



TELOMERASE

Sílvia Castany, Helena Costa i Paula Garcia

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- Telomerase

2. TERT

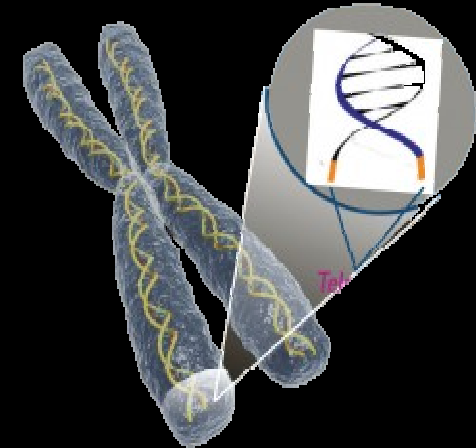
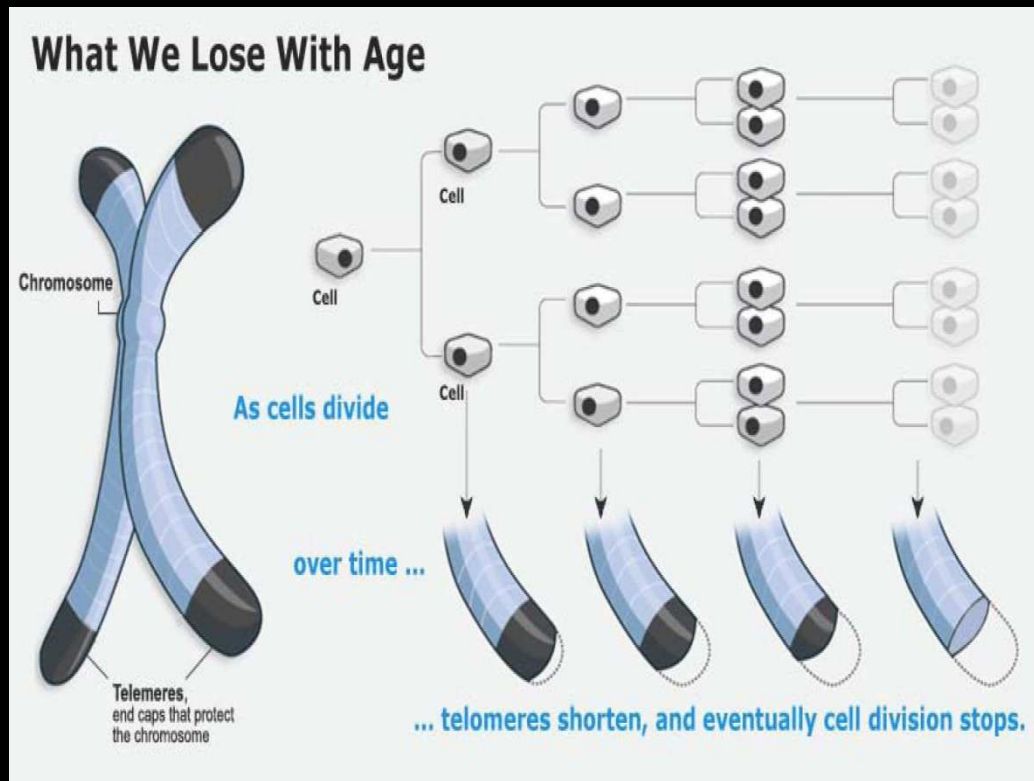
- N-TER, TRBD, RT and CTE domains
- TERT with RNA+DNA hybrid - TERT free superimposition

3. TER

- Pseudoknot/template, CR4/5, H/ACA box and CR7 domains
- Vertebrate TER comparison

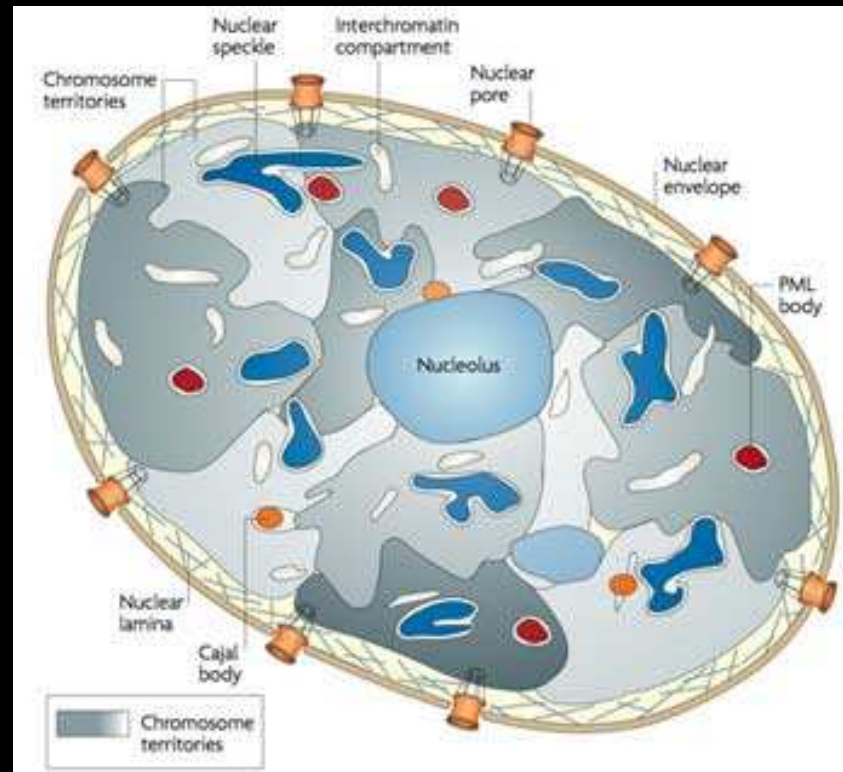
Introduction: **telomeres**

Telomeres: protective DNA-protein complex



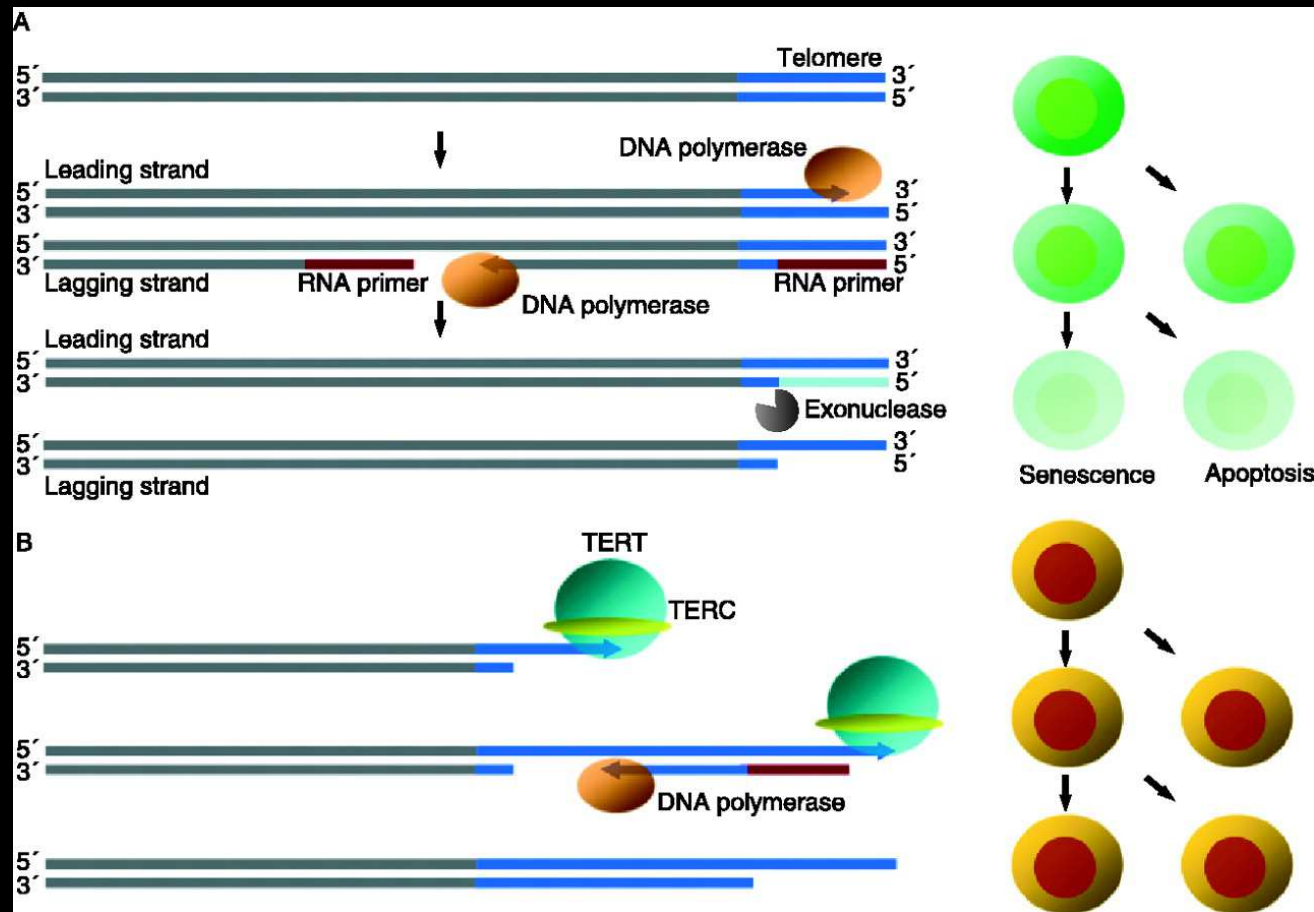
Introduction: telomeres

Telomere replication takes place in Cajal Bodies



Introduction: telomeres

DNA duplication is incomplete → shortening of chromosomes
Telomerase adds telomeric DNA repeats on ends to solve the problem

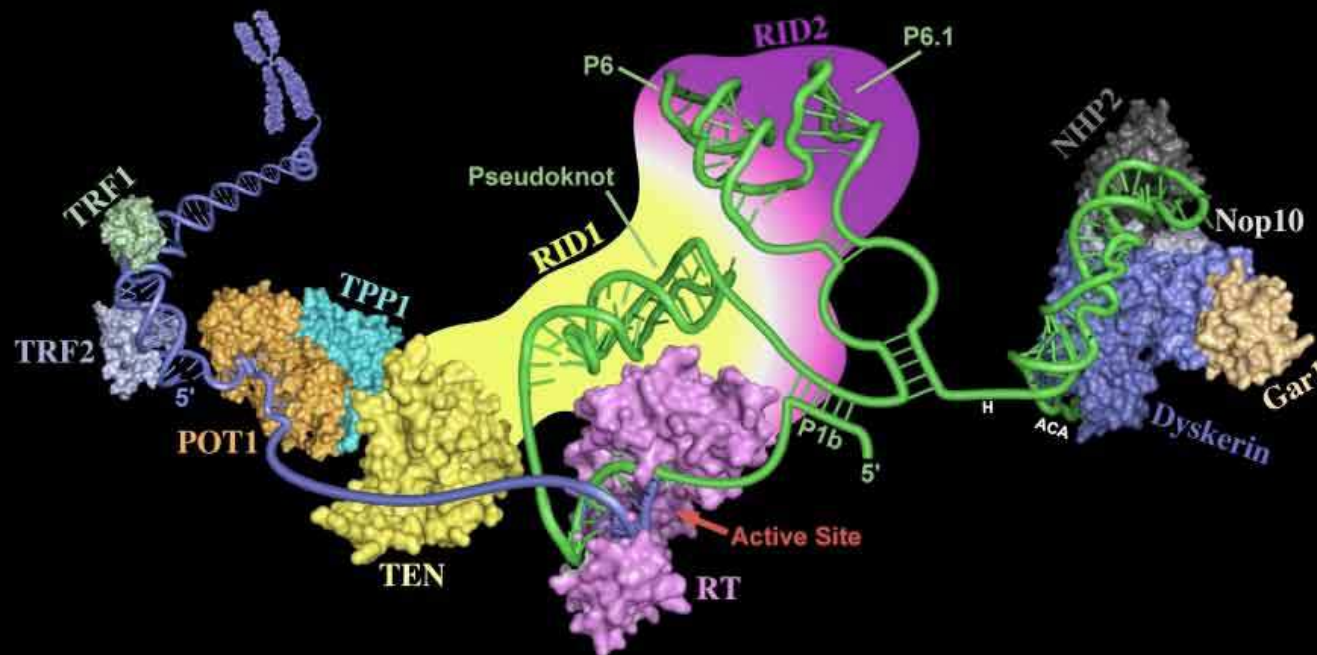


Introduction: **telomerase**

Telomerase functions as a ribonucleoprotein

1. **TERT** (telomerase reverse transcriptase) → Catalytic site for DNA synthesis
Assembles with TER
1. **TER/TR** (RNA) → Provides the template

Accessory proteins play crucial roles in telomerase biogenesis, localization and regulation.



Introduction: **telomerase reaction**

- a) Synthesis of two telomere repeats onto 3' end of telomeric DNA primer.
- b) DNA-pol can extend the previous 5' end of DNA → Shortening problem solved

