Thermus aquaticus DNA polymerase

Unseal the secret of thermostable from structure

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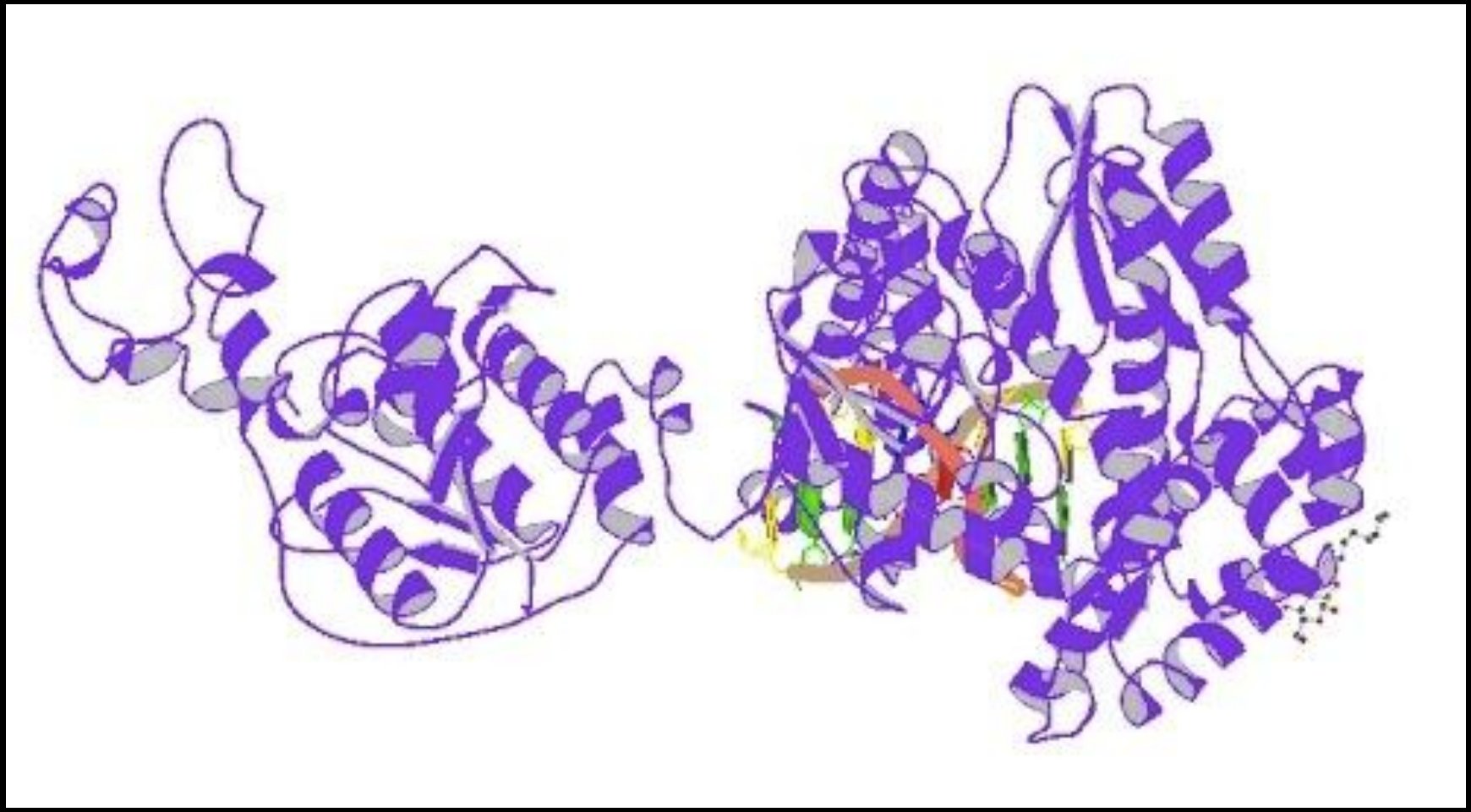
1TAU-The foundation of molecular-bio



From PDB

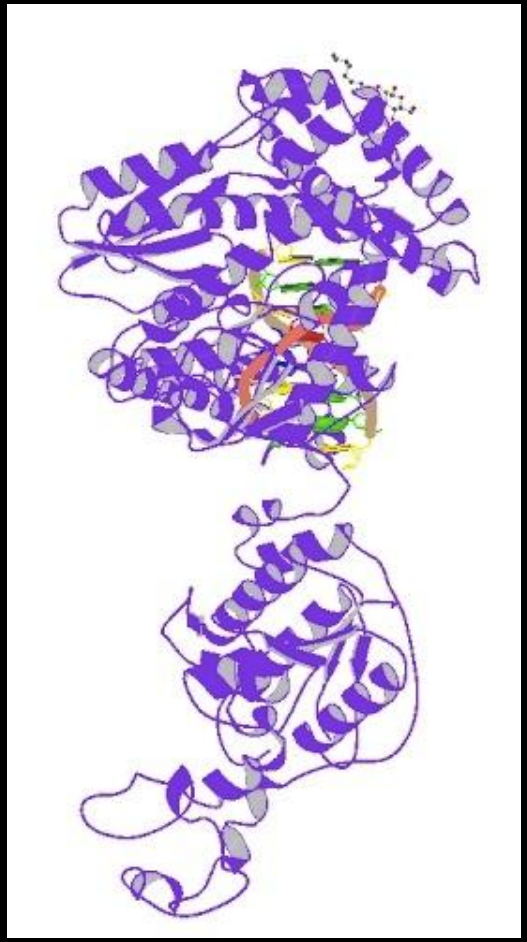
Something about history

- 1980s, Kary Mullis working on the PCR
- Isolated by Thomas D. Brock in 1965 from the thermophilic bacterium Thermus aquaticus “ life at high temperatures” → Taq polymerase
- The structure was first resloved in 1995
- These days, the protein is isolated completely from unique strain YT-1, which is a T. aquaticus culture gathered from a hot spring known as Mushroom Spring. It is considered one of the biggest hot springs found in the Geyser Basin.

Something about structure

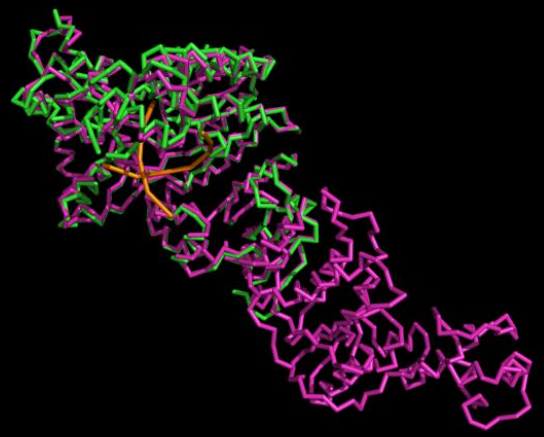
- N terminal: 5’ nuclease activity
- C terminal: polymerase activity

