Universitat Pompeu Fabra Barcelona

TOPOISOMERASES

TOPOISOMERASE I AND II

Júlia Antolí, Aina Encinas, Elias Ravji Smith, Berta Xiqués

TABLE OF CONTENTS

01 DNA TOPOLOGY

DNA topological problems & solutions

02 TOPOISOMERASES

Function and classification

03 TOPOISOMERASE I

Mechanism of action, structure, SCOP, interaction with DNA

04 TOPOISOMERASE II

Mechanism of action, structure

05 COMPARISONS

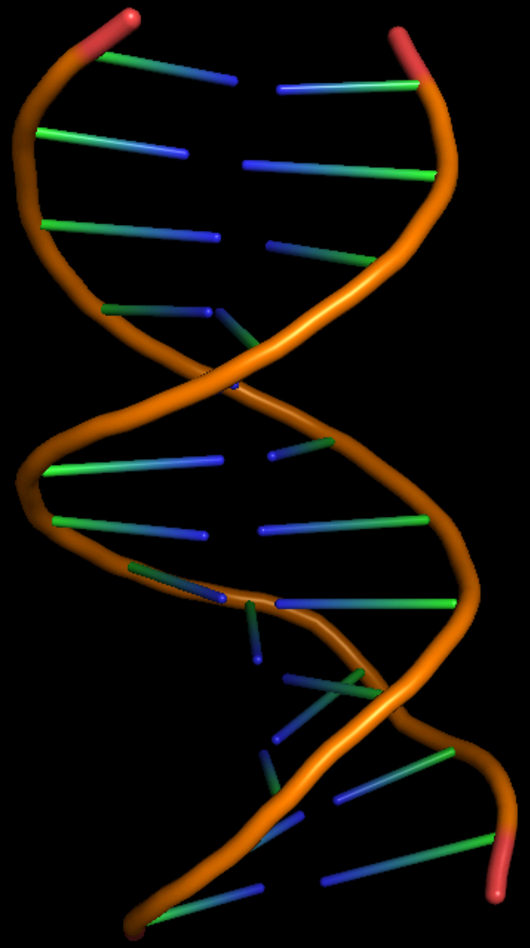
MSA, conserved regions, structural superimpositions, phylogenomics

06 CONCLUSIONS

07 APPENDIX

01 DNA TOPOLOGY

DNA TOPOLOGY - The core problem



PDB ID: 1BNA | Res: 1.90 Å

DNA topology is defined as the three-dimensional spatial arrangement of DNA molecules.

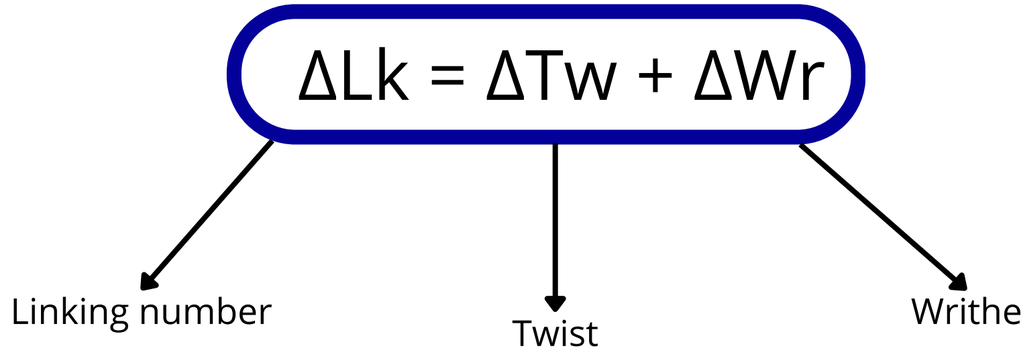
Closed domains of DNA can topologically limit its rotational movement.

DNA TOPOLOGY - Twist and writhe

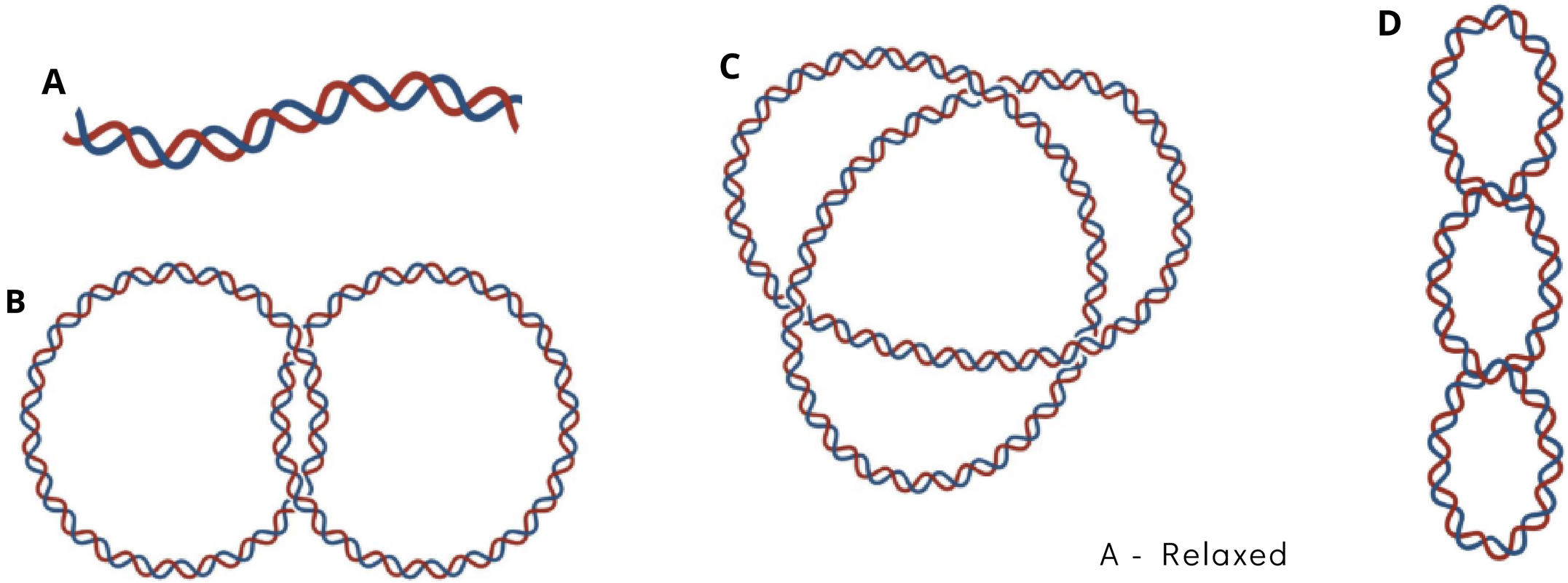
TWIST- the number of helical turns of one strand around another

WRITHE- the number of times a double helix crosses over itself

"the sum of twist and writhe describing a given DNA molecule is equal to the linking number".



DNA TOPOLOGY - Different states

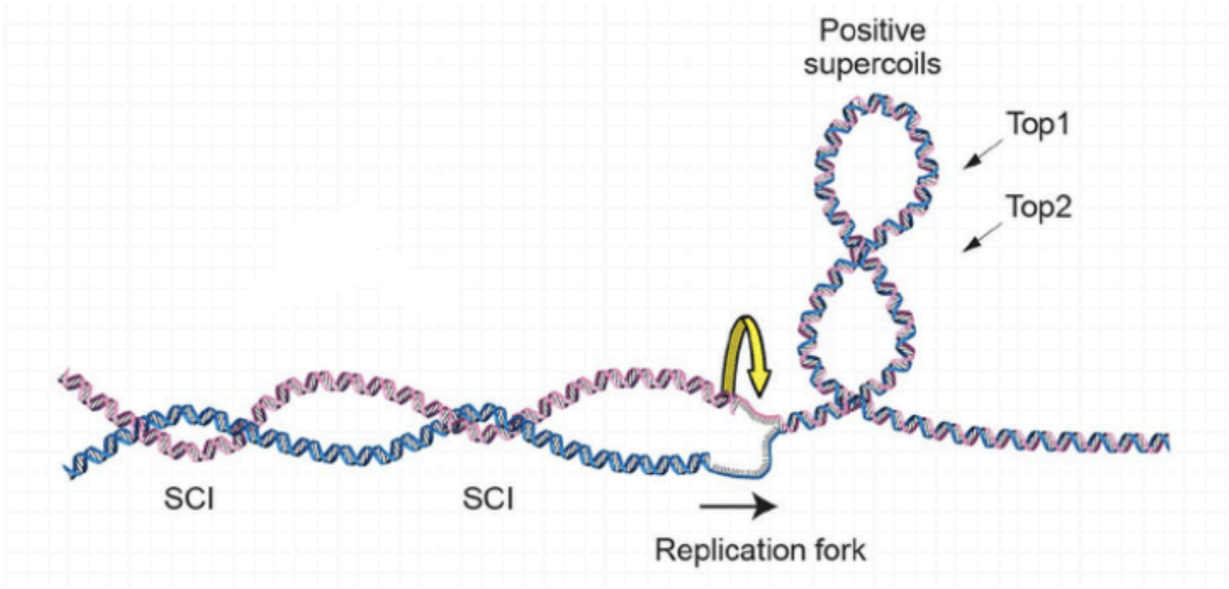


A - Relaxed
B - Catenated
C - Knotted
D - Supercoiled

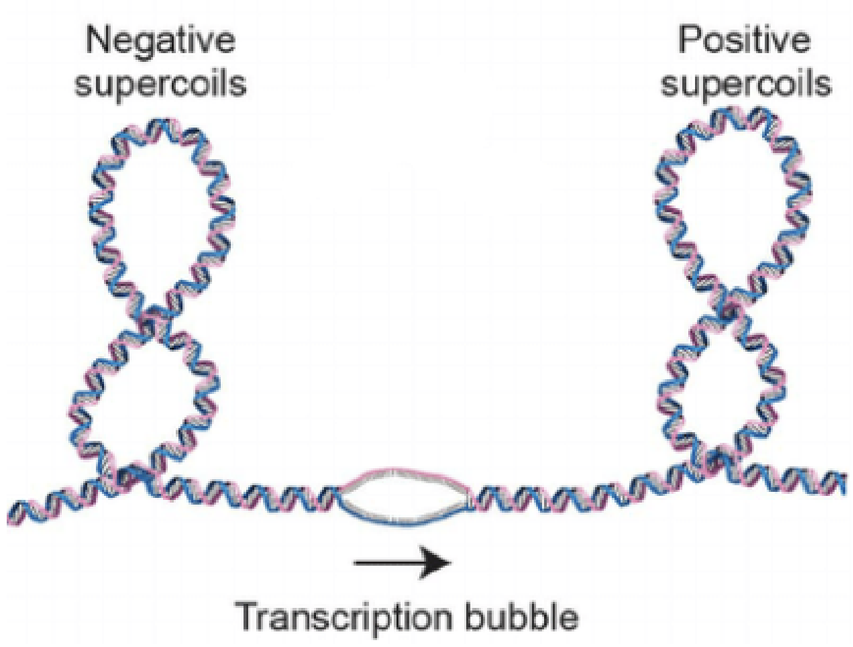
Created using Biorender

DNA TOPOLOGY - Supercoils

Replication: The DNA helicase enzyme separates strands, forcing the DNA ahead of the replication fork to become supercoiled.



Transcription: As RNA polymerase moves along DNA, it generates positive supercoiling (overwinding) ahead and negative supercoiling (underwinding) behind it.



Adapted from (G Bush, 2015)

DNA TOPOLOGY - Consequences

If supercoiling develops in the DNA structure and remains unresolved, it may disrupt cellular homeostasis for the following reasons:

1. Transcription blockage
2. Replication failure
3. Genome instability
4. Topological barriers
5. DNA melting



Created using Biorender

02 TOPOISOMERASES

WHAT ARE TOPOISOMERASES?



PDB ID: 1A36 | Res: 2.80 Å

Enzymes that alter the topological state of DNA.

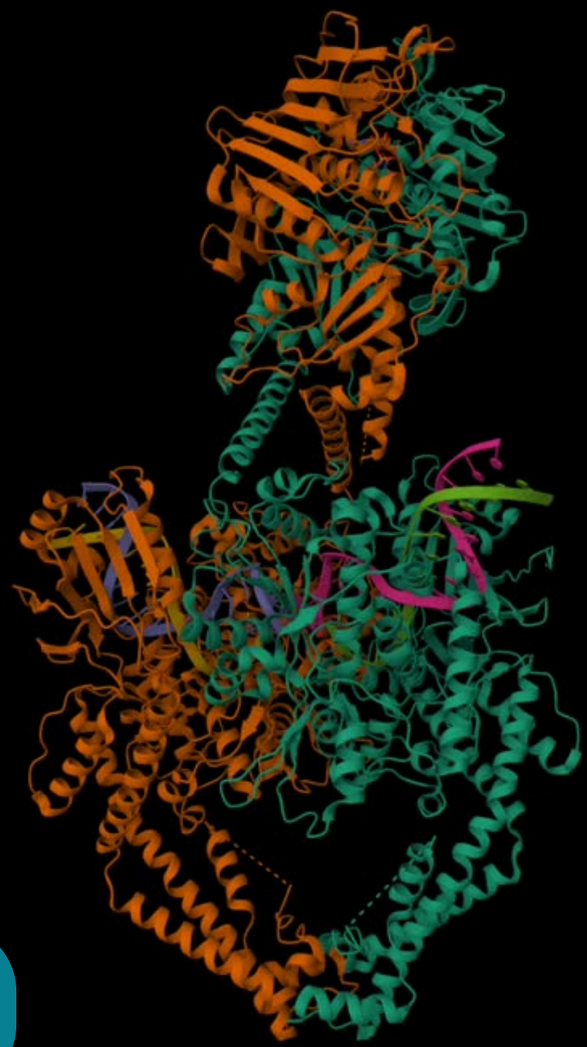
↓

Add a transient break in the DNA backbone + uncoil.

↓

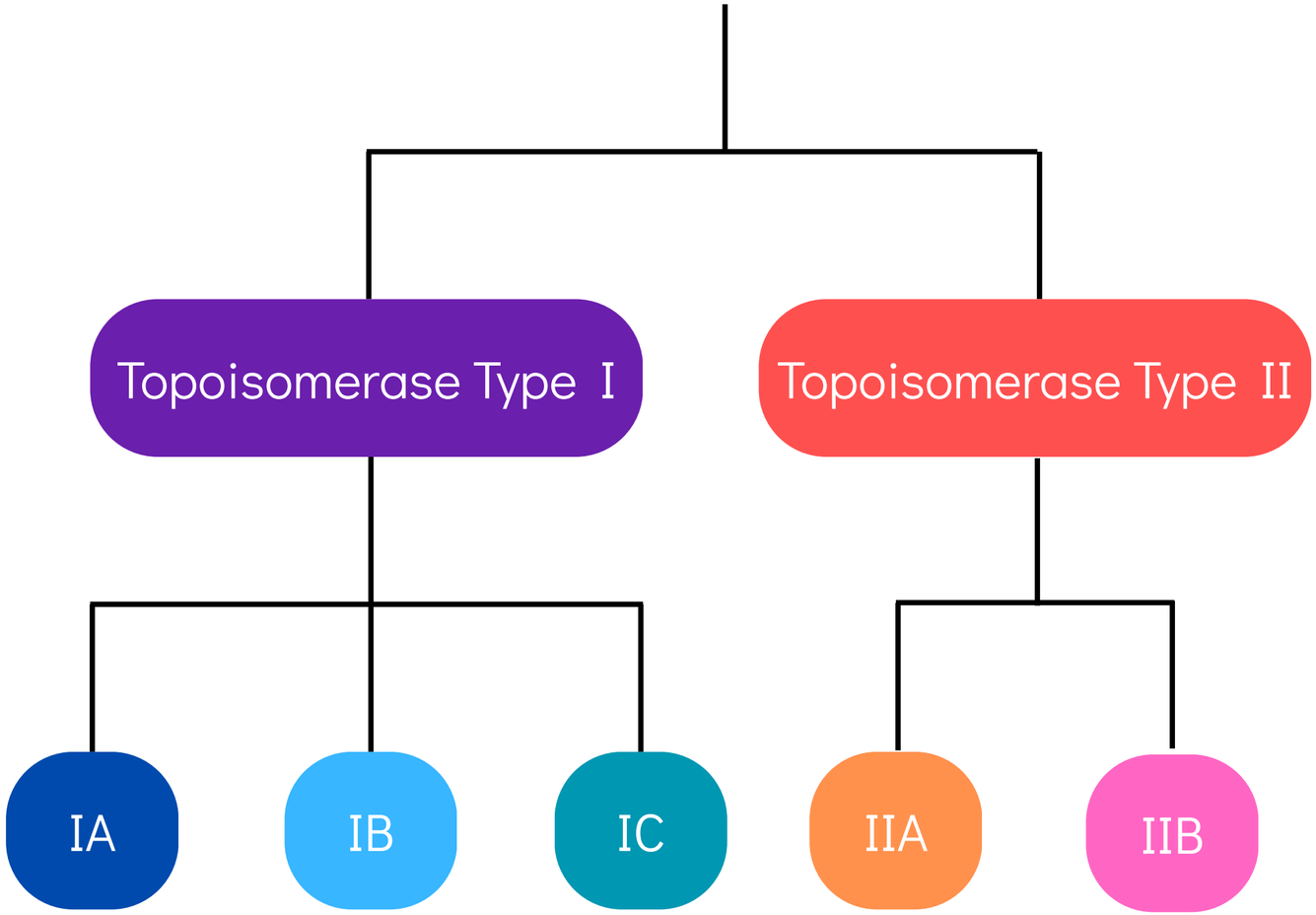
Through a covalent bond between Tyr and DNA backbone.

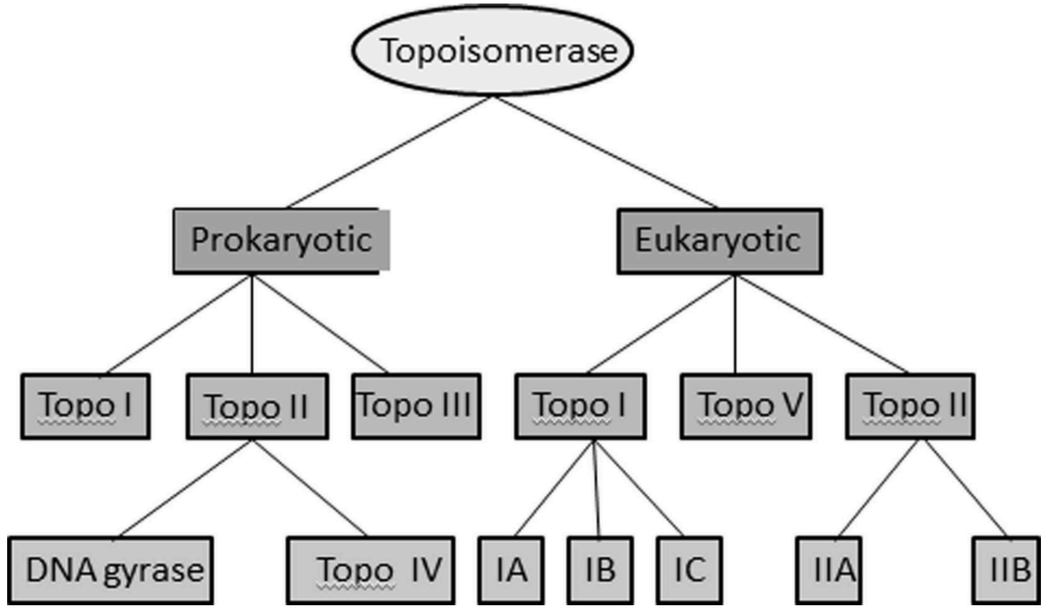
Topoisomerases resolve topological stress generated during DNA metabolism



PDB ID: 6ZY7 | Res: 4.64 Å

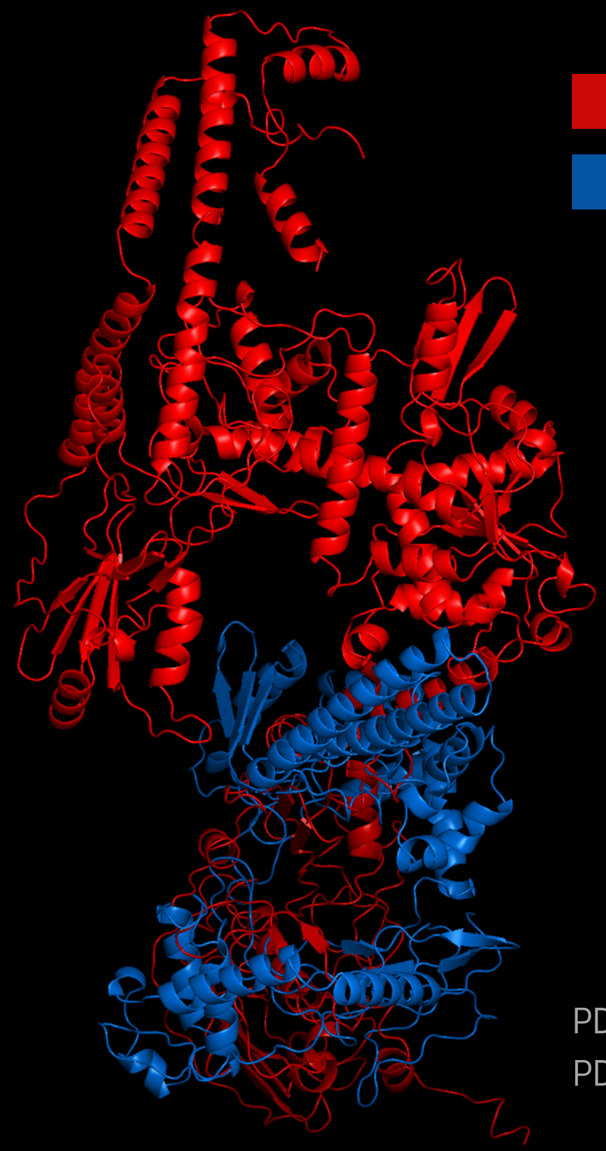
BASIC CLASSIFICATION





Jaswal S., Nehra B., Kumar S., Monga V. (2020). Recent advancements in the medicinal chemistry of bacterial type II topoisomerase inhibitors. Bioorganic Chemistry.

STAMP TOPO I VS TOPO II



Human topoisomerase IIA (chain A)

Human topoisomerase IB

PDB ID: 6ZY7 | Res: 4.64 Å

PDB ID: 1A36 | Res: 2.80 Å

NO SUPERIMPOSITION!

03 TOPOISOMERASE I

TOPOISOMERASES TYPE I

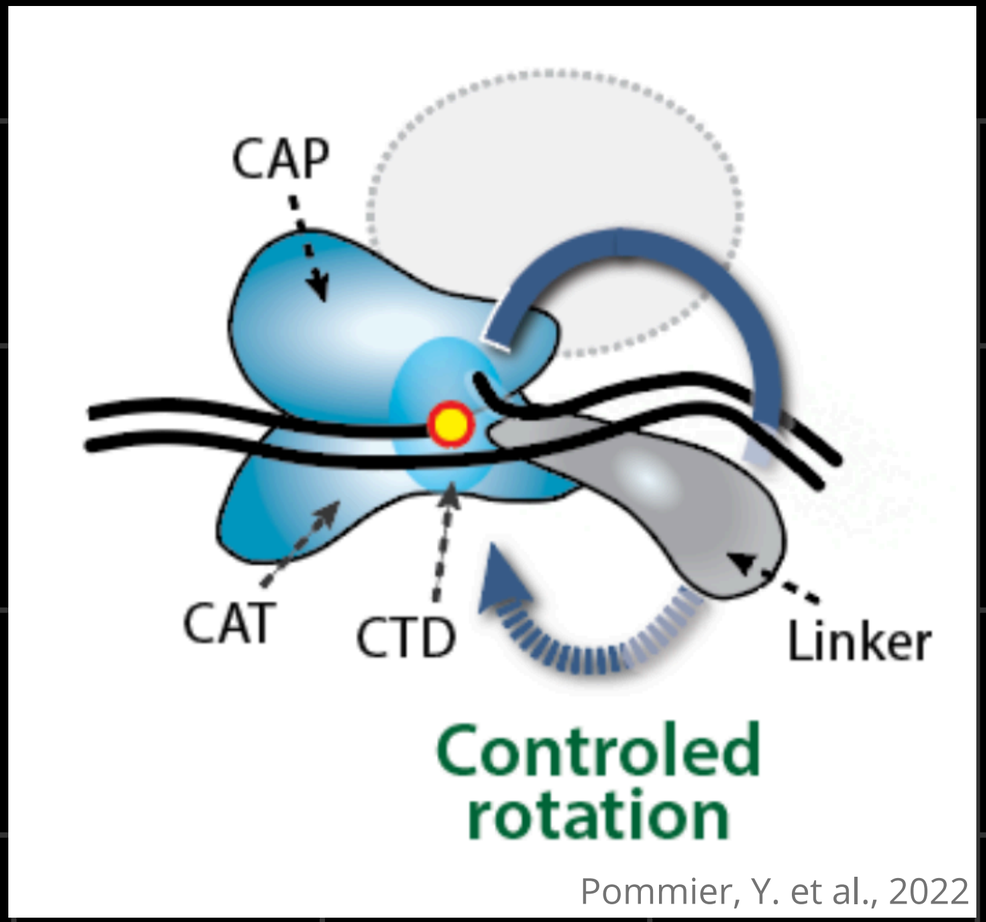
Cut one strand of DNA

	ORGANISM	STRUCTURE	5' or 3' BOND	MECHANISM	RELAXATION	Mg^{2+} DEPENDENT
Topo IA	Bacteria Archaea Eukarya	Monomer	5'	Strand passage	Negatively supercoiled DNA	Yes
Topo IB	Eukaryotic Some archaea		3'	Controlled rotation	Negatively and positively supercoiled DNA	No
Topo IC	Rare archaea					