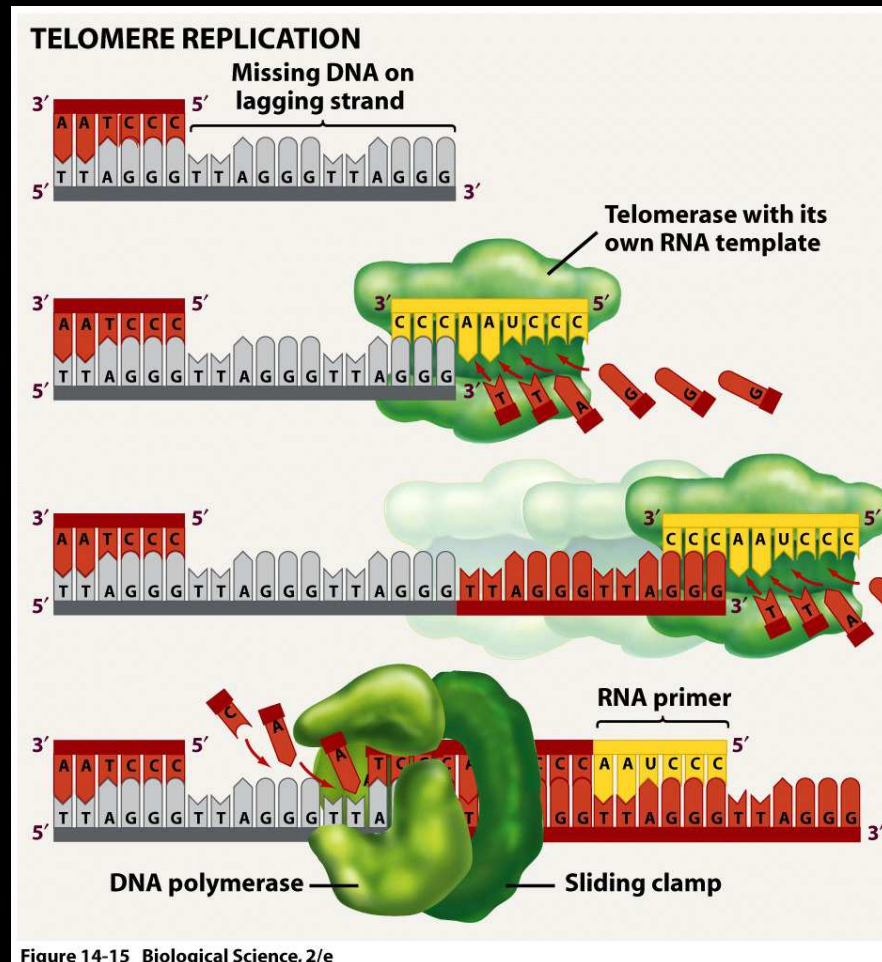
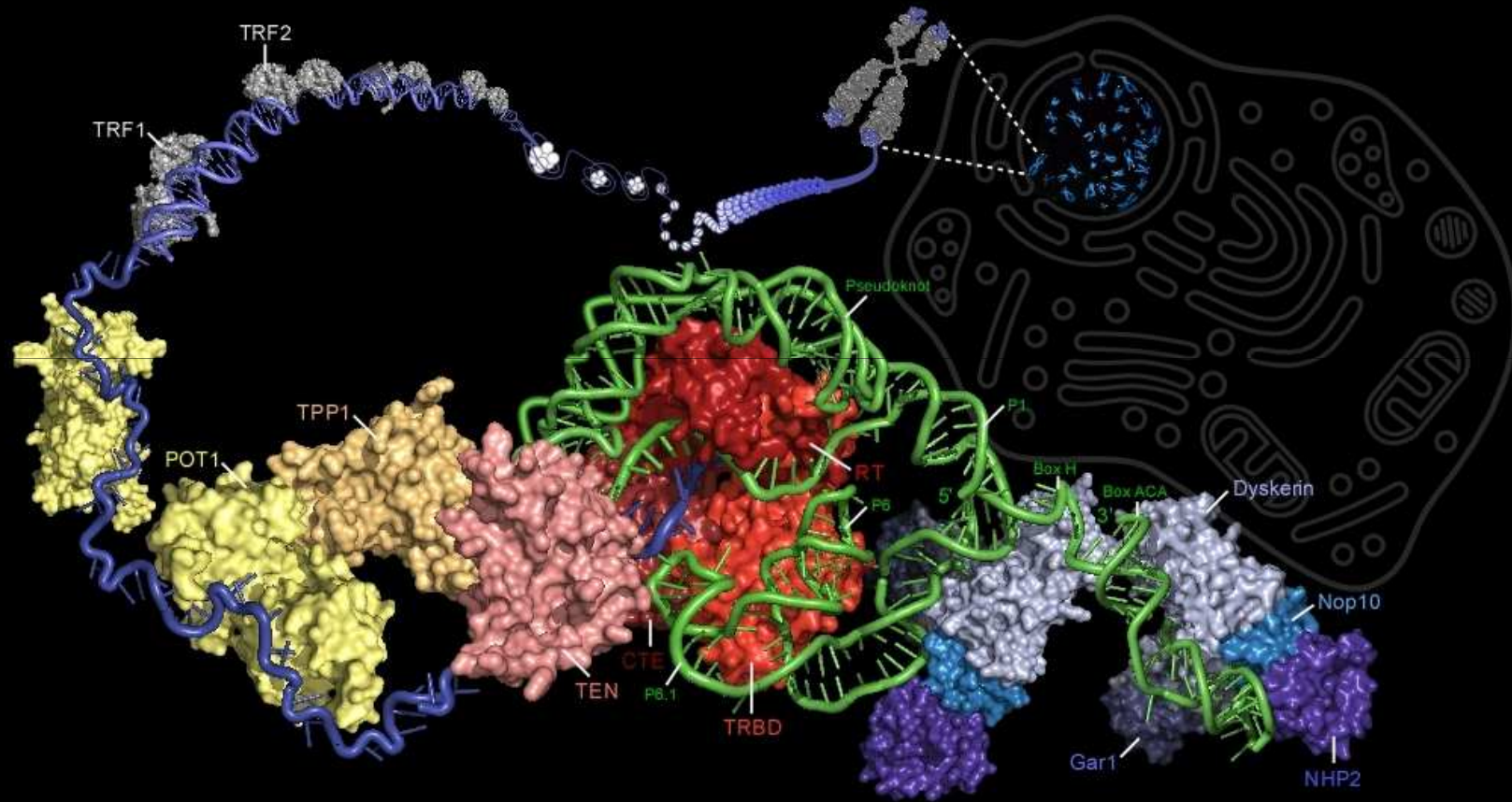


Figure 14-15 Biological Science, 2/e

Introduction: telomerase



Catalytic subunit of telomerase, **TERT**

Catalytic subunit of telomerase, **TERT**

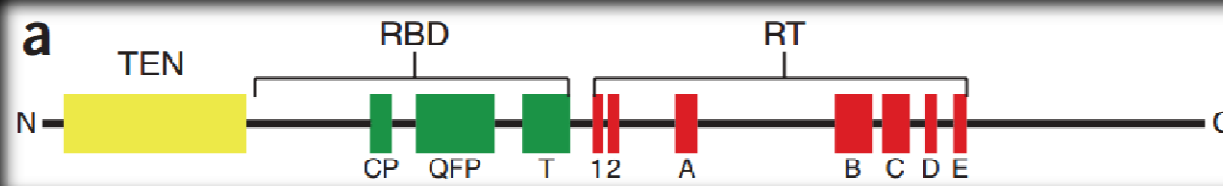
TERT domains

- Nt
- TRBD
- RT
- CTE

Tetrahymena thermophila

Tribolium castaneum

Tetrahymena thermophila N-terminal domain

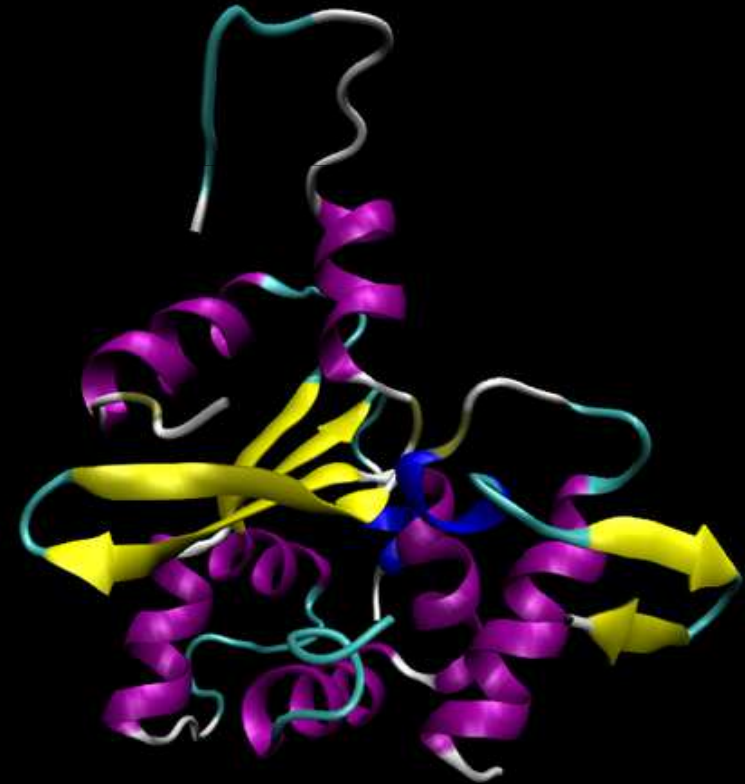


Functions

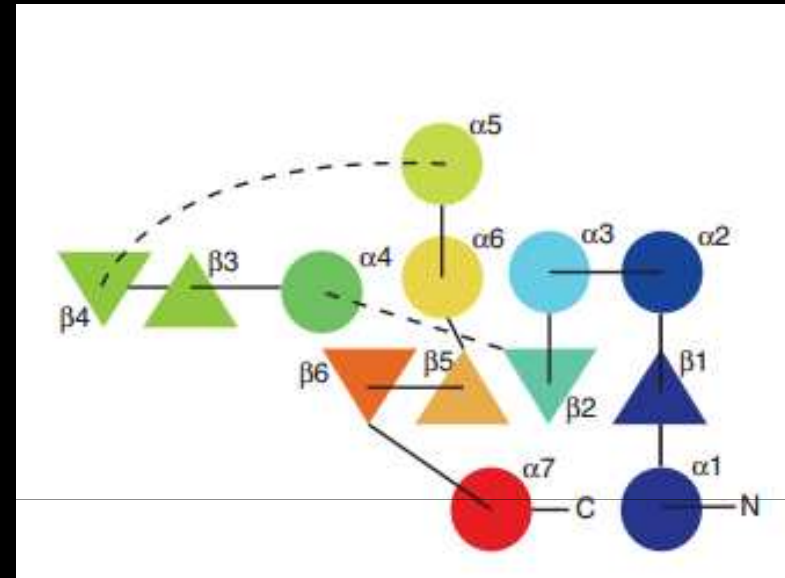
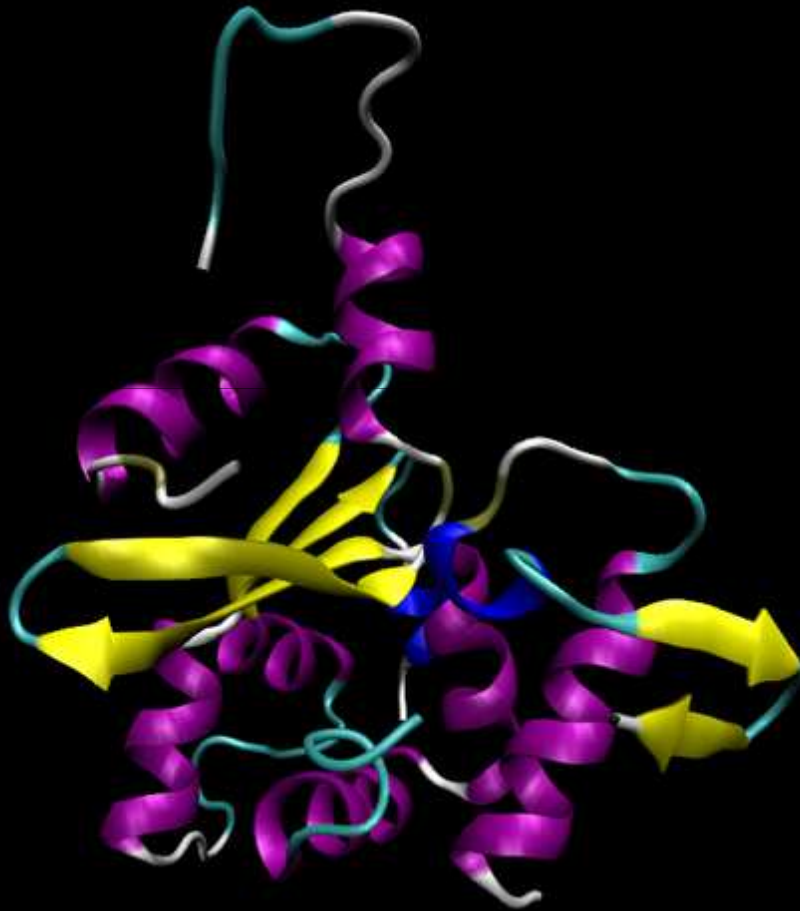
- Providing anchor site for telomeric DNA
- Involved in binding the RNA subunit
- Recruiting some accessory protein into complex

Mixed $\alpha\beta$ structure

- 4 β -sheet
- 7 α -helices
- short β hairpin



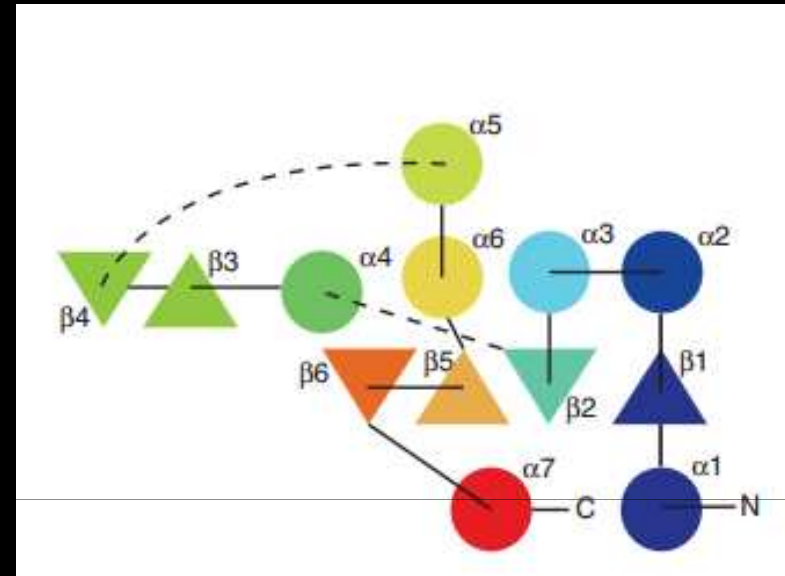
Tetrahymena thermophila N-terminal domain



N-terminal region
(α1, α2, α3, β1, β2)

C-terminal region
(α4, α5, α6, α7, β2, β3, β4, β5, β7)

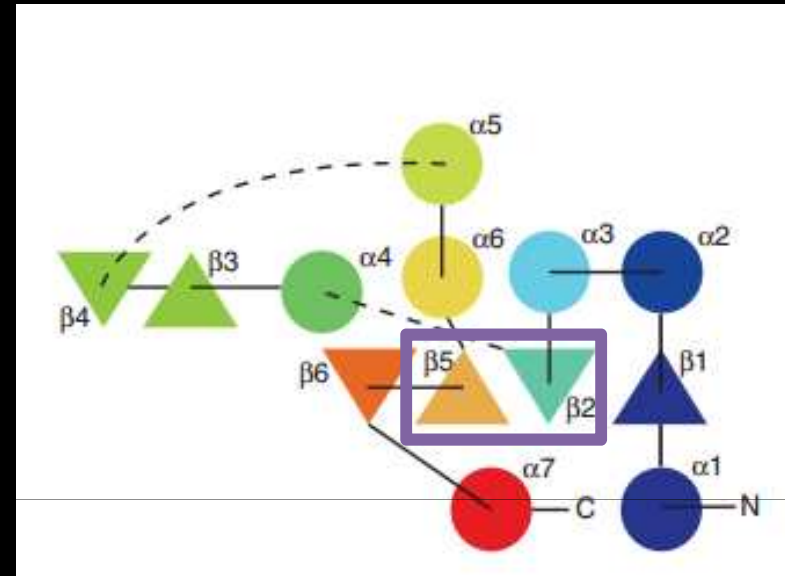
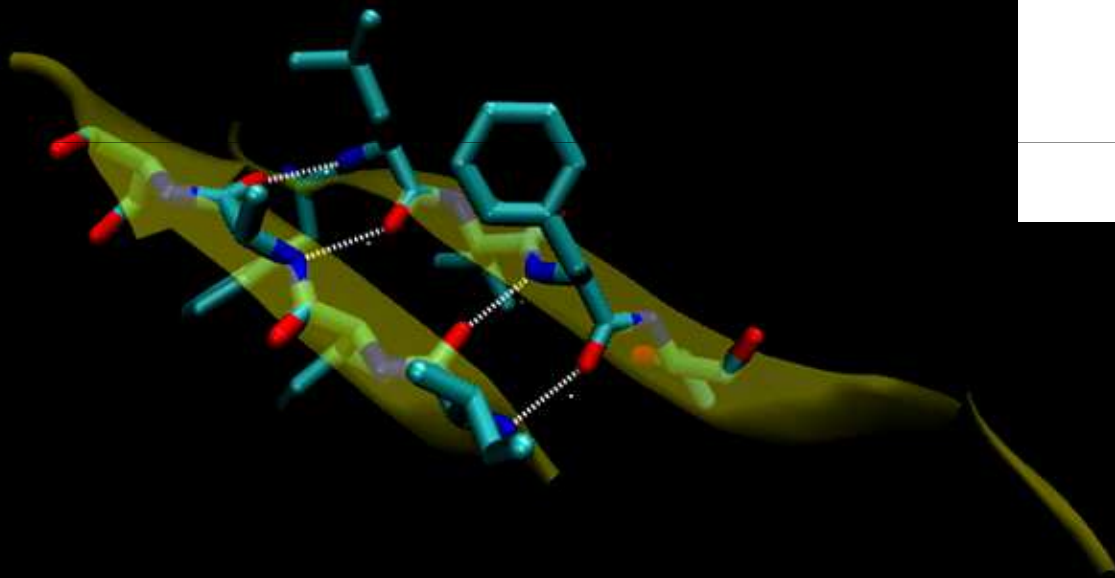
Tetrahymena thermophila N-terminal domain



N-terminal region
(α1, α2, α3, β1, β2)

C-terminal region
(α4, α5 α6 , α7, β2, β3, β4, β5, β7)

