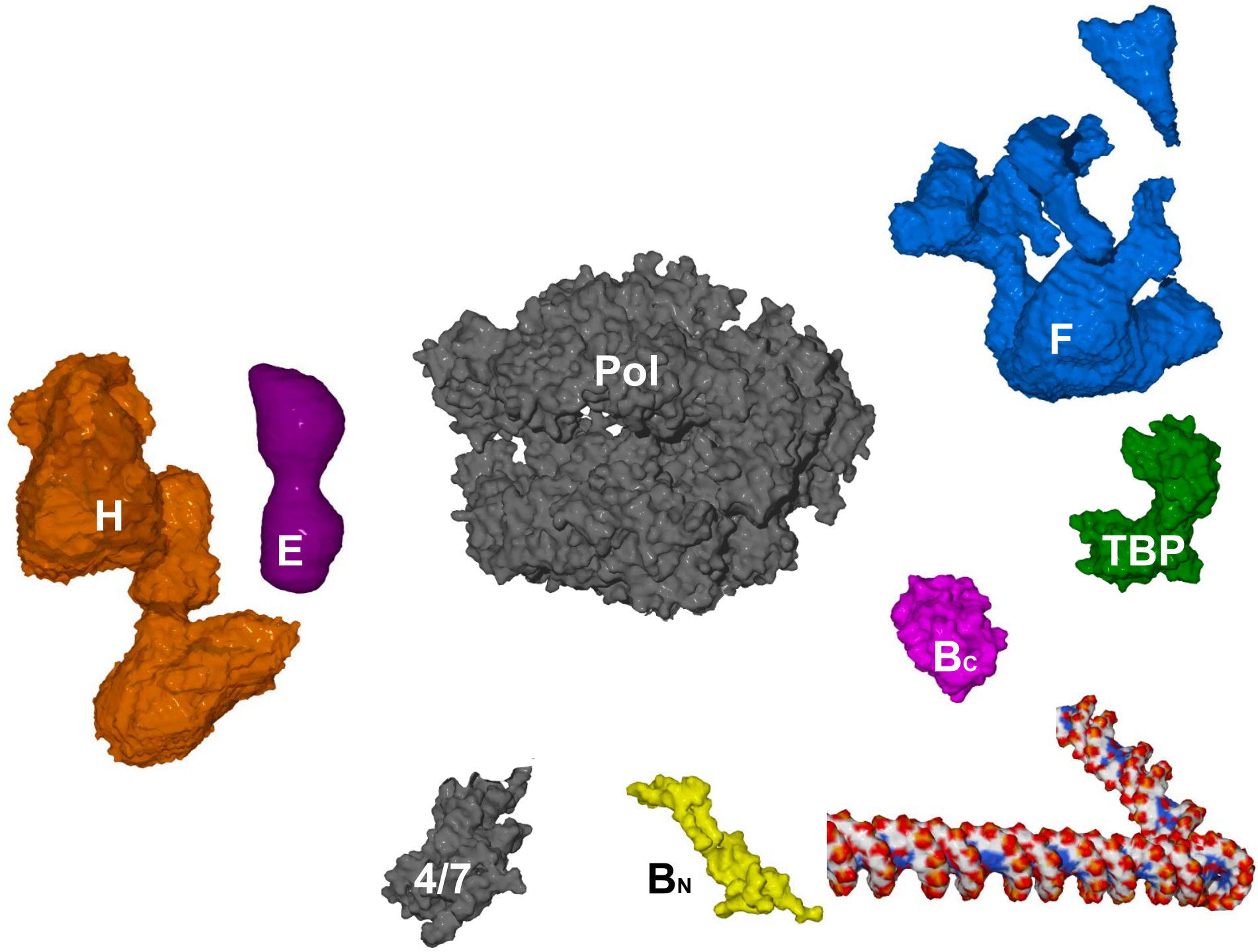


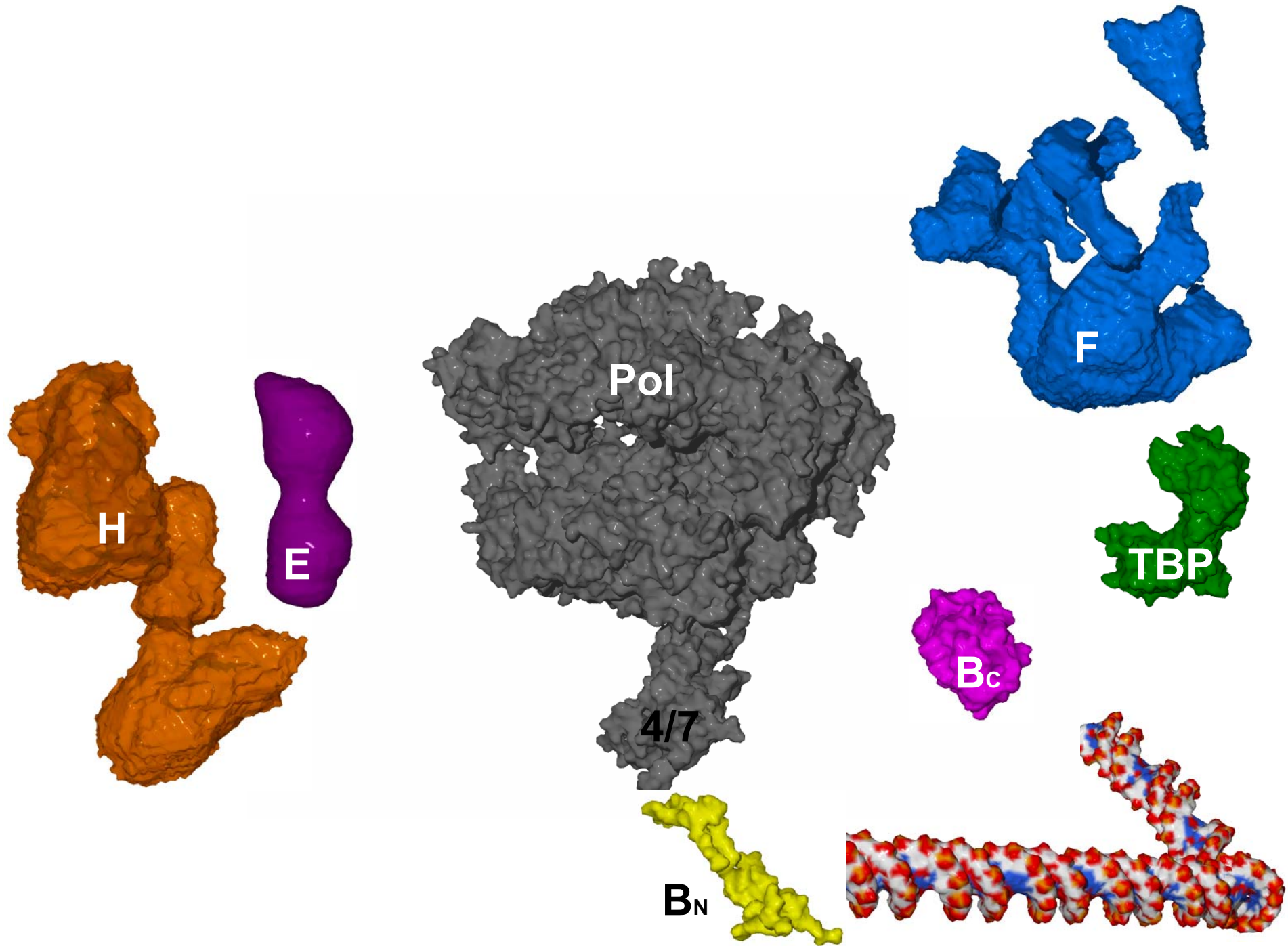
Complete transcription bubble in TFIIIF-2 complex