TOPOISOMERASES TYPE I

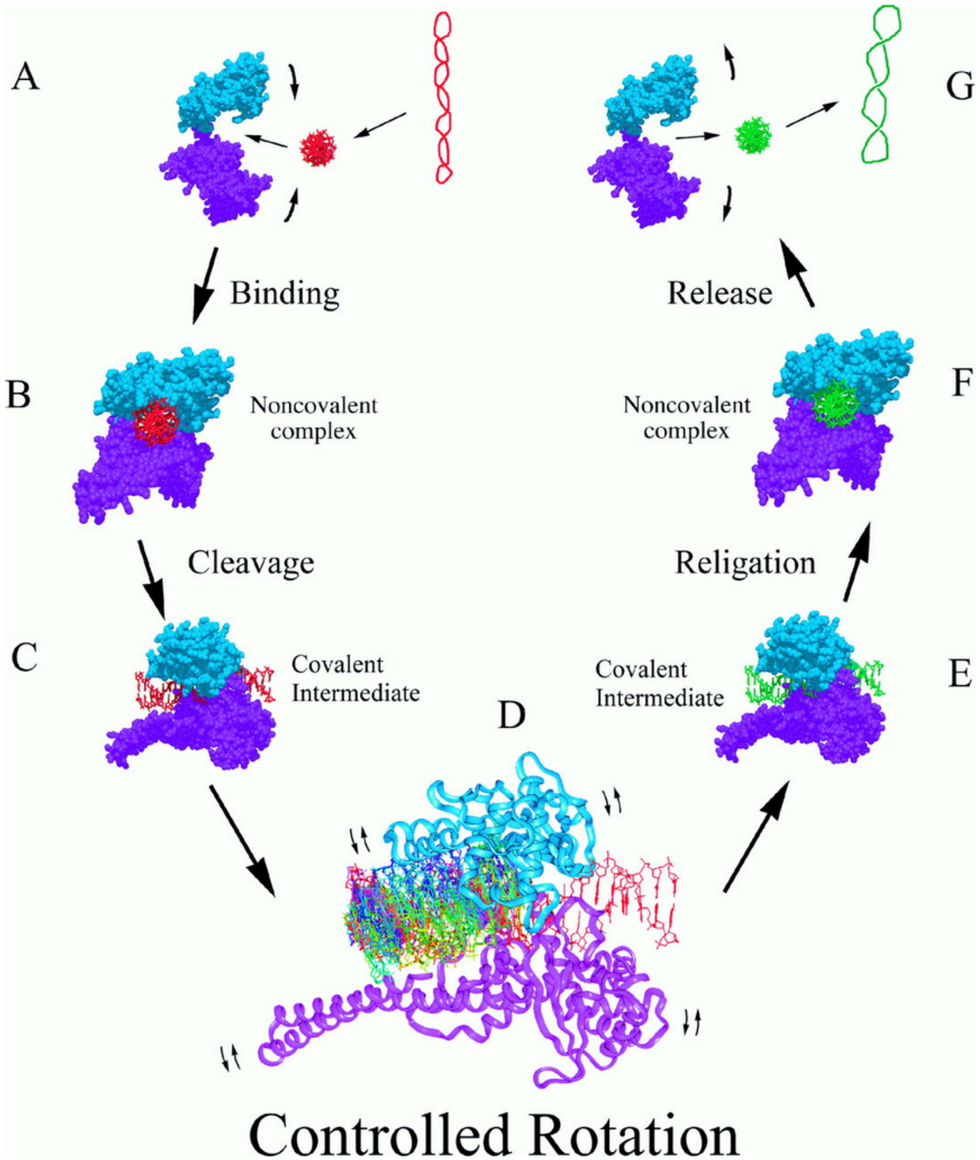
Cut one strand of DNA

	ORGANISM				RELAXATION	Mg^{2+} DEPENDENT
Topo IA	Bacteria Archaea Eukarya				Negatively supercoiled DNA	Yes
Topo IB	Eukaryotic Some archaea					No
Topo IC	Rare archaea					



Pommier, Y. et al., 2022

MECHANISM OF ACTION



Lance Stewart et al., 1998

3.1 TOPOISOMERASE IB

DOMAINS

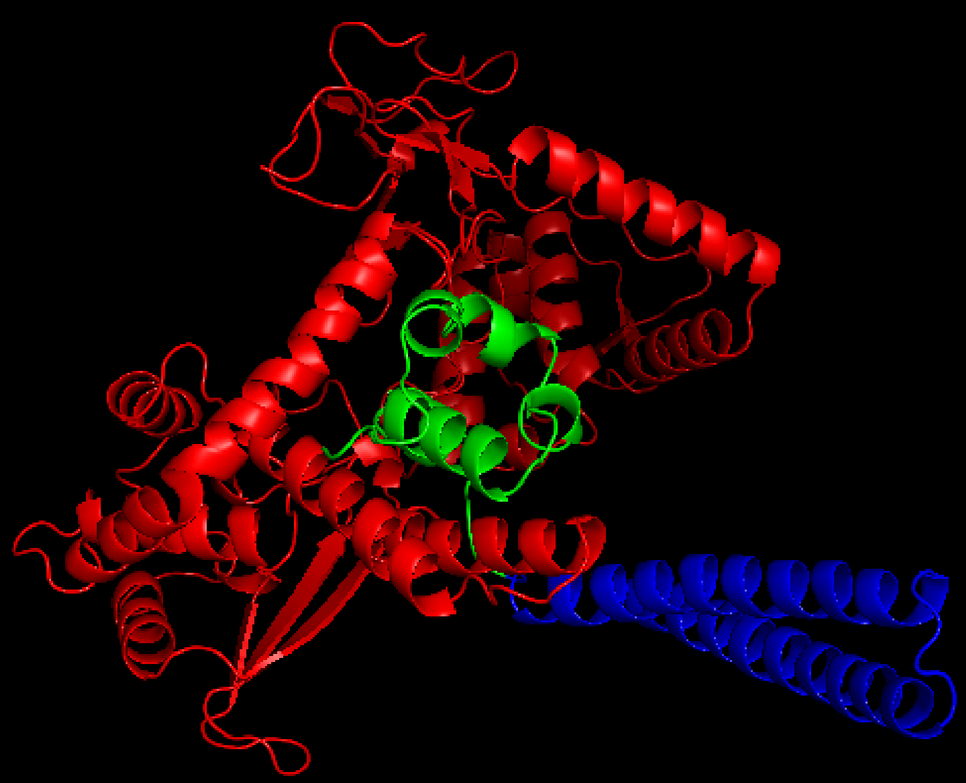
Core Domain (res 214-635)

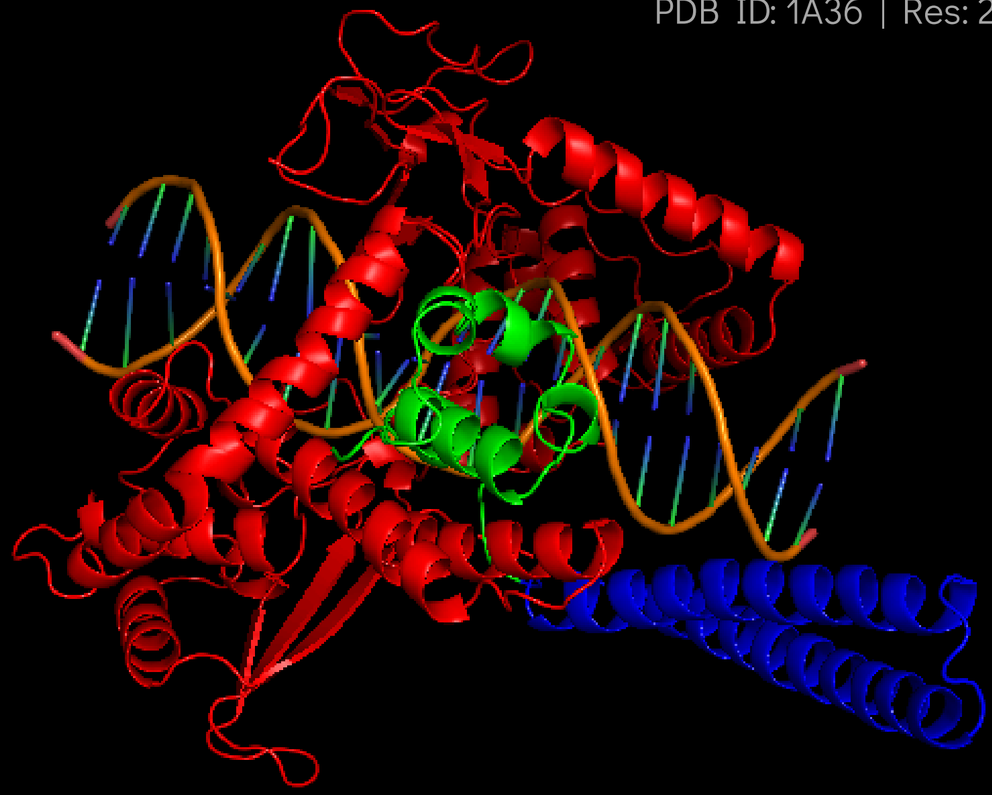
Linker Domain (res 636-712)

C-terminal Domain (res 713-765)

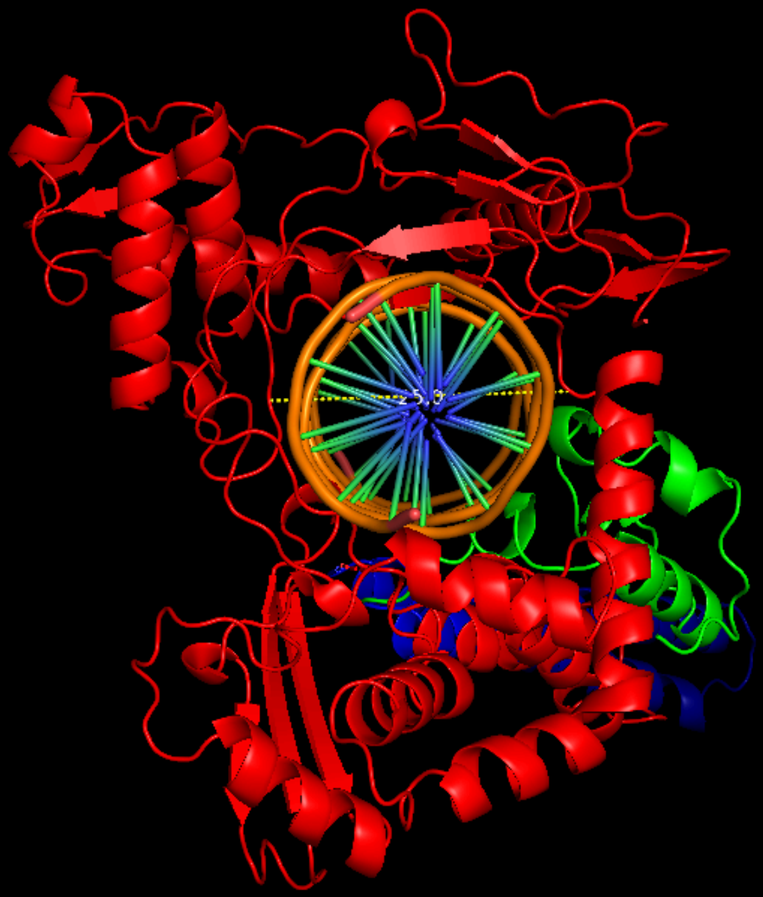
* N-terminal Domain (res 1-213)

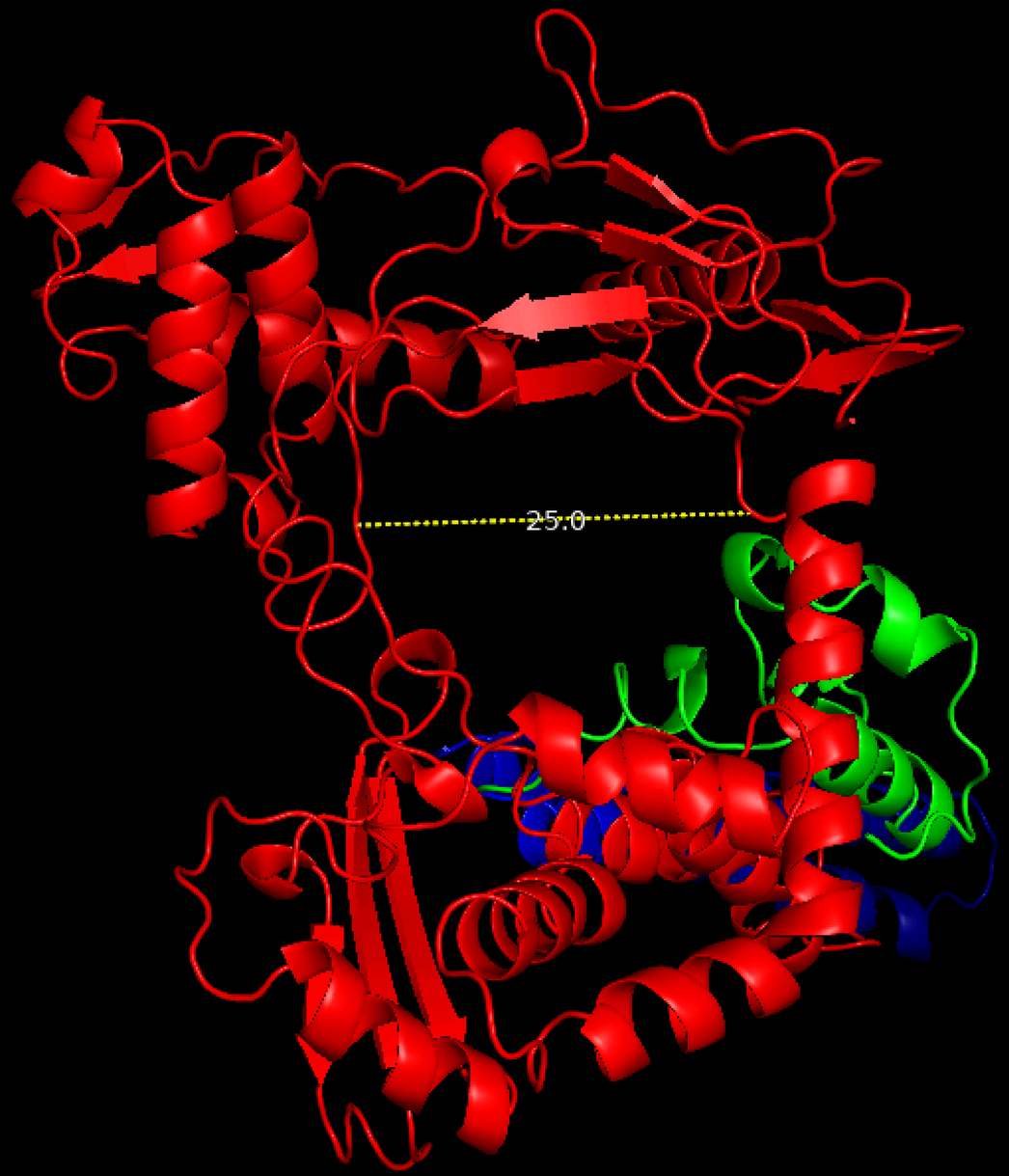
PDB ID: 1A36 | Res: 2.80 Å





PORE 15 - 25 Å





PDB ID: 1A36 | Res: 2.80 Å

PORE 15 - 25 Å

Lysine

Arginine



PDB ID: 1A36 | Res: 2.80 Å

CORE SUBDOMAINS



Subdomain I (res 214-232, 320-433)

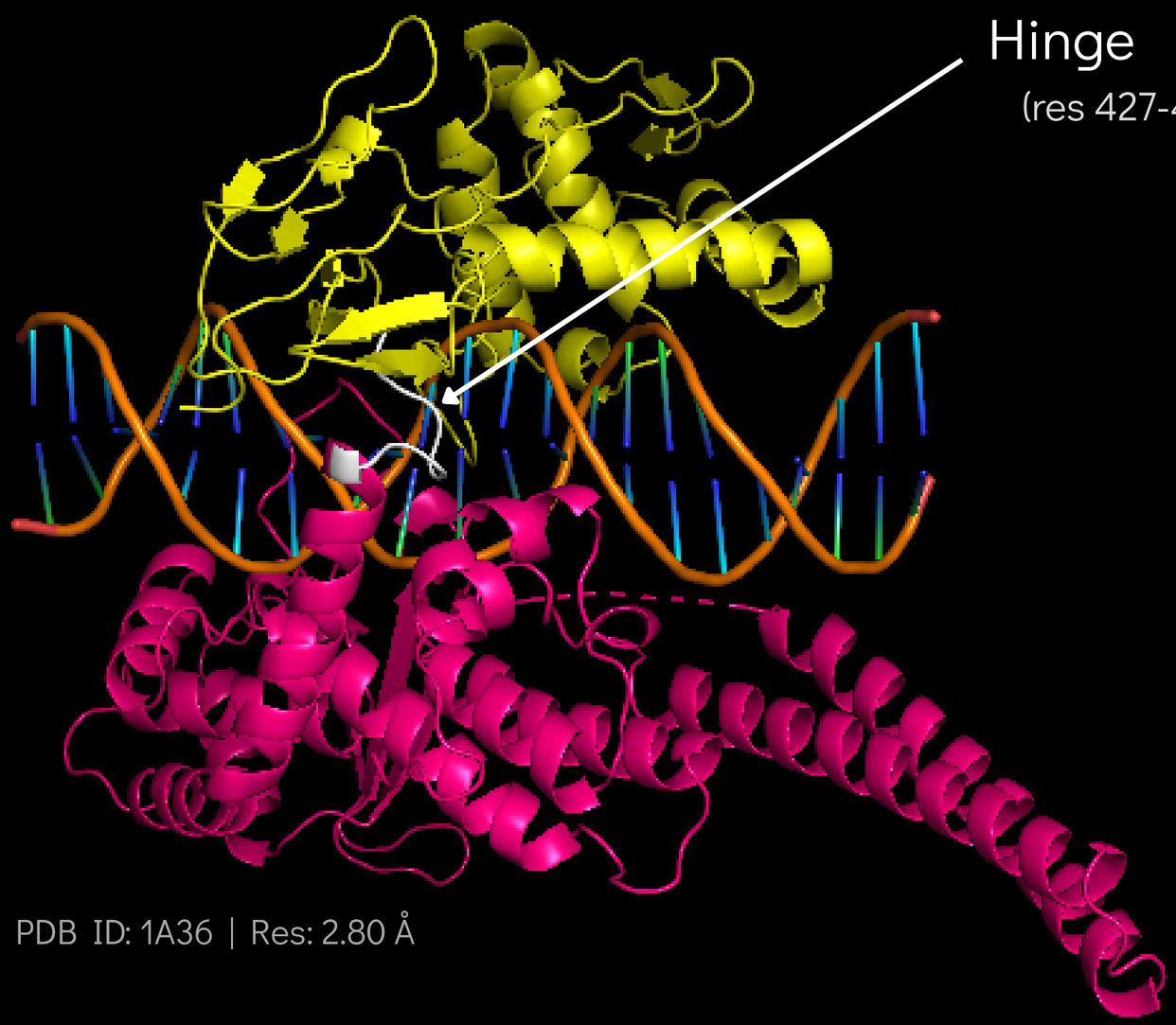
Subdomain II (res 233-319)

Subdomain III (res 434-635)

PDB ID: 1A36 | Res: 2.80 Å



LOBES



PDB ID: 1A36 | Res: 2.80 Å

CAP lobe

- Subdomain I
- Subdomain II

(res 214-426)

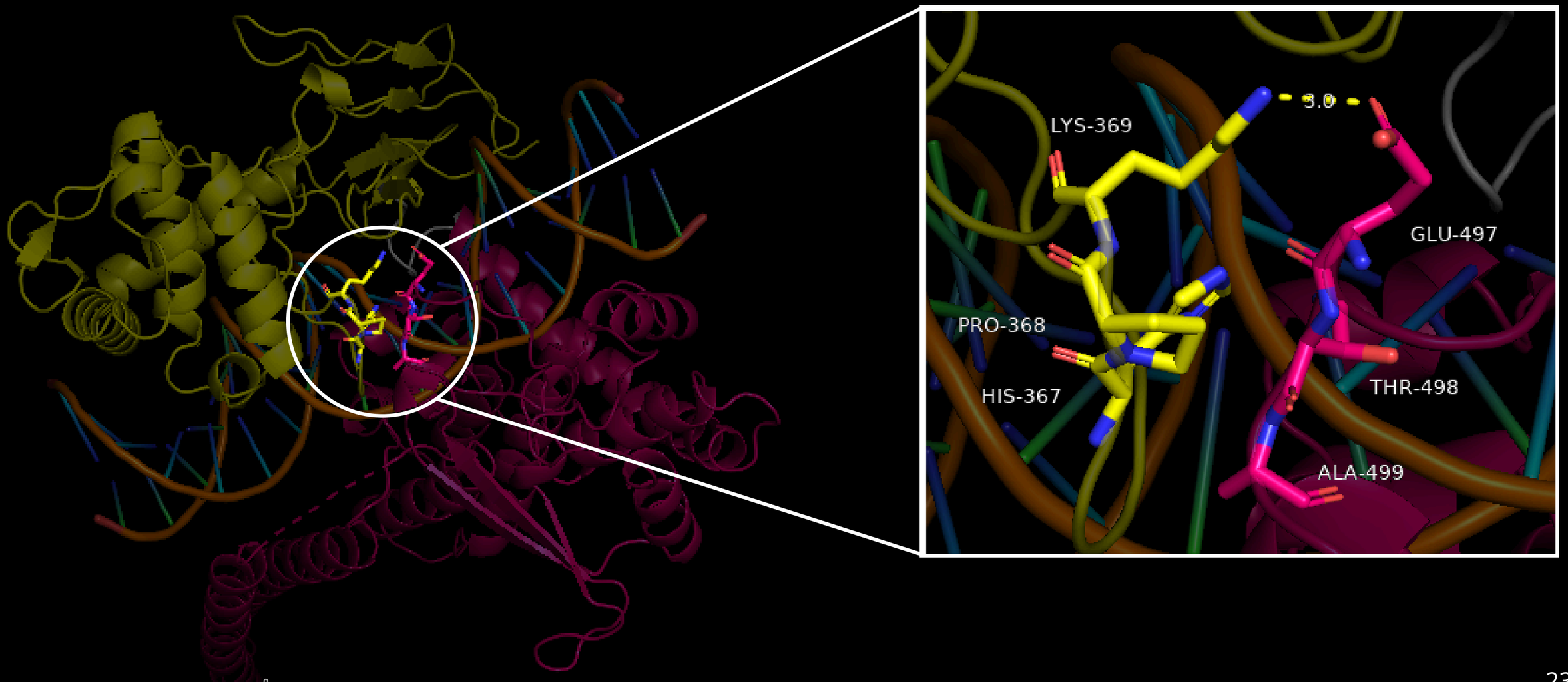
DNA

CAT lobe

- Subdomain III
- Linker domain
- C-terminal domain

(res 435-765)

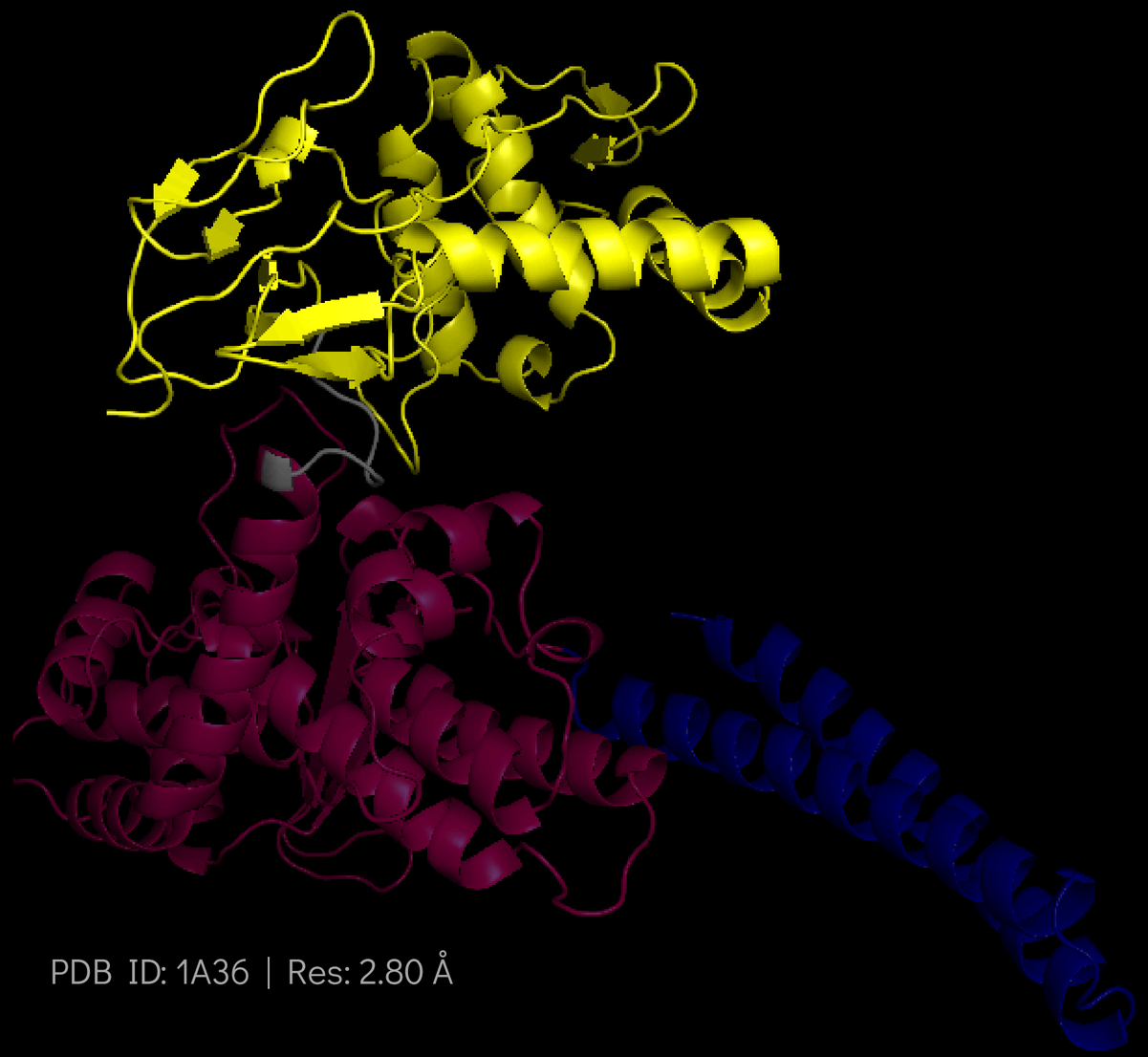
LIPS



PDB ID: 1A36 | Res: 2.80 Å

SCOP CLASSIFICATION

CAP lobe

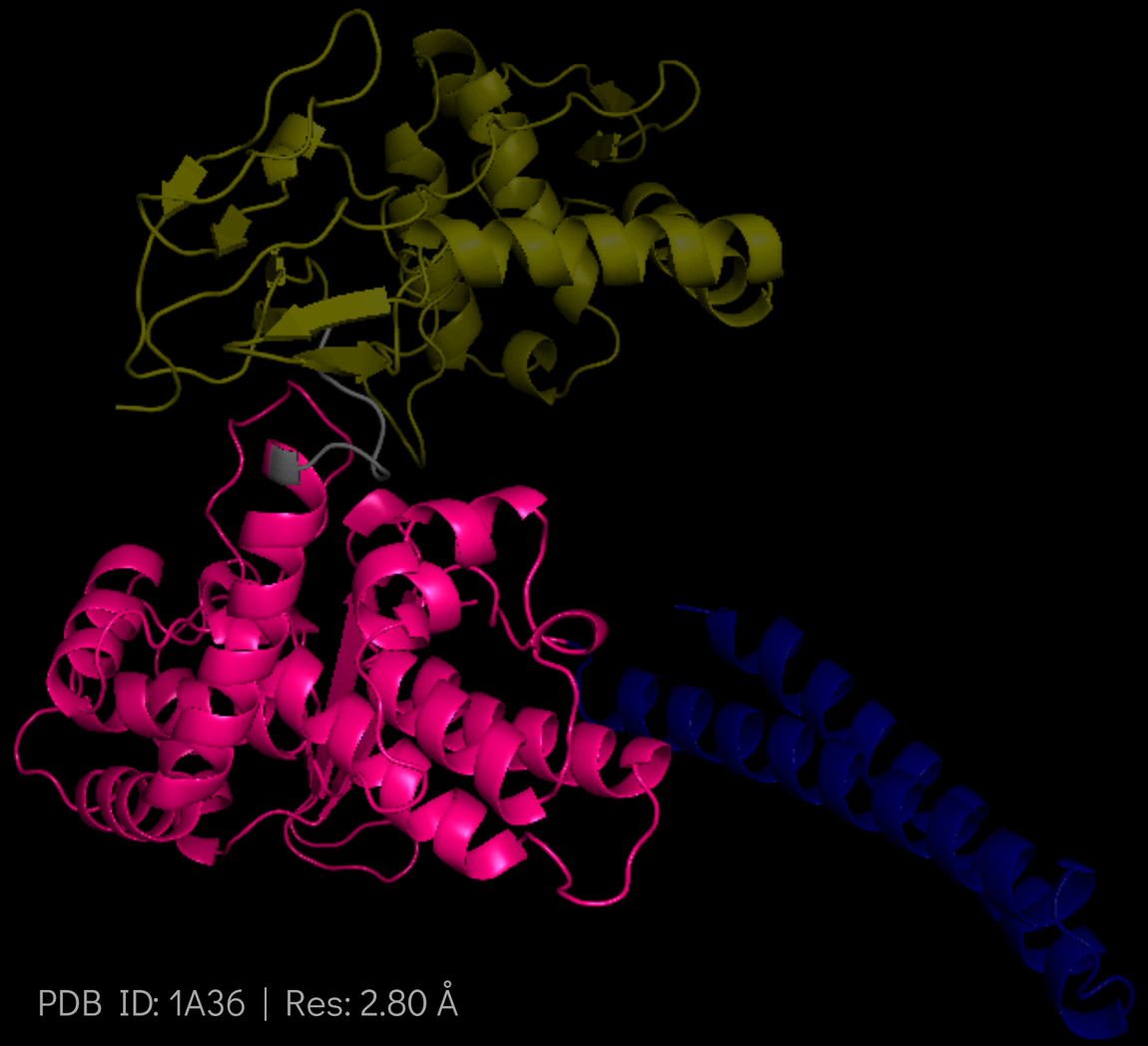


PDB ID: 1A36 | Res: 2.80 Å

CLASS	α and β proteins
FOLD	Eukaryotic DNA topoisomerase I N-terminal DNA-binding domain-like
SUPERFAMILY	
FAMILY	