Tetrahymena thermophila N-terminal domain

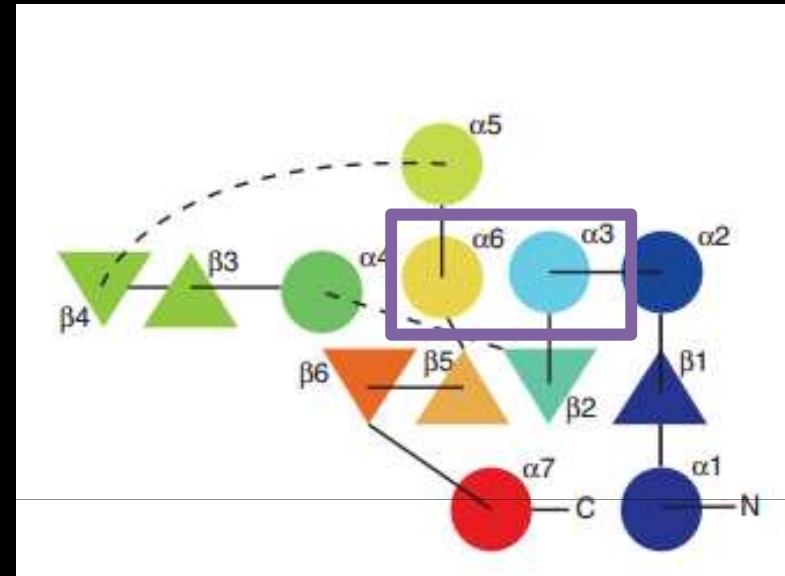
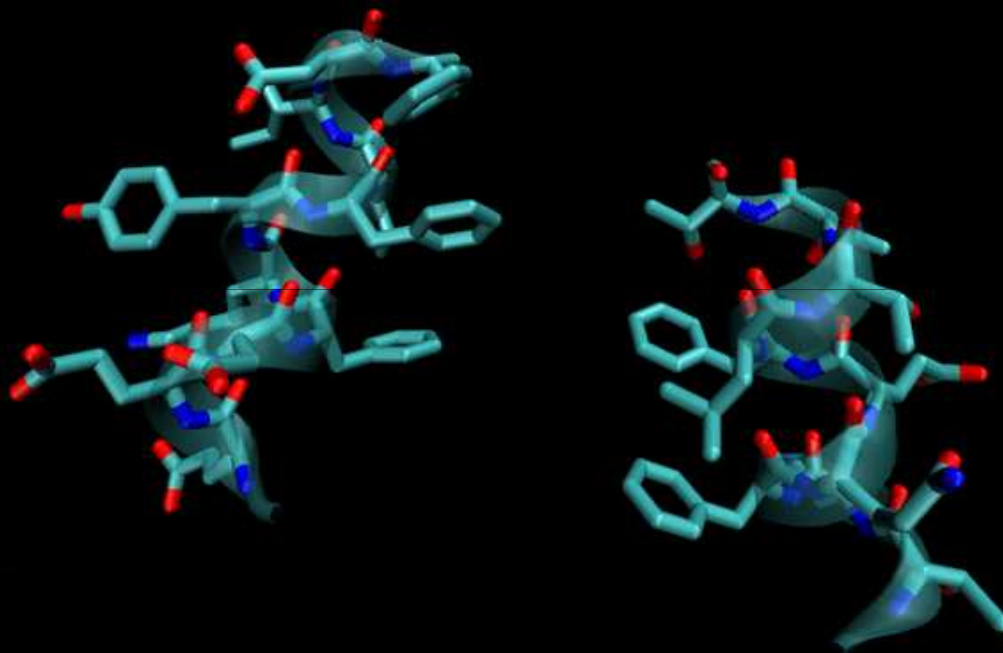


$\beta 2 \rightarrow \beta 5$

$\alpha 3 \rightarrow \alpha 6$

$\alpha 2 \rightarrow \alpha 5$

Tetrahymena thermophila N-terminal domain

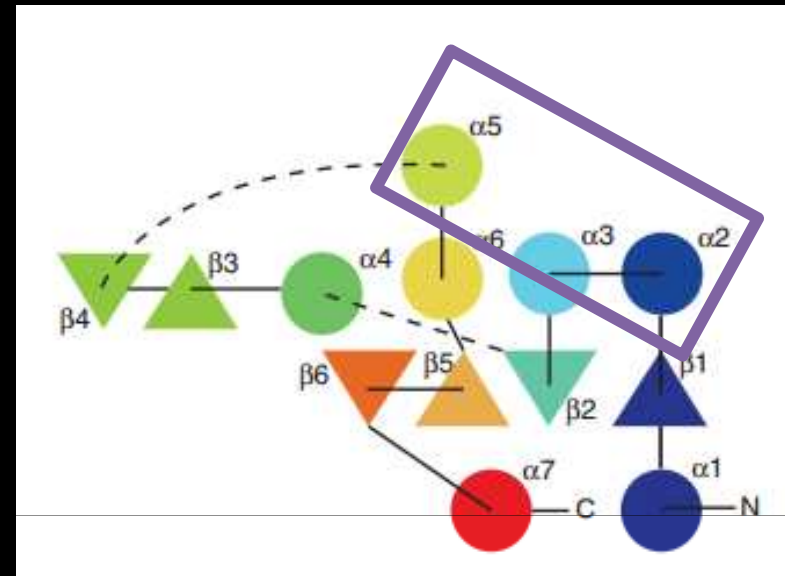
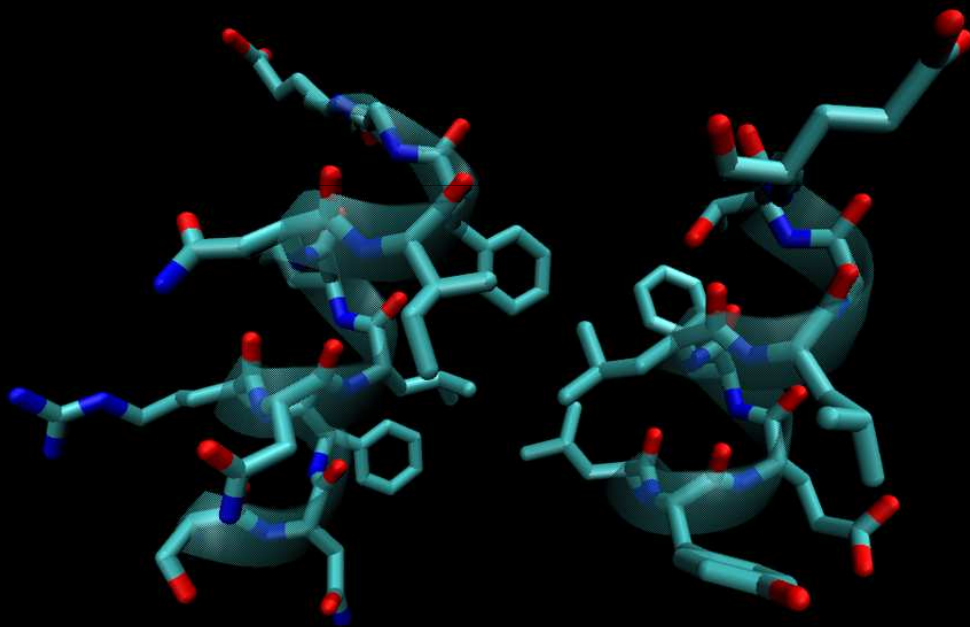


$\beta 2 \rightarrow \beta 5$

$\alpha 3 \rightarrow \alpha 6$

$\alpha 2 \rightarrow \alpha 5$

Tetrahymena thermophila N-terminal domain



$\beta 2 \rightarrow \beta 5$

$\alpha 3 \rightarrow \alpha 6$

$\alpha 2 \rightarrow \alpha 5$

Tetrahymena thermophila N-terminal domain

CLUSTAL W(1.60) multiple sequence alignment



Domain strongly conserved
<20% sequence identity
Structural conservation

Conserved residues

Gly144
Gln168
Gly171

Tetrahymena thermophila **N-terminal** domain



Domain strongly conserved
<20% sequence identity
Structural conservation

Conserved residues

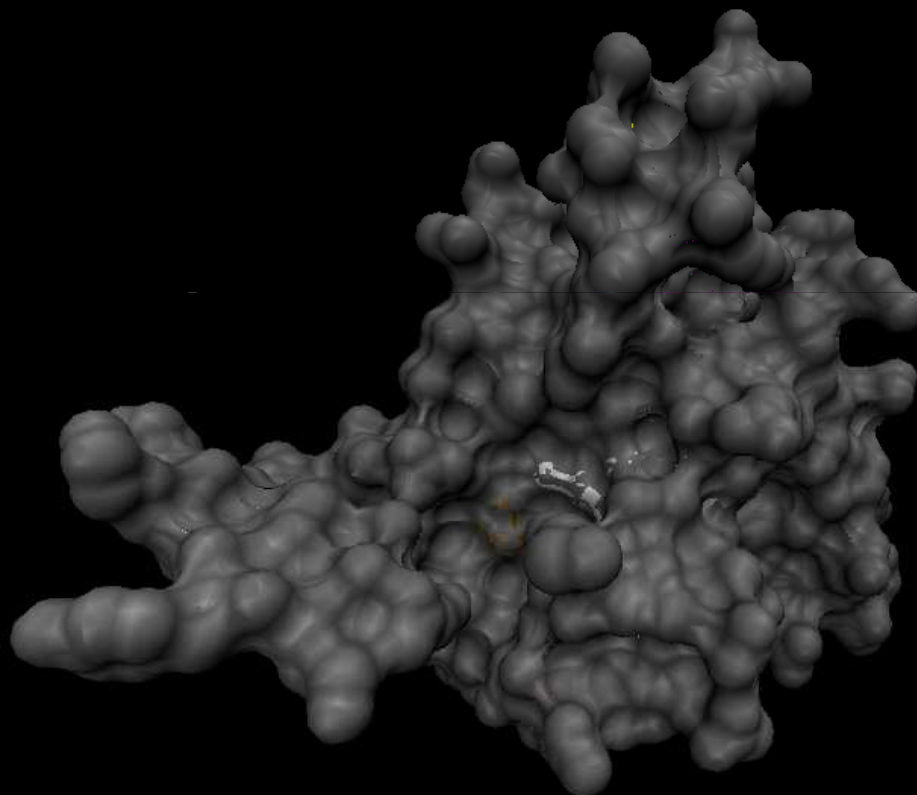
Gly144 → $\alpha 5$ and $\alpha 6$

Gln168

Gly171 → $\beta 6$ and $\alpha 7$

Tetrahymena thermophila **N-terminal** domain

“Deep groove”



Phe158

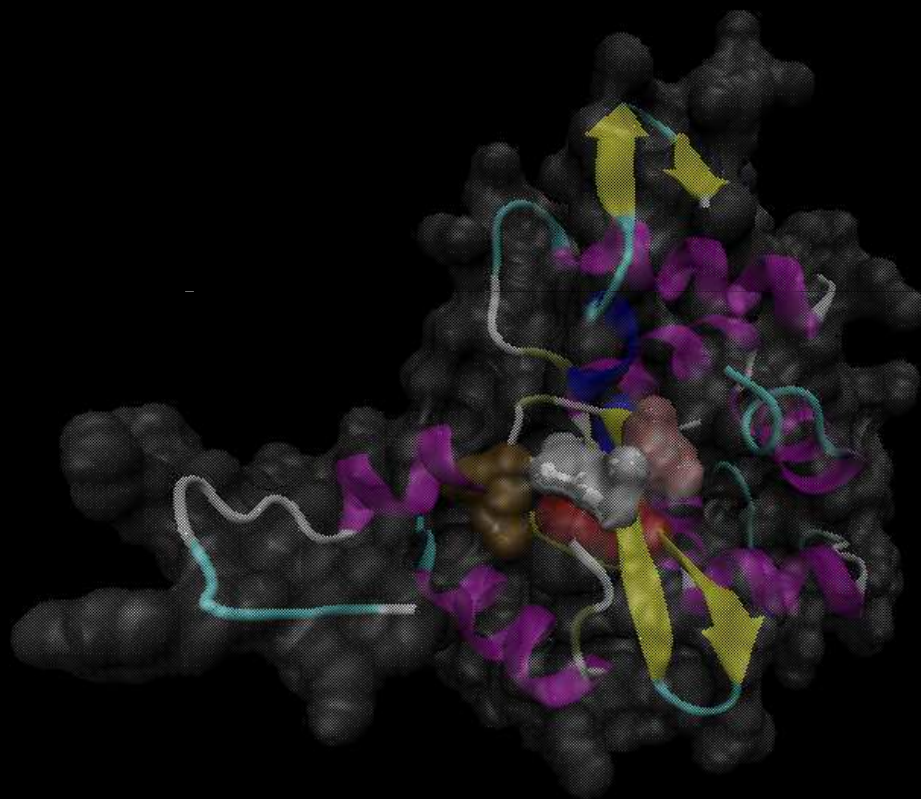
Gln168

Leu174

Val169

Tetrahymena thermophila N-terminal domain

“Deep groove”



Phe158

Gln168

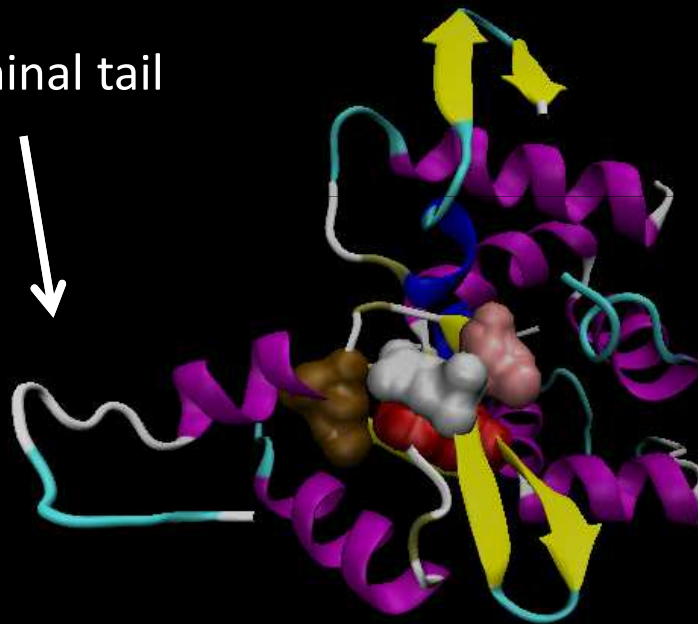
Leu174

Val169

Tetrahymena thermophila N-terminal domain

“Deep groove”

C terminal tail



Phe158

Gln168

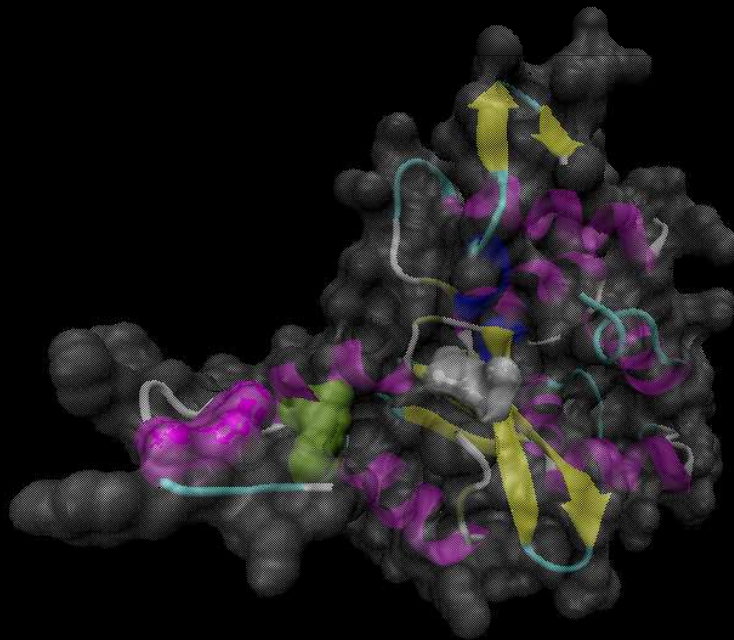
Leu174

Val169

Tetrahymena thermophila N-terminal domain

Functions

- Providing anchor site for telomeric DNA
- Involved in binding of the RNA subunit
- Recruiting some accessory protein into complex