- Proofreading



1TAU vs 1KTQ vs 1KLN

- 1TAU vs 1KTQ: RMS=1.236

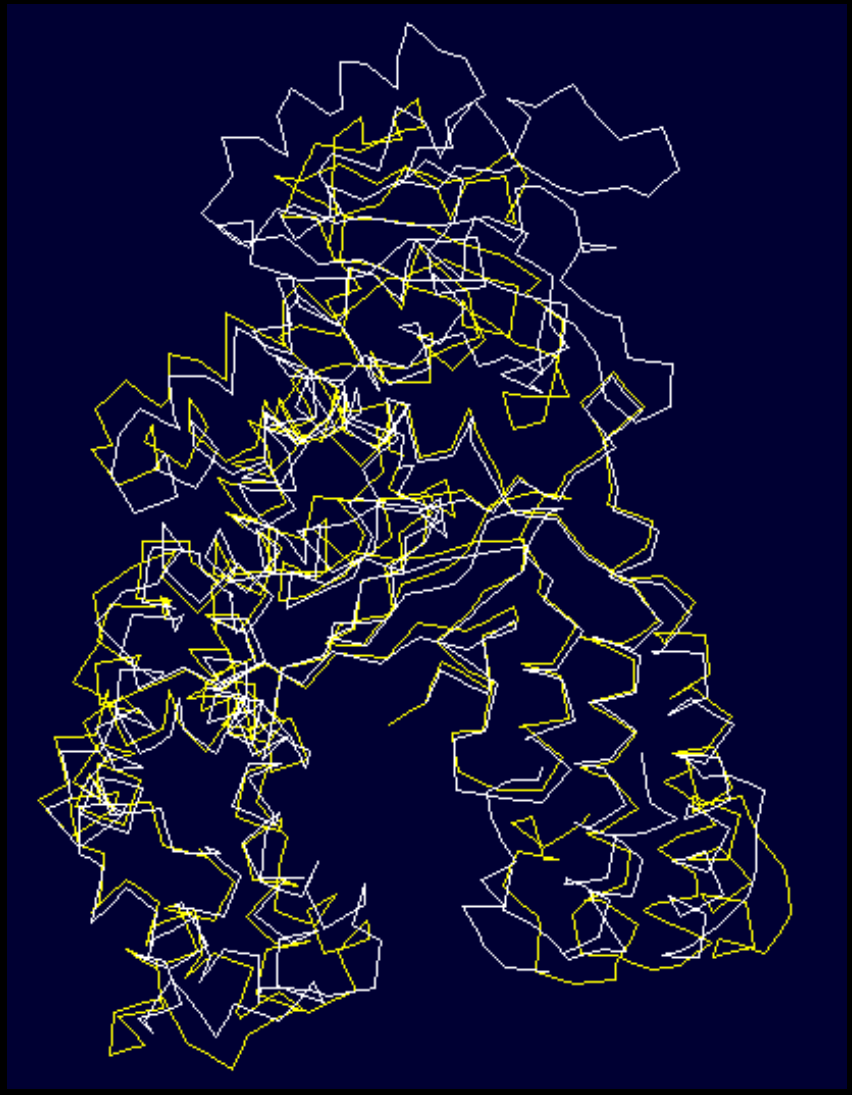


- 1KLN vs 1TAU RMS=3.687

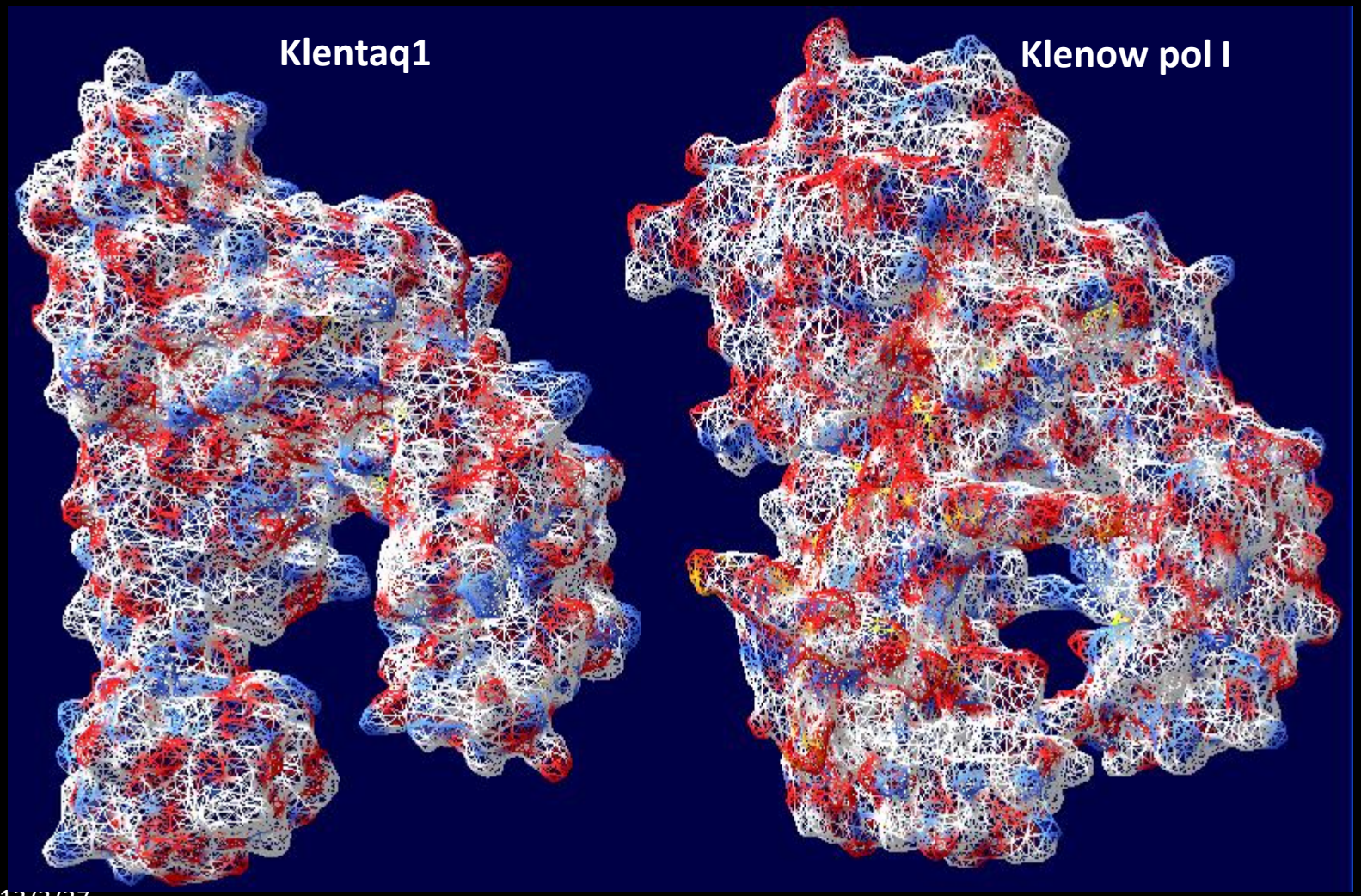
- 1KLN vs 1KTQ RMS=2.162

Superimposed stereodiagram of Ca

- Yellow： taq
- White： Pol I

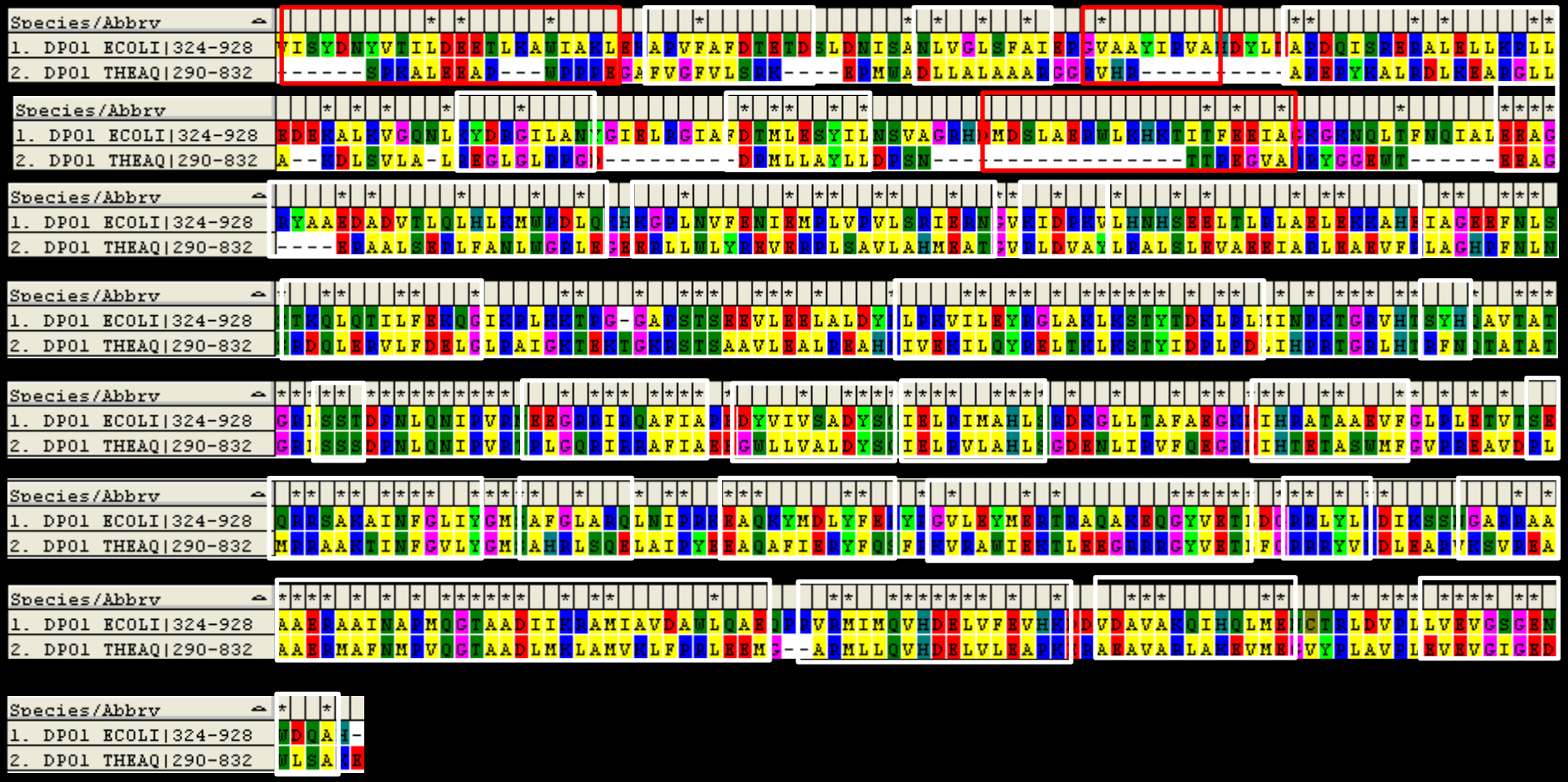


The compare between surfaces



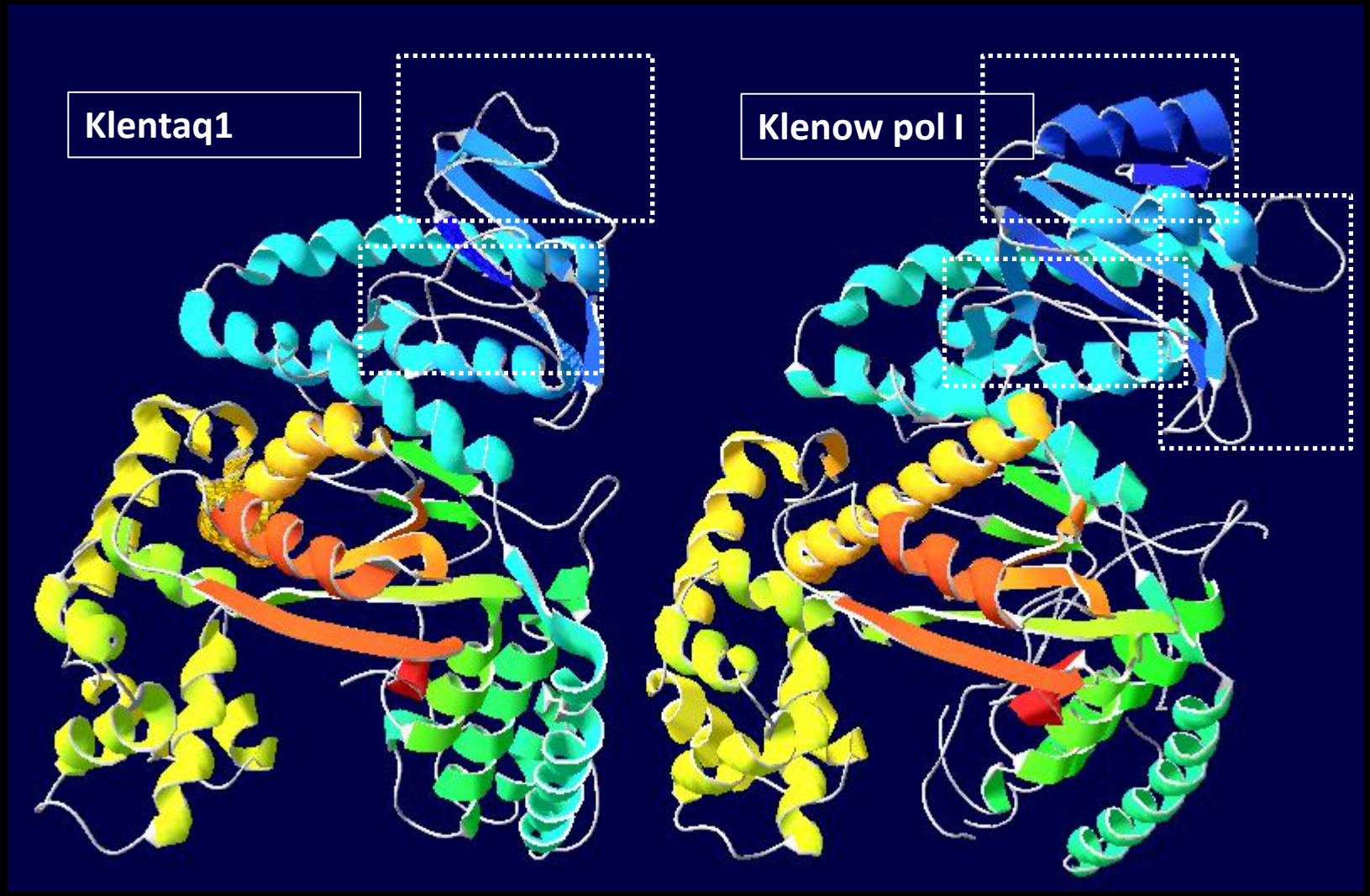
Residue	Klenow pol I		Klentaq1	
	Number	Mole%	Number	Mole%
Ala	62	10.248	63	11.602
Cys	1	0.165	0	0
Asp	35	5.785	20	3.683
Glu	54	8.926	61	11.234
Phe	16	2.645	17	3.131
Gly	33	5.455	36	6.63
His	16	2.645	10	1.842
Ile	39	6.446	17	3.131
Lys	38	6.281	20	3.683
Leu	69	11.405	75	13.812
Met	14	2.314	13	2.394
Asn	23	3.802	10	1.842
Pro	27	4.463	33	6.077
Gln	25	4.132	12	2.21
Arg	36	5.95	54	9.945
Ser	27	4.463	22	4.052
Thr	29	4.793	21	3.867
Val	35	5.785	36	6.63
Trp	5	0.826	9	1.657
Tyr	21	3.471	14	2.578