SCOP CLASSIFICATION

CAT lobe

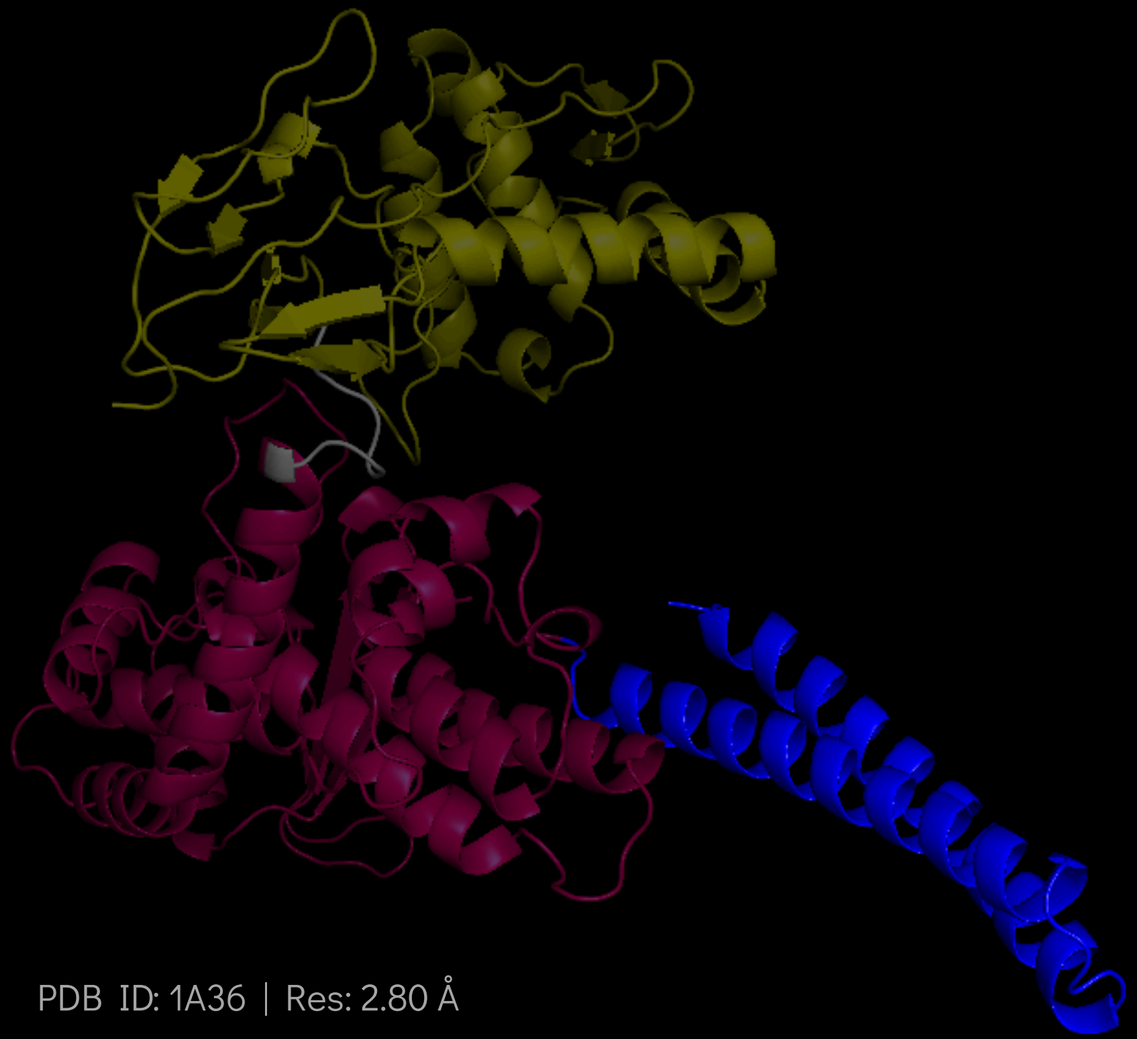


PDB ID: 1A36 | Res: 2.80 Å

CLASS	α and β proteins
FOLD	DNA breaking-rejoining enzymes
SUPERFAMILY	
FAMILY	Eukaryotic DNA topoisomerase I, catalytic core

SCOP CLASSIFICATION

Linker domain

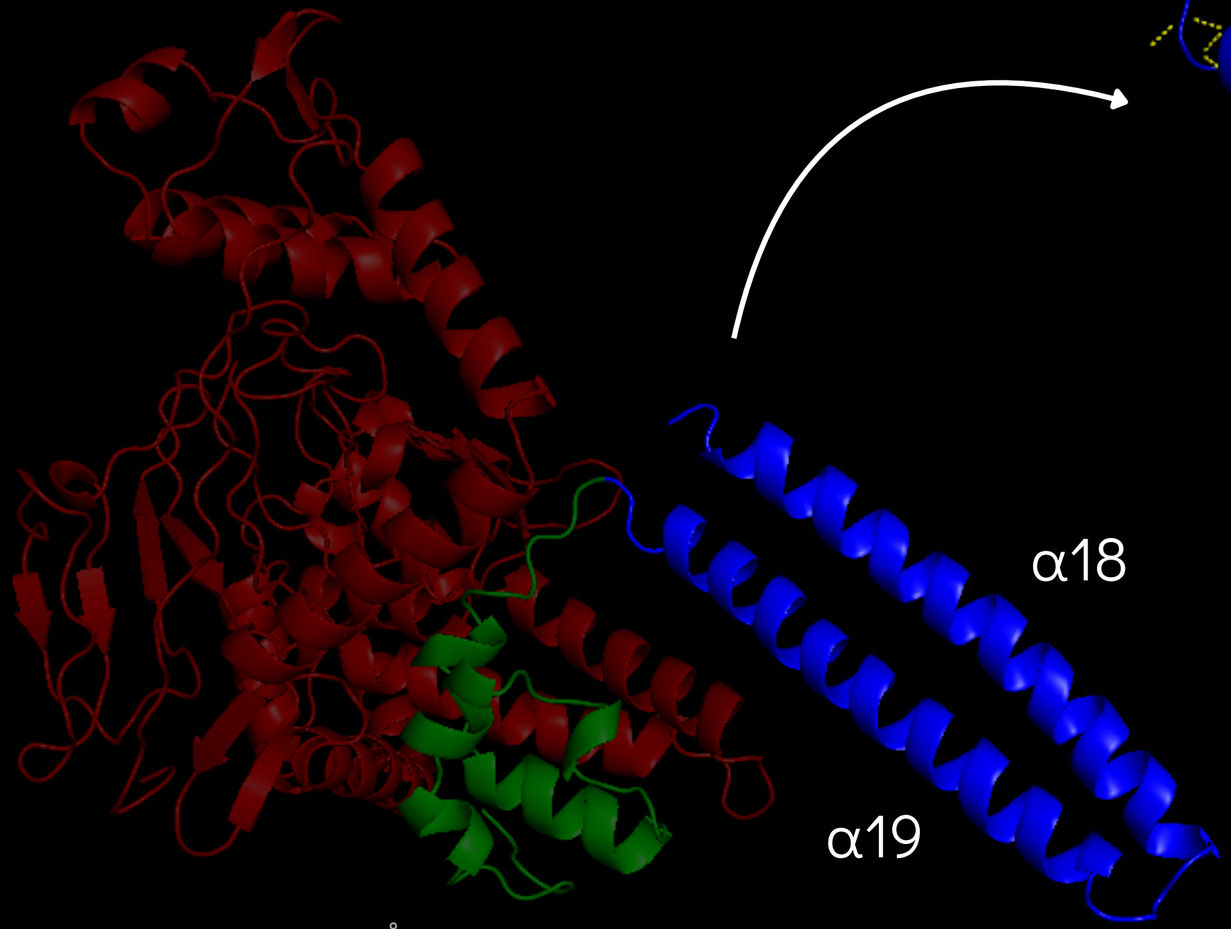


PDB ID: 1A36 | Res: 2.80 Å

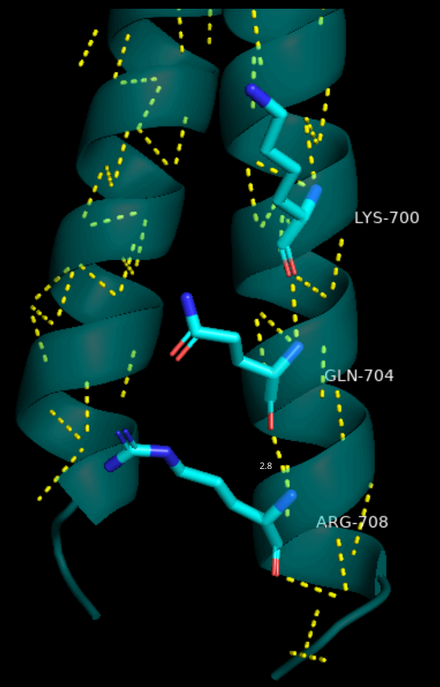
CLASS	All α proteins
FOLD	Long α-hairpin
SUPERFAMILY	Eukaryotic DNA topoisomerase I, dispensable insert domain
FAMILY	

LINKER DOMAIN

Antiparallel α-helical hairpin



Hydrogen Bonds

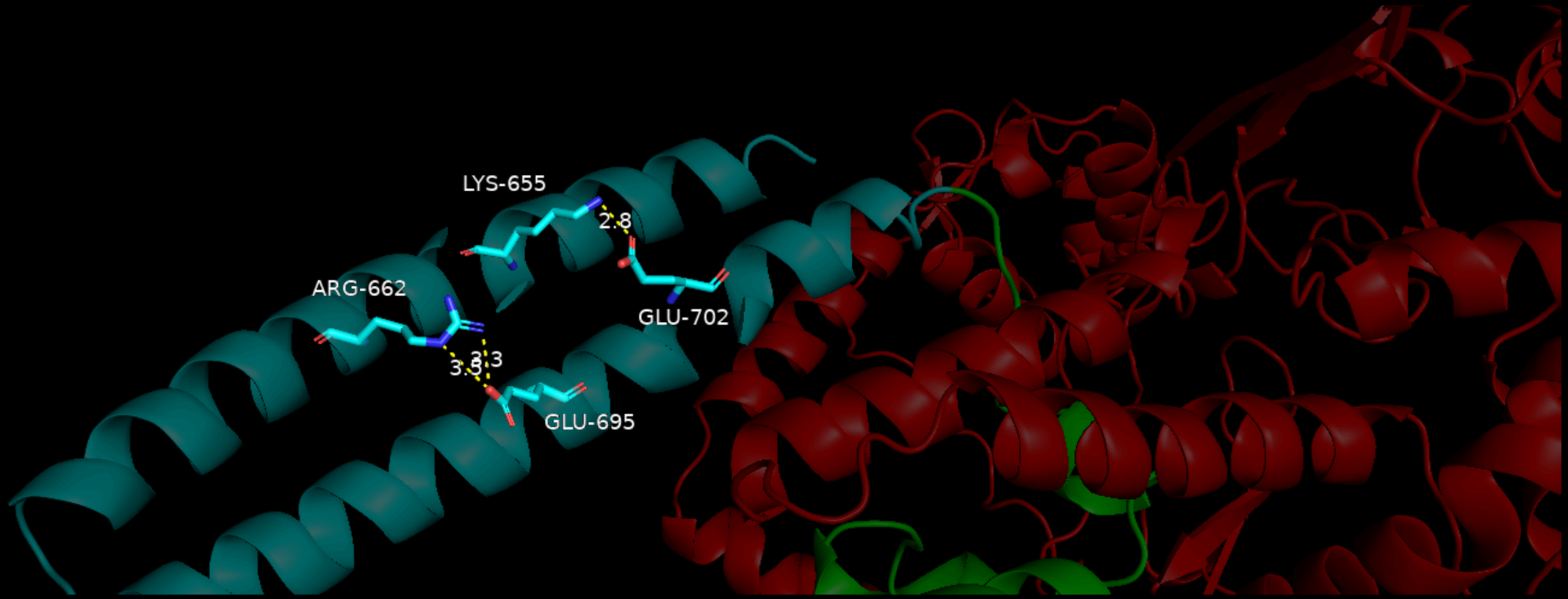


PDB ID: 1A36 | Res: 2.80 Å

N-terminal | Core | Linker | C-terminal

LINKER DOMAIN

Salt-bridges

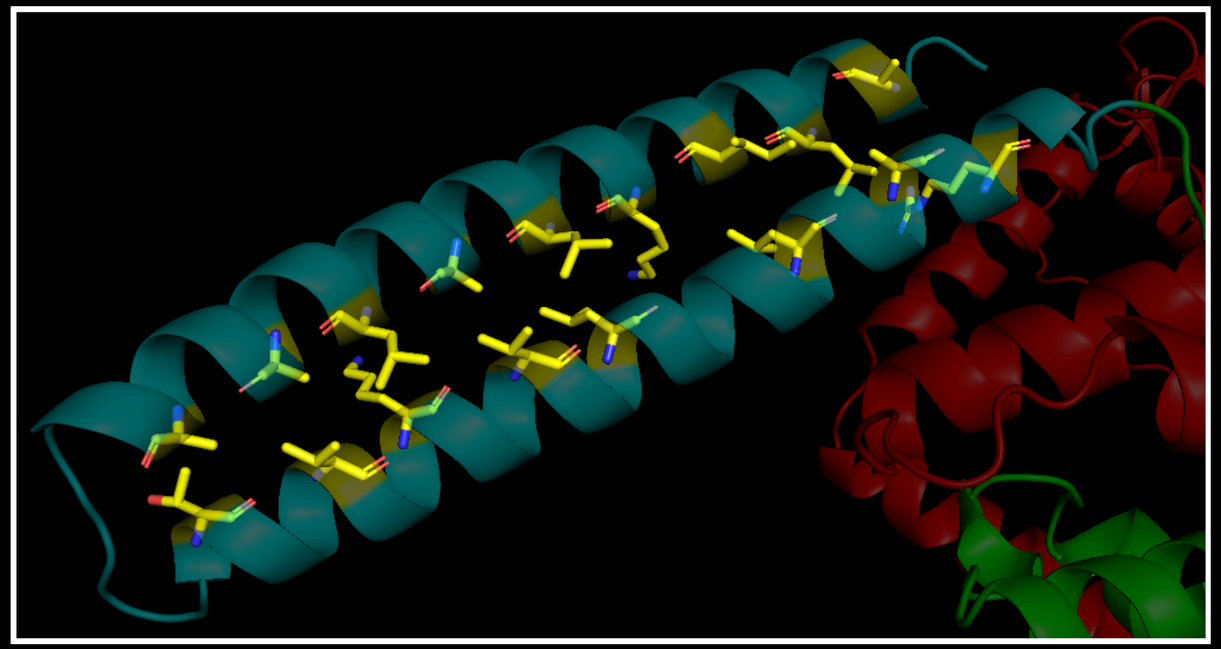


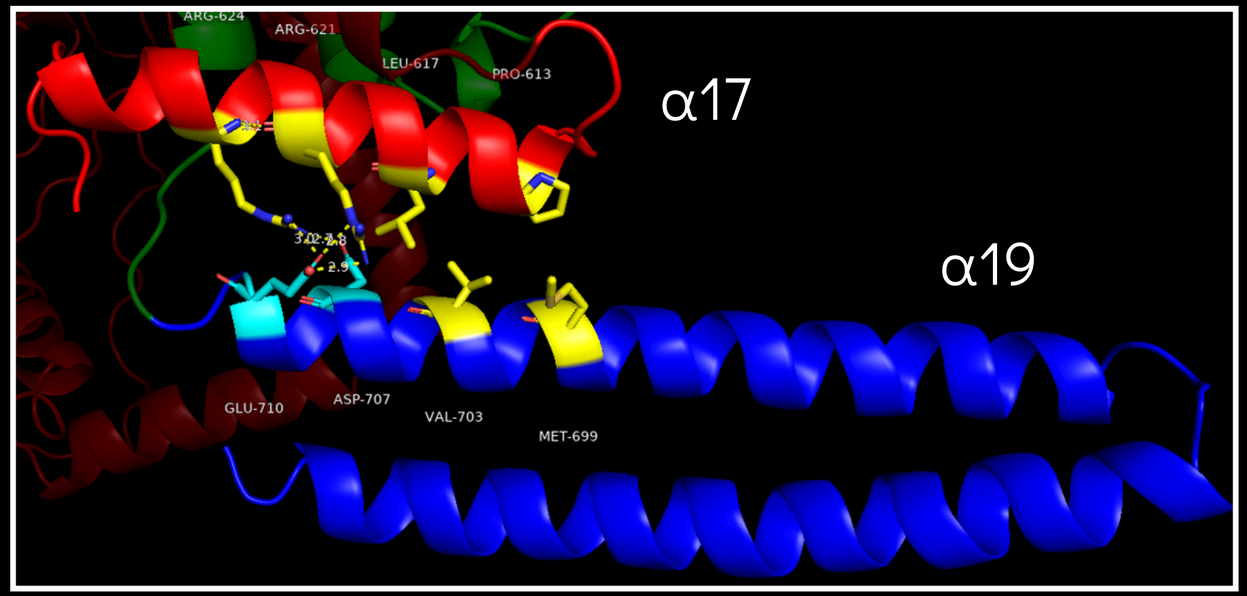
PDB ID: 1A36 | Res: 2.80 Å

N-terminal | Core | Linker | C-terminal

LINKER DOMAIN

Hydrophobic interactions



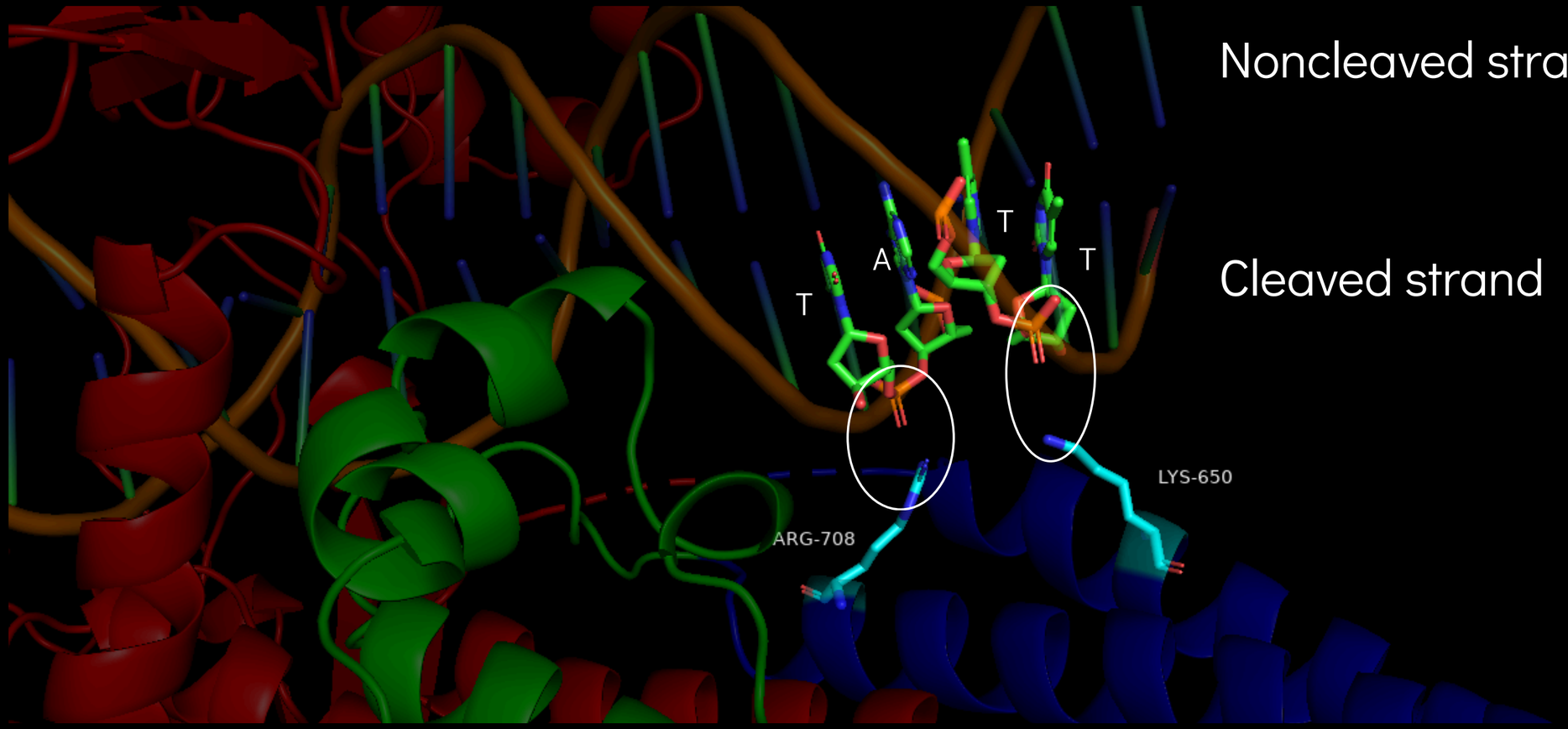


PDB ID: 1A36 | Res: 2.80 Å

N-terminal | Core | Linker | C-terminal

DNA INTERACTION

Protein-DNA phosphate interactions



Noncleaved strand

Cleaved strand

PDB ID: 1A36 | Res: 2.80 Å

DNA INTERACTION

Protein-DNA phosphate interactions



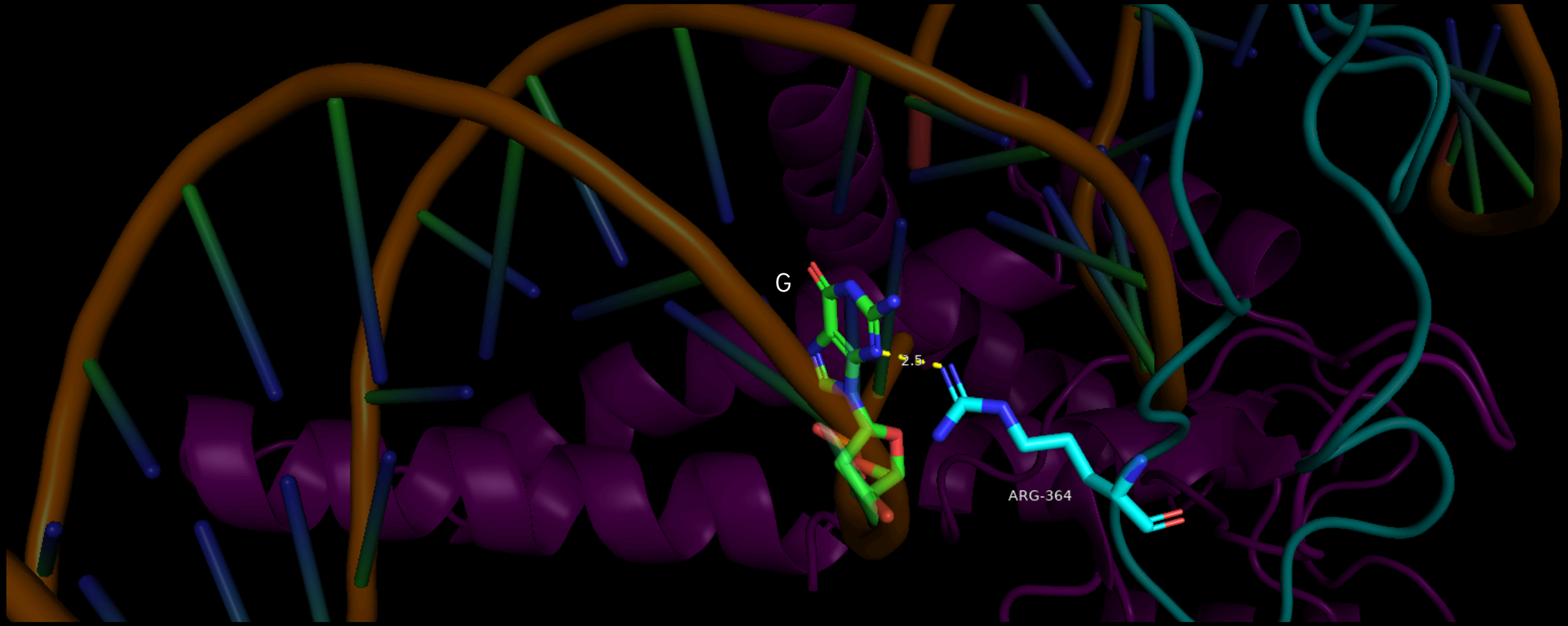
"Nose-cone"