Gln168

Leu178

Trp187

Tetrahymena thermophila N-terminal domain

Functions

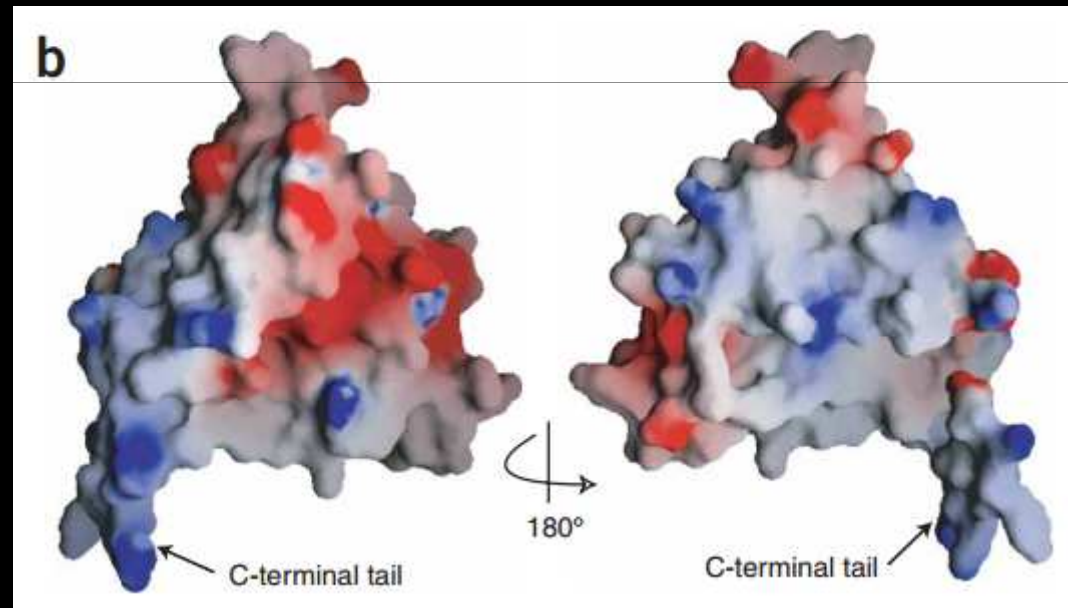
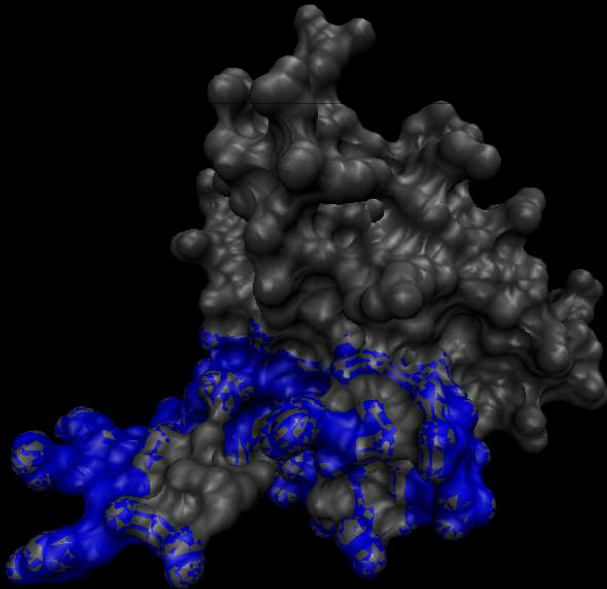
-Providing anchor site for telomeric DNA

-Involved in binding of the RNA subunit

- Recruiting some accesory protein into complex

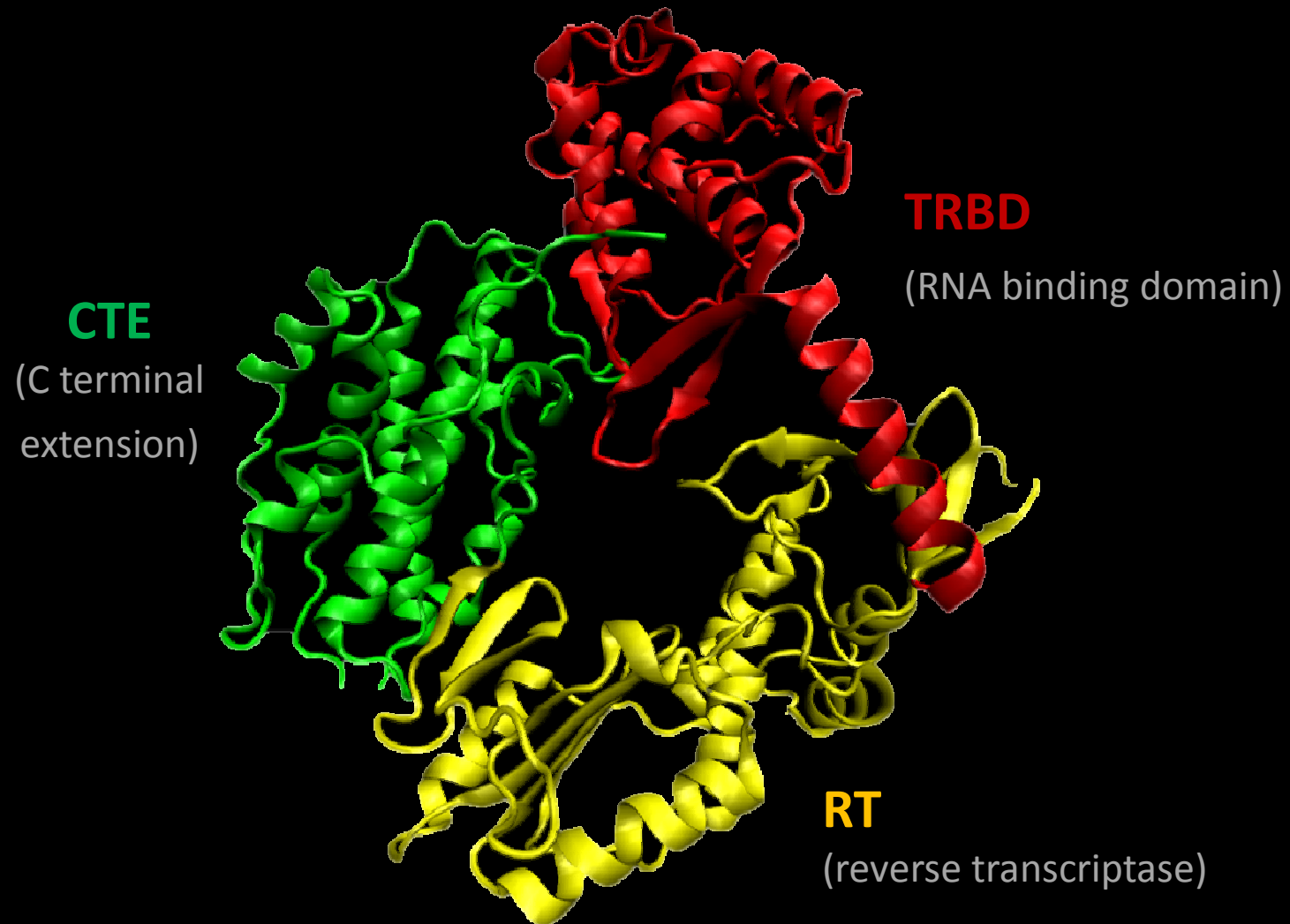


Low affinity protein – RNA interaction domain



Tribolium castaneum catalytic subunit of telomerase, **TERT**

3 highly conserved domains, organized into a ring-like structure

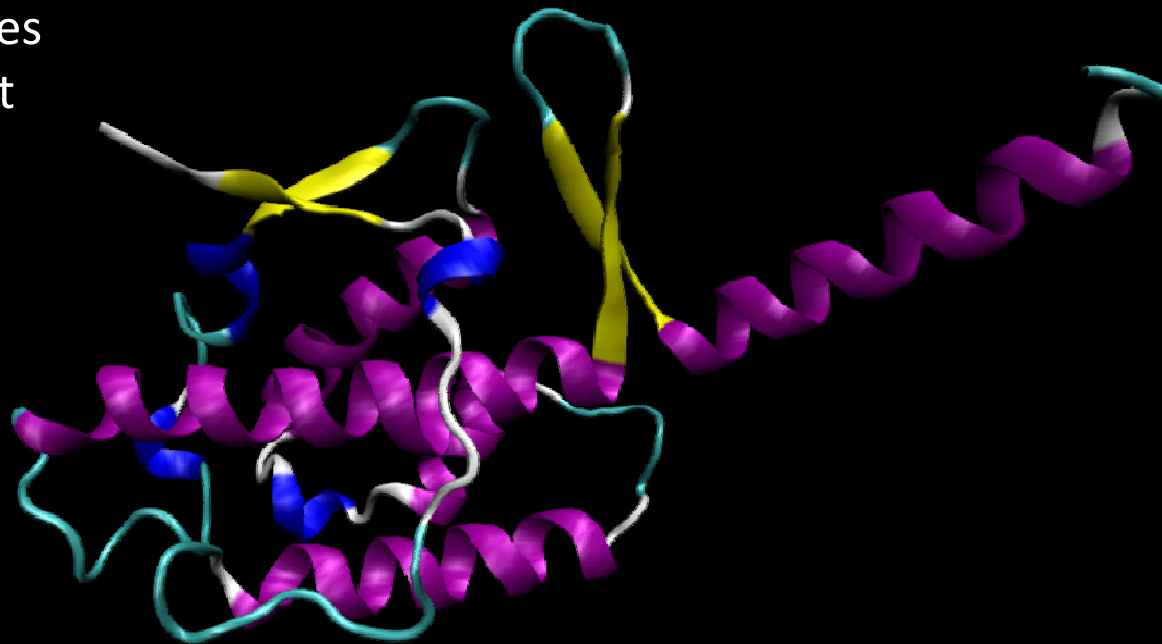


Tribolium castaneum RNA binding domain, **TRBD**

TRBD interactions with TBE are important for proper assembly and stabilization of the TERT/TER complex → promotes repeat addition processivity

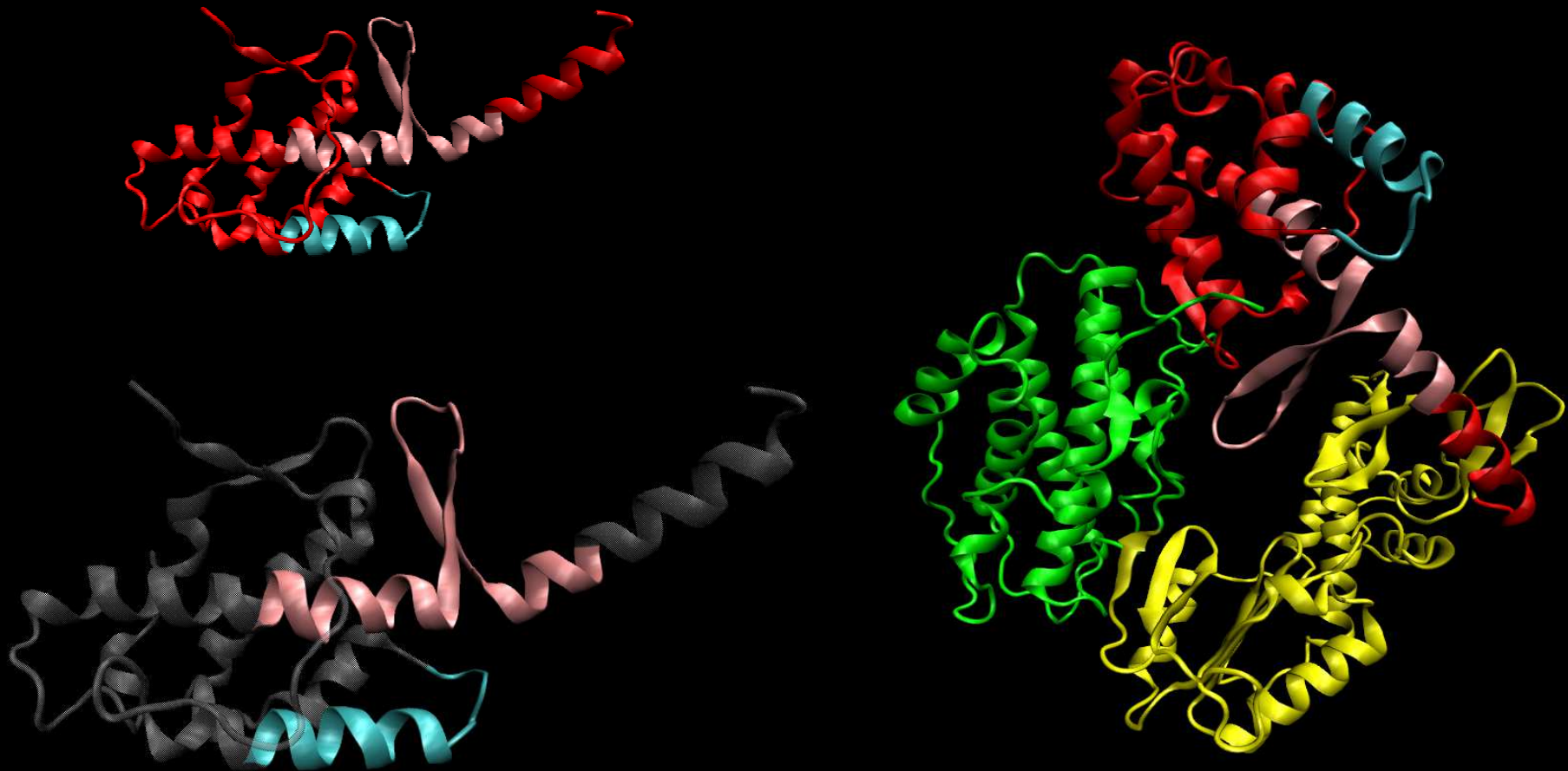
Mostly helical

- 9 α helices
- 4 β -sheet



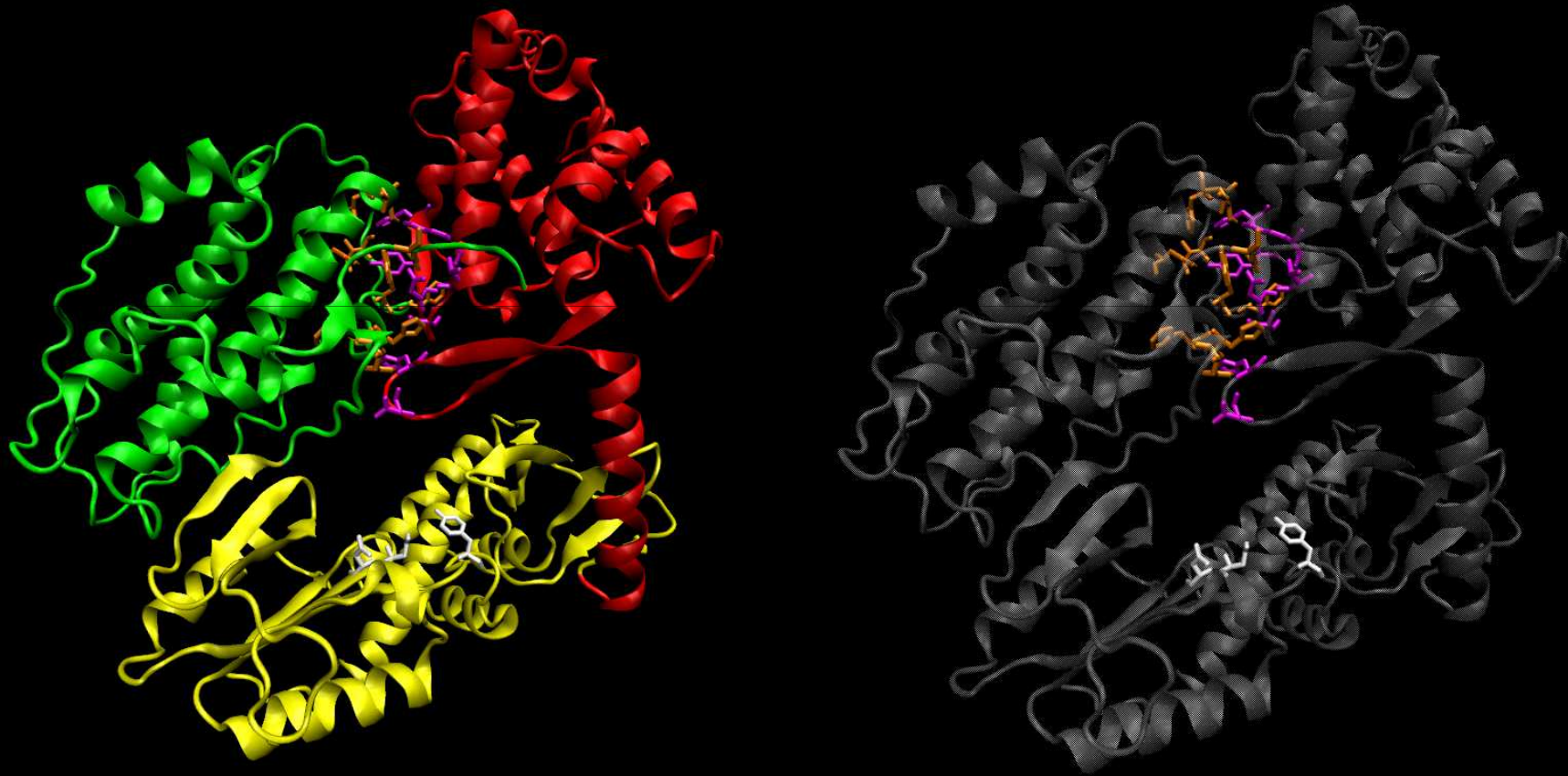
Tribolium castaneum RNA binding domain, **TRBD**