Klentaq1和Klenow pol I序列比对



N端变异大，C端保守性高

Klentaq1和Klenow pol I结构比较

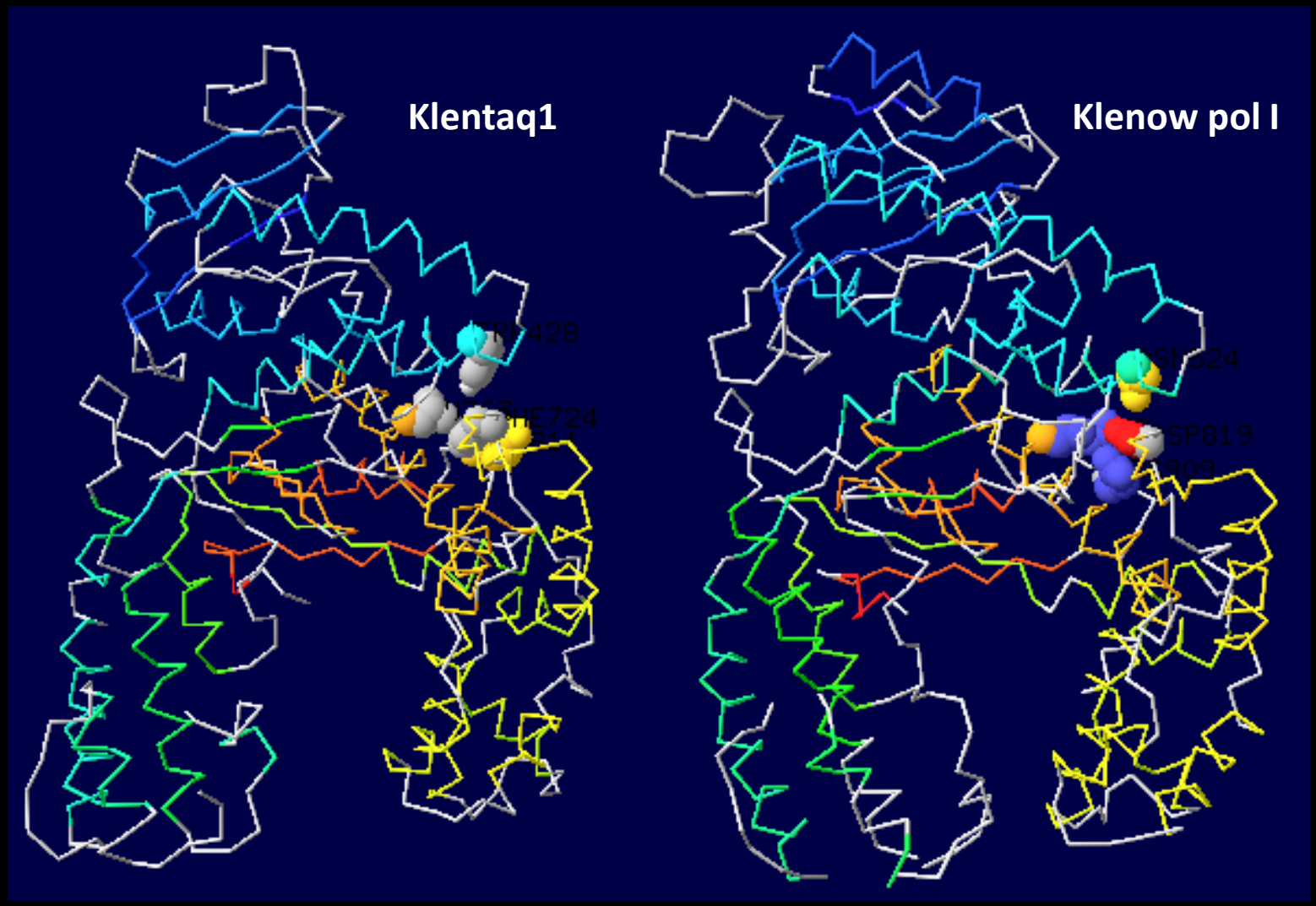


1. 3个pro相连，使得Klentaq1的N端少了一个α螺旋
2. 二级结构间连接的loop更短

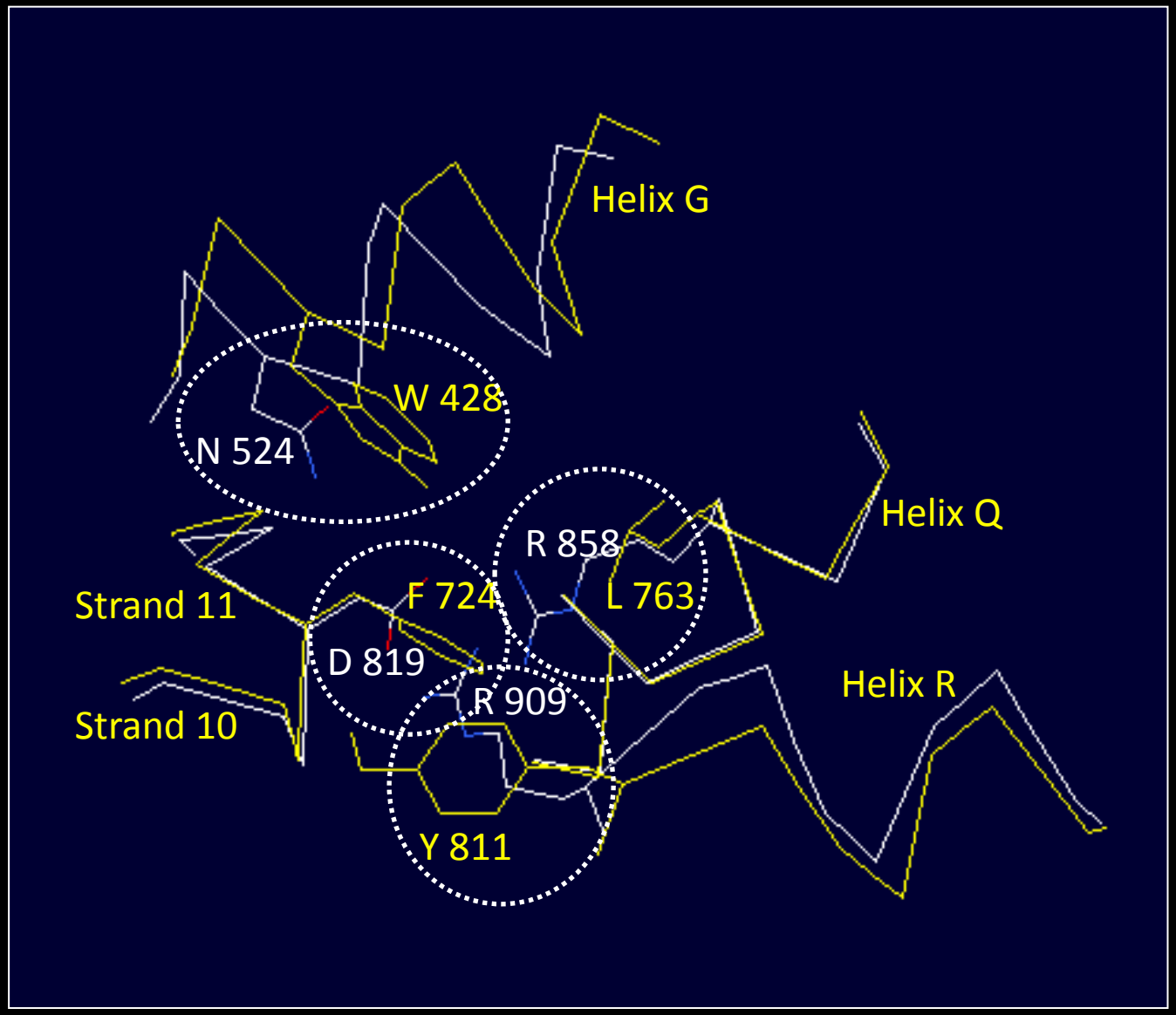
Amino acids constitution

Property	Residues	Klenow pol I		Klentaq1	
		Number	Mole%	Number	Mole%
Non-polar	(A+C+F+G+I+L+M+P+V+W+Y)	322	53.223	313	57.643
Polar	(D+E+H+K+N+Q+R+S+T+Z)	283	46.777	230	42.357
Charged	(B+D+E+H+K+R+Z)	179	29.587	165	30.387
Basic	(H+K+R)	90	14.876	84	15.47
Acidic	(B+D+E+Z)	89	14.711	81	14.917

N端与C端结构域的界面处残基发生变化



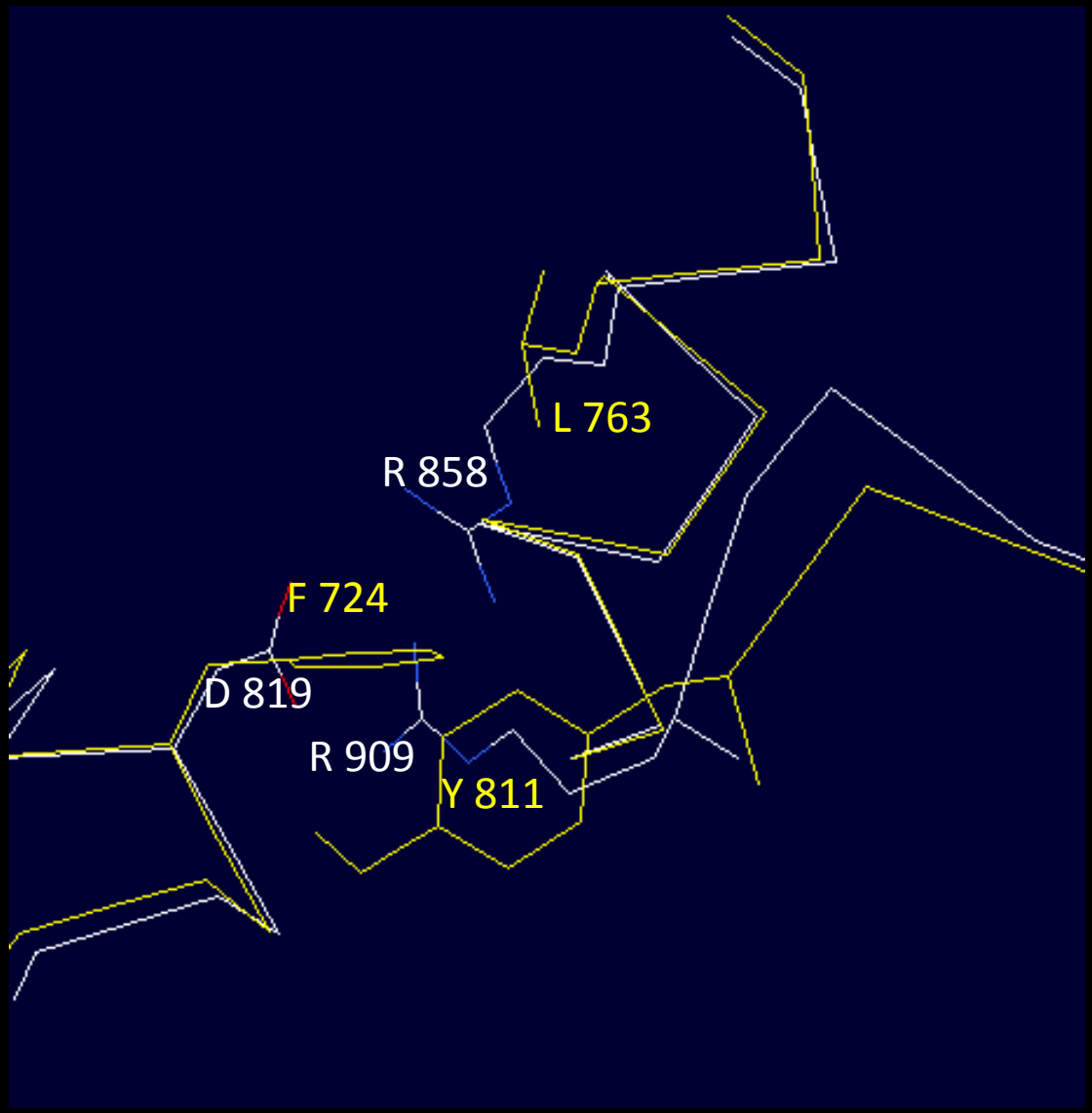
Klentaq1的芳香、杂环残基簇形成疏水核



N (Asn) 524 – W(Trp) 428
D (Asp-)819 – F(Phe) 724
R(Arg+) 858 – L763
R 909 – Y(Tyr) 811

黄线： Klentaq1
白线： Klenow pol I

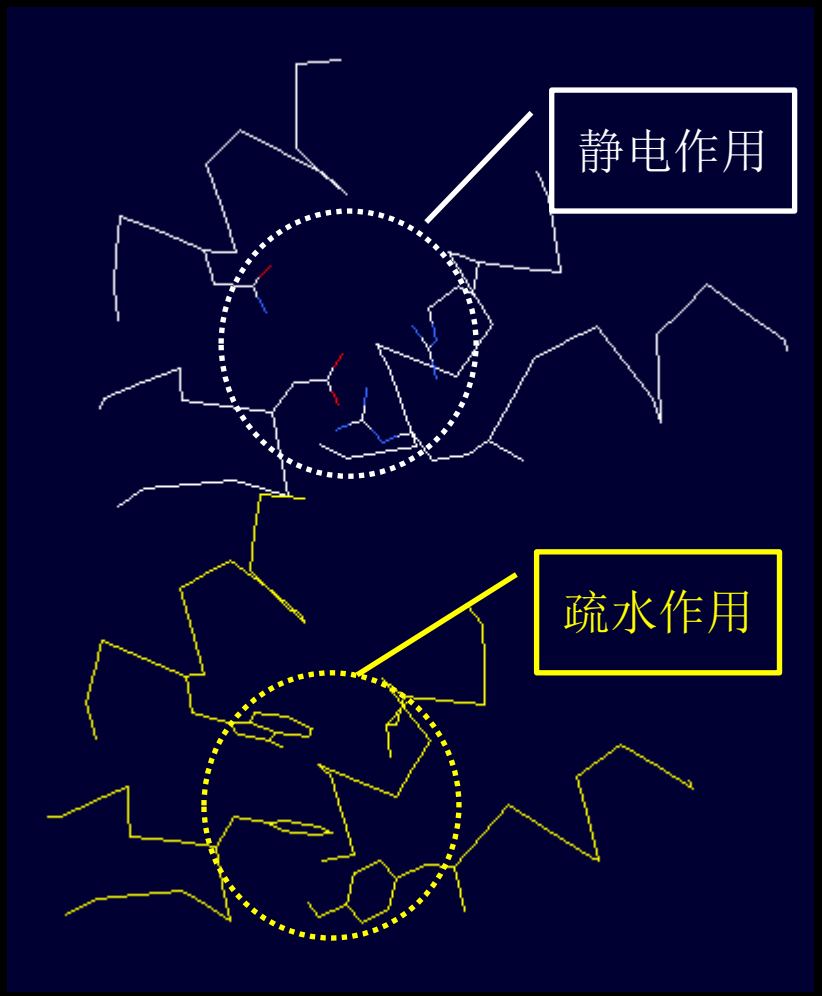
Klentaq1的芳香、杂环残基簇形成疏水核



三个疏水残基 **F724** 、**L763**、 **Y811**取代了**Klenow pol I**的带电残基，从而消除了**R858**和**R 909**的静电排斥作用，且增加疏水作用力