PDB ID: 1A36 | Res: 2.80 Å

N-terminal | Core | Linker | C-terminal

DNA INTERACTION

Protein-DNA base interactions

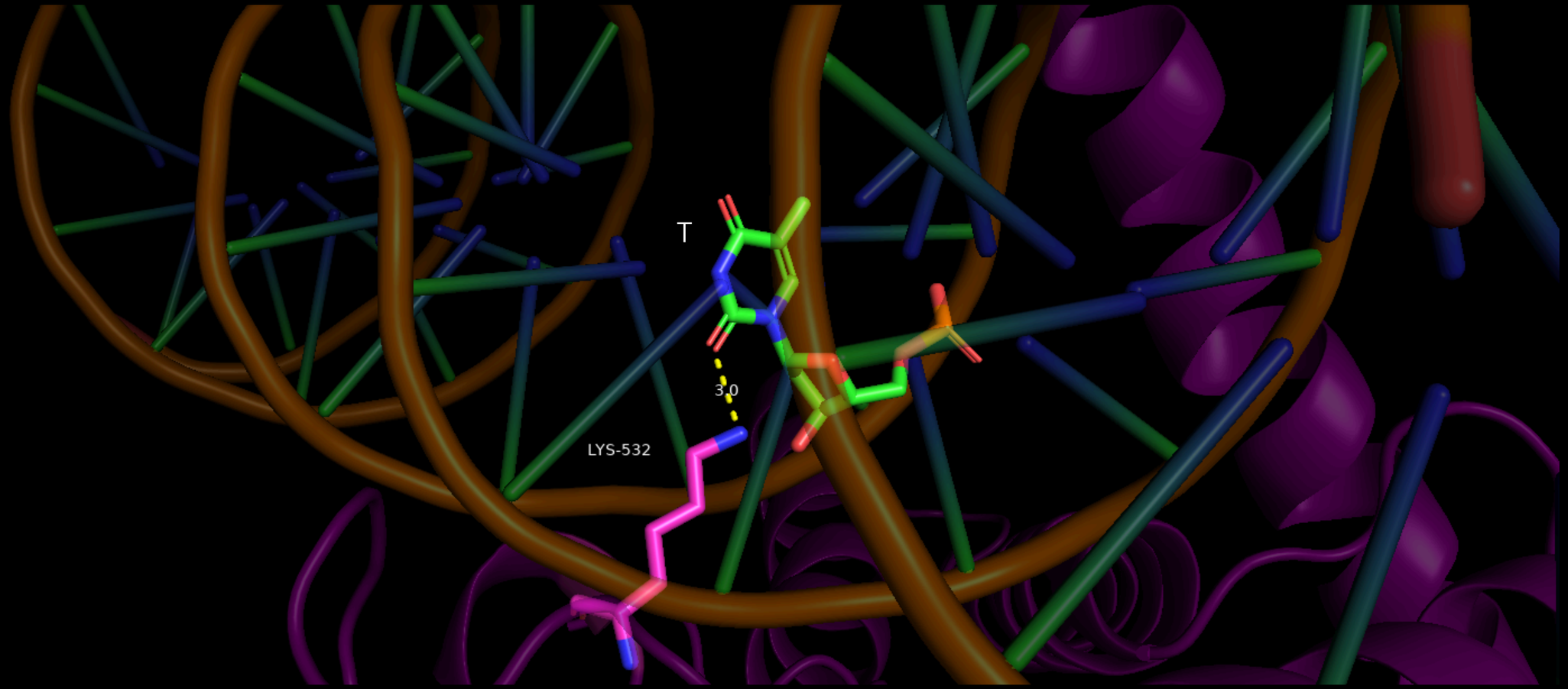


PDB ID: 1A36 | Res: 2.80 Å

N-terminal | Core | Linker | C-terminal

DNA INTERACTION

Protein-DNA base interactions



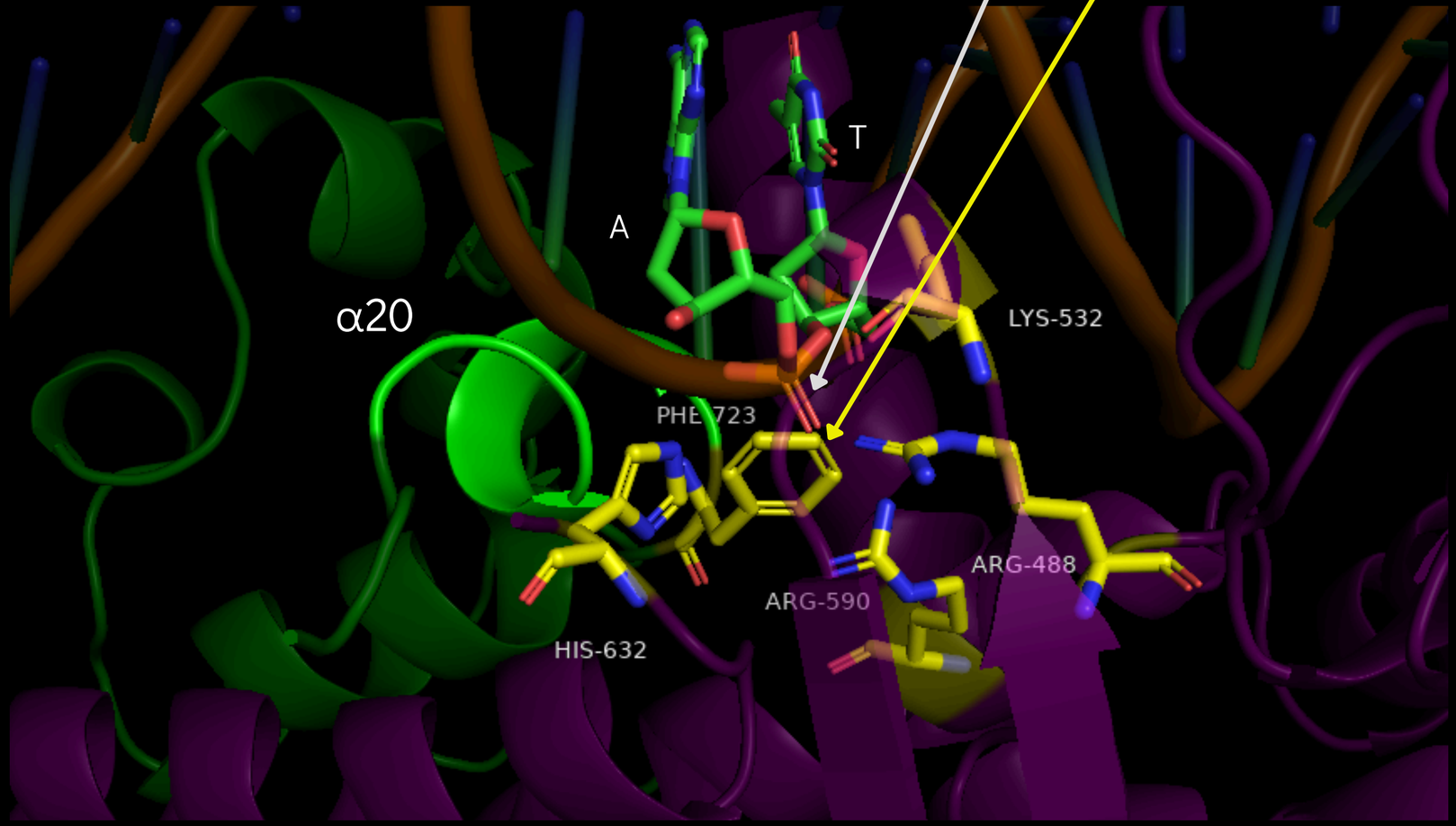
PDB ID: 1A36 | Res: 2.80 Å

N-terminal | Core | Linker | C-terminal

ACTIVE SITE

Catalytic pentad

* Y723F

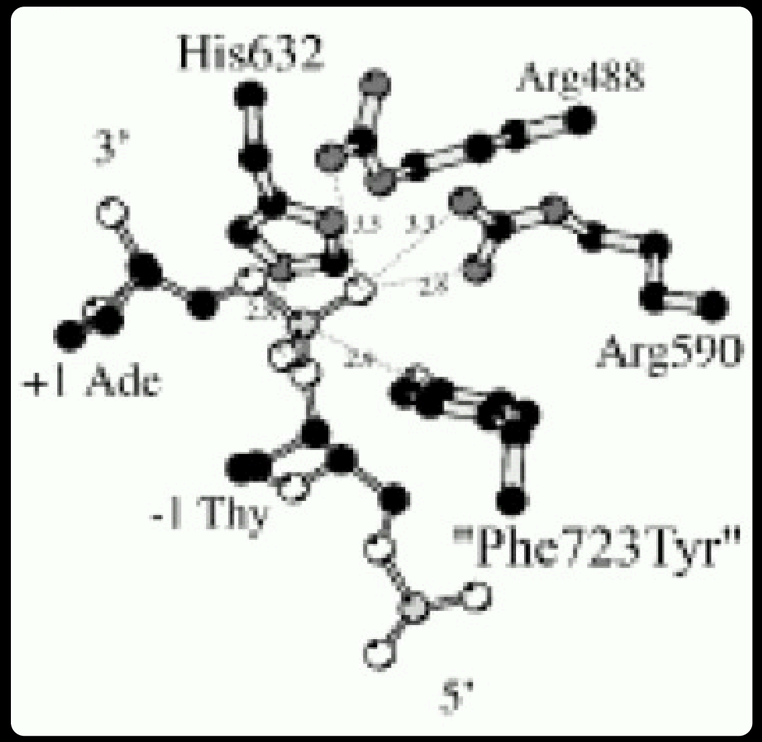


PDB ID: 1A36 | Res: 2.80 Å

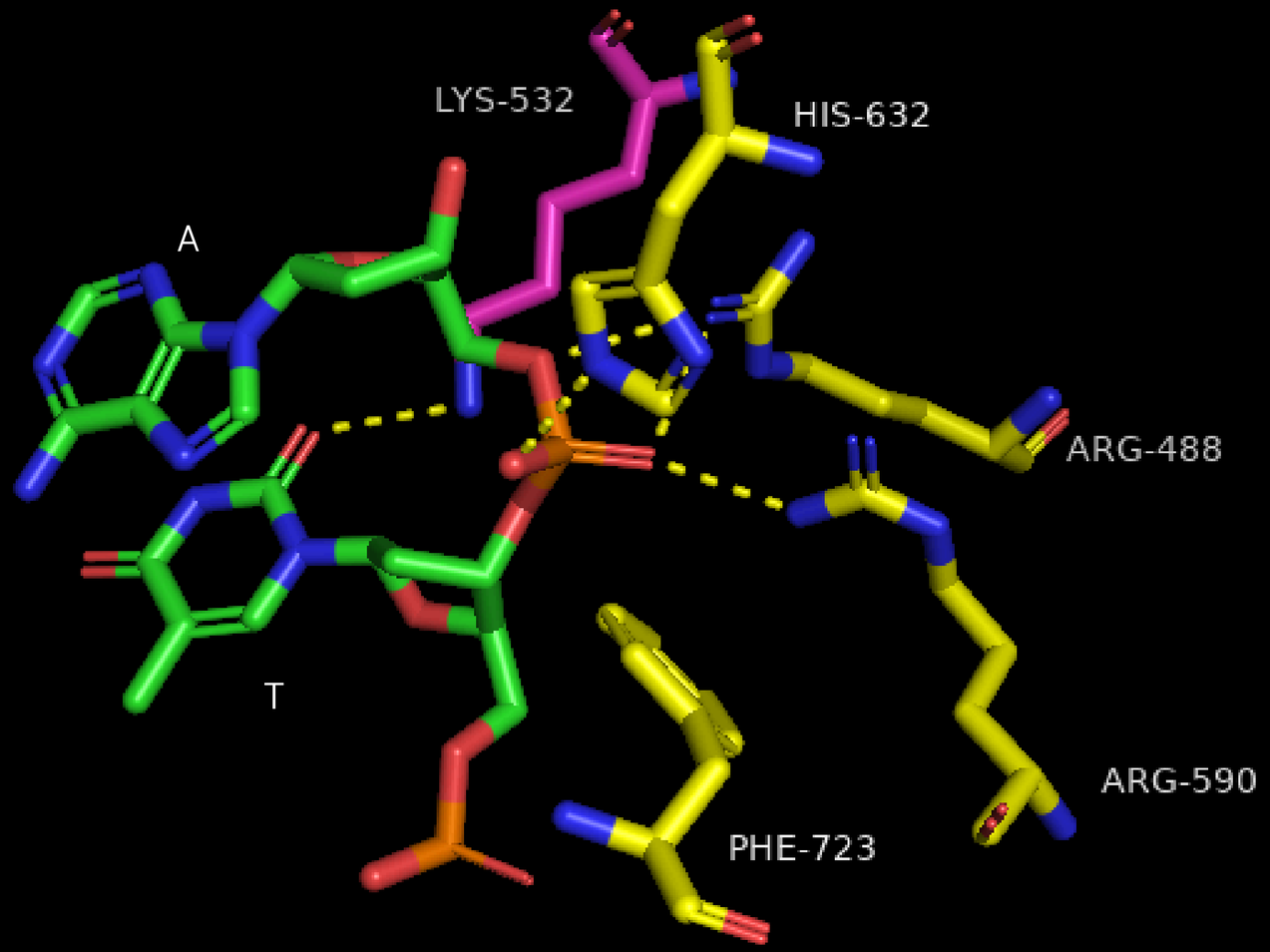
N-terminal | Core | Linker | C-terminal

ACTIVE SITE

Catalytic pentad



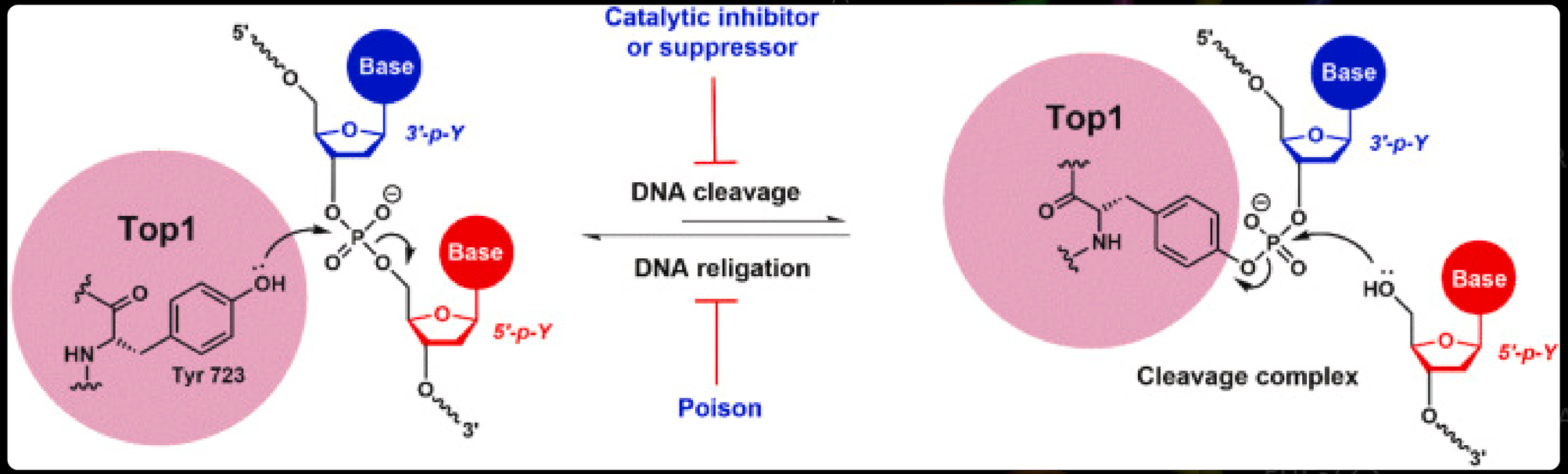
Lance Stewart et al., 1998



PDB ID: 1A36 | Res: 2.80 Å

ACTIVE SITE

Catalytic pentad



Talukdar, A. et al., 2022

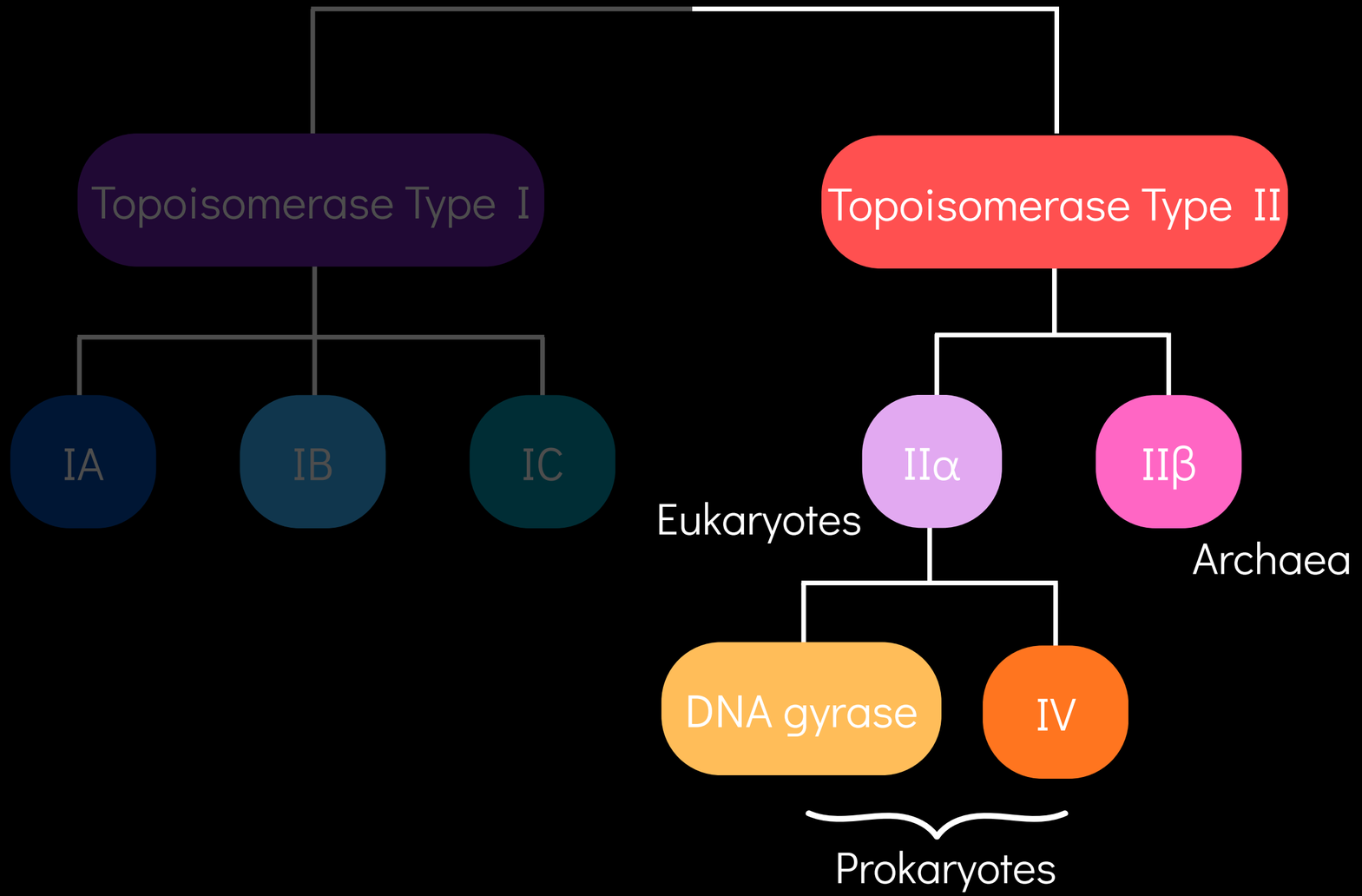
PDB ID: 1A36 | Res: 2.80 Å

N-terminal | Core | Linker | C-terminal

04 TOPOISOMERASE II

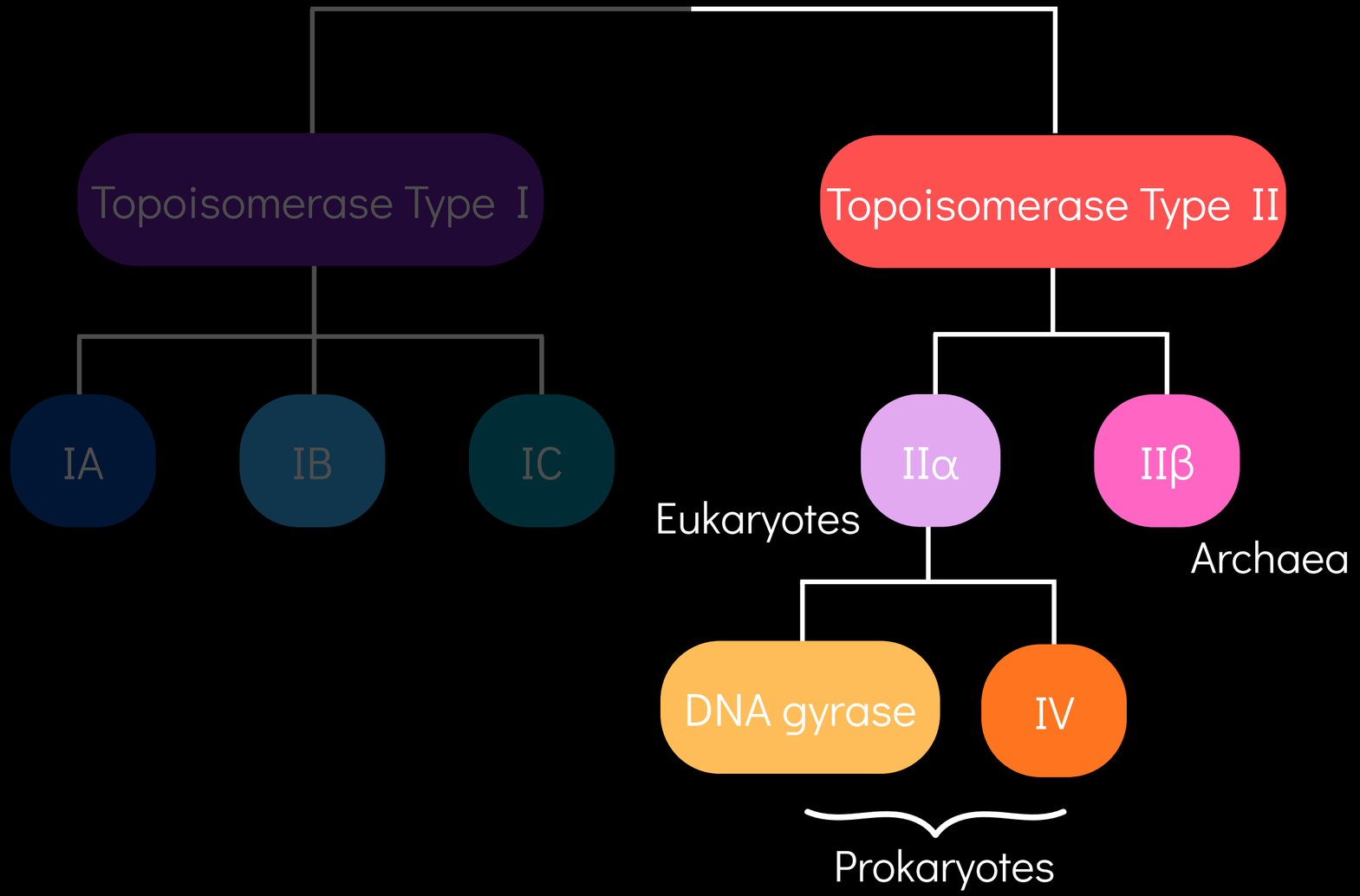
TOPOISOMERASES II

Classification



TOPOISOMERASES II

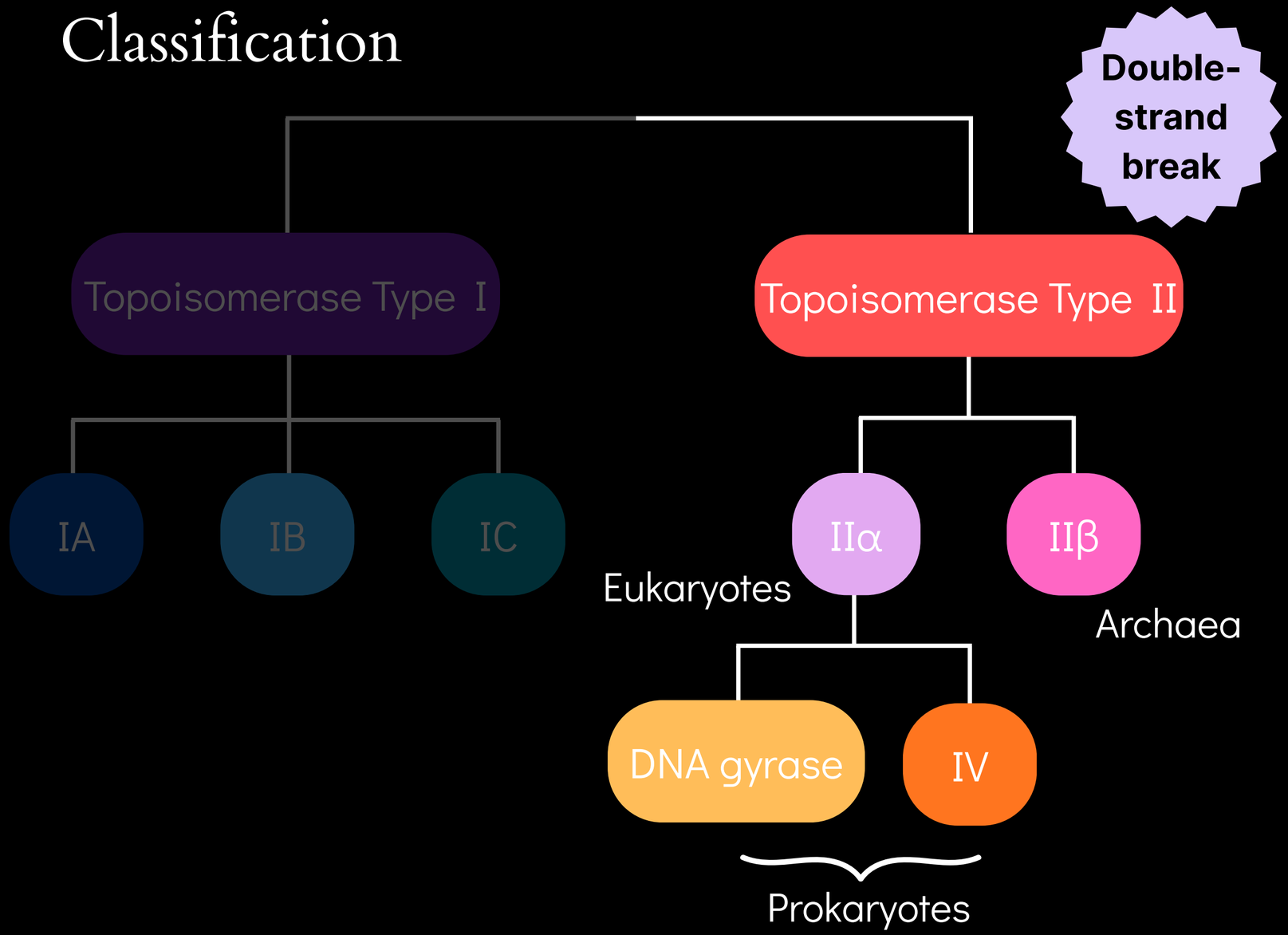
Classification



- Bacteria topoisomerases domains:
  - GyrA / ParE
  - GyrB / ParC
- Eukaryotes topoisomerases domains:
  - Carboxy-terminal domains.
  - Amino-terminal domains.