2 conserved motifs on its surface: **CP motif** → binds dsRNA
T motif → binds ssRNA

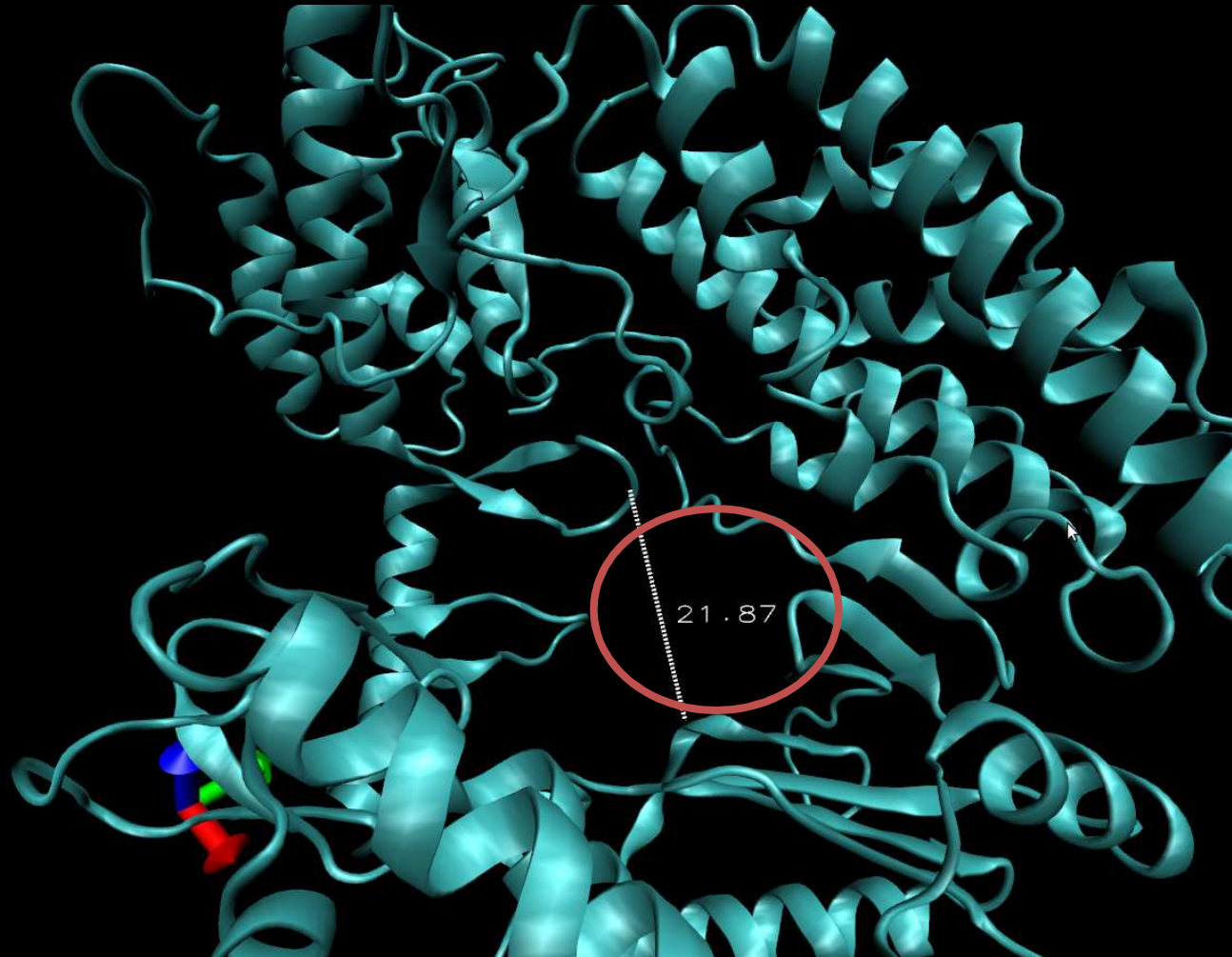


Tribolium castaneum RNA binding domain, **TRBD**

TERT domains arrangement → **TRBD** - **thumb** get close → ring-like structure



Tribolium castaneum RNA binding domain, **TRBD**



Tribolium castaneum RNA binding domain, **TRBD**

T motif β -hairpin makes extensive contacts with thumb loop and motifs 1 and 2



TRBD-RT domain organization forms a deep cavity on the surface of the protein



Placement of RNA template in the interior of the ring
(where the enzyme's active site is located)



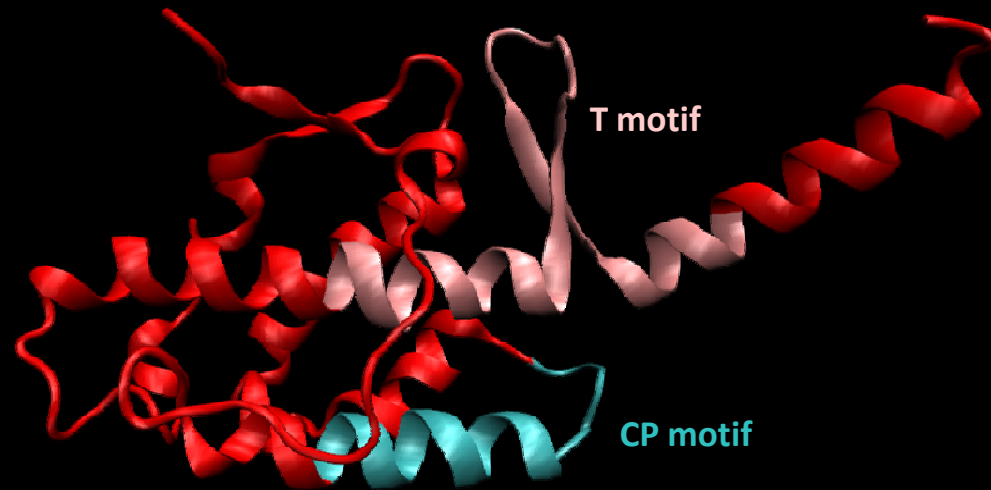
Pairing with incoming DNA substrate → RNA-DNA hybrid
(required for telomere elongation)



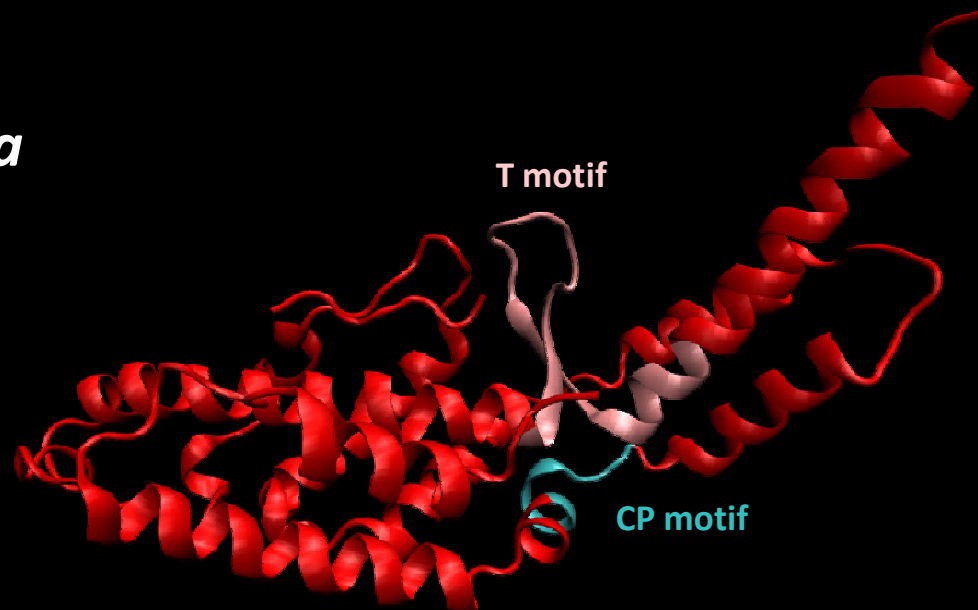
Brings 3' end of DNA primer in proximity to the active site for nucleotide addition

TRBD is a conserved domain

T. castaneum



T. thermophila



TRBD is a conserved domain

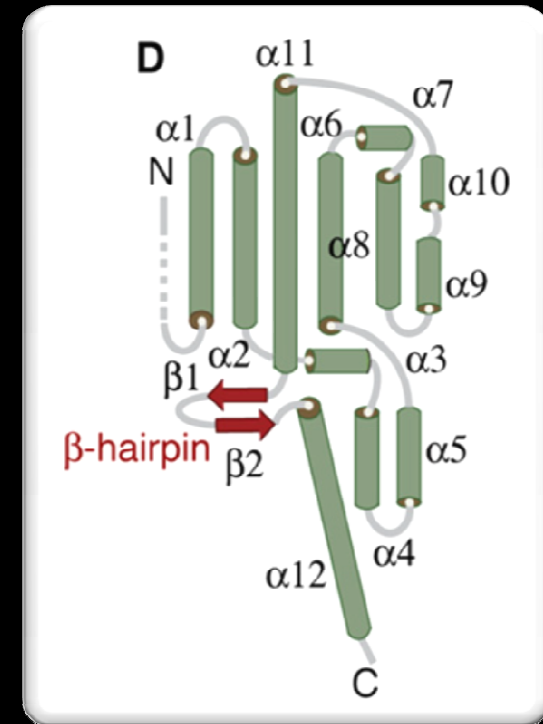
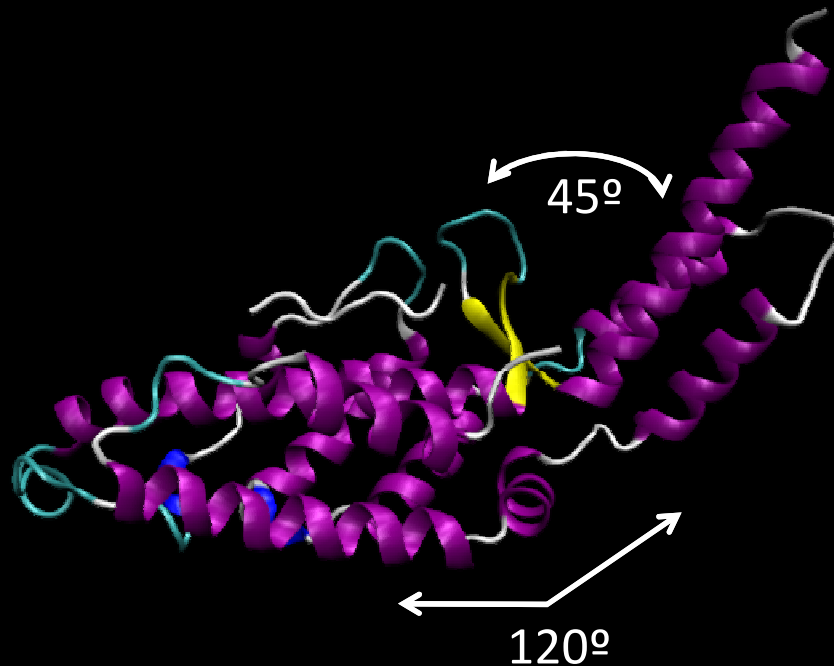
T. CASTANEUM	16	-----PKIVNSKYNSILNIALKNFRLCK---K-----	39
HUMAN	378	--RLPRLPQ---RY-WQMR---PLFLELLGNHAQCPYGVLLKTH--CPLRAAVTPAA-GVG---	426
MOUSE	383	--RTHRLSR---RY-WQMR---PLFQQLLVNHAECQYVRLLRSH--CRFRTANQQVT-D-----	429
A. THALIANA	363	PSGSICL-----Y-HSLL---KSLKNLIGKTKSSHLKMLLDKH--CPVLLLQEDALKSGTTSQ	414
YEAST	285	VSQSTVVPK---RL-LKVY---PLIEQTAKRLHRISLSKVYNHY--CPYIDTH-----	328
S. POMBE	286	KKTLLKEYQSKNFSC-QEERDLFLEFTEKIVQNFHNINFNLYLLKKF--CKLPENYQSLK-S----	342
S.	225	--NLVKIPQ---RLKIRMN---LTLQKLLKRHKRLNYVSILNSI--CPPLEGTVL-----	269
E. AEDICULATUS	291	-----DKVIEKIAYMLEKVKDFNFNYLTKS--CPLPENWRERK-O-----	328
T. THERMOPHYLA	286	KKTLLKEYQSKNFSC-QEERDLFLEFTEKIVQNFHNINFNLYLLKKF--CKLPENYQSLK-S----	342
cons	529		594
CP Motif			
T. CASTANEUM	40	-----HK-TKKPVO---ILALLOEIIPKSYFGTTTNLKR	69
HUMAN	427	--AREKPQGSVAA--PEE-EDTDPR-RLVQLLRQHSSPWQVYGFVRACLRRLVPPGLWGSRHNNRR	486
MOUSE	430	-----A-----LNTSPP-HLMDLLRLHSSPWQVYGFRLACLCVVSASLWGRHNERR	476
A. THALIANA	415	SSRRQKADKLPHGSSSSQTGKPKCP-SVEE-RKLYCTNDQVVSFIWAICRYIVPESLLGTTTHOMRV	478
YEAST	329	-----DDEKILSYSLKPNQVFAFLRSILVRVFPKLIWGNQRIFEI	368
S. POMBE	343	-----QVKQIVQS--ENKANQQSCENLFNSLYDTEISYKQITNFLRQIIQNCVNPQLLGK-KNFKV	400
S.	270	-----DLSHLSROSPKERVLFKIIVILQKLLPOEMFGSKKNKGK	308
E. AEDICULATUS	329	-----KIENLINK--TREEKSKYYEE--LFSYTTDNKCVTQFINEFFYNILPKDFTG-RNRKN	382
T. THERMOPHYLA	343	-----QVKQIVQS--ENKANQQSCENLFNSLYDTEISYKQITNFLRQIIQNCVNPQLLGK-KNFKV	400
cons	595		660
T. CASTANEUM	70	FYKVVEKILTQSSFECHLSVLHKCYDYDAIPWLQNV-----PNLRPKLLLKHNL	120
HUMAN	487	FLRNTKKFISLQGHAKLSLQELTWKMSVRDCAWLRSSPGVG-----CVPA-AEHLREEILAKFLH	546
MOUSE	477	FFKNLKKFISLQGYGKLSLOELMWKMKVEDCHWLSSPGKD-----RVPA-AEHLRERILATFLF	536
A. THALIANA	479	LRKNIAWVFSRRRNEKCTVNQFLHKVKPSDFFPFARKELCCMVNGHELOSESIRSTQMLCTKWIS	544
YEAST	369	ILKDLETFLKLSRYESFSLHYLSNLIKISEIEWLVGKRSH---A-KMCL-SDFEKRKQIFAEFIY	429
S. POMBE	401	FLEKLYEFVOMKRFENQKVLQDYICFMDVFDVEVFDLKNQK-----FTQKRKYISDKRKILGDLIV	461
S.	309	IIKNLNLNLLSLPLNGYLPFDSLKKLRLKDFRWLFISDI-----WFTK-HNFENLNQLAICFIS	366
E. AEDICULATUS	383	FQKKVKKYVELNKHHLHKNLLLEKINTREISWMQVETSAK-----HFYY--FDHENIYVLWKLRL	441
T. THERMOPHYLA	401	FLEKLYEFVQMKRFENQKVLQDYICFMDVFDVEVFDLKNQK-----FTQKRKYISDKRKILGDLIV	461
cons	661		726
T Motif			
T. CASTANEUM	121	FLDNIVKPIIA--FYYKPIKTLNGHEIKFIRKEEYISFESKVFH-KLKMKYLVLEVQD-----	176
HUMAN	547	WLMSVYVVLLRSFFYVTETTFQKNRLFFYRKSVWSKLOSIGIROHLKRV-OLRELSEAEVROHRE	611
MOUSE	537	WLMDTVYVQLLRSSFFYITESTFQKNRLFFYRKSVWSKLOSIGVRQHLERV-RLRELSQEEVRHHQD	601
A. THALIANA	545	WLFLIVKKLVHFNFYATESQGRLNIYYRKRKSWERLISKEISKALDGY-VLVDDAAEAS-----	604
YEAST	430	WLYNSFIIPILQSFFYITESSDLRNRVTYFRKDIWKLRCRPFIT-SMKME-AFEKINENNVRMDT-	492
S. POMBE	462	FIINKIVIPVLRNPFYITEKHKEGSQIFYRKPINKLVSKLTIV-KLEEE-NLEKVEEKLIPED--	523
S.	367	WLFROLIPKIIQTFFYCTEISST-VTIVYFRHDTWNKLITPFIVEYFKT--YLVENNVCRN-HNSY	428
E. AEDICULATUS	442	WIFEDLVVSLIRCFFYVTEOKSYSKTYYYRKNINDVIMKMSIA-DLKKE-TLAEVOEKEVEEWK-	504
T. THERMOPHYLA	462	FIINKIVIPVLRNPFYITEKHKEGSQIFYRKPINKLVSKLTIV-KLEEE-NLEKVEEKLIPED--	523