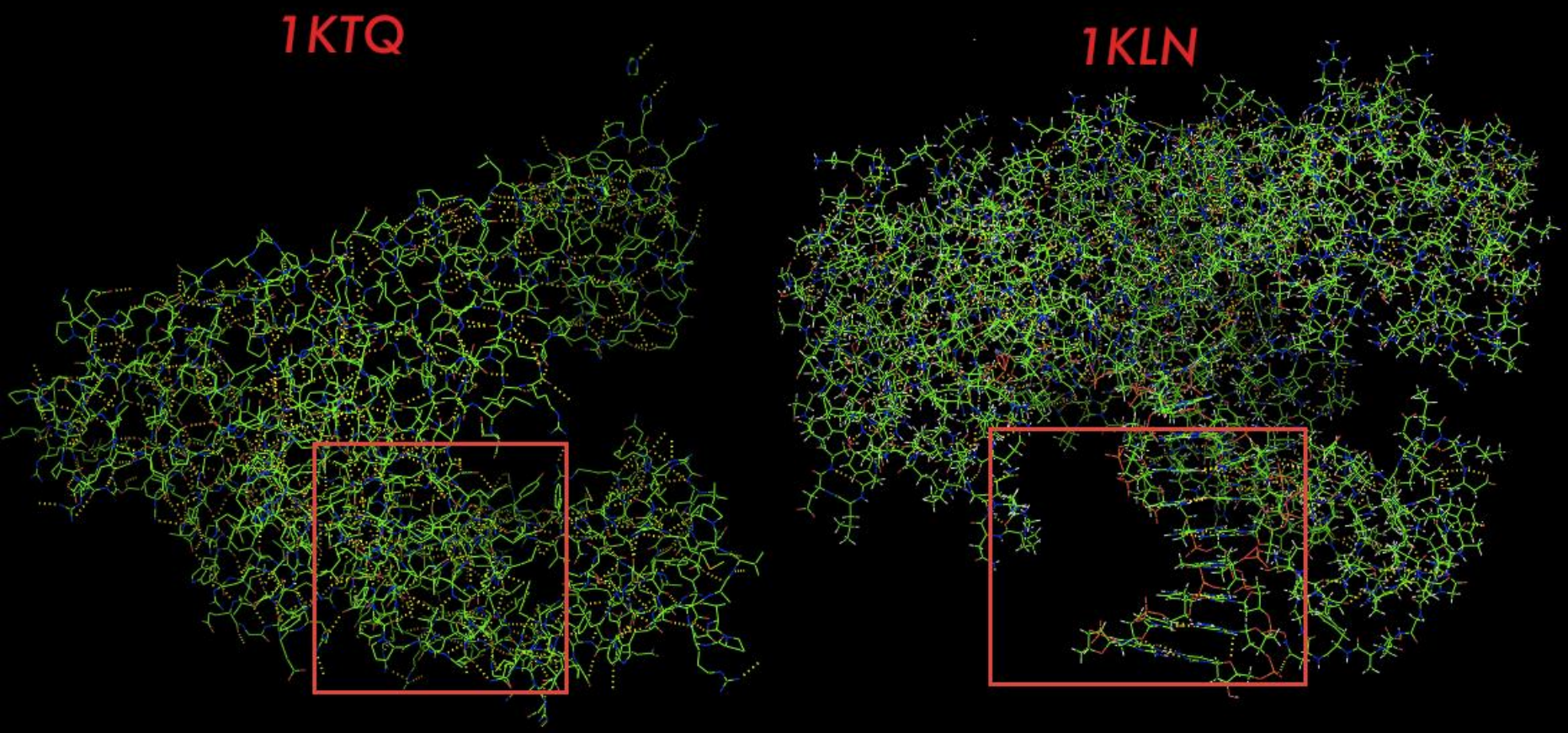
黄线： **Klentaq1**
白线： **Klenow pol I**

Klentaq1的芳香、杂环残基簇形成疏水核



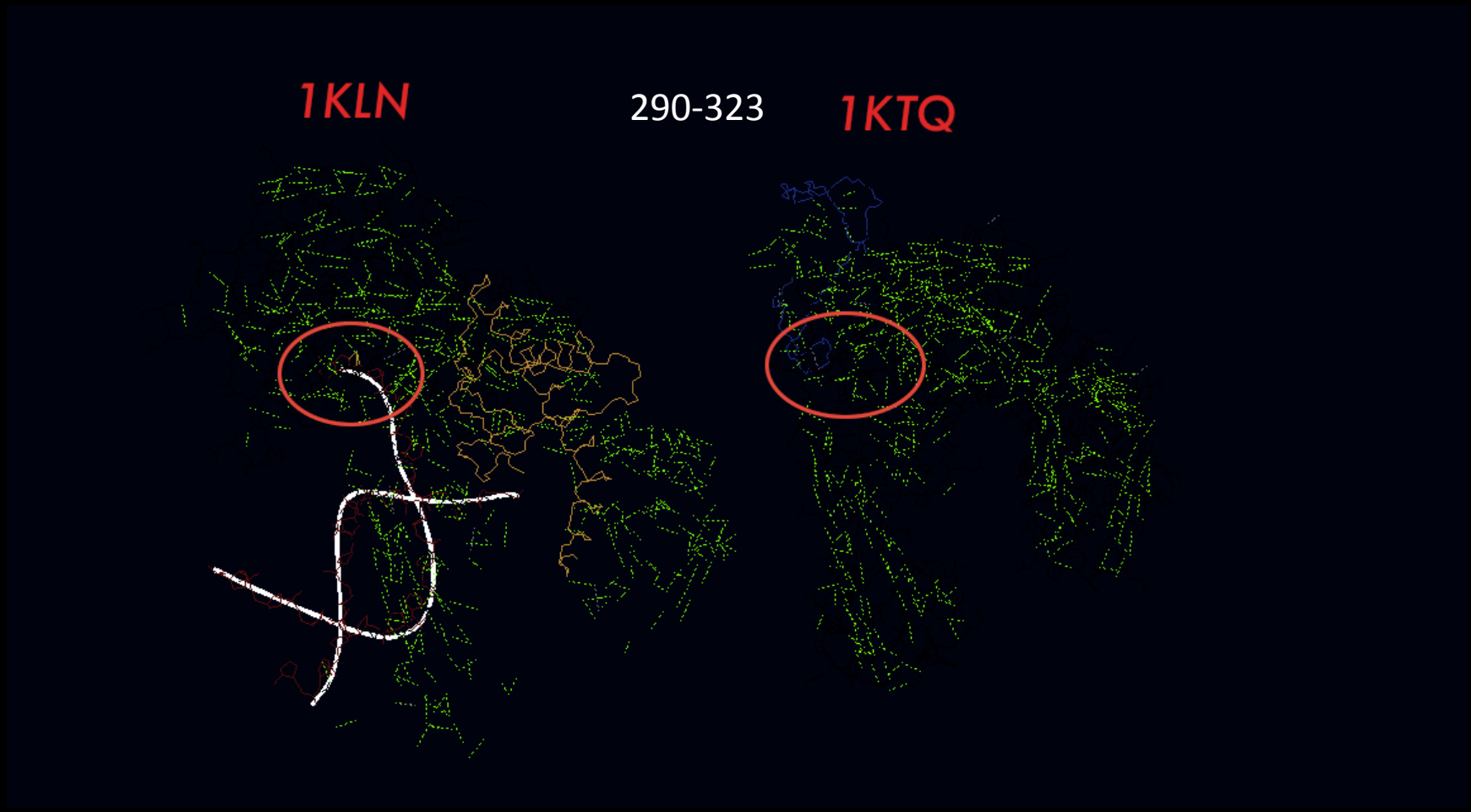
黄线： **Klentaq1**
白线： **Klenow pol I**

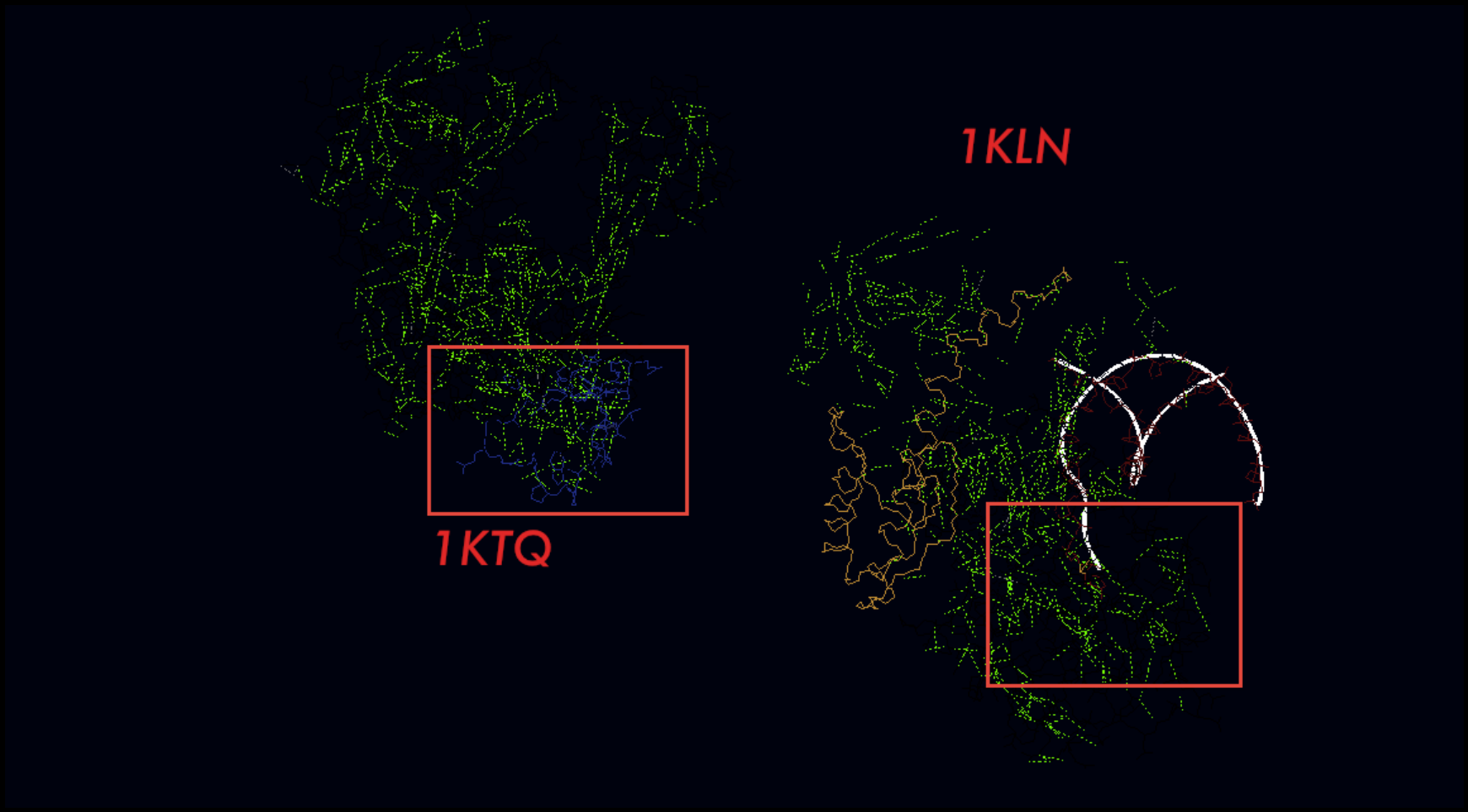