TOPOISOMERASES II

Classification



- Bacteria topoisomerases domains:
  - GyrA / ParE
  - GyrB / ParC
- Eukaryotes topoisomerases domains:
  - Carboxy-terminal domains.
  - Amino-terminal domains.

- Heart-shaped dimeric protein with a large central hole.
- Regulate topology.
- ATP-dependent.

4.1 TOPOISOMERASE IIα

TOPOISOMERASE IIα

- Large homodimeric enzyme.
- Highly expressed during mitosis.
- Allosteric connection between ATPase and DNA binding/cleavage domains.

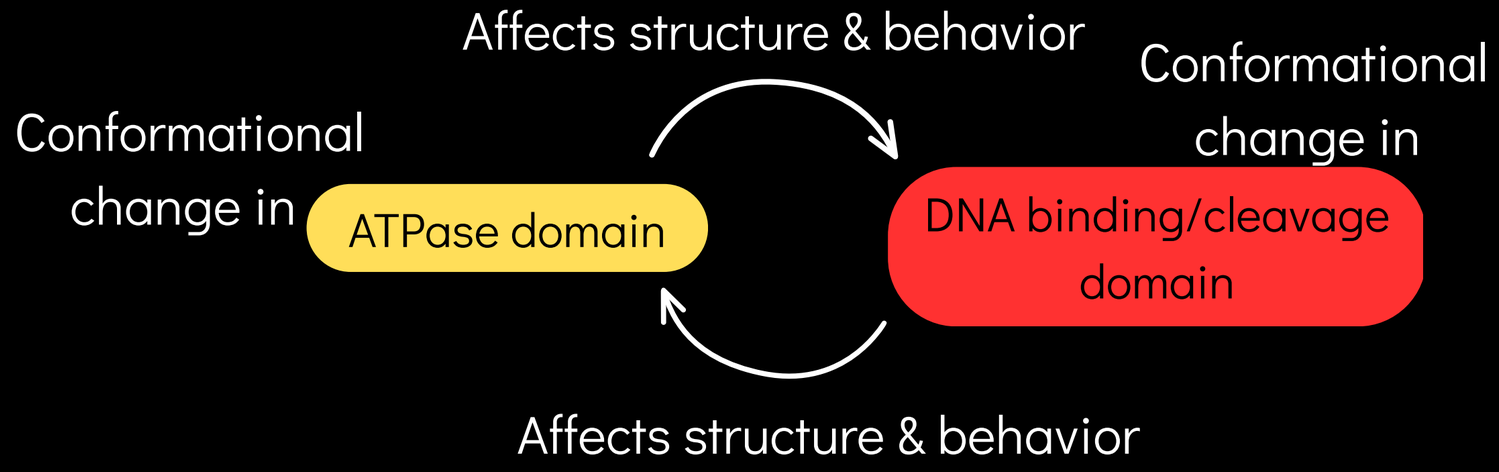
No SCOP neither CATH classification



PDB ID: 6ZY7 | Res: 4.64 Å

TOPOISOMERASE IIα

- Allosteric connection between ATPase and DNA binding/cleavage domains.



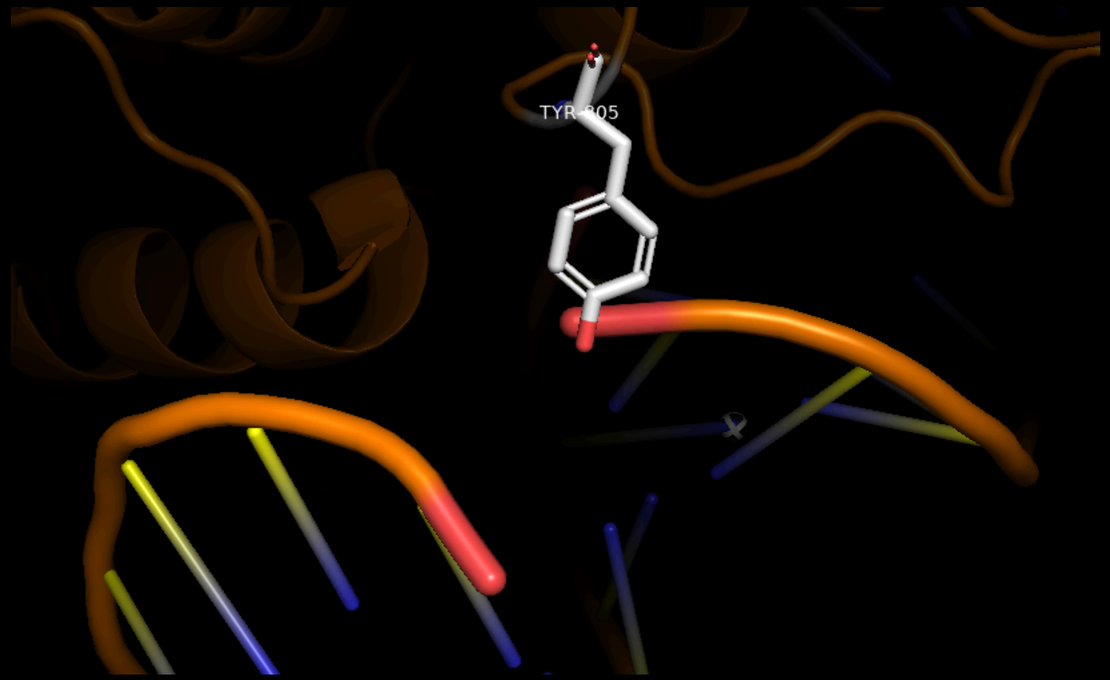


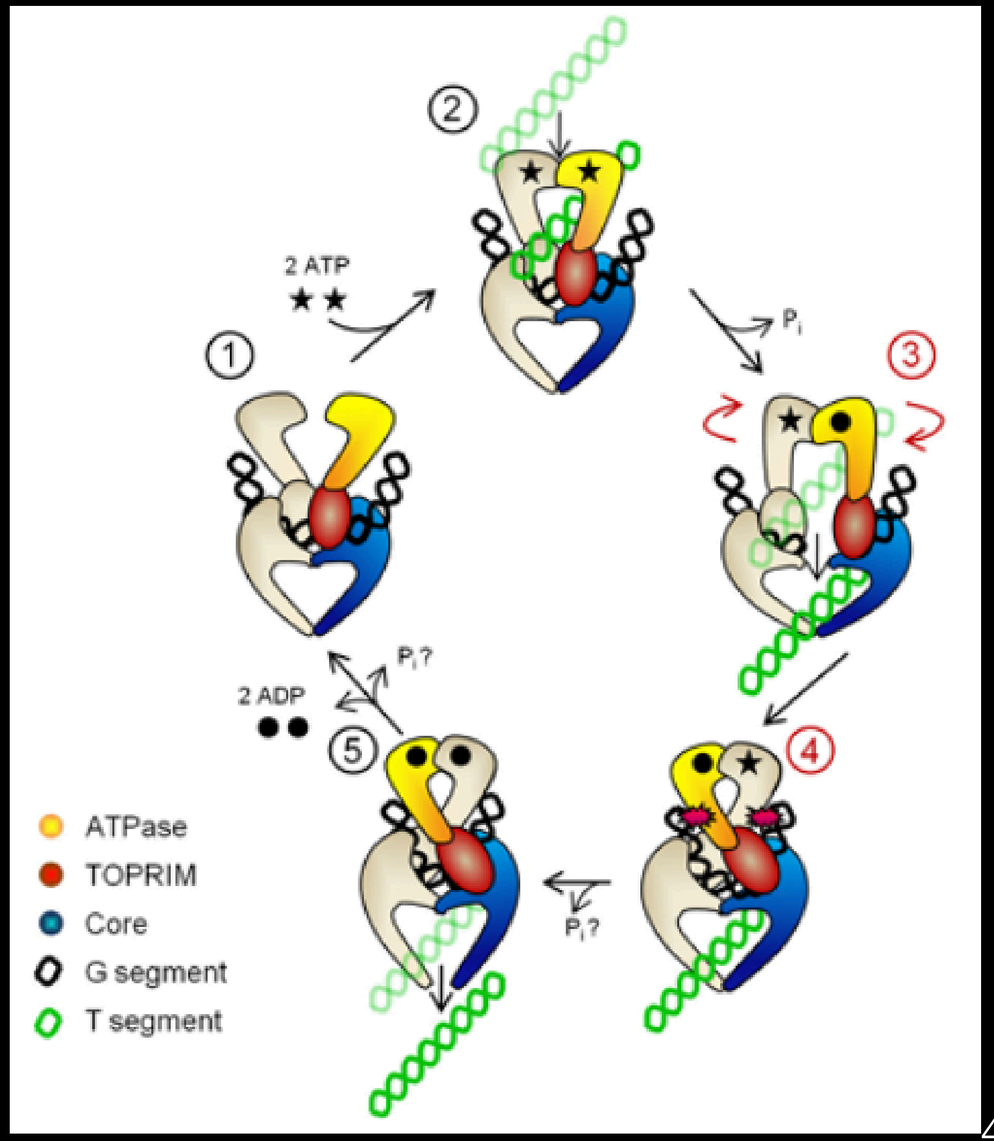
PDB ID: 6ZY7 | Res: 4.64 Å

TOPOISOMERASES IIα

Mechanism of action

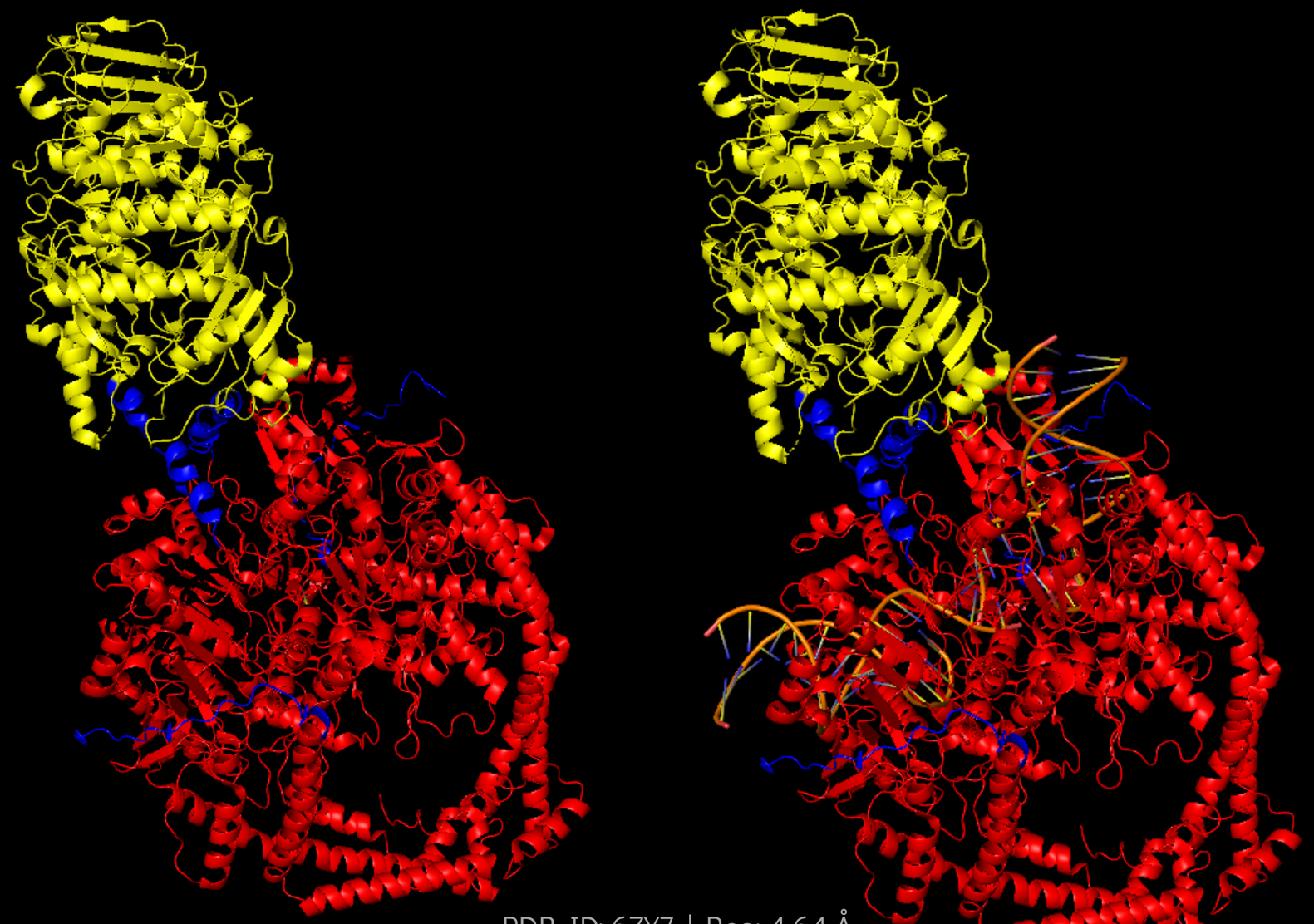
Tyr805





Schmidt, B. H., et al. (2012).

DOMAINS



PDB ID: 6ZY7 | Res: 4.64 Å

ATPase domain

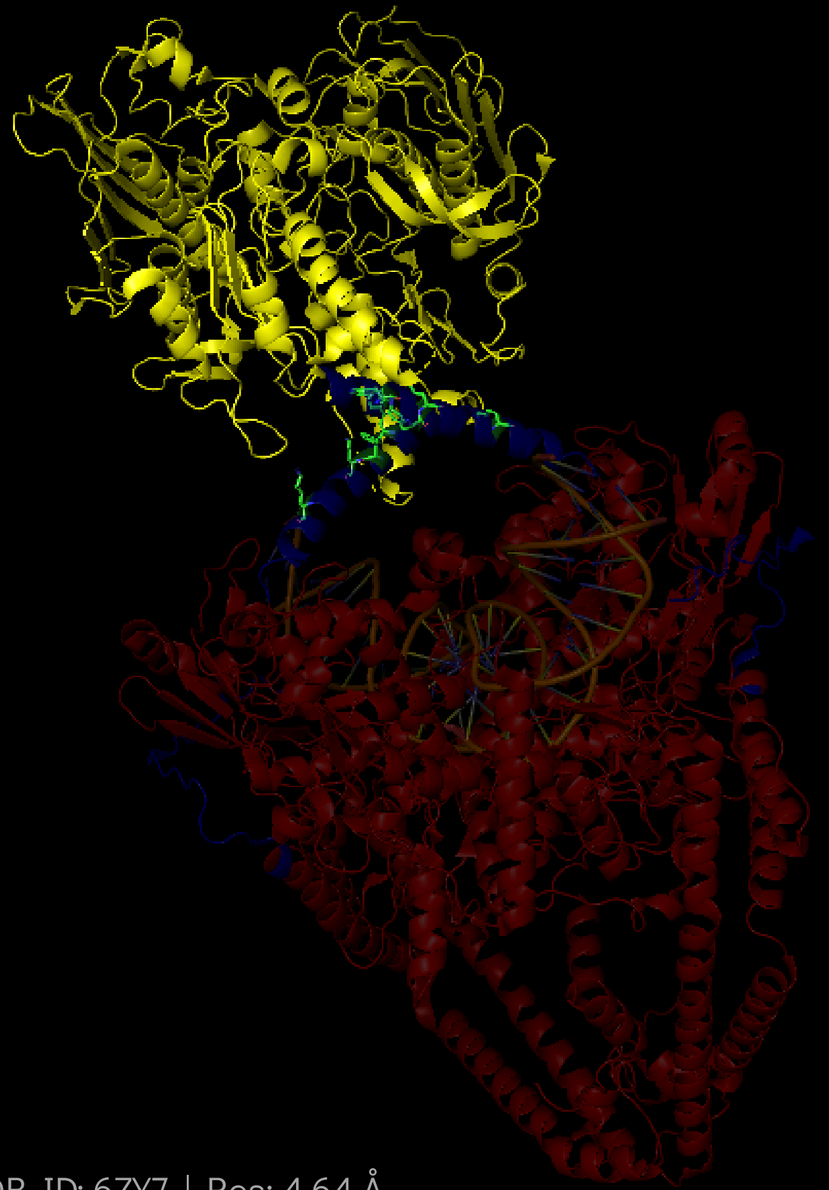
DNA binding/cleavage domain

CTD

DOMAINS

ATPase domain or N-gate

- Residues 1-407
- Binds and hydrolazes ATP → closes the N-gate.
- Not required for G-segment cleavage.
- Stimulated by DNA binding.



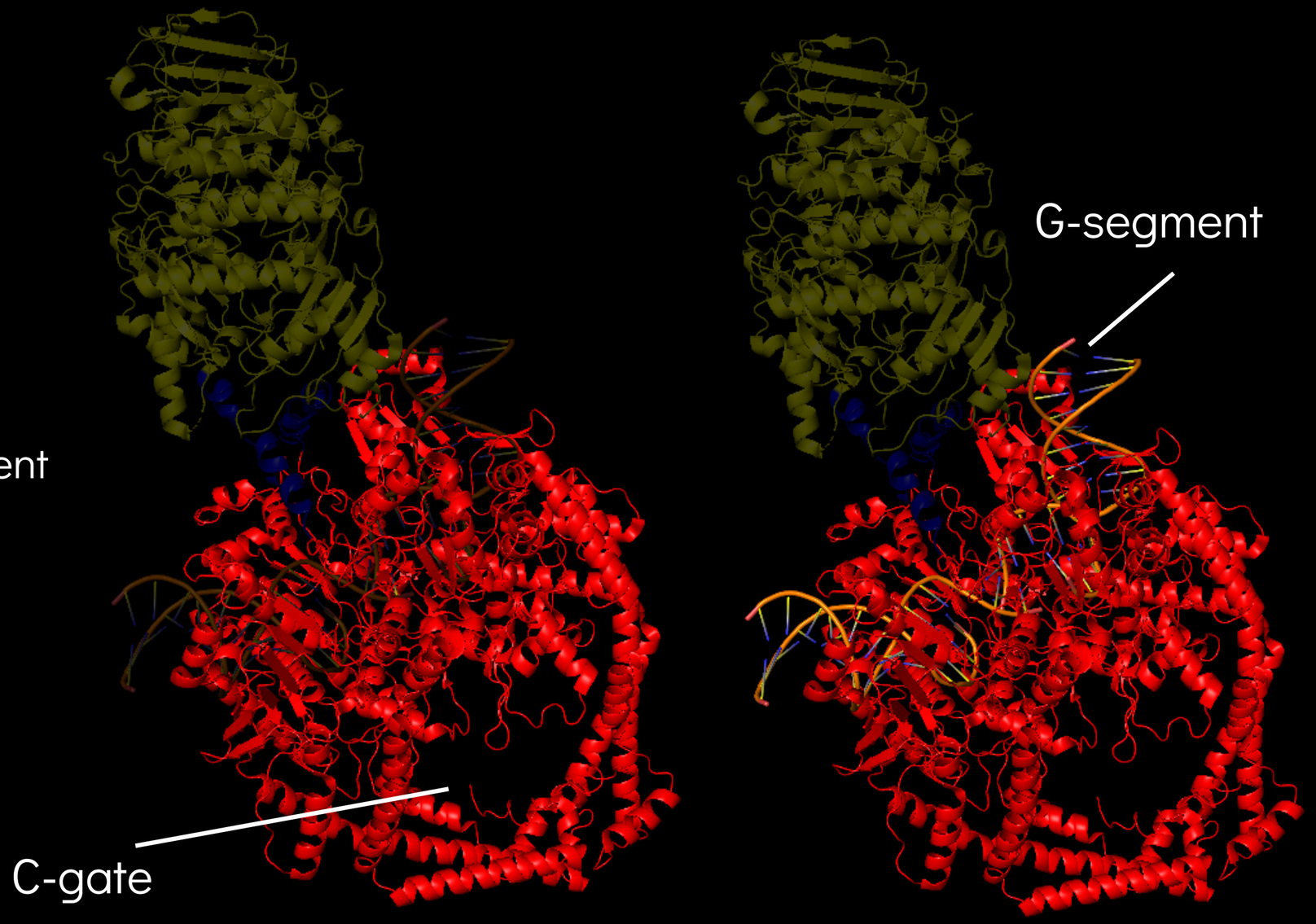
PDB ID: 6ZY7 | Res: 4.64 Å



DOMAINS

DNA-binding/cleavage domain

- Residues 434-1191
- It forms two gates:
  - DNA-gate: where G-segment cleavage occurs.
  - C-gate: exit gate.