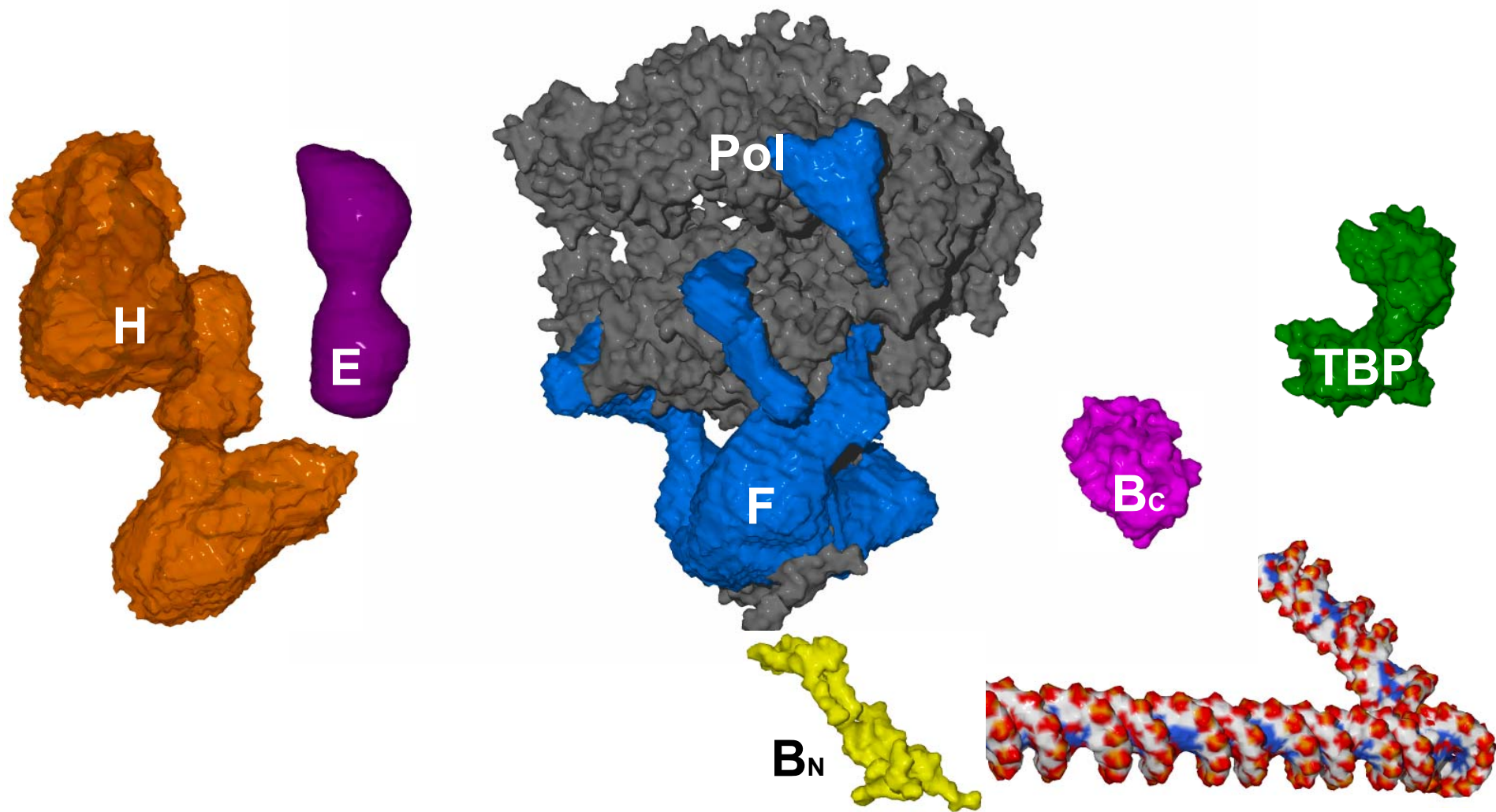


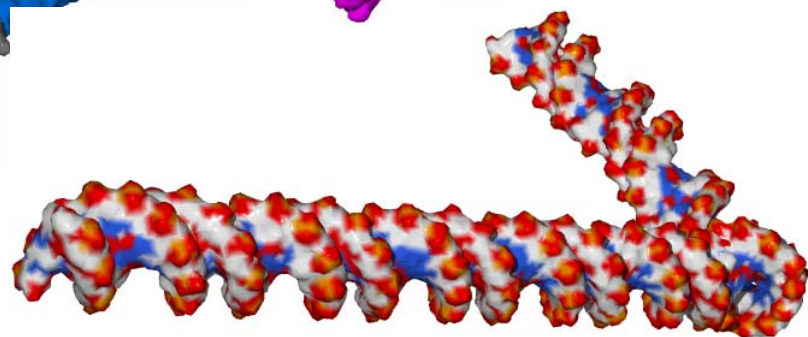
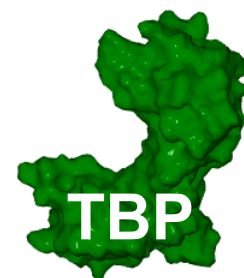