Hydron bonds

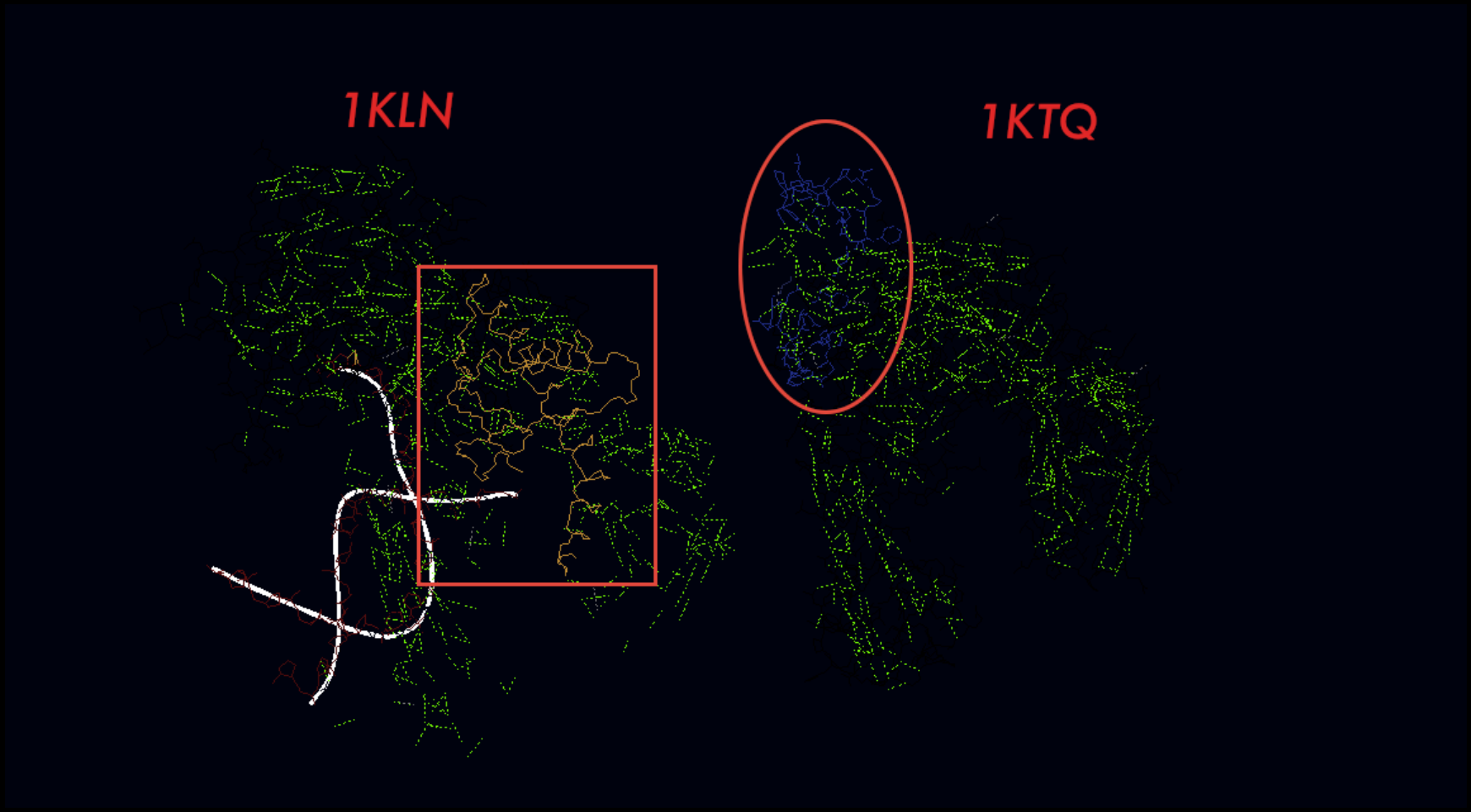


Some numbers

- 1KLN(324-929)：1312 H-bonds
- 1KTQ(290-832)：1380 H-bonds
- 1KTQ had even more H-bonds with fewer residues involved.
- H-bonds model: Length: <=3.2; Angle: <=55.