PDB ID: 6ZY7 | Res: 4.64 Å



DNA-binding/cleavage subdomains

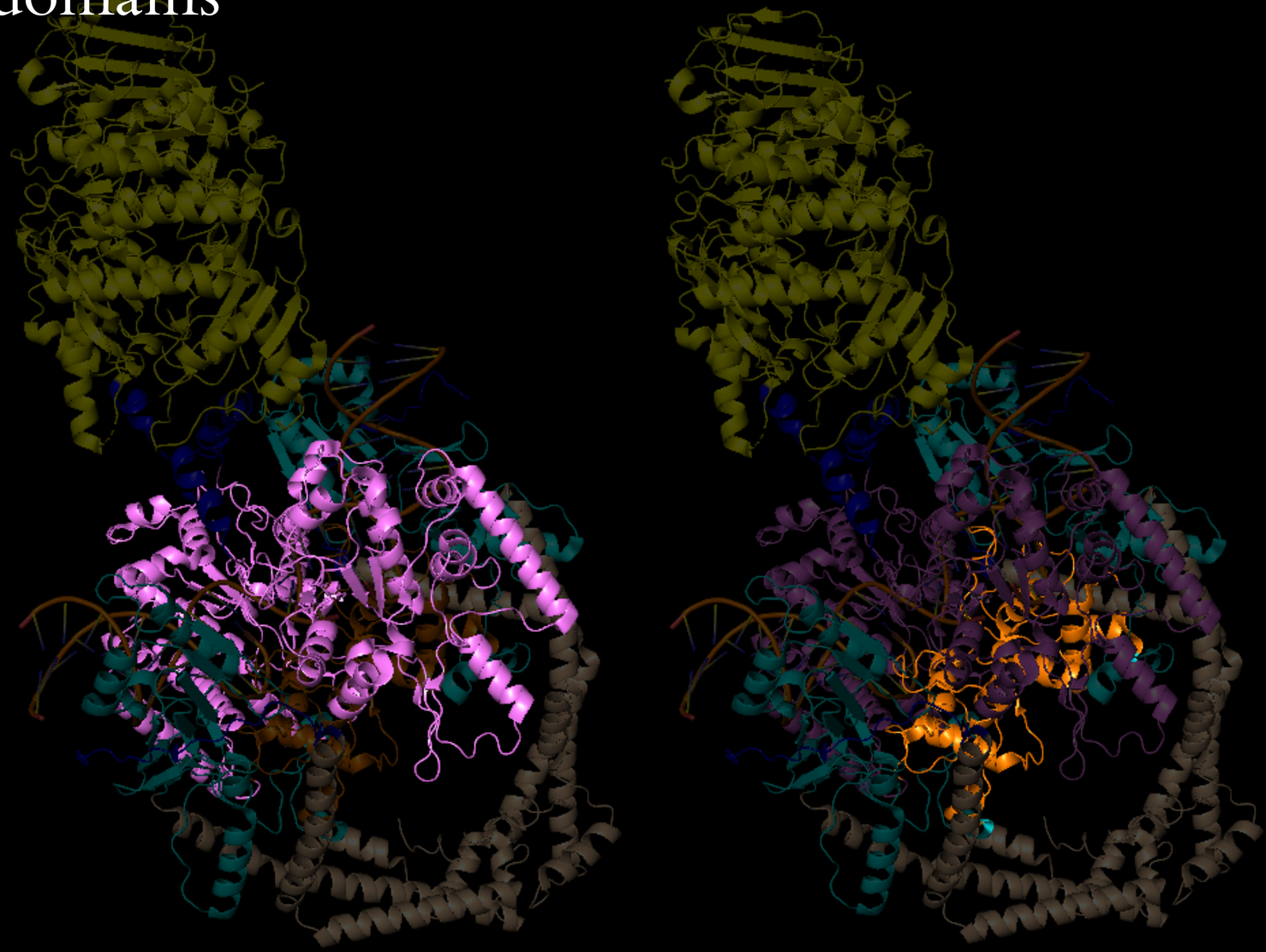
PDB ID: 6ZY7 | Res: 4.64 Å

TOPRIM residues 434-721

WHD residues 721-820

Tower residues 820-1013

Coiled Coil residues 1013-1191

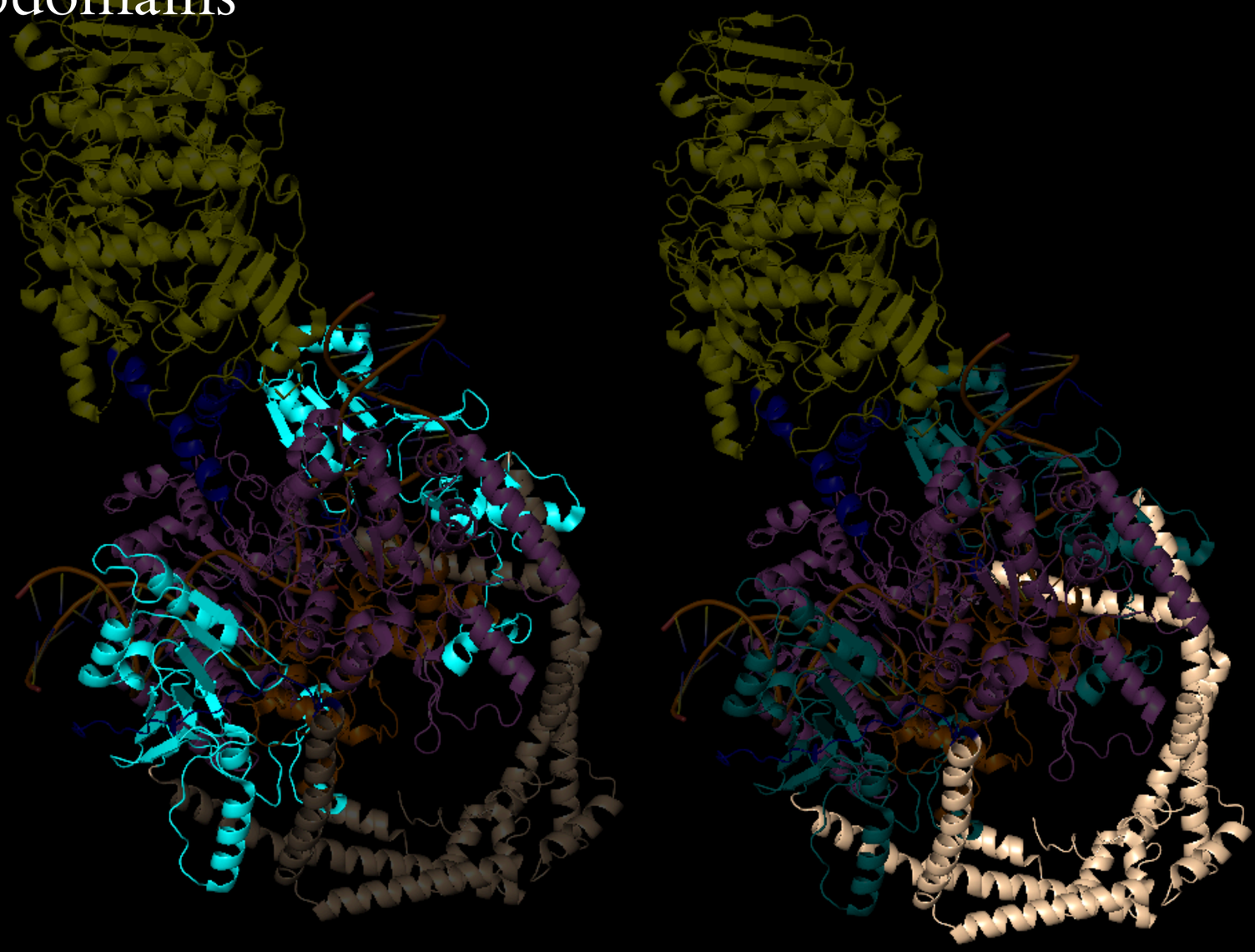


ATPase domain | DNA binding/cleavage domain | CTD

DNA-binding/cleavage subdomains

PDB ID: 6ZY7 | Res: 4.64 Å

- TOPRIM — residues 434-721
- WHD — residues 721-820
- Tower — residues 820-1013
- Coiled Coil — residues 1013-1191

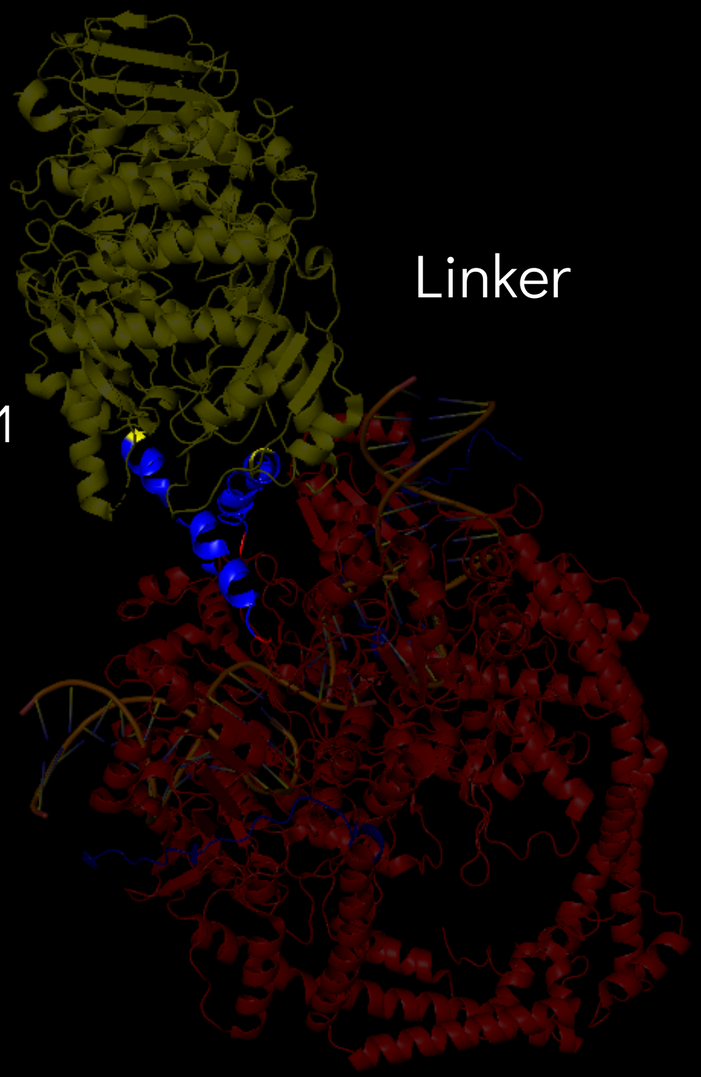


ATPase domain | DNA binding/cleavage domain | CTD

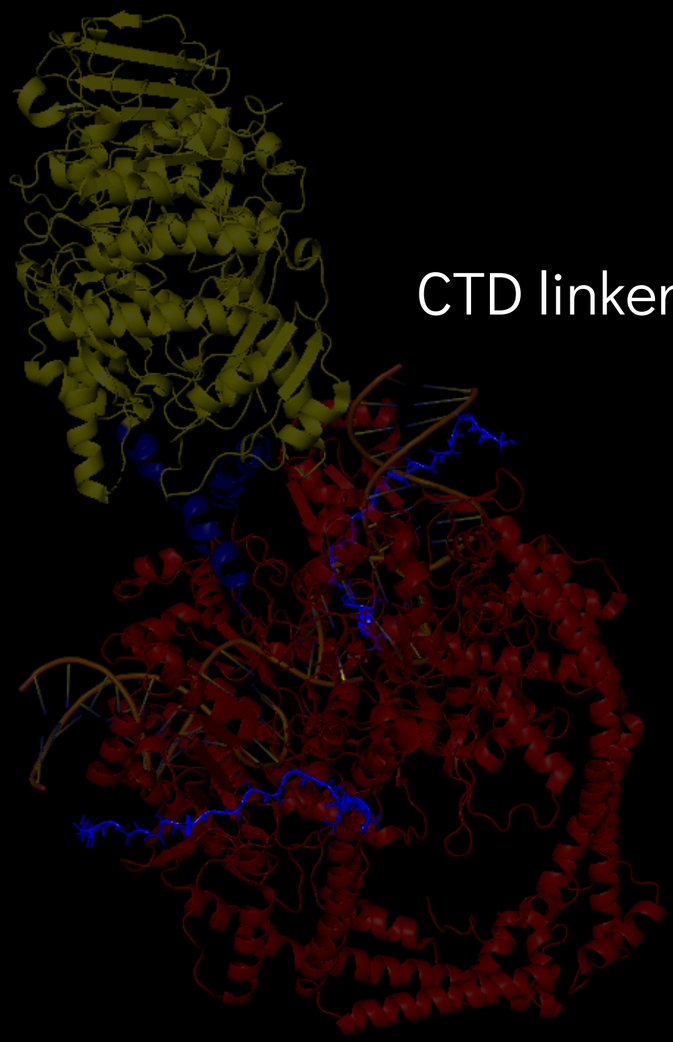
DOMAINS

C-terminal & linker

- Linker: residues 407-434
- CTD linker: residues 1191-1217
- *CTD (predicted unfolded): 1217-1531



Linker



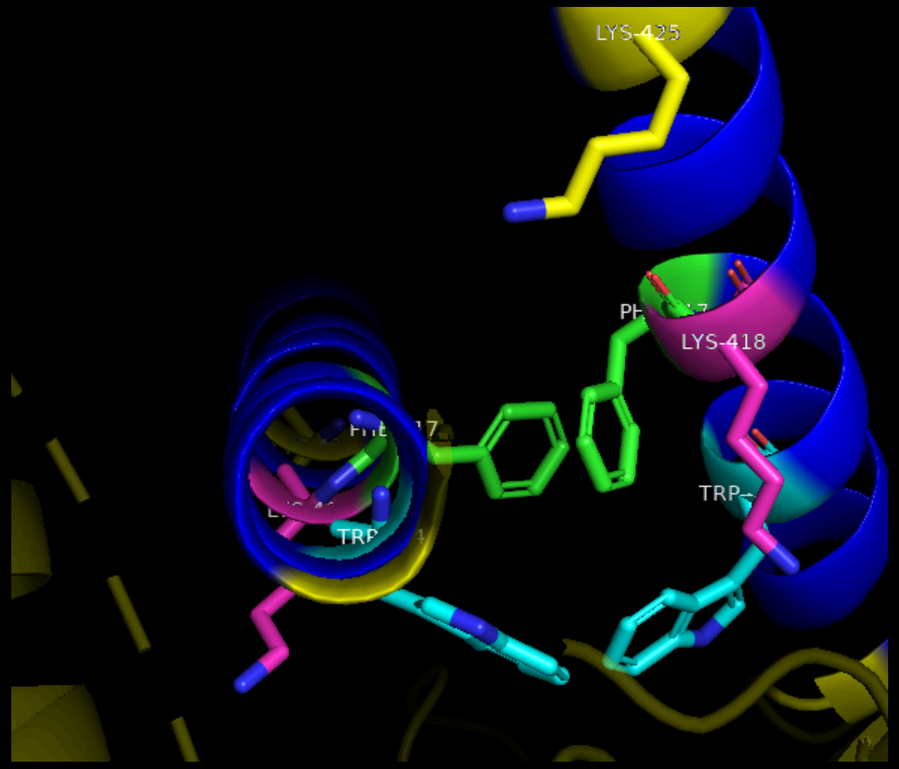
CTD linker

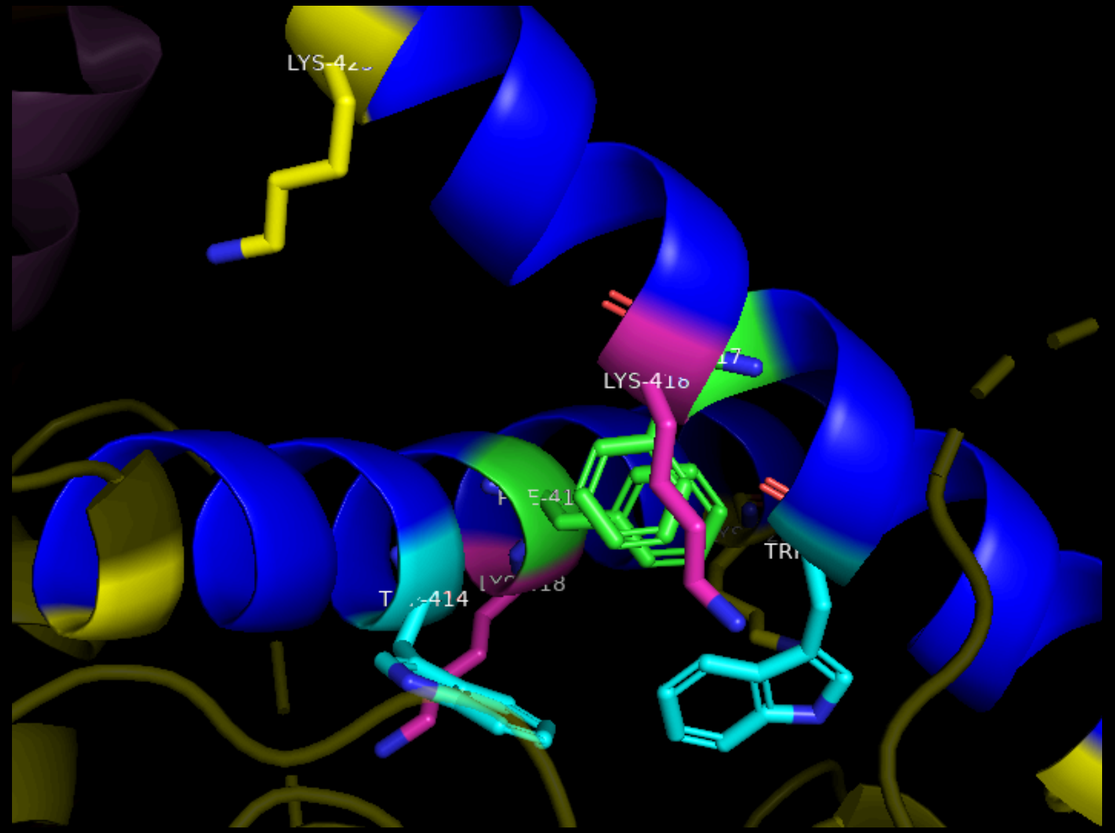
PDB ID: 6ZY7 | Res: 4.64 Å



Highly conserved residues of the linker domain in superior eukaryotes

- Hydrophobic patch: F417 and W414
- Close to the K-loop (resi 342-344): K418
- Towards the entrance of TOPRIM: K425



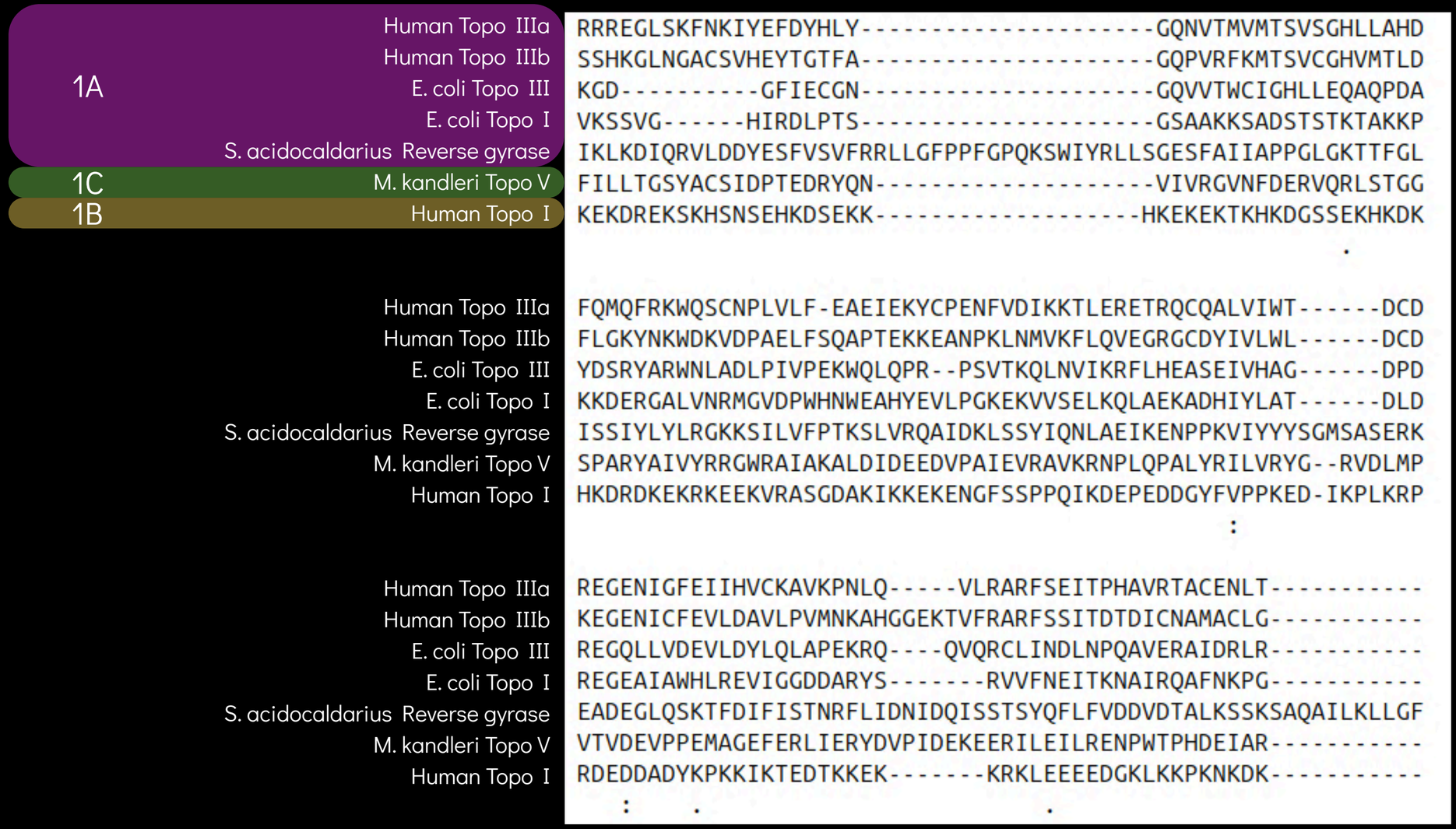


PDB ID: 6ZY7 | Res: 4.64 Å

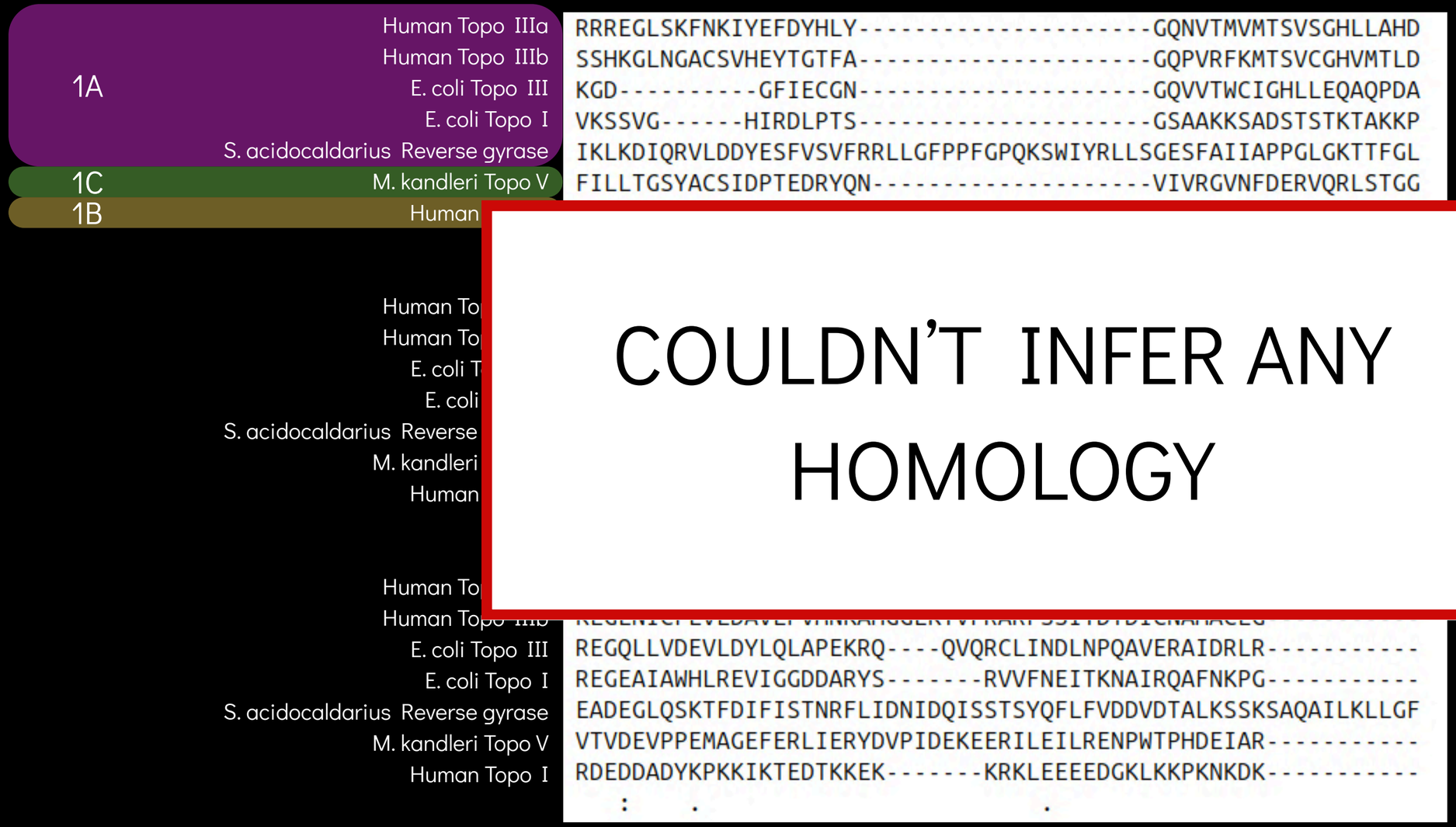
ATPase domain | DNA binding/cleavage domain | CTD

05 COMPARISONS

MSA TOPOISOMERASE I



MSA TOPOISOMERASE I

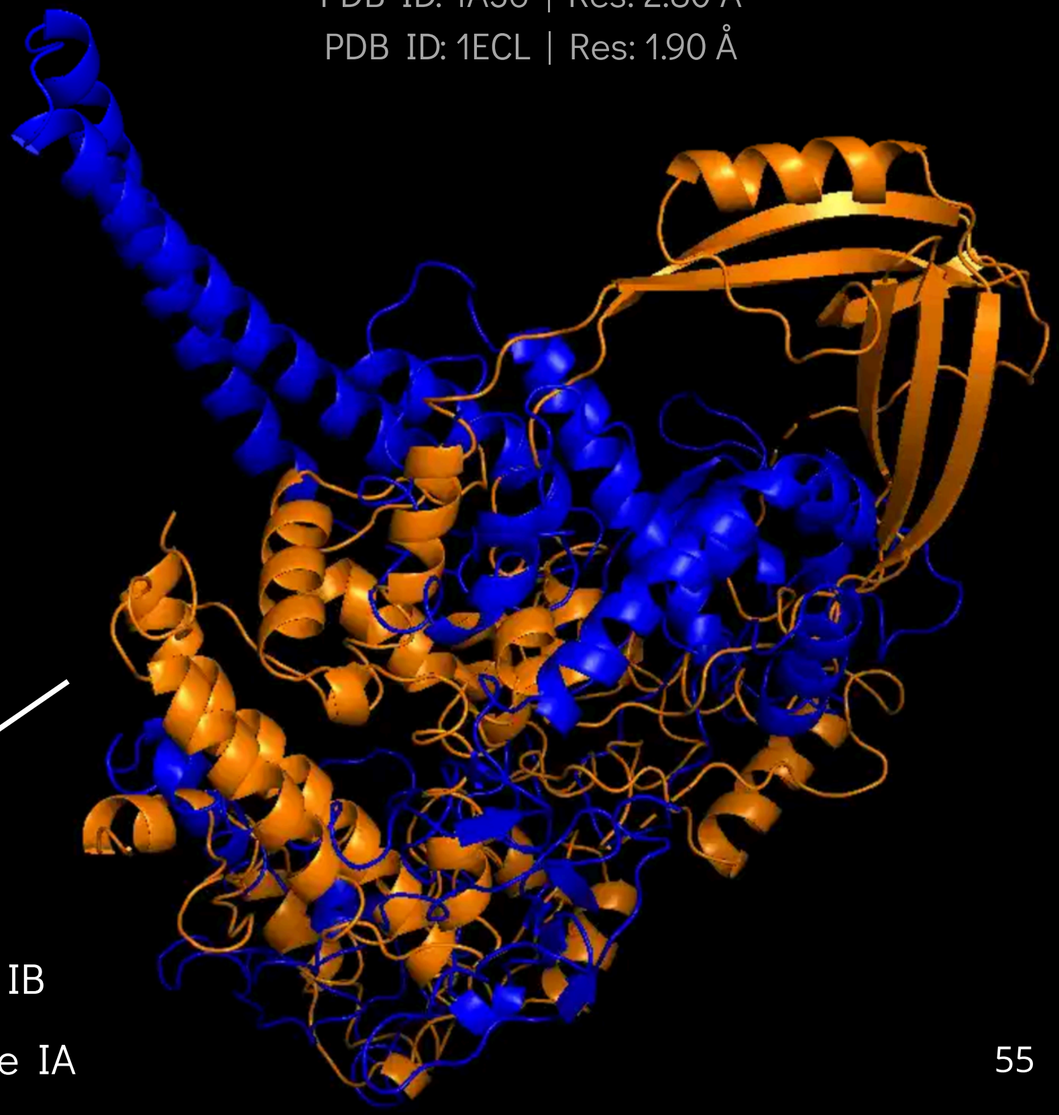


STAMP – EUKARYOTE VS PROKARYOTE

```
 2 STAMP Structural Alignment of Multiple Proteins
 3
 4 Version 4.4 (May 2010)
 5  by Robert B. Russell & Geoffrey J. Barton
 6  Please cite PROTEINS, v14, 309-323, 1992
 7
 8 Running roughfit.
 9
10     Sc = STAMP score, RMS = RMS deviation, Align = alignment length
11     Len1, Len2 = length of domain, Nfit = residues fitted
12     Secs = no. equivalent sec. strucs. Eq = no. equivalent residues
13     %I = seq. identity, %S = sec. str. identity
14     P(m)  = P value (p=1/10) calculated after Murzin (1993), JMB, 230, 689-694
15             (NC = P value not calculated - potential FP overflow)
16
17      No.  Domain1         Domain2         Sc     RMS    Len1 Len2  Align NFit Eq. Secs.   %I   %S   P(m)
18 Pair   1  TOP1_1A36_A     TOP1_1ECL_A     0.18  15.27    544  552   754  306  93    0   2.15 100.00 1.00e+00  LOW
   SCORE
19 Reading in matrix file IBvsIA.mat...
20 Doing cluster analysis...
21 Cluster:  1 (TOP1_1A36_A  & TOP1_1ECL_A ) Sc  0.33 RMS   2.48 Len 931 nfit  24  LOW SCORE
22  See file IBvsIA.1 for the alignment and transformations
```