Tetrahymena thermophila RNA binding domain, **TRBD**

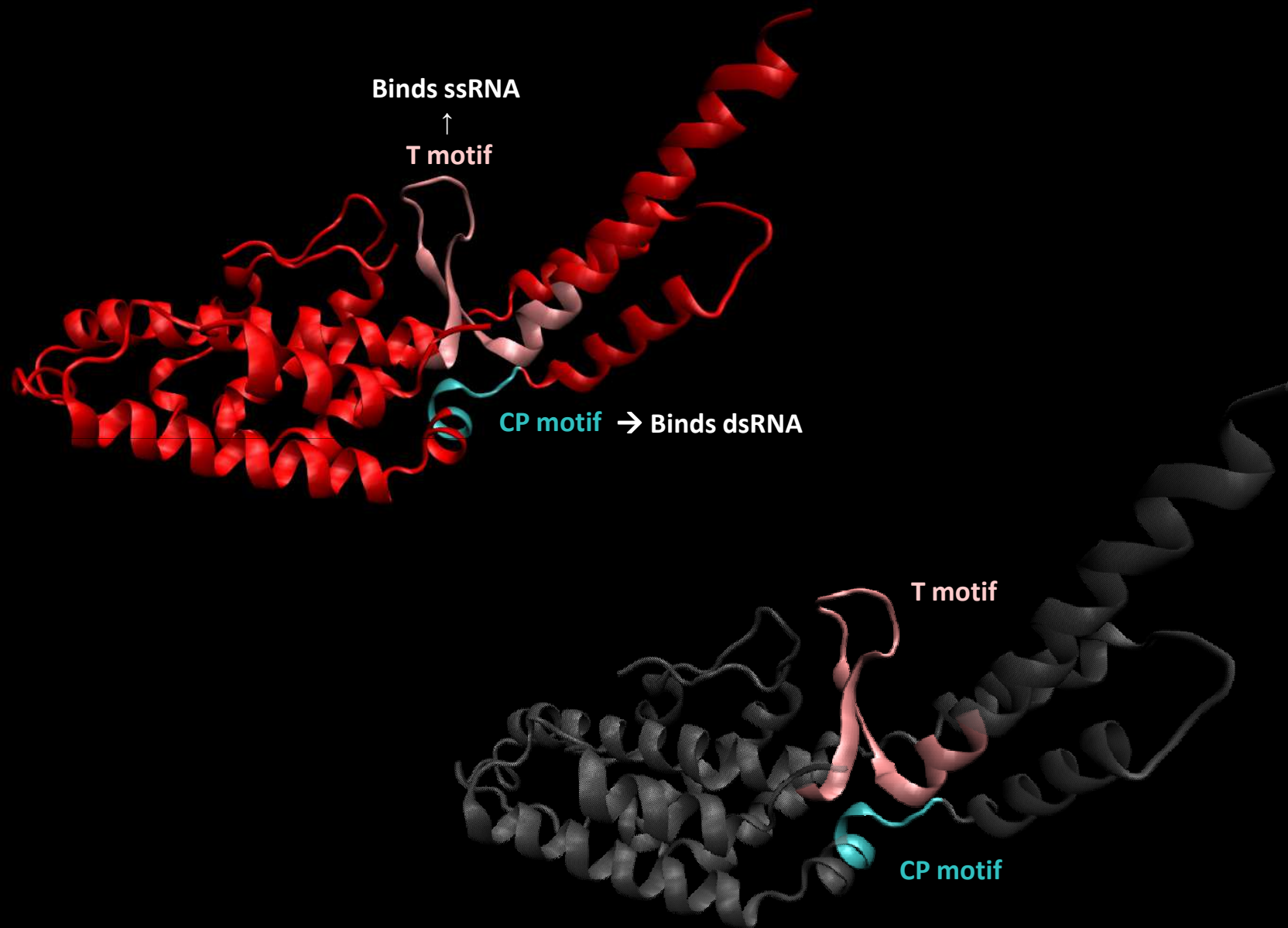
12 α helices linked with loops
2 short β strands (15 residues)

2 asymmetric halves

- Larger $\rightarrow$ 9 α
- Smaller $\rightarrow$ 3 α
 - More flexible (absence of observable contacts with RNA)



Interaction TRBD-TER → CP and T



TRBD motifs

CP motif

$\alpha 3$ and following loop

Shallow, wide (20Å) cavity high positively charged $\rightarrow$ adjacent and beneath T pocket

Several conserved residues buried –

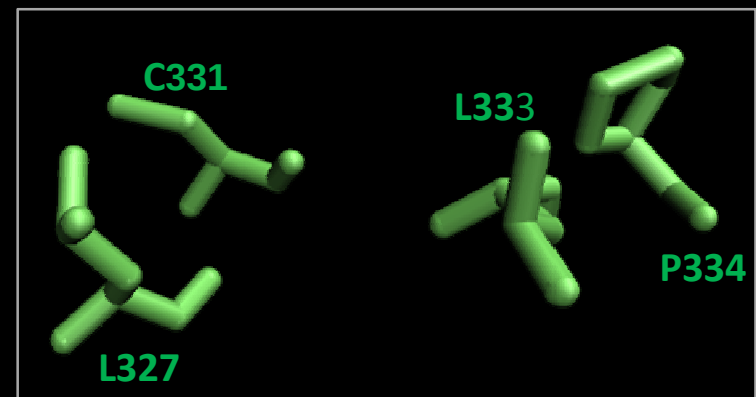
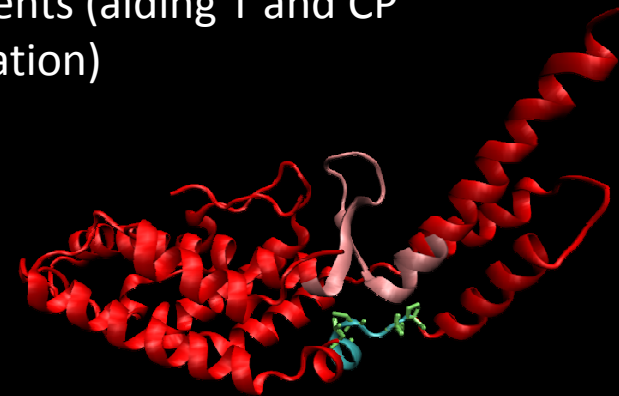
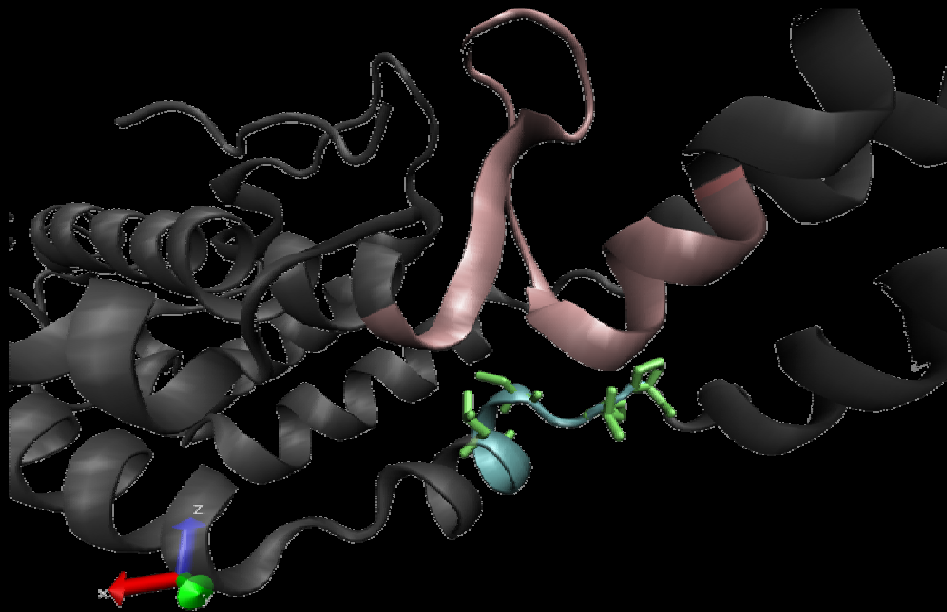
L327

C331

L333

P334

Direct contacts with T
elements (aiding T and CP
formation)



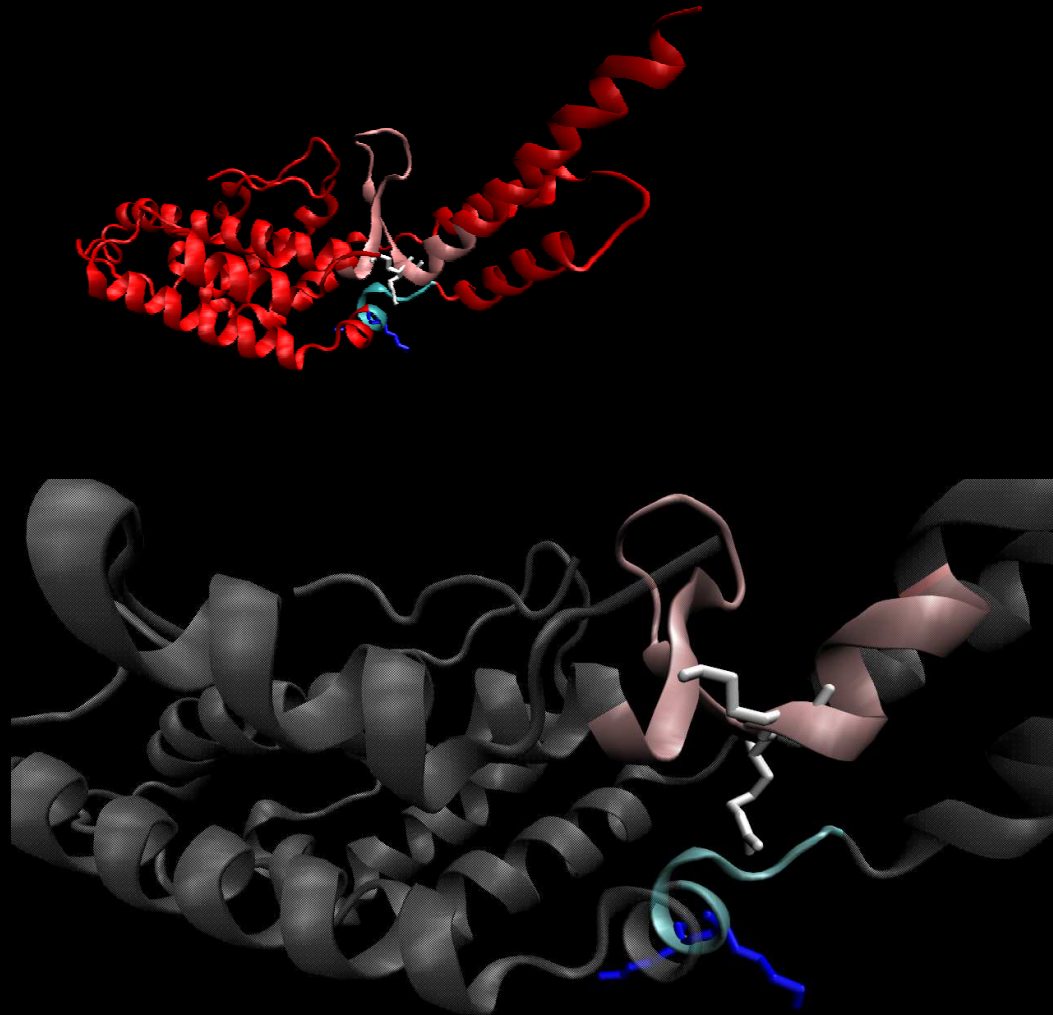
TRBD motifs

CP motif

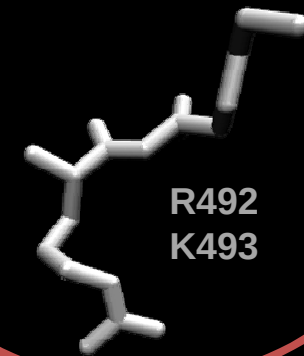
Also several surface-exposed conserved residues (mainly hydrophilic) –

K328
K329

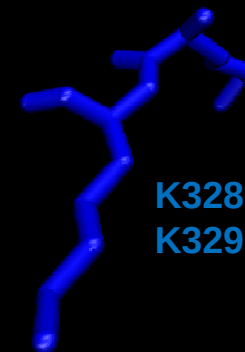
Close to R492 and K493
(form a single large positively
charged surface area)



T motif



R492
K493



K328
K329

TRBD motifs

T motif

In the centre (where the 2 halves meet)

Residues from β -sheet and $\alpha 12 \rightarrow$ Narrow pocket (10Å)

Several solvent-exposed and highly conserved residues

Invariant residues of particular note –

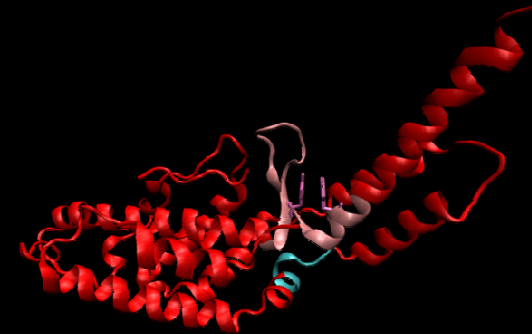
Y477 (β part)

W496 (α part)