1KLN
290-323
1KTQ

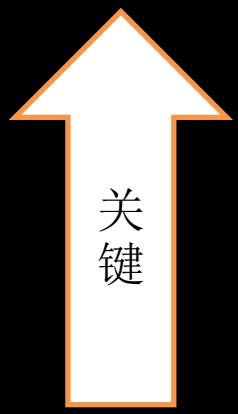

1KLN
1KTQ

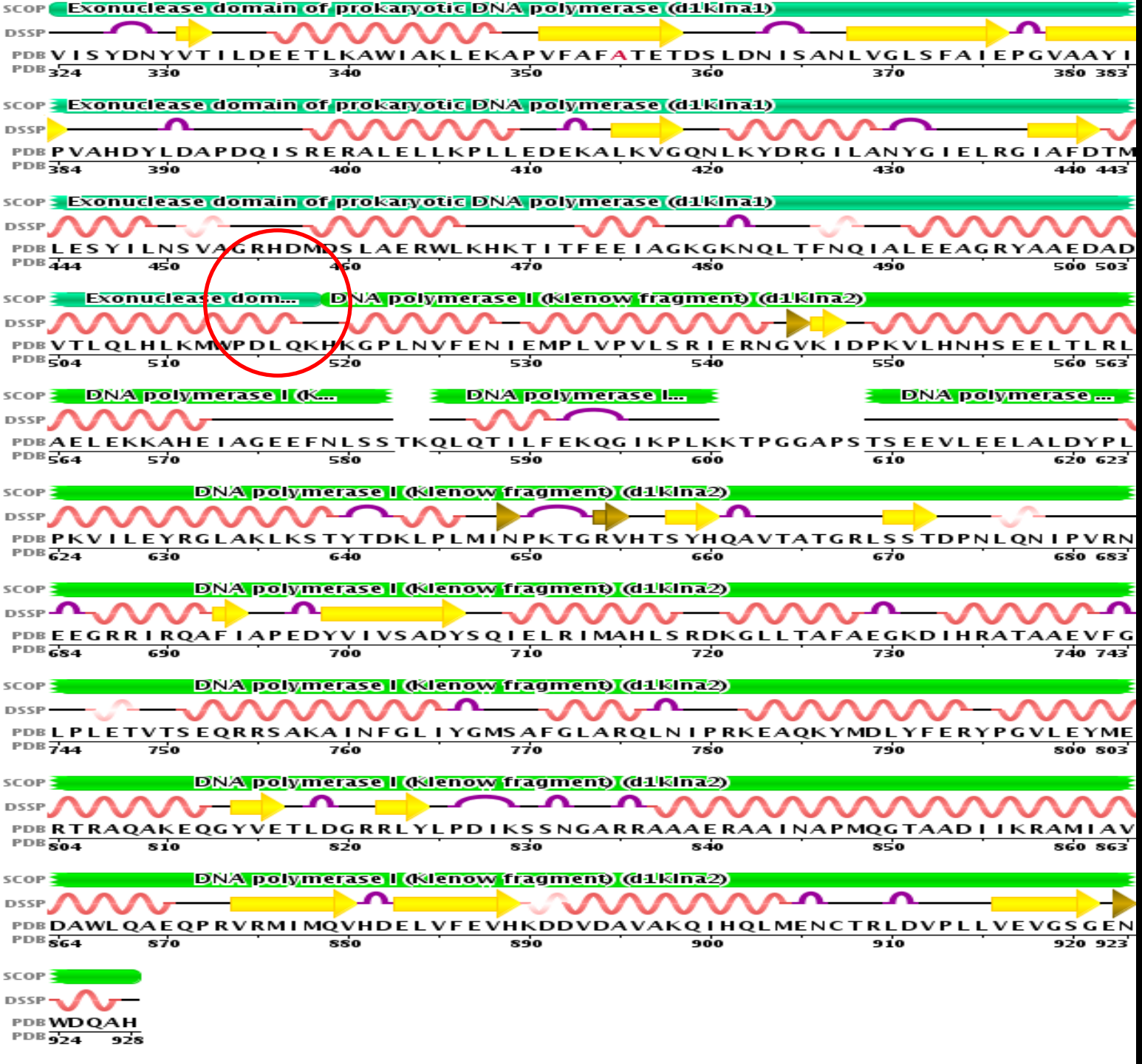

1KLN
1KTQ

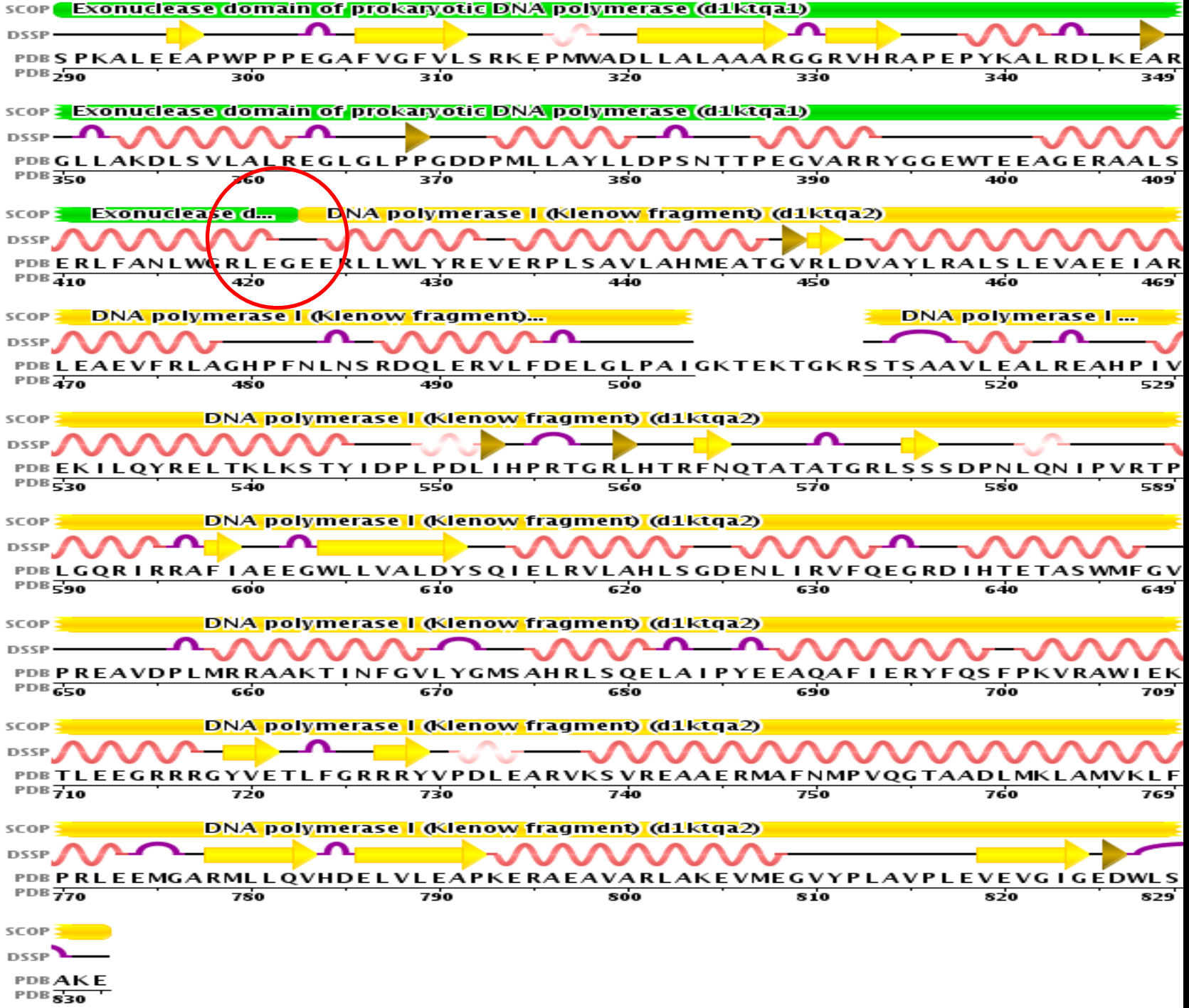
Salt bridges

- 通常由以下氨基酸构成：
  - 带阴离子的羧基（RCOOH-）：Asp-和Glu-
  - 带阳离子的铵(RNH3+)：Lys+
  - 带阳离子的胍基(RNHC(NH2)2+)：Arg+
- 偶尔也有以下氨基酸参与：
  - His，Try，Ser
- 但是距离必须得小于4埃

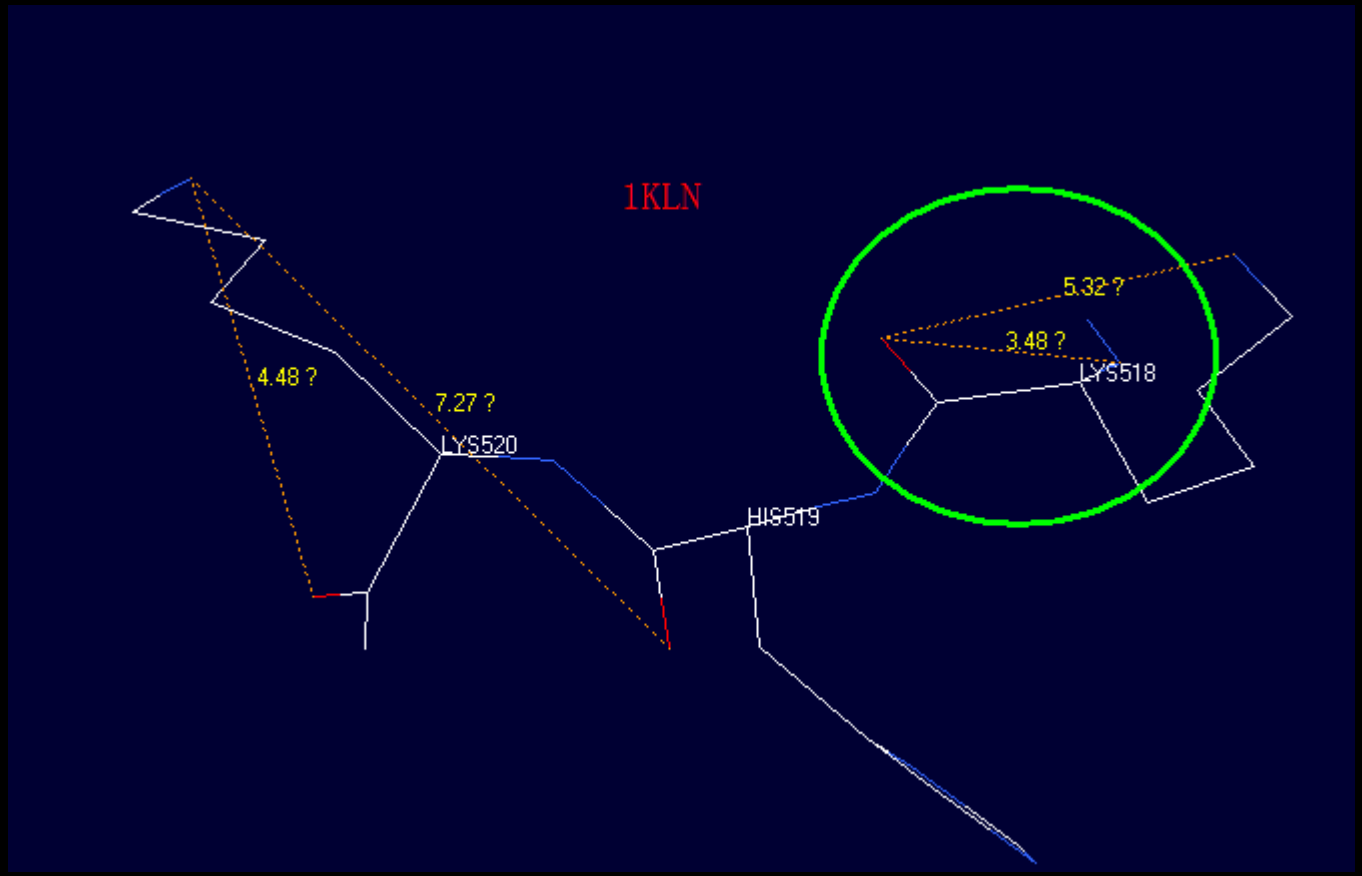
PDB	taxon	Feature	The number of special animo acids	The salt bridges between subdomain
1KLN	Escherichia coli	Not hot stable	223	Lys518His519Lys520
1KTQ	Thermus aquaticus	Hot stable	196	Glu421Gly422Glu423