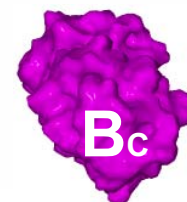
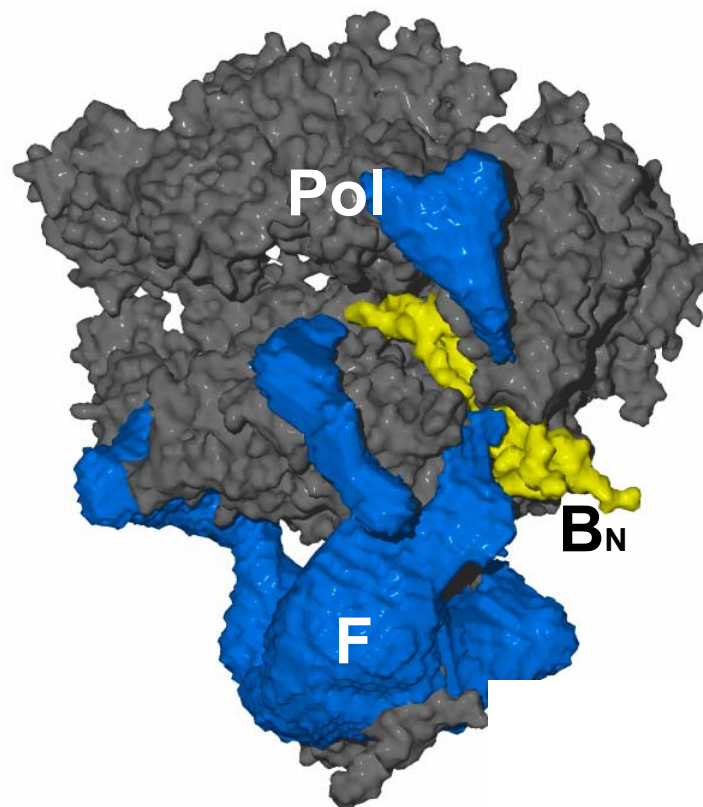
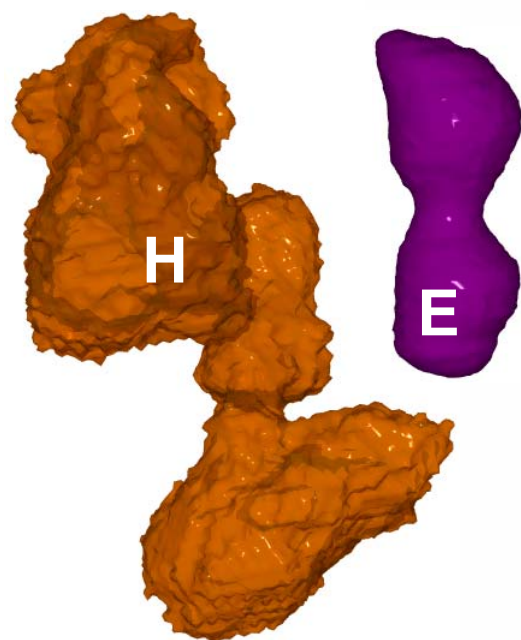