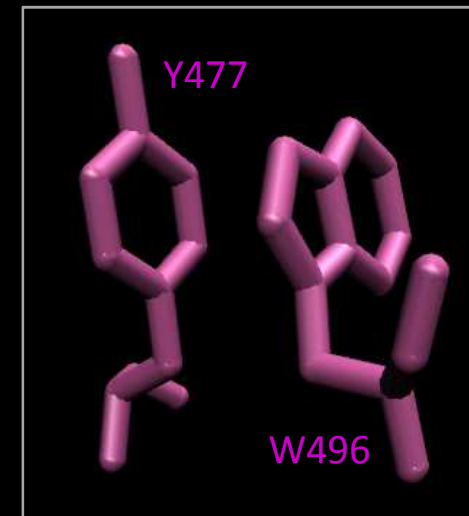
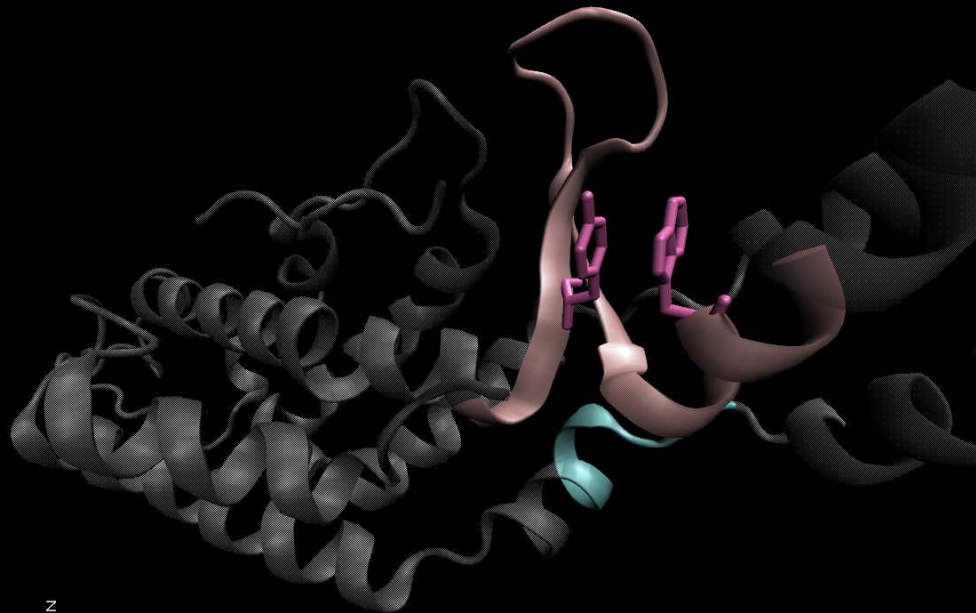
Several hydrophilic residues –

R492

K493



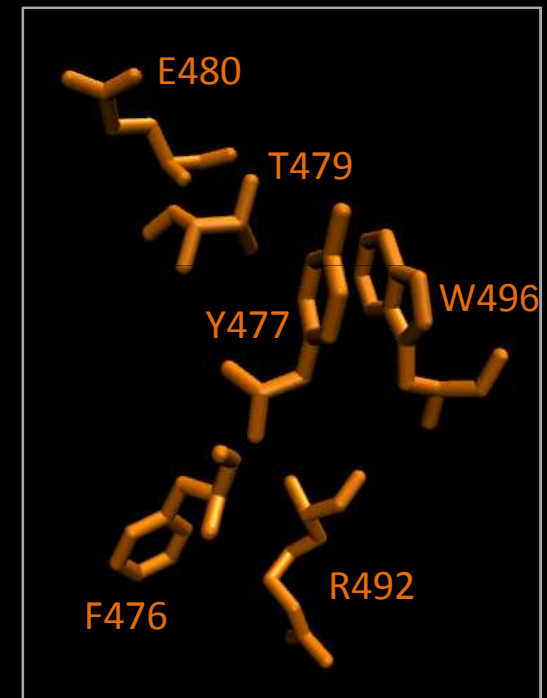
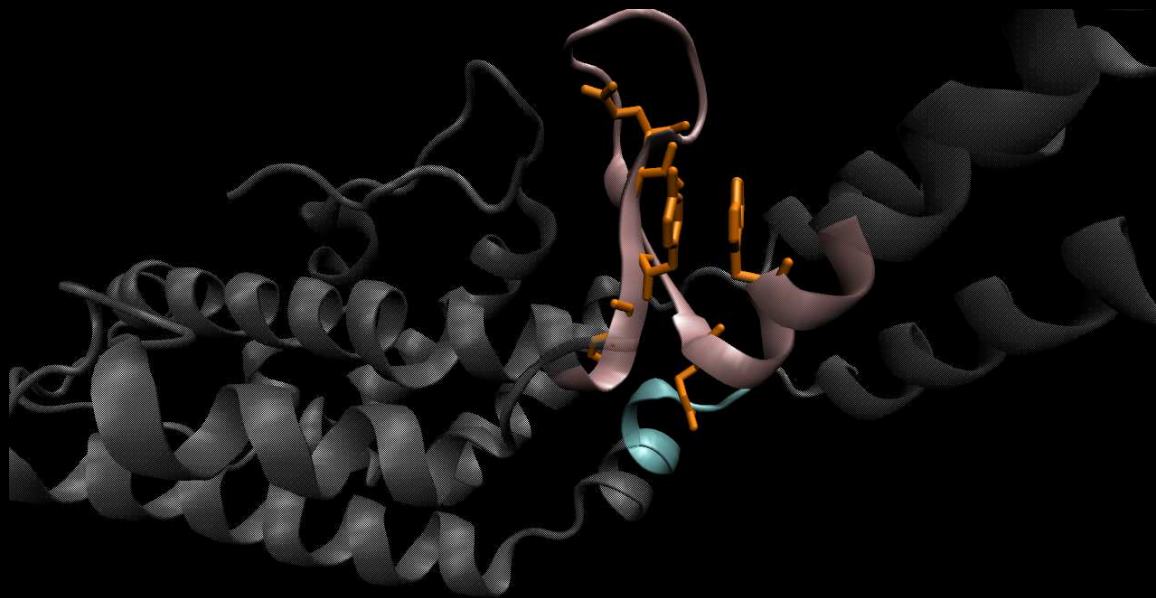
Hydrophobic pincer 4Å apart
(not sufficient to accommodate a nucleotide base)



T motifs **mutants**: how do they affect to TRBD activity?

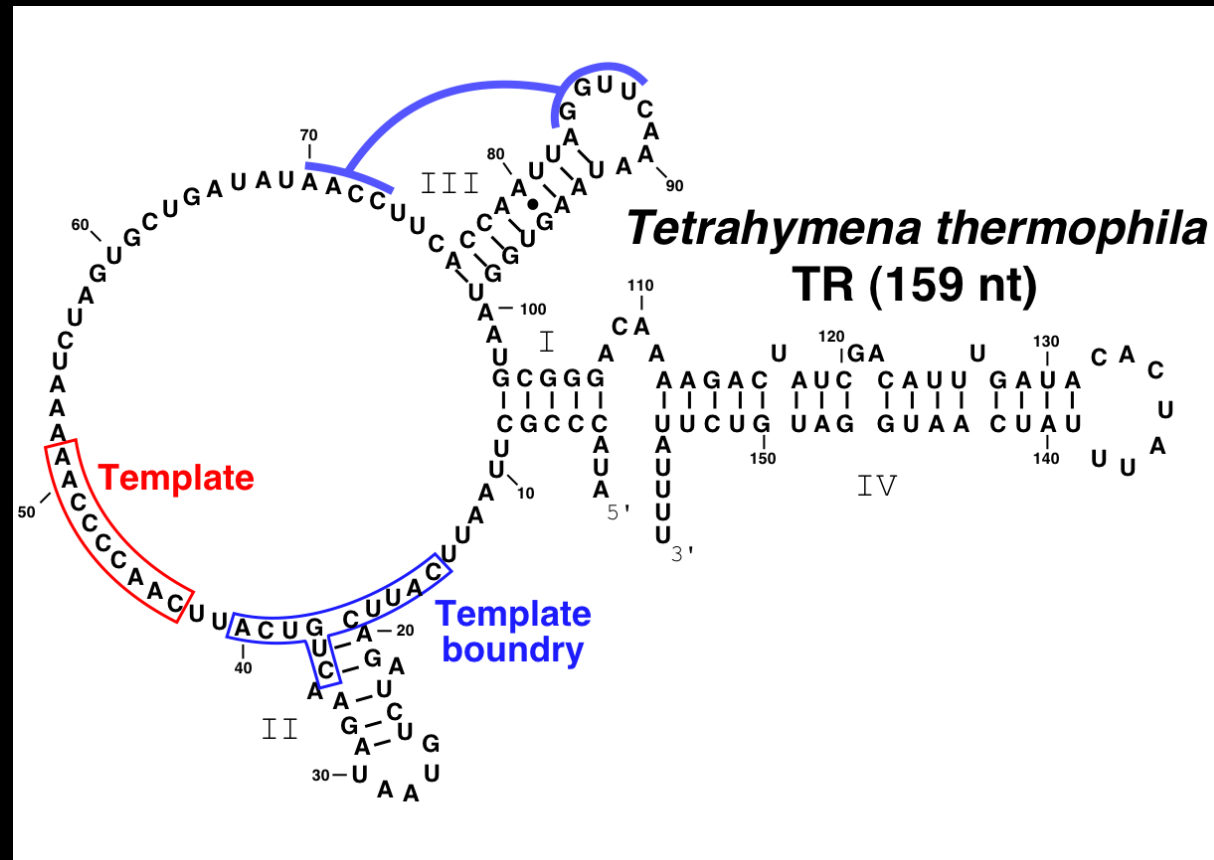
One set of mutants showed severe loss (80%-100%) of RNA binding affinity and telomerase activity –

F476A
Y477A
T479A
E480A
R492A
W496A



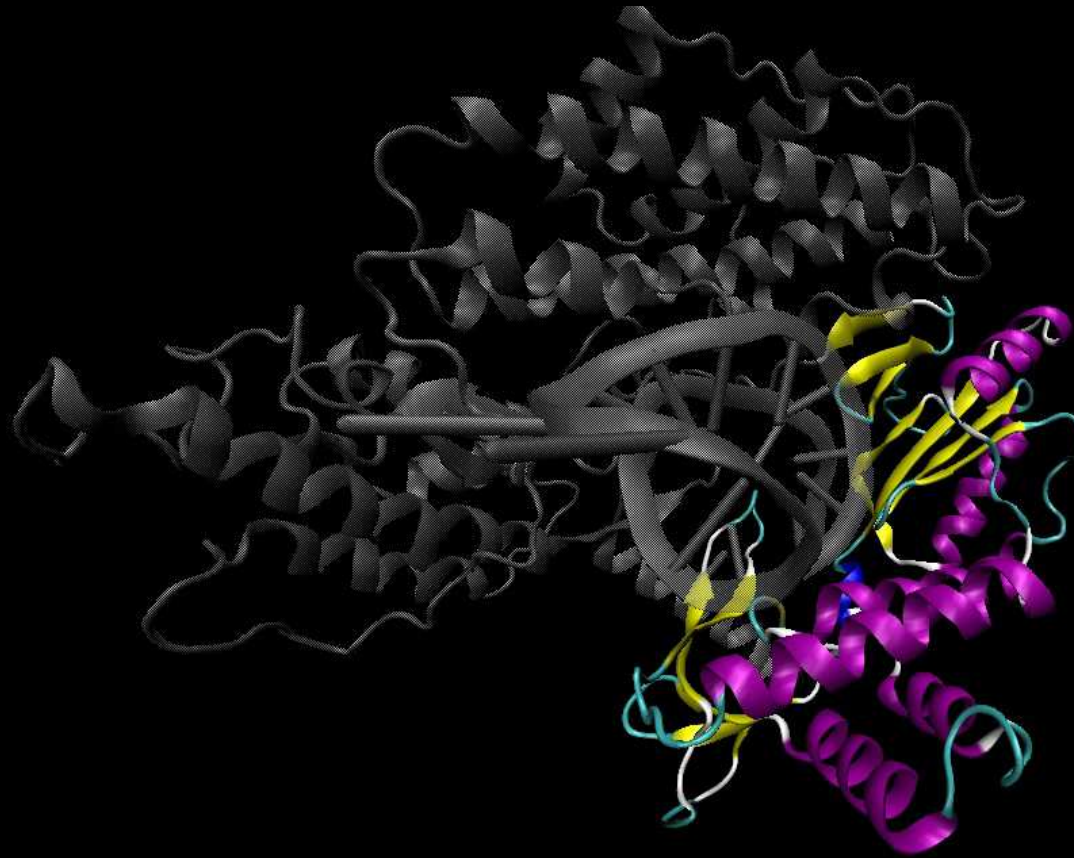
Tetrahymena thermophila RNA binding domain, **TRBD**

TRBD binds to TBE



Tribolium castaneum retrotranscriptase domain, RT

Mixture of α -helix and β -strands in two subdomains
Contains the active site



Tribolium castaneum retrotranscriptase domain, RT

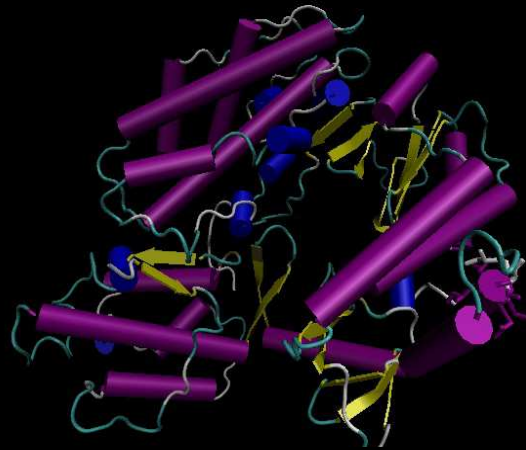
Fingers subdomain are in an open configuration respect to the palm subdomain



Tribolium castaneum retrotranscriptase domain, RT

Similar to the “fingers” and “palm” subdomains of: Retroviral RT
Viral RNA-pol
B-family DNA-pol