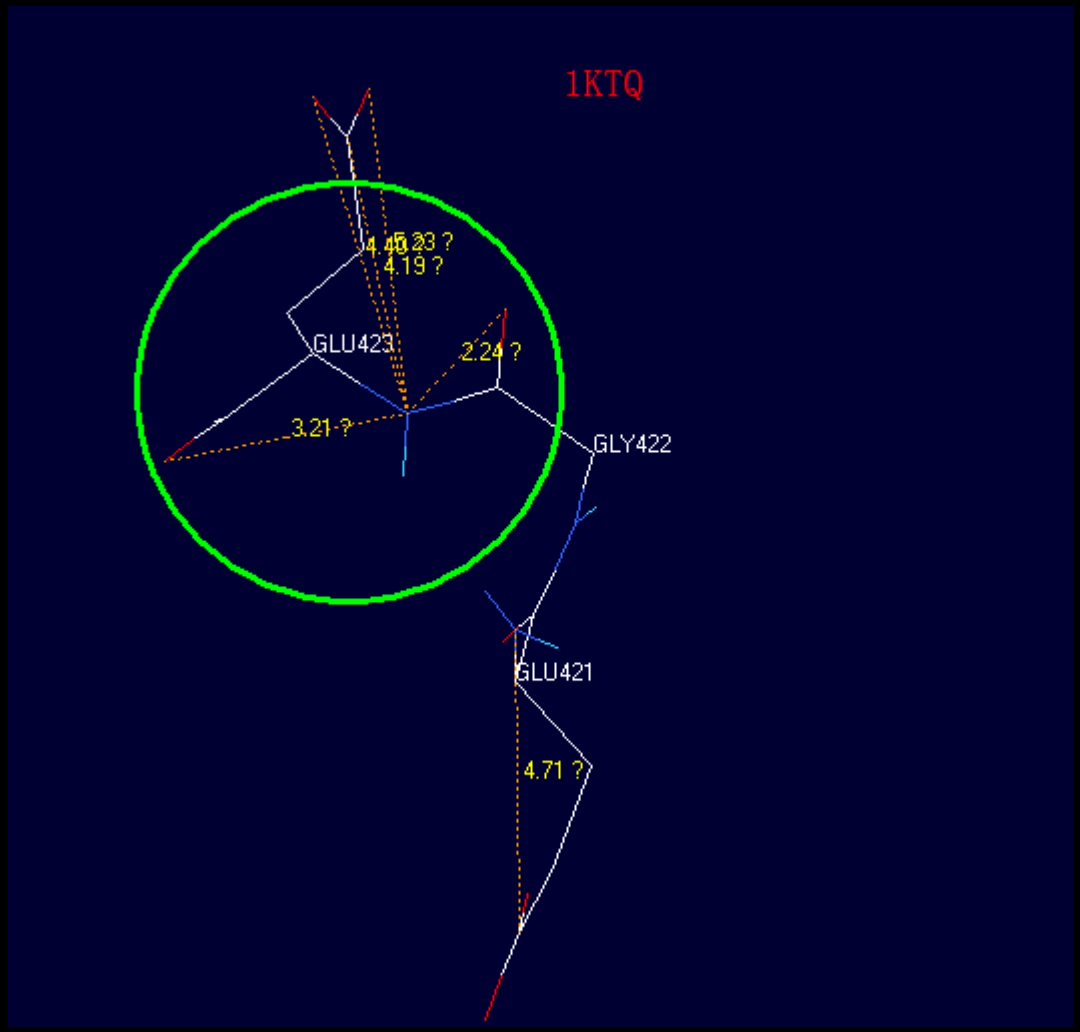

SCOP Exonuclease domain of prokaryotic DNA polymerase (d1klna1)
DSSP
PDB VISYDNYVTILDEETLKAWIAKLEKAPVFAFATETDSLDNISANLVGLSFAIEPGVAAYI
PDB 324 330 340 350 360 370 380 383
SCOP Exonuclease domain of prokaryotic DNA polymerase (d1klna1)
DSSP
PDB PVAHDYLDAPDQISRERALELLKPLLEDEKALKVGQNLKYDRGILANYGIELRGIAFDTM
PDB 384 390 400 410 420 430 440 443
SCOP Exonuclease domain of prokaryotic DNA polymerase (d1klna1)
DSSP
PDB LESYILNSVAGRHDMDSLAERWLKHKTITFEEIAGKGKNQLTFNQIALEEAGRYAAEDAD
PDB 444 450 460 470 480 490 500 503
SCOP Exonuclease dom... DNA polymerase I (Klenow fragment) (d1klna2)
DSSP
PDB VTLQLHLKMVPDLQKHKGPLNVFENIEMPLVPVLSRIERNGVKIDPKVLHNHSEELTLRL
PDB 504 510 520 530 540 550 560 563
SCOP DNA polymerase I (K... DNA polymerase I... DNA polymerase ...
DSSP
PDB AELEKKAHEIAGEEFNLSSTKQLQTILFEKQGIKPLKKTPGGAPSTSEEVLEELALDYPL
PDB 564 570 580 590 600 610 620 623
SCOP DNA polymerase I (Klenow fragment) (d1klna2)
DSSP
PDB PKVILEYRGLAKLKSTYTDKLPLMINPKTGRVHTSYHQAVTATGRLSSTDPNLQNIPVRN
PDB 624 630 640 650 660 670 680 683
SCOP DNA polymerase I (Klenow fragment) (d1klna2)
DSSP
PDB EEGRRIRQAFIAPEDYVIVSADYSQIELRIMAHLSRDKGLLTAFAEGKDIHRATAAEVFG
PDB 684 690 700 710 720 730 740 743
SCOP DNA polymerase I (Klenow fragment) (d1klna2)
DSSP
PDB LPLETVTSEQRRSAKAINFGLIYGMSAFGLARQLNIPRKEAQKYMDLYFERYPGVLEYME
PDB 744 750 760 770 780 790 800 803
SCOP DNA polymerase I (Klenow fragment) (d1klna2)
DSSP
PDB RTRAQAKEQGYVETLDGRRLYLPDIKSSNGARRAAAERAAINAPMQGTAADIIKRAMIAV
PDB 804 810 820 830 840 850 860 863
SCOP DNA polymerase I (Klenow fragment) (d1klna2)
DSSP
PDB DAWLQAEQPRVRMIMQVHDELVFEVHKDDVDAVAKQIHQLMENCTRLDVPLLVEVGSGEN
PDB 864 870 880 890 900 910 920 923
SCOP
DSSP
PDB WDQAH
PDB 924 928


SCOP
Exonuclease domain of prokaryotic DNA polymerase (d1ktqa1)
DSSP
PDB S P K A L E E A P W P P P E G A F V G F V L S R K E P M W A D L L A L A A A R G G R V H R A P E P Y K A L R D L K E A R
PDB 290 300 310 320 330 340 349
SCOP
Exonuclease domain of prokaryotic DNA polymerase (d1ktqa1)
DSSP
PDB G L L A K D L S V L A L R E G L G L P P G D D P M L L A Y L L D P S N T T P E G V A R R Y G G E W T E E A G E R A A L S
PDB 350 360 370 380 390 400 409
SCOP
Exonuclease d...
DNA polymerase I (Klenow fragment) (d1ktqa2)
DSSP
PDB E R L F A N L W G R L E G E E R L L W L Y R E V E R P L S A V L A H M E A T G V R L D V A Y L R A L S L E V A E E I A R
PDB 410 420 430 440 450 460 469
SCOP
DNA polymerase I (Klenow fragment)...
DNA polymerase I ...
DSSP
PDB L E A E V F R L A G H P F N L N S R D Q L E R V L F D E L G L P A I G K T E K T G K R S T S A A V L E A L R E A H P I V
PDB 470 480 490 500 520 529
SCOP
DNA polymerase I (Klenow fragment) (d1ktqa2)
DSSP
PDB E K I L Q Y R E L T K L K S T Y I D P L P D L I H P R T G R L H T R F N Q T A T A T G R L S S S D P N L Q N I P V R T P
PDB 530 540 550 560 570 580 589
SCOP
DNA polymerase I (Klenow fragment) (d1ktqa2)
DSSP
PDB L G Q R I R R A F I A E E G W L L V A L D Y S Q I E L R V L A H L S G D E N L I R V F Q E G R D I H T E T A S W M F G V
PDB 590 600 610 620 630 640 649
SCOP
DNA polymerase I (Klenow fragment) (d1ktqa2)
DSSP
PDB P R E A V D P L M R R A A K T I N F G V L Y G M S A H R L S Q E L A I P Y E E A Q A F I E R Y F Q S F P K V R A W I E K
PDB 650 660 670 680 690 700 709
SCOP
DNA polymerase I (Klenow fragment) (d1ktqa2)
DSSP
PDB T L E E G R R R G Y V E T L F G R R R Y V P D L E A R V K S V R E A A E R M A F N M P V Q G T A A D L M K L A M V K L F
PDB 710 720 730 740 750 760 769
SCOP
DNA polymerase I (Klenow fragment) (d1ktqa2)
DSSP
PDB P R L E E M G A R M L L Q V H D E L V L E A P K E R A E A V A R L A K E V M E G V Y P L A V P L E V E V G I G E D W L S
PDB 770 780 790 800 810 820 829
SCOP
DSSP
PDB A K E
PDB 830

1KLN中只有一个可以形成盐键，且原子之间距离由于His的空间位阻变得较大



1KTQ有2个可以形成盐键，且原子之间距离都相对较小



Our conclusion

- Amino acid composition
- Amino acids change to form hydrophobic core
- Hydrogen bond
- Salt bridges

Reference

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Acknowledgements

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Thank you !