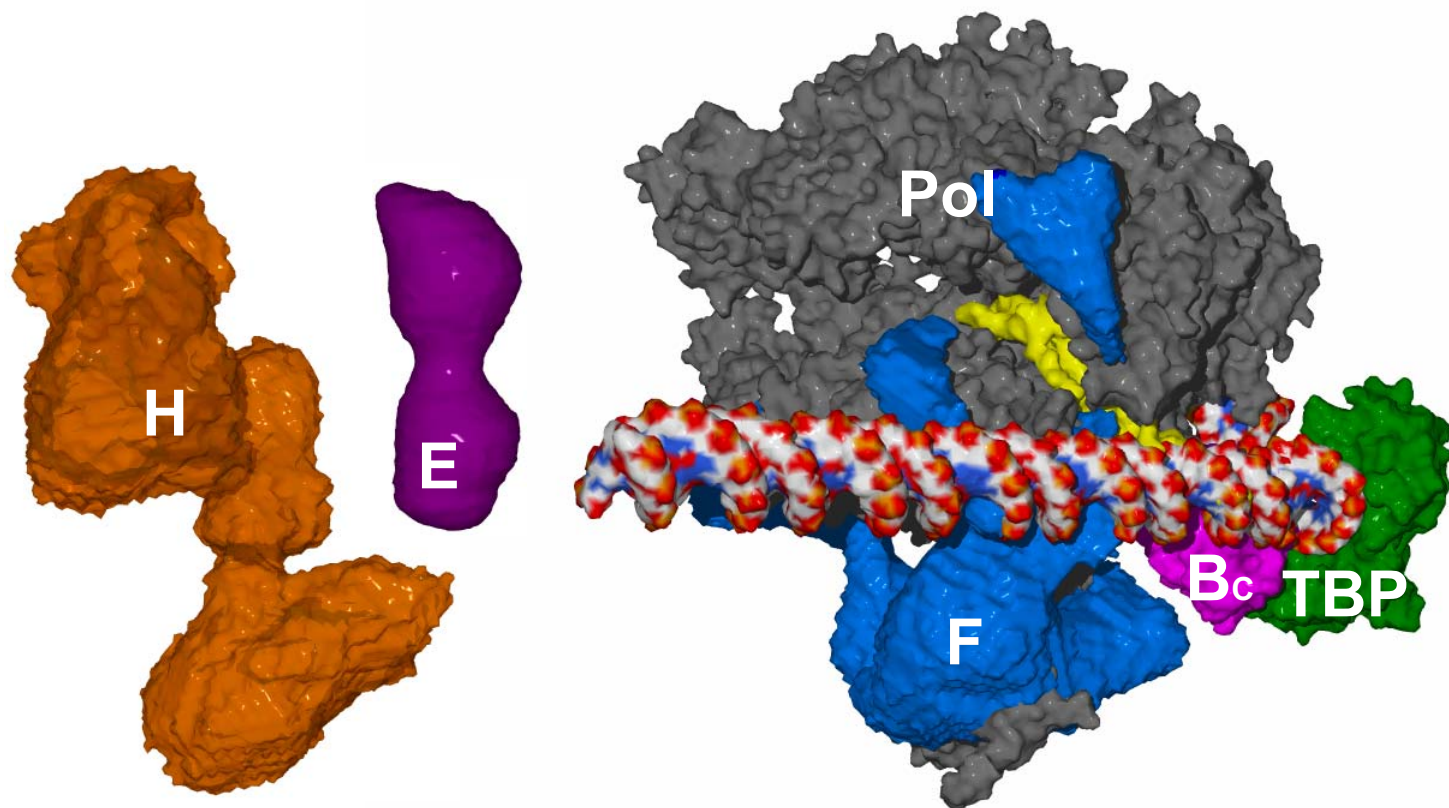


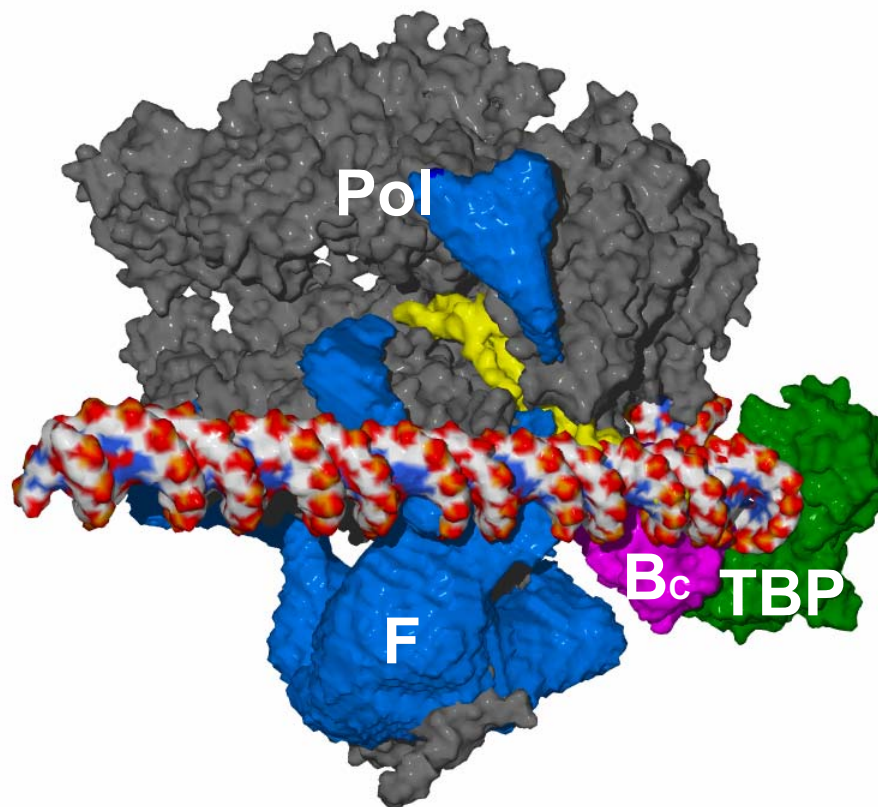
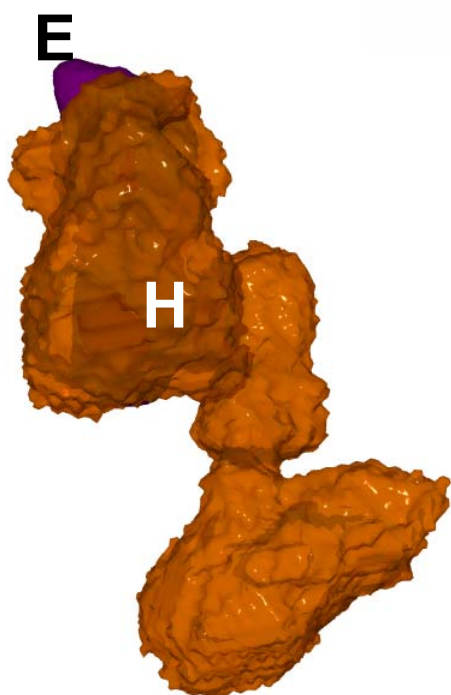