PDB ID: 1A36 | Res: 2.80 Å

PDB ID: 1ECL | Res: 1.90 Å



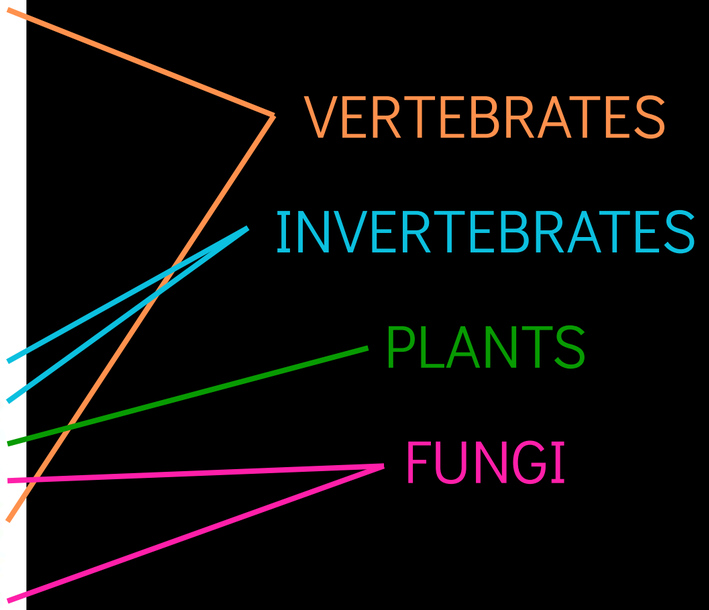
Belong to distinct evolutionary and structural families

Human topoisomerase IB

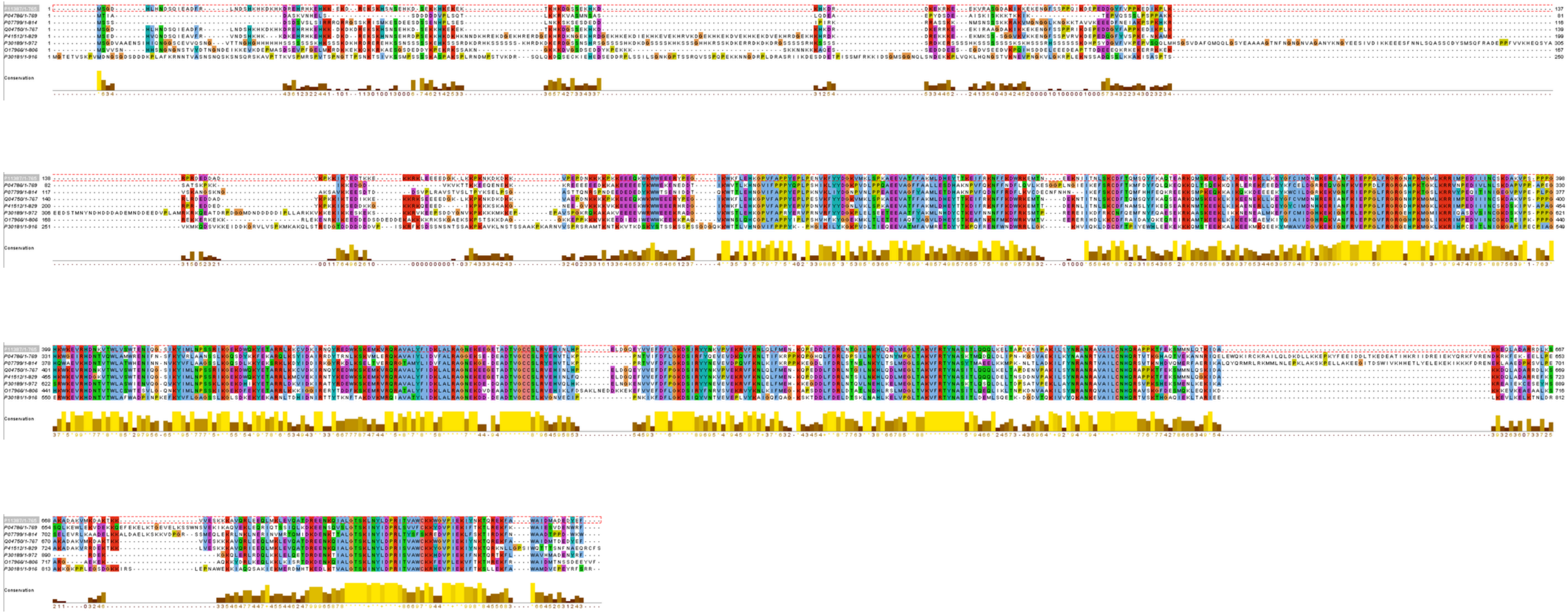
Bacterial topoisomerase IA

MSA TOPOISOMERASE I

```
30533 Results from round 5
30534
30535
30536 Query= P11387
30537
30538 Length=765
30539                                                                  Score     E
30540 Sequences producing significant alignments:                      (Bits)  Value
30541 Sequences used in model and found again:
30542
30543 sp|Q8R4U6|TOP1M_MOUSE DNA topoisomerase I, mitochondrial OS=Mus m...  639     0.0
30544 sp|Q6IM78|TOP1M_RAT DNA topoisomerase I, mitochondrial OS=Rattus ...  636     0.0
30545 sp|A9Q1D5|TOP1M_PANTR DNA topoisomerase I, mitochondrial OS=Pan t...  628     0.0
30546 sp|Q969P6|TOP1M_HUMAN DNA topoisomerase I, mitochondrial OS=Homo ...  626     0.0
30547 sp|Q7YR26|TOP1_CHLAE DNA topoisomerase 1 OS=Chlorocebus aethiops ...  609     0.0
30548 sp|P11387|TOP1_HUMAN DNA topoisomerase 1 OS=Homo sapiens GN=TOP1 ...  606     0.0
30549 sp|Q9WUL0|TOP1_RAT DNA topoisomerase 1 OS=Rattus norvegicus GN=To...  606     0.0
30550 sp|Q04750|TOP1_MOUSE DNA topoisomerase 1 OS=Mus musculus GN=Top1 ...  604     0.0
30551 sp|Q07050|TOP1_CRIGR DNA topoisomerase 1 OS=Cricetulus griseus GN...  601     0.0
30552 sp|P30189|TOP1_DROME DNA topoisomerase 1 OS=Drosophila melanogast...  547     0.0
30553 sp|O17966|TOP1_CAEEL DNA topoisomerase 1 OS=Caenorhabditis elegan...  525     7e-175
30554 sp|P30181|TOP1_ARATH DNA topoisomerase 1 OS=Arabidopsis thaliana ...  515     1e-169
30555 sp|P07799|TOP1_SCHPO DNA topoisomerase 1 OS=Schizosaccharomyces p...  495     2e-163
30556 sp|P41512|TOP1_XENLA DNA topoisomerase 1 OS=Xenopus laevis GN=top...  490     4e-161
30557 sp|Q54RC3|TOP1_DICDI DNA topoisomerase 1 OS=Dictyostelium discoid...  486     7e-159
30558 sp|P04786|TOP1_YEAST DNA topoisomerase 1 OS=Saccharomyces cerevis...  476     2e-156
```



MSA TOPOISOMERASE I



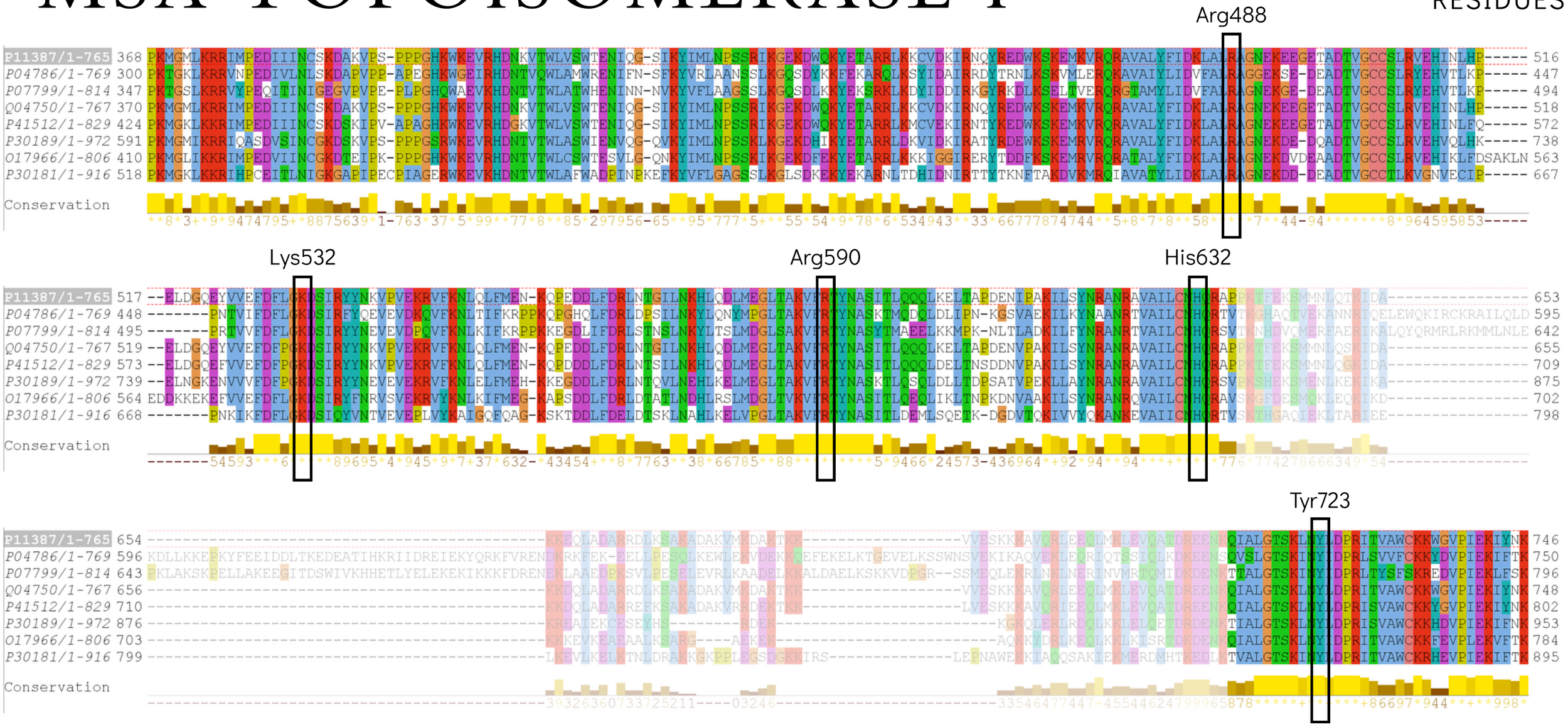
MSA TOPOISOMERASE I



N-terminal | Core | Linker | C-terminal

MSA TOPOISOMERASE I

ACTIVE SITE RESIDUES

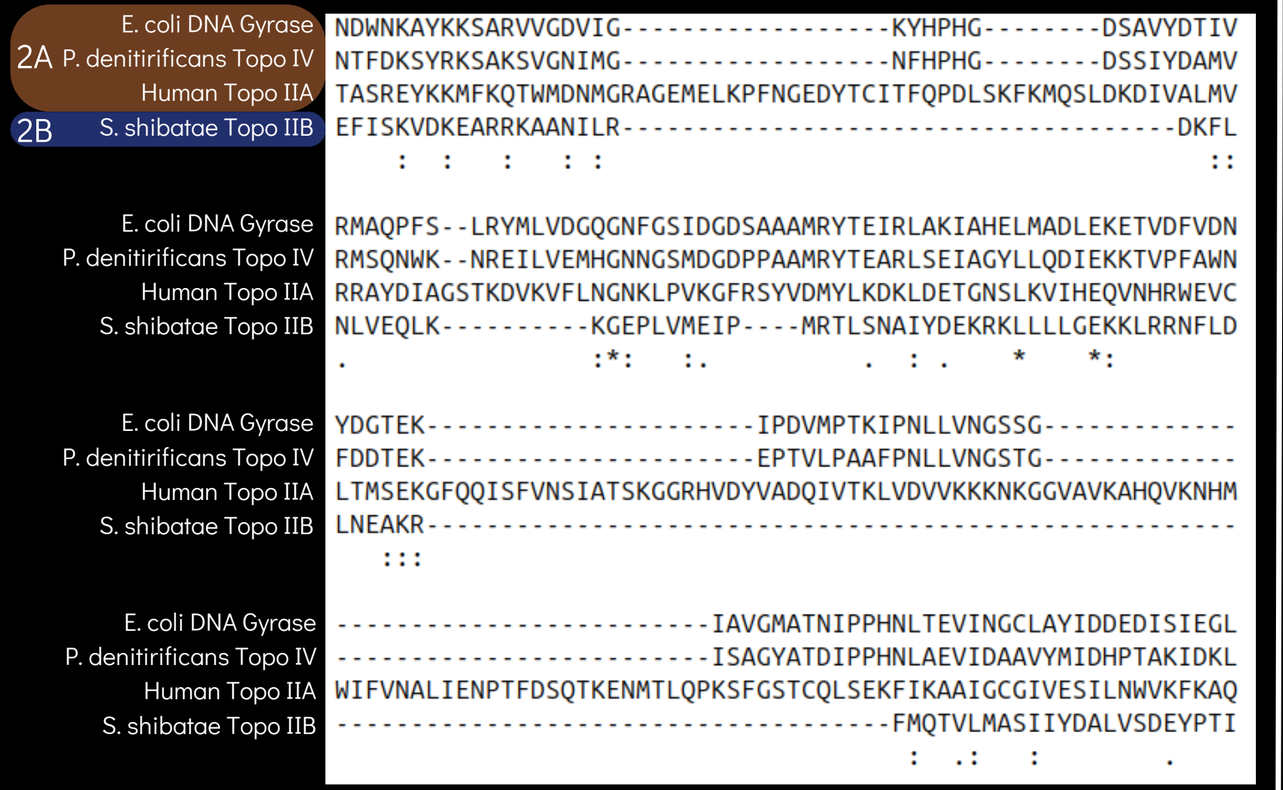


N-terminal | Core | Linker | C-terminal

MSA TOPOISOMERASE II

Subunit A

Subunit B

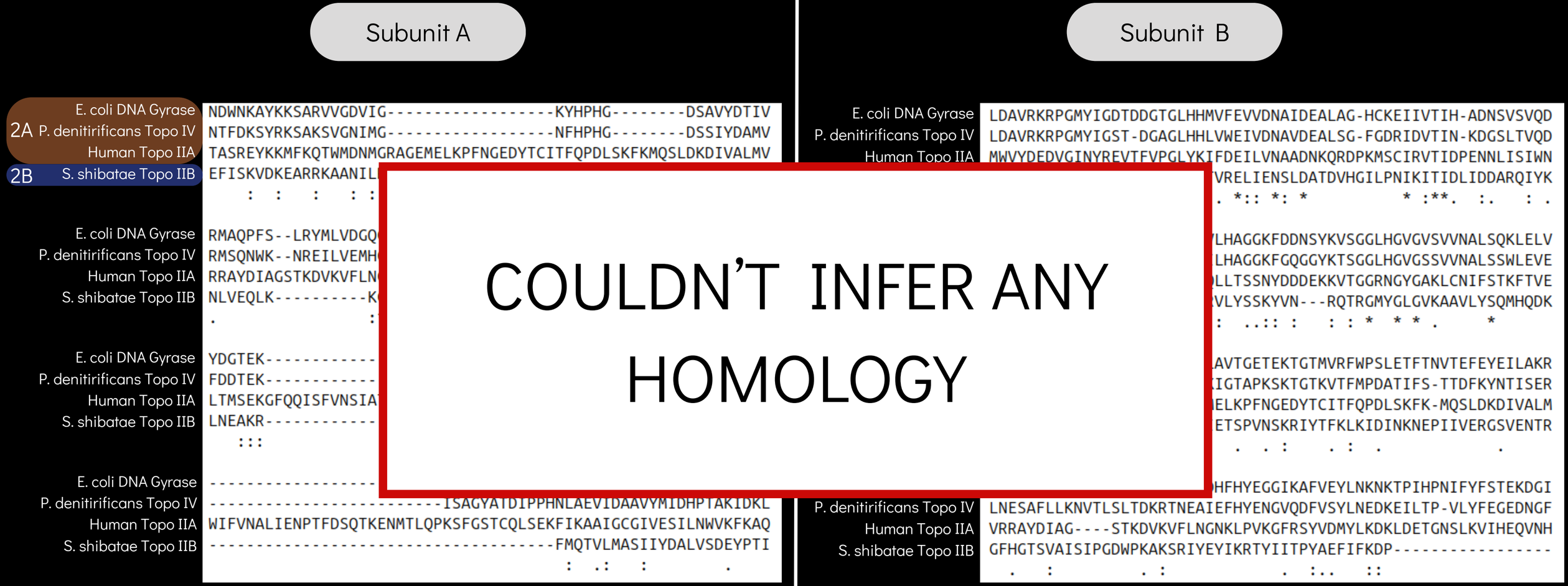




MSA TOPOISOMERASE II

Subunit A

Subunit B



MSA TOPOISOMERASE II

```
58811 Results from round 5
58812
58813
58814 Query= P11388
58815
58816 Length=1531
58817                                                                  Score     E
58818 Sequences producing significant alignments:                      (Bits)  Value
58819 Sequences used in model and found again:
58820
58821 sp|P11388|TOP2A_HUMAN DNA topoisomerase 2-alpha OS=Homo sapiens G...  1591    0.0
58822 sp|P41515|TOP2A_CRIGR DNA topoisomerase 2-alpha OS=Cricetulus gri...  1586    0.0
58823 sp|O46374|TOP2A_PIG DNA topoisomerase 2-alpha OS=Sus scrofa GN=TO...  1581    0.0
58824 sp|P41516|TOP2A_RAT DNA topoisomerase 2-alpha OS=Rattus norvegicu...  1566    0.0
58825 sp|Q01320|TOP2A_MOUSE DNA topoisomerase 2-alpha OS=Mus musculus G...  1559    0.0
58826 sp|O42130|TOP2A_CHICK DNA topoisomerase 2-alpha OS=Gallus gallus ...  1476    0.0
58827 sp|Q64399|TOP2B_CRILO DNA topoisomerase 2-beta OS=Cricetulus long...  1459    0.0
58828 sp|O42131|TOP2B_CHICK DNA topoisomerase 2-beta OS=Gallus gallus G...  1459    0.0
58829 sp|Q64511|TOP2B_MOUSE DNA topoisomerase 2-beta OS=Mus musculus GN...  1457    0.0
58830 sp|Q02880|TOP2B_HUMAN DNA topoisomerase 2-beta OS=Homo sapiens GN...  1453    0.0
58831 sp|P15348|TOP2_DROME DNA topoisomerase 2 OS=Drosophila melanogast...  1346    0.0
58832 sp|Q23670|TOP2_CAEEL Probable DNA topoisomerase 2 OS=Caenorhabdit...  1320    0.0
58833 sp|O16140|TOP2_BOMMO DNA topoisomerase 2 OS=Bombyx mori GN=TOP2 P...  1305    0.0
58834 sp|P08096|TOP2_SCHPO DNA topoisomerase 2 OS=Schizosaccharomyces p...  1246    0.0
58835 sp|P87078|TOP2_CANAX DNA topoisomerase 2 OS=Candida albicans GN=T...  1239    0.0
58836 sp|Q01879|TOP2_PICGU DNA topoisomerase 2 OS=Meyerozyma guilliermo...  1231    0.0
58837 sp|P30182|TOP2_ARATH DNA topoisomerase 2 OS=Arabidopsis thaliana ...  1231    0.0
58838 sp|O93794|TOP2_CANGA DNA topoisomerase 2 OS=Candida glabrata (str...  1226    0.0
58839 sp|Q9Y8G8|TOP2_PENCH DNA topoisomerase 2 OS=Penicillium chrysogen...  1224    0.0
58840 sp|P41001|TOP2_PLAFK DNA topoisomerase 2 OS=Plasmodium falciparum...  1221    0.0
58841 sp|O24308|TOP2_PEA DNA topoisomerase 2 OS=Pisum sativum GN=TOP2 P...  1219    0.0
58842 sp|P06786|TOP2_YEAST DNA topoisomerase 2 OS=Saccharomyces cerevis...  1202    0.0
```

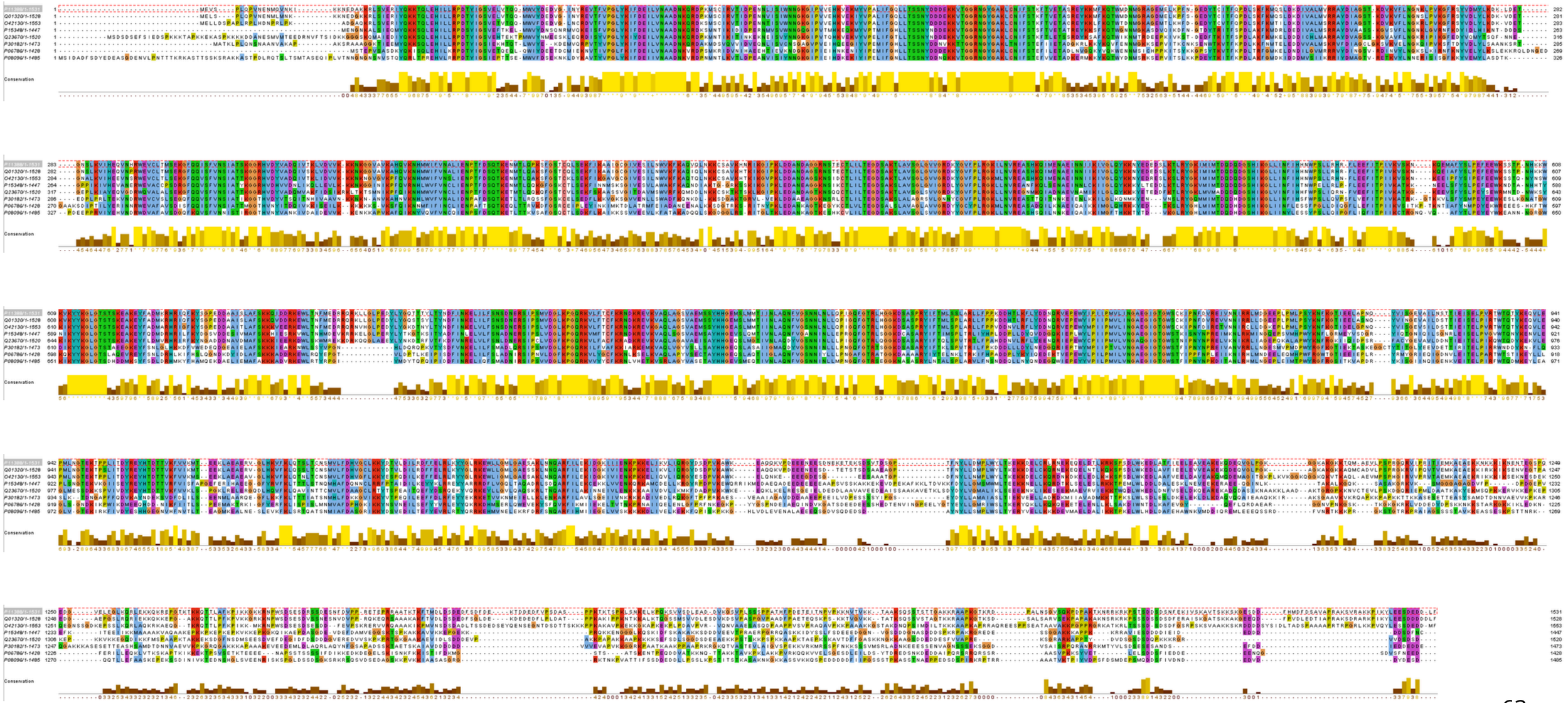
VERTEBRATES

INVERTEBRATES

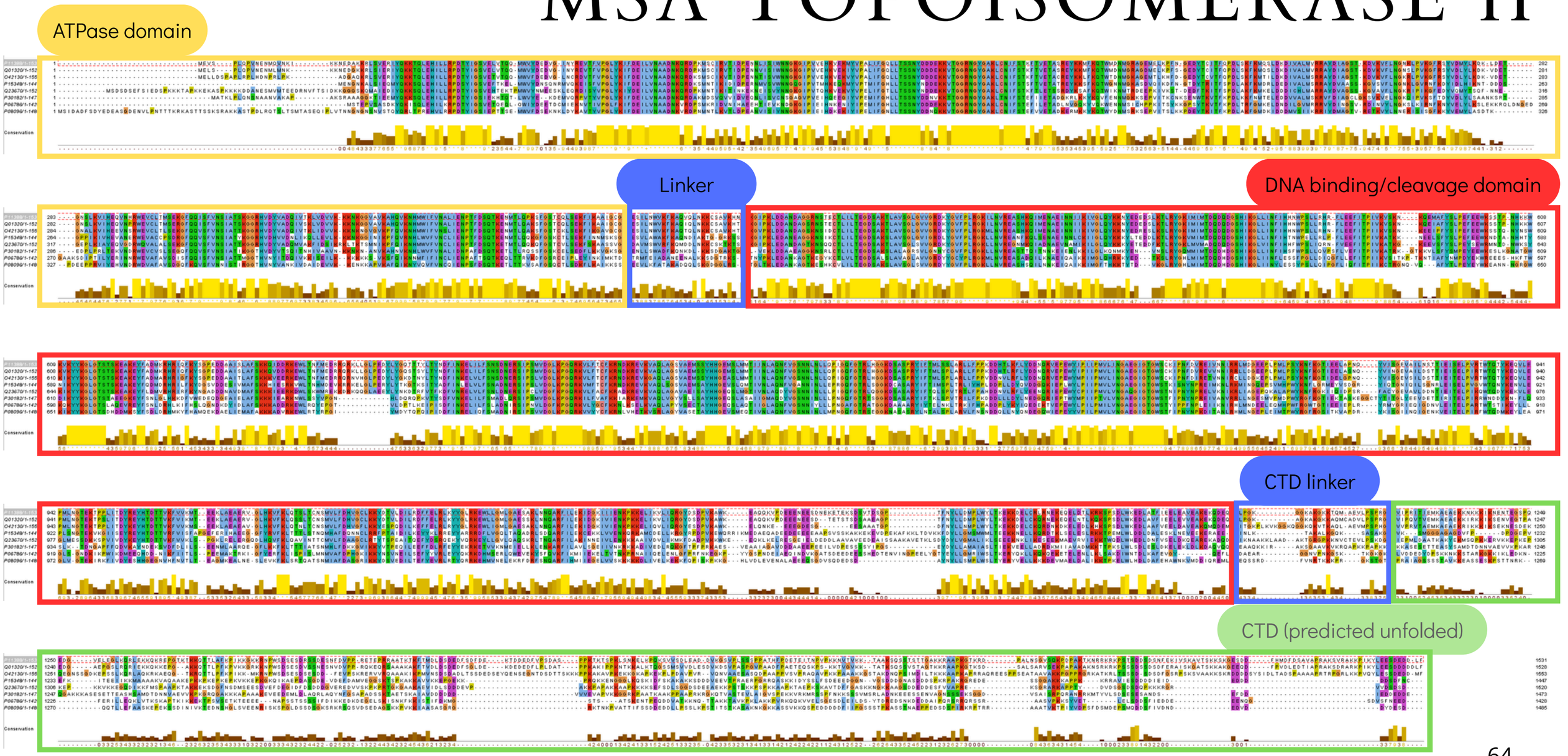
PLANTS

FUNGI

MSA TOPOISOMERASE II



MSA TOPOISOMERASE II



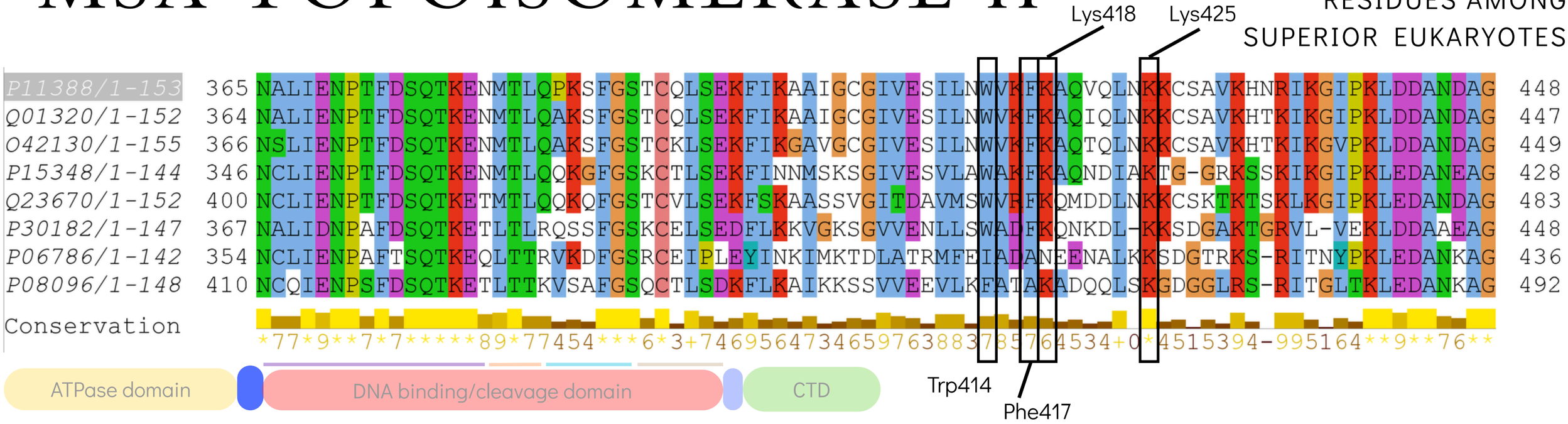
MSA TOPOISOMERASE II

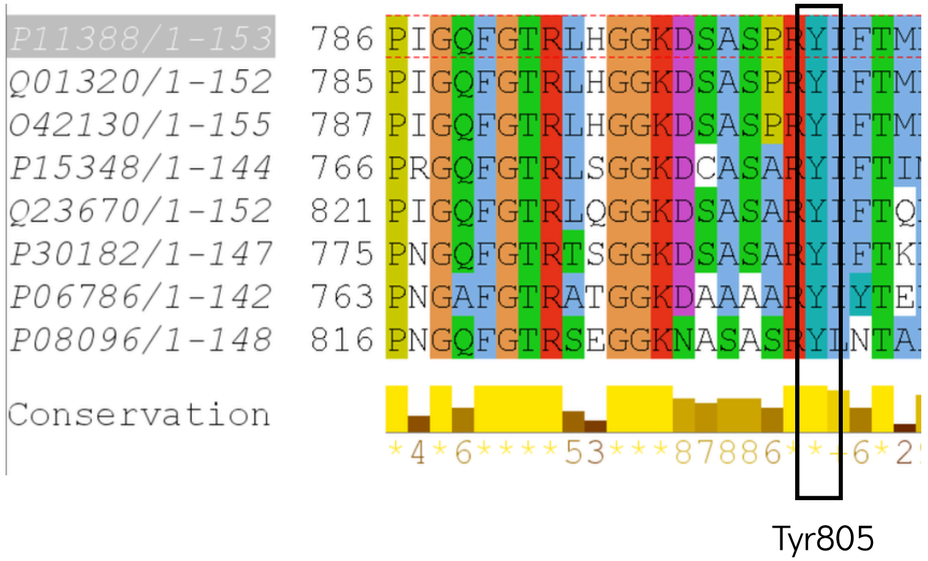


ATPase domain | DNA binding/cleavage domain | CTD

MSA TOPOISOMERASE II

HIGHLY CONSERVED RESIDUES AMONG SUPERIOR EUKARYOTES







STAMP - TOPO II PARALOGS

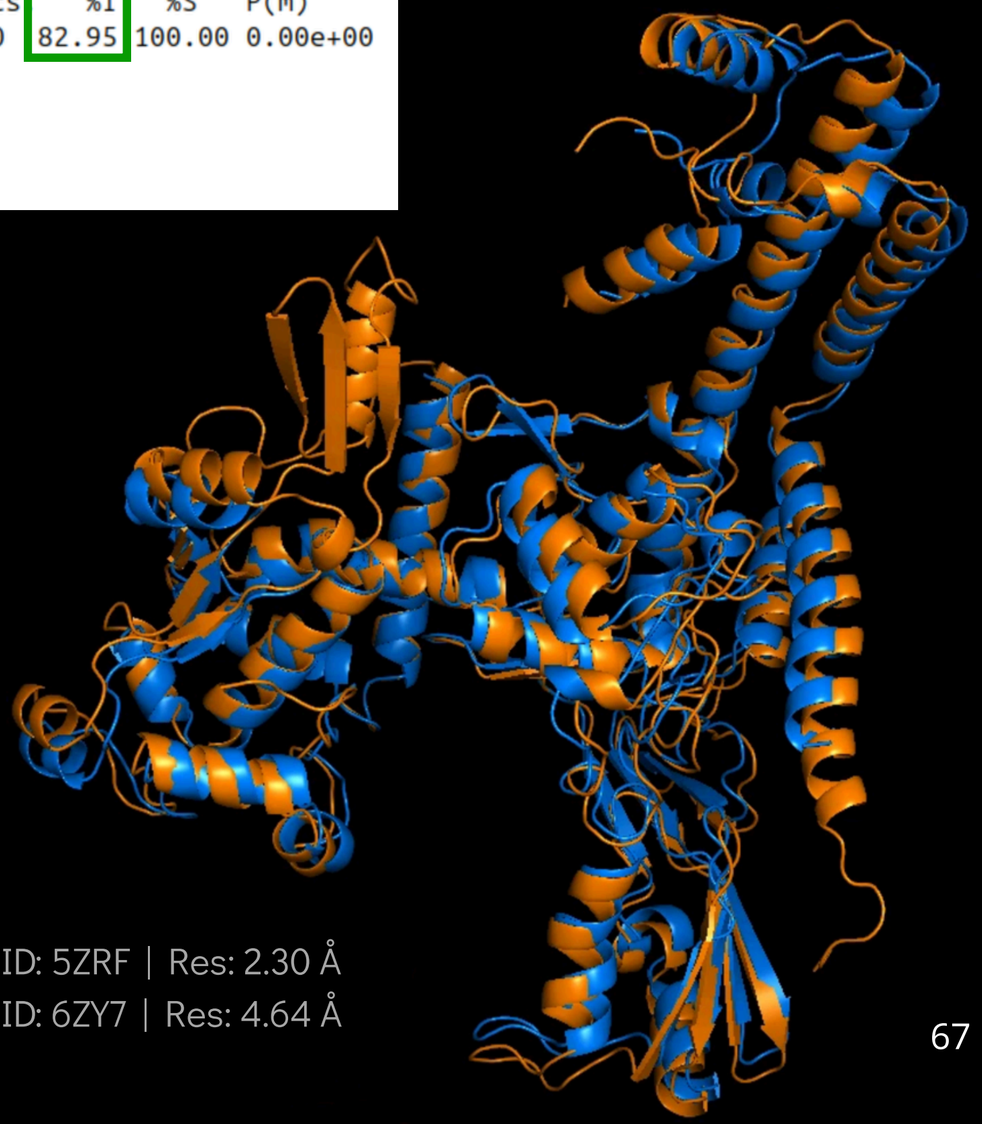
```
17      No.  Domain1          Domain2          Sc     RMS     Len1 Len2  Align NFit Eq. Secs    %I   %S    P(m)
18 Pair   1  TOP2B_HUMAN_A    TOP2A_HUMAN_A    7.44   1.80     616  694   680  605 604    0  82.95 100.00 0.00e+00
19 Reading in matrix file topo2_para.mat...
20 Doing cluster analysis...
21 Cluster:  1 (TOP2B_HUMAN_A  & TOP2A_HUMAN_A ) Sc  7.44 RMS   1.80 Len 680 nfit 605
22  See file topo2_para.1 for the alignment and transformations
```

Alignment of 3 shared Pfam domains:

- Toprim domain
- C-terminal associated domain of Toprim
- DNA gyrase/topoIV subunit A

Human topoisomerase IIB (A chain)

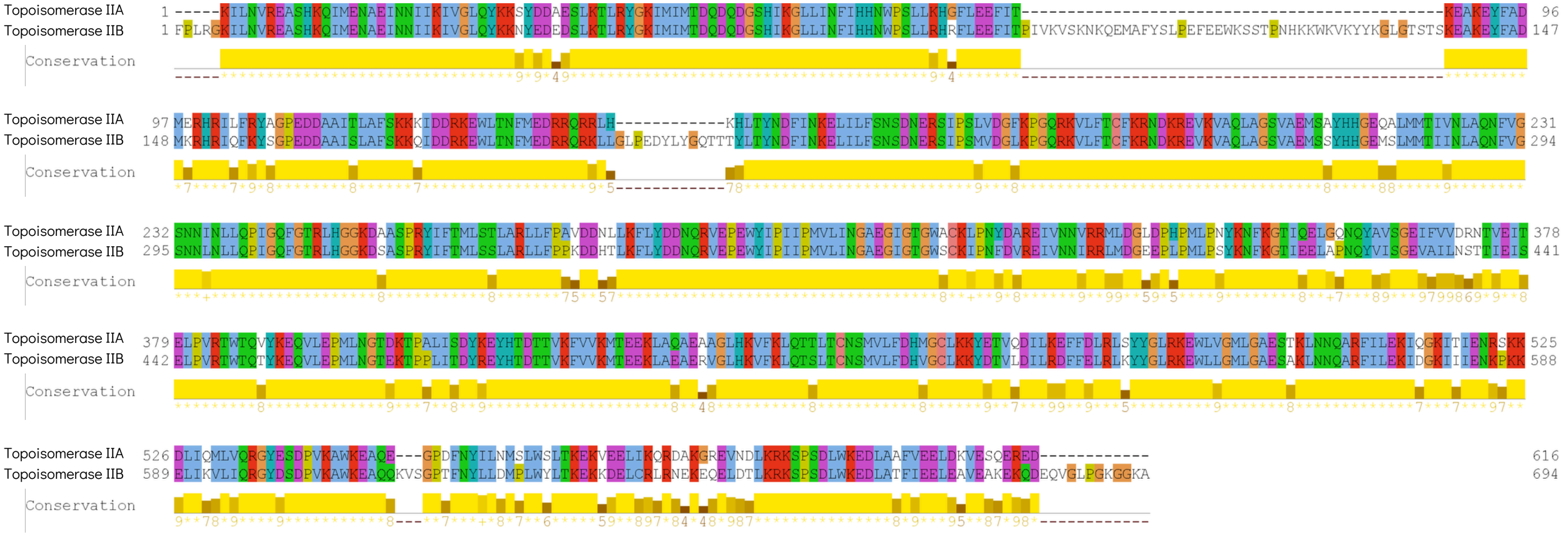
Human topoisomerase IIA (A chain)



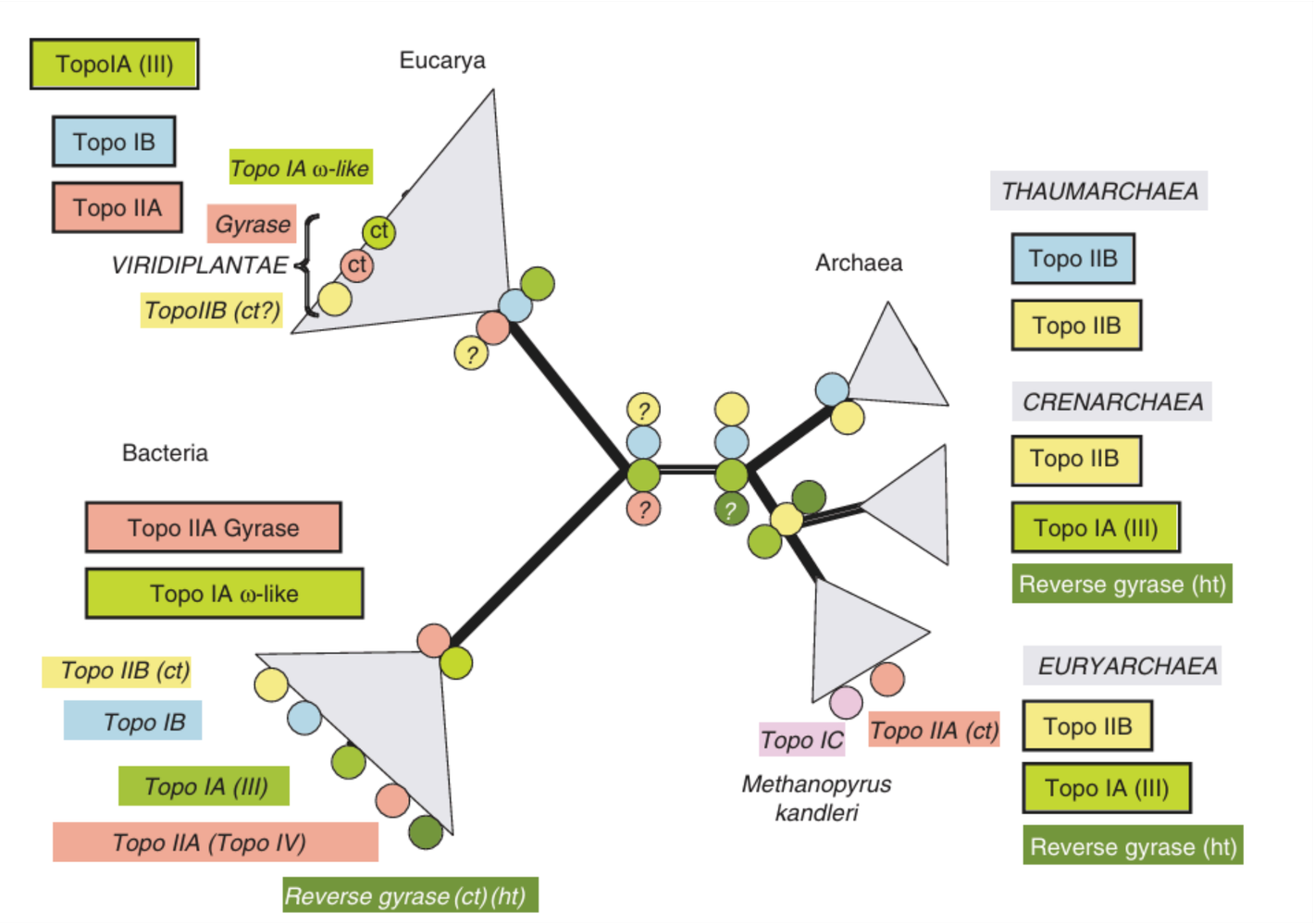
PDB ID: 5ZRF | Res: 2.30 Å

PDB ID: 6ZY7 | Res: 4.64 Å

MSA - TOPO II PARALOGS



PHYLOGENOMICS



- IA: Bacteria, Archaea, Eukarya
- IB: Eukarya (+ some Archaea)
- IC: Rare Archaea
- IIA: Bacteria, Eukarya
- IIB: Archaea

- Topoisomerase I and II are mechanistic categories
- Not single evolutionary lineages
- Multiple independent origins
- Mosaic distribution across domains

Forterre P., Gadelle D. (2009). Phylogenomics of DNA topoisomerases: their origin and putative roles in the emergence of modern organisms. Nucleic Acids Research.

06 CONCLUSIONS

- DNA topological problems arise from torsional stress and supercoiling.
- Topoisomerases are ubiquitous, essential enzymes that resolve these entanglements by creating transient breaks in the DNA helix.
- Topoisomerase IB performs a single-strand break and relaxes the DNA through a controlled rotation mechanism.
- Topoisomerase IB contains a catalytic pentad composed of five well-conserved residues from the C-terminal and core domains, with Tyr723 being the most important.
- Topoisomerase II performs a double strand break through Tyr805 and requires Mg2+ ions.
- The DNA-gate of the DNA-binding/cleavage domain of topoisomerase IIα is where the G-segment is cleaved.
- Functional classification does not imply evolutionary homology.
- Evolutionary conservation reflects functional similarity across topoisomerases.



THANK YOU FOR YOUR TIME

07 APPENDIX

BIBLIOGRAPHY

1. Berger JM, Gamblin SJ, Harrison SC, Wang JC. Structure and mechanism of DNA topoisomerase II. Nature. 1996;379(6562):225-232. doi:10.1038/379225a0

2. Berger JM, Wang JC. Recent developments in DNA topoisomerase II structure and mechanism. Curr Opin Struct Biol. 1996;6(1):84-90. doi:10.1016/s0959-440x(96)80099-6

3. Bergerat A, de Massy B, Gadelle D, Varoutas PC, Nicolas A, Forterre P. An atypical topoisomerase II from Archaea with implications for meiotic recombination. Nature. 1997;386(6623):414-417. doi:10.1038/386414a0

4. Bush NG, Evans-Roberts K, Maxwell A. DNA Topoisomerases. EcoSal Plus. 2015;6(2):10.1128/ecosalplus.ESP-0010-2014. doi:10.1128/ecosalplus.ESP-0010-2014

5. Deweese JE, Osheroff N. The DNA cleavage reaction of topoisomerase II: wolf in sheep's clothing. Nucleic Acids Res. 2009;37(3):738-748. doi:10.1093/nar/gkn937

6. Forterre, P., & Gadelle, D. (2009). Phylogenomics of DNA topoisomerases: their origin and putative roles in the emergence of modern organisms. Nucleic acids research, 37(3), 679-692. https://doi.org/10.1093/nar/gkp032

7. Jaswal S, Nehra B, Kumar S, Monga V. Recent advancements in the medicinal chemistry of bacterial type II topoisomerase inhibitors. Bioorg Chem. 2020;104:104266. doi:10.1016/j.bioorg.2020.104266

8. Lance Stewart et al. A Model for the Mechanism of Human Topoisomerase I.Science279,1534-1541(1998).DOI:10.1126/science.279.5356.1534

9. Matthew R. Redinbo et al. ,Crystal Structures of Human Topoisomerase I in Covalent and Noncovalent Complexes with DNA.Science279,1504-1513(1998).DOI:10.1126/science.279.5356.1504

BIBLIOGRAPHY

10. Molecule of the Month PDB: https://pdb101.rcsb.org/motm/73

11. Nicole M. Baker, Rakhi Rajan, Alfonso Mondragn, Structural studies of type I topoisomerases, Nucleic Acids Research, Volume 37, Issue 3, 1 February 2009, Pages 693-701, https://doi.org/10.1093/nar/gkn1009

12. Pommier, Y., Nussenzweig, A., Takeda, S. et al. Human topoisomerases and their roles in genome stability and organization. Nat Rev Mol Cell Biol 23, 407-427 (2022). https://doi.org/10.1038/s41580-022-00452-3

13. Roca J. Topoisomerase II: a fitted mechanism for the chromatin landscape. Nucleic Acids Res. 2009;37(3):721-730. doi:10.1093/nar/gkn994

14. Schmidt BH, Osheroff N, Berger JM. Structure of a topoisomerase II-DNA-nucleotide complex reveals a new control mechanism for ATPase activity. Nat Struct Mol Biol. 2012;19(11):1147-1154. doi:10.1038/nsmb.2388

15. Seol Y, Neuman KC. The Dynamic Interplay Between DNA Topoisomerases and DNA Topology. Biophys Rev. 2016;8(3):221-231. doi:10.1007/s12551-016-0206-x

16. Talukdar, A., Kundu, B., Sarkar, D., Goon, S., & Mondal, M. A. (2022). Topoisomerase I inhibitors: Challenges, progress and the road ahead. European journal of medicinal chemistry, 236, 114304. https://doi.org/10.1016/j.ejmech.2022.114304

17. Vanden Broeck A, Lotz C, Drillien R, Haas L, Bedez C, Lamour V. Structural basis for allosteric regulation of Human Topoisomerase II?. Nat Commun. 2021;12(1):2962. Published 2021 May 20. doi:10.1038/s41467-021-23136-6

18. Wang JC. Cellular roles of DNA topoisomerases: a molecular perspective. Nat Rev Mol Cell Biol. 2002;3(6):430-440. doi:10.1038/nrm831

19. Wei H, Ruthenburg AJ, Bechis SK, Verdine GL. Nucleotide-dependent domain movement in the ATPase domain of a human type IIA DNA topoisomerase. J Biol Chem. 2005;280(44):37041-37047. doi:10.1074/jbc.M506520200

MULTIPLE CHOICE QUESTIONS

1. Which statement best explains why topoisomerases are essential during replication?
A) They relieve torsional stress generated ahead of replication forks.
B) They prevent mutations by proofreading newly synthesized DNA.
C) They synthesize new DNA strands.
D) They stabilize histone–DNA interactions.
E) They increase DNA supercoiling to compact chromosomes.

2. Which mechanistic difference correctly distinguishes Type I from Type II topoisomerases?
A) Type I requires ATP; Type II does not.
B) Type I changes linking number in steps of two; Type II in steps of one.
C) Type I forms 5' phosphotyrosyl intermediates exclusively.
D) Type II introduces double-strand breaks and requires ATP hydrolysis.
E) Type II acts only on negatively supercoiled DNA.

3. In human Topoisomerase IB, which domain is dispensable for catalysis but contributes to regulation and flexibility?
A) Core domain
B) C-terminal domain
C) N-terminal domain
D) Subdomain III
E) Active-site pocket

MULTIPLE CHOICE QUESTIONS

4. The catalytic pentad of human Topoisomerase IB includes Tyr723. What is the specific role of this residue?
A) Stabilizes Mg^{2+} coordination
B) Forms a 5' phosphotyrosyl linkage
C) Stabilizes the DNA minor groove
D) Deprotonates the leaving group
E) Performs nucleophilic attack on the scissile phosphate

5. Which interaction stabilizes the closed conformation of Topoisomerase IB lobes?
A) Disulfide bond between subdomains I and III
B) Salt bridge between residues from opposing lobes
C) ATP-dependent dimerization
D) Mg^{2+}-mediated bridging
E) Hydrophobic collapse of the linker

6. In Topoisomerase IIα, what is the functional role of the ATPase (N-gate) domain?
A) Drives strand passage through ATP-dependent conformational changes
B) Coordinates Mg^{2+} ions for catalysis
C) Directly catalyzes DNA cleavage
D) Forms the C-gate exit channel
E) Provides sequence specificity for DNA binding

MULTIPLE CHOICE QUESTIONS

7. During Topoisomerase II–mediated cleavage, the catalytic tyrosines become covalently linked to which DNA end?
A) 3' hydroxyl end
B) 3' phosphate end
C) 5' phosphate end
D) 5' hydroxyl end
E) Both 3' and 5' ends simultaneously

8. The highly conserved residues W414 and F417 in the linker region primarily contribute to:
A) Direct DNA cleavage
B) Mg^{2+} coordination
C) Recognition of the T-segment
D) ATP binding
E) Formation of a hydrophobic patch stabilizing transducer helices

9. The failure of the naïve MSA combining Type IA, IB, and IC enzymes suggests that:
A) All Type I enzymes share recent common ancestry.
B) Mechanistic classification does not guarantee evolutionary homology.
C) Sequence alignment tools are unreliable for large proteins.
D) Topoisomerases are universally conserved at sequence level.
E) Structural similarity is always independent of sequence conservation.

10. According to phylogenomic analysis, why do human Topoisomerase I and II not structurally superimpose?
A) They evolved from the same ancestor but diverged structurally.
B) They belong to the same structural fold but differ in regulation.
C) They represent distinct evolutionary families despite functional similarity.
D) One enzyme is viral in origin.
E) They share identical ATPase domains but different catalytic cores.