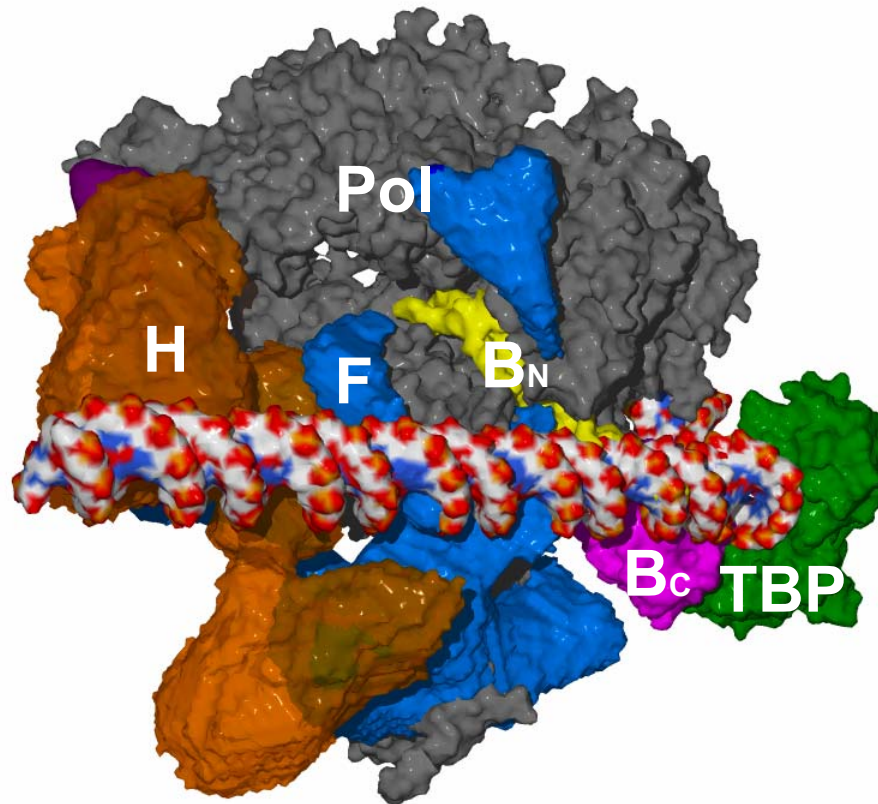






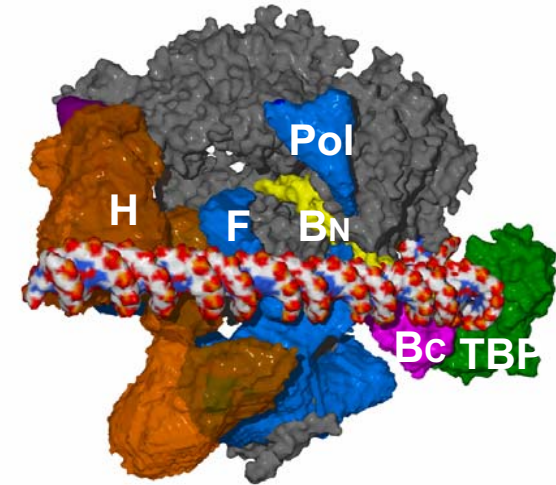


Complete minimal RNA polymerase II transcription initiation complex



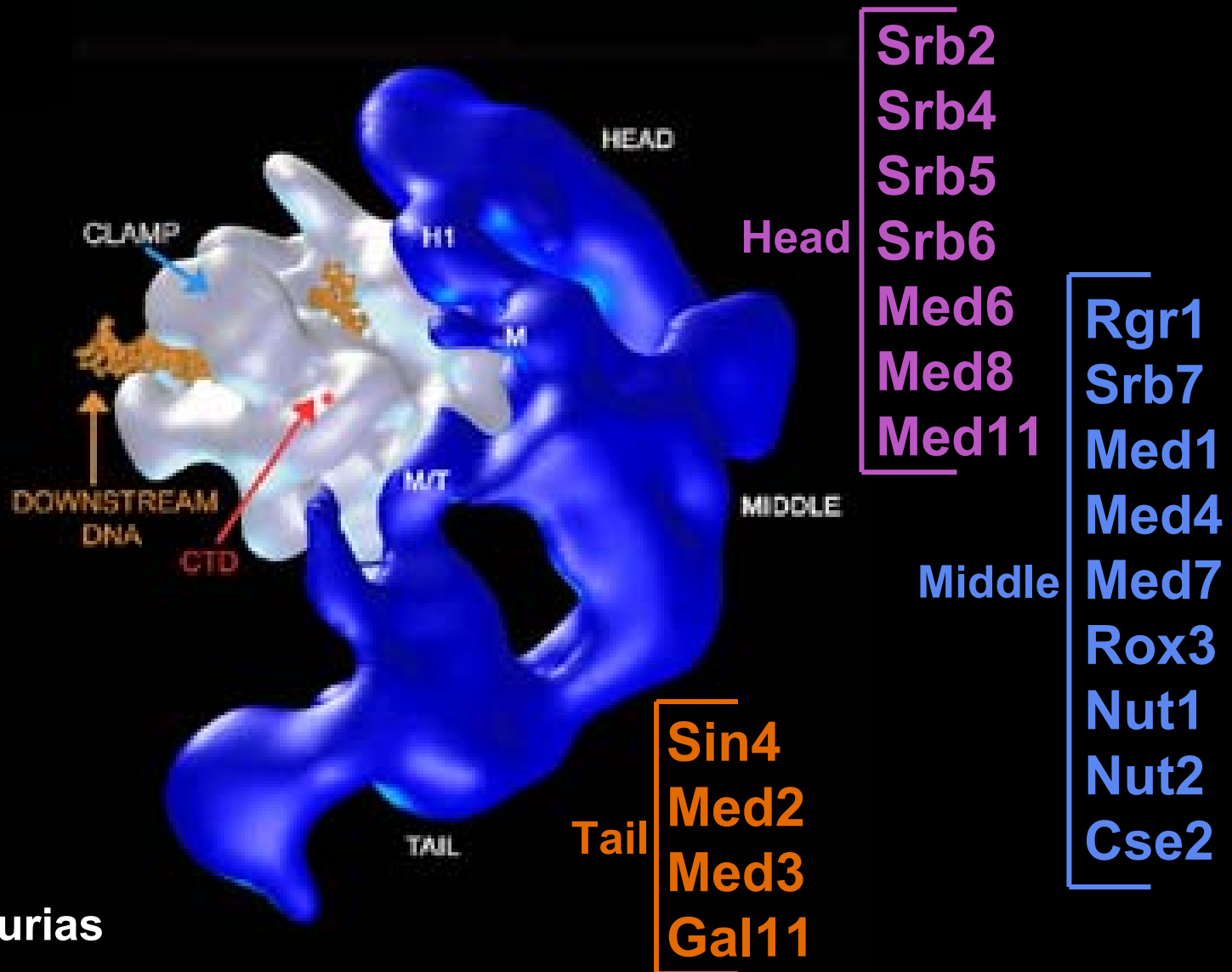


Mechanism of initiation of RNA polymerase II transcription



Factor	Role
TBP	Configure DNA to the pol II surface
TFIIB	Direct DNA path, stabilize early transcribing complex
TFIIE	Recognize closed complex, recruit TFIIH
TFIIF	Capture nontemplate strand upon melting
TFIIH	Introduce negative superhelicity in promoter DNA

Modular structure of Mediator



Head module crystals



proteins: 7

MW: 223 kDa

space group: C222

diffraction limit: 4 Å

$a=236$ Å, $b=435$ Å, $c=328$ Å