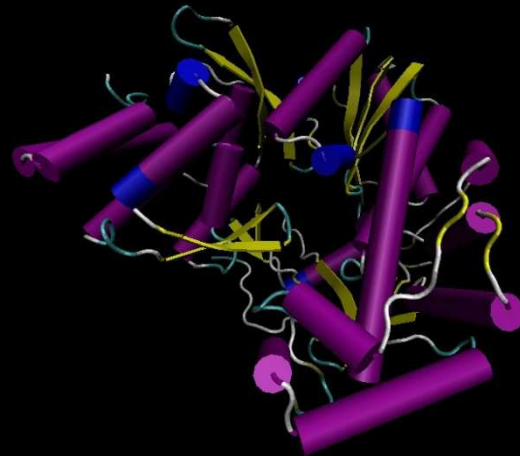
TERT



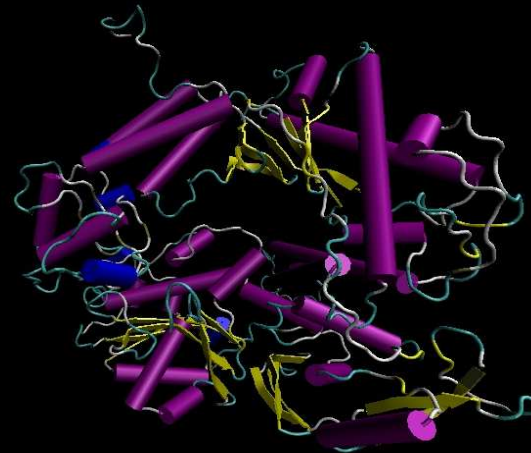
RT HIV



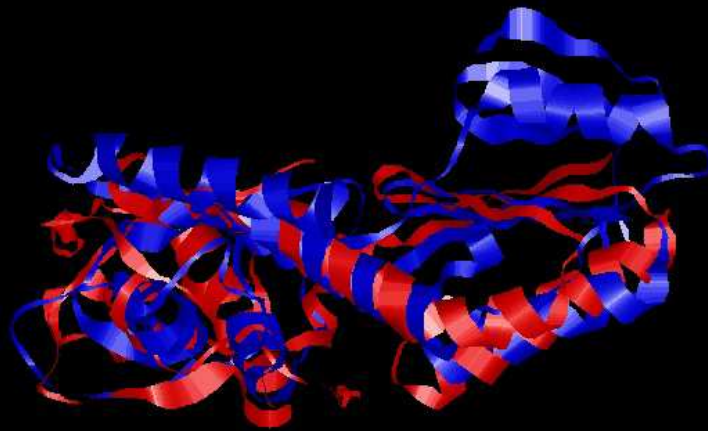
RNA-POL



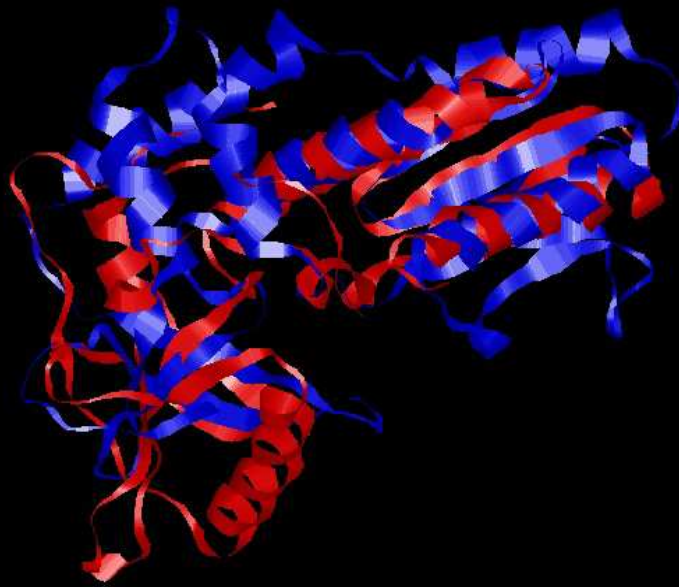
B-FAM
DNA-POL



TERT RT – HIV RT superimposition



RSMD 3.436

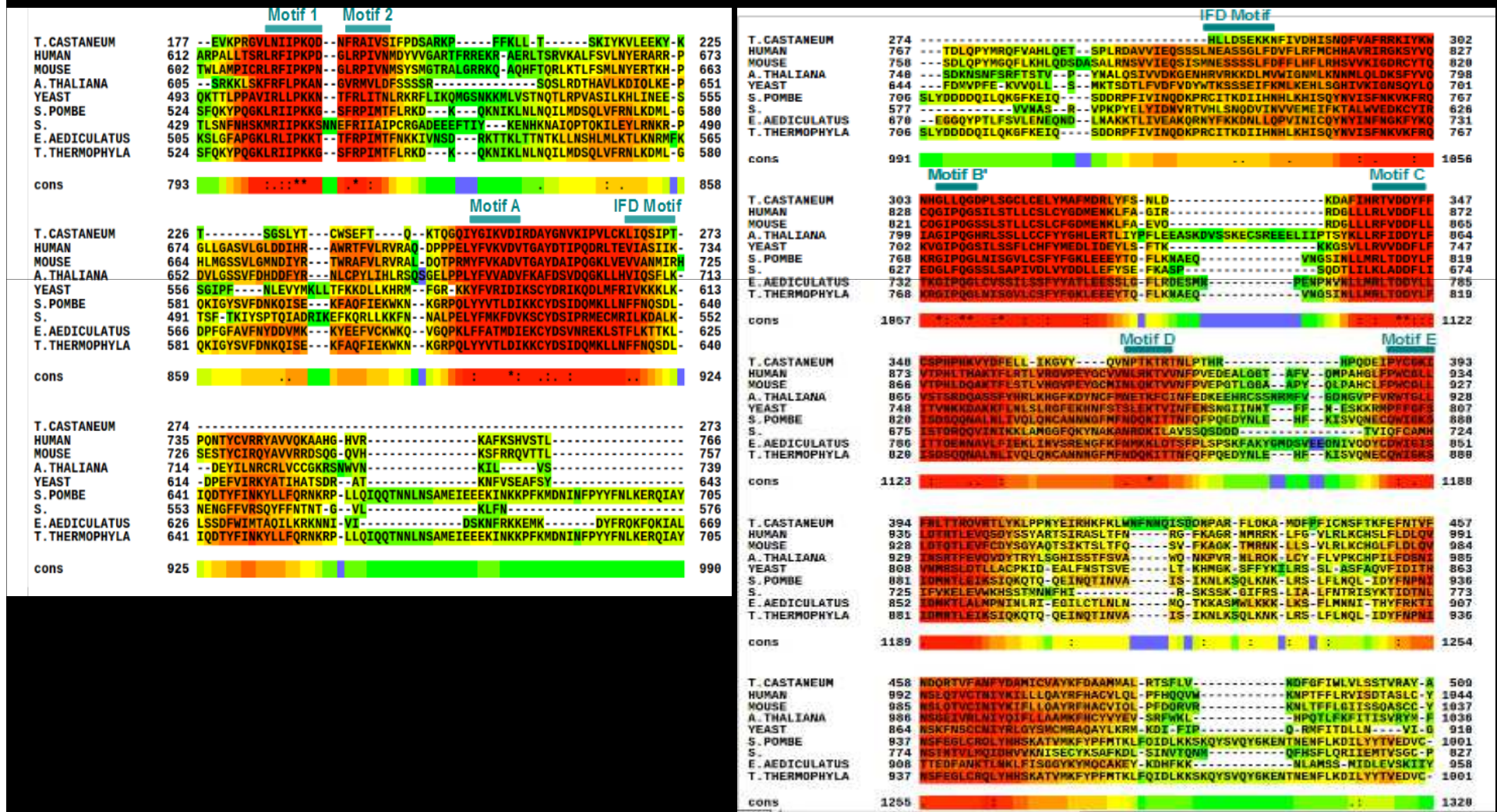


RT HIV (finger & palm domains)

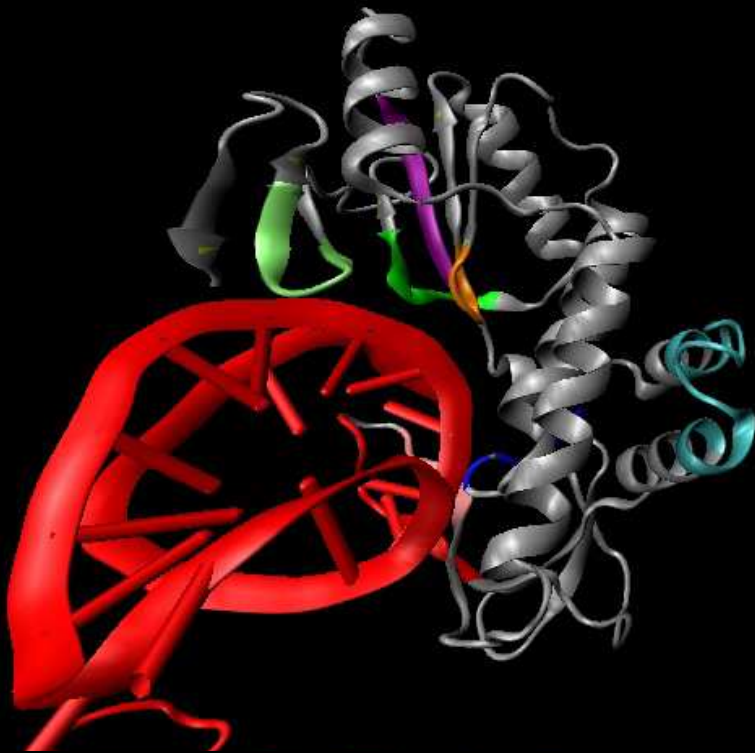
TERT RT domain

RT is a conserved domain

Important conserved motifs: 1, 2, A, IFD, B', C, D and E



Tribolium castaneum RT motifs



Motif 1

Motif 2

Motif A

Motif IFD

Motif B'

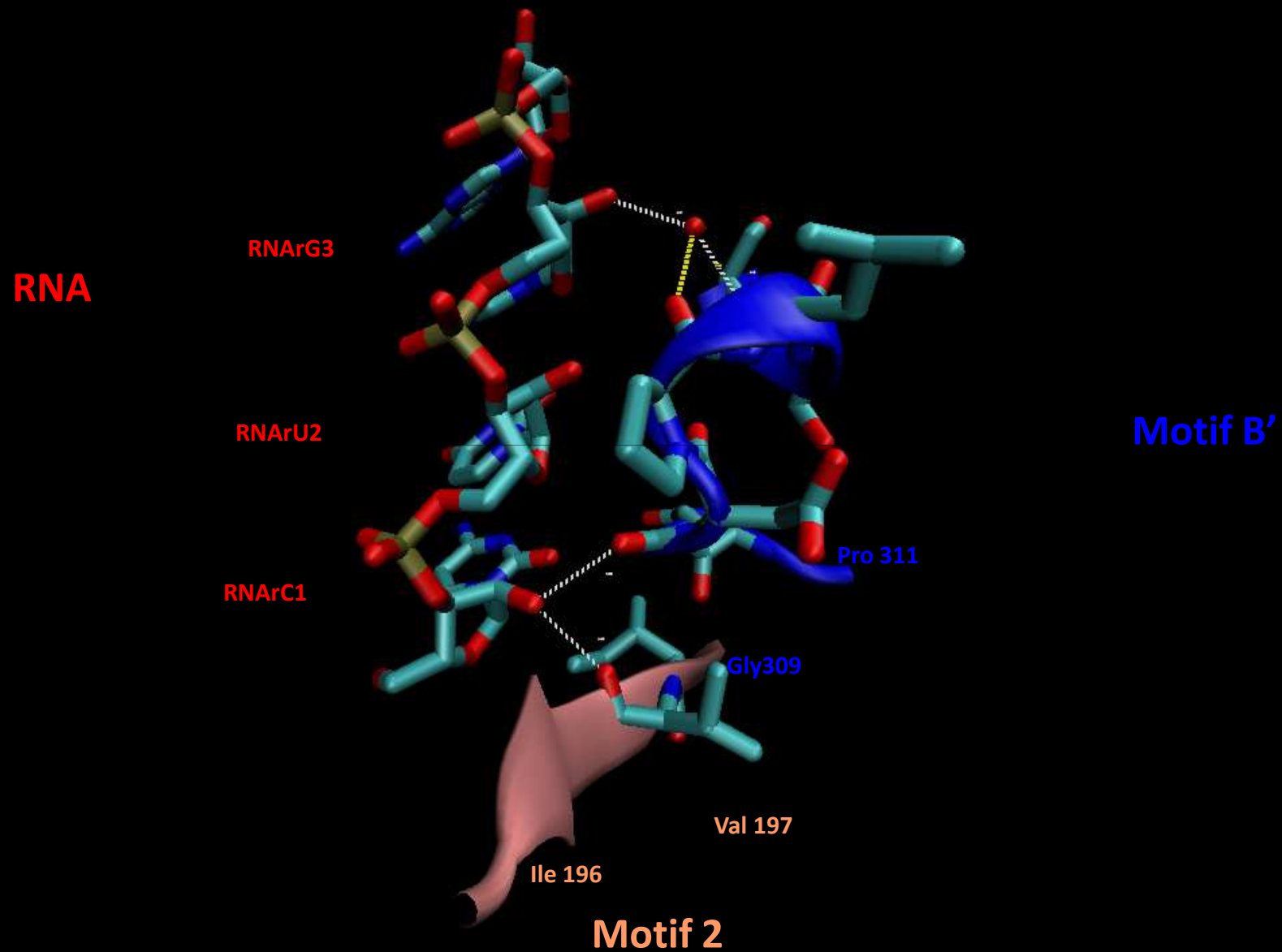
Motif C

Motif D

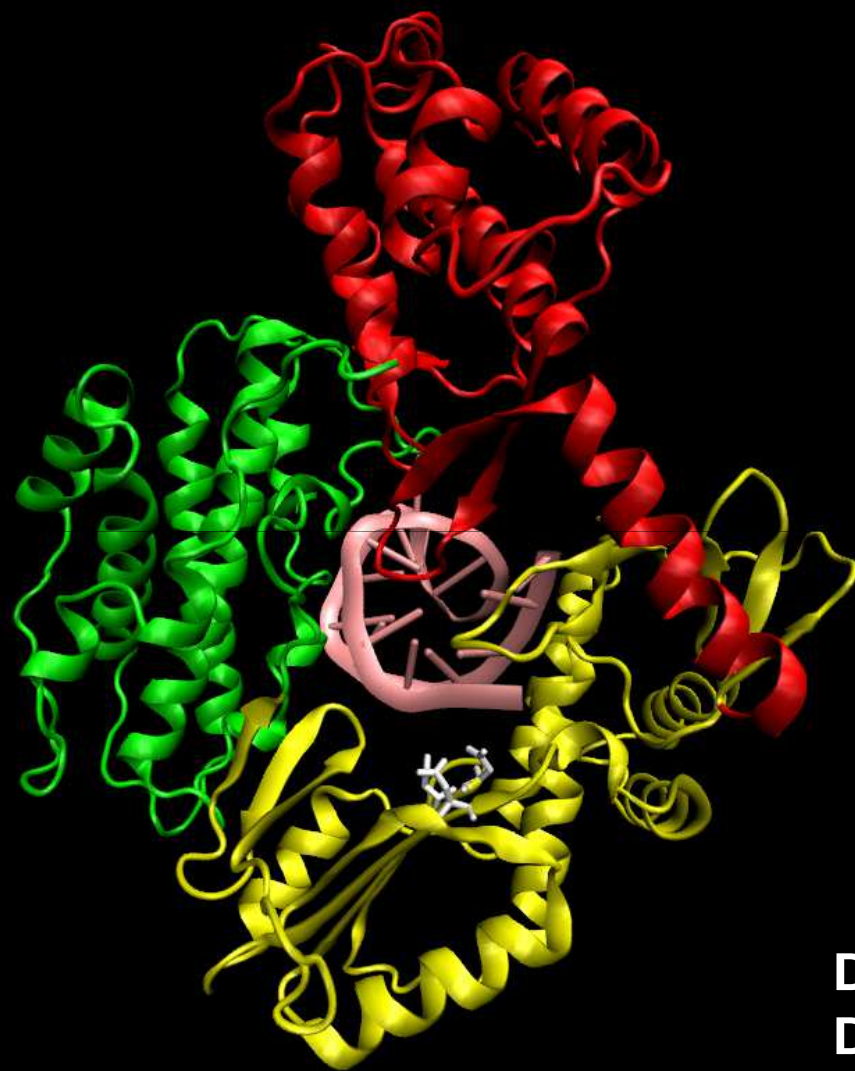
Motif E



Tribolium castaneum retrotranscriptase domain, RT



Tribolium castaneum RT active site



D251
D343
D344

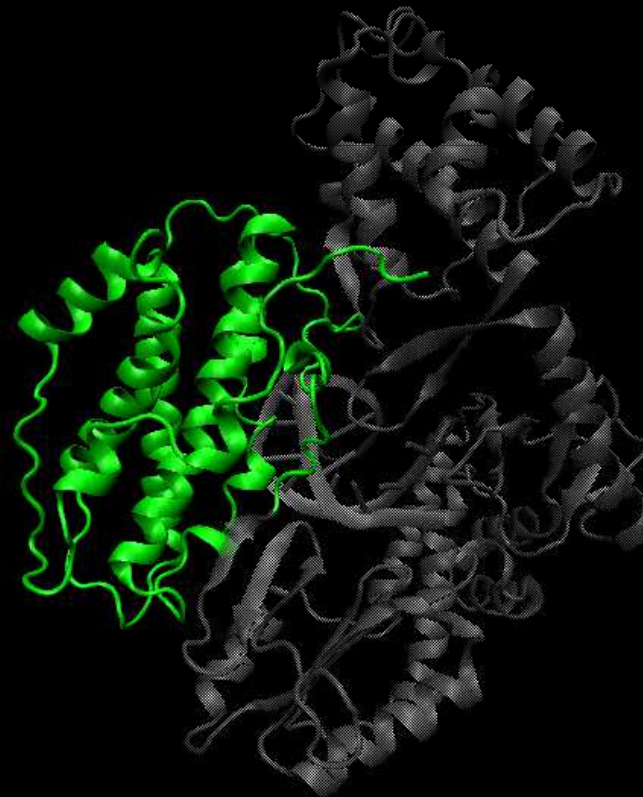


Tribolium castaneum thumb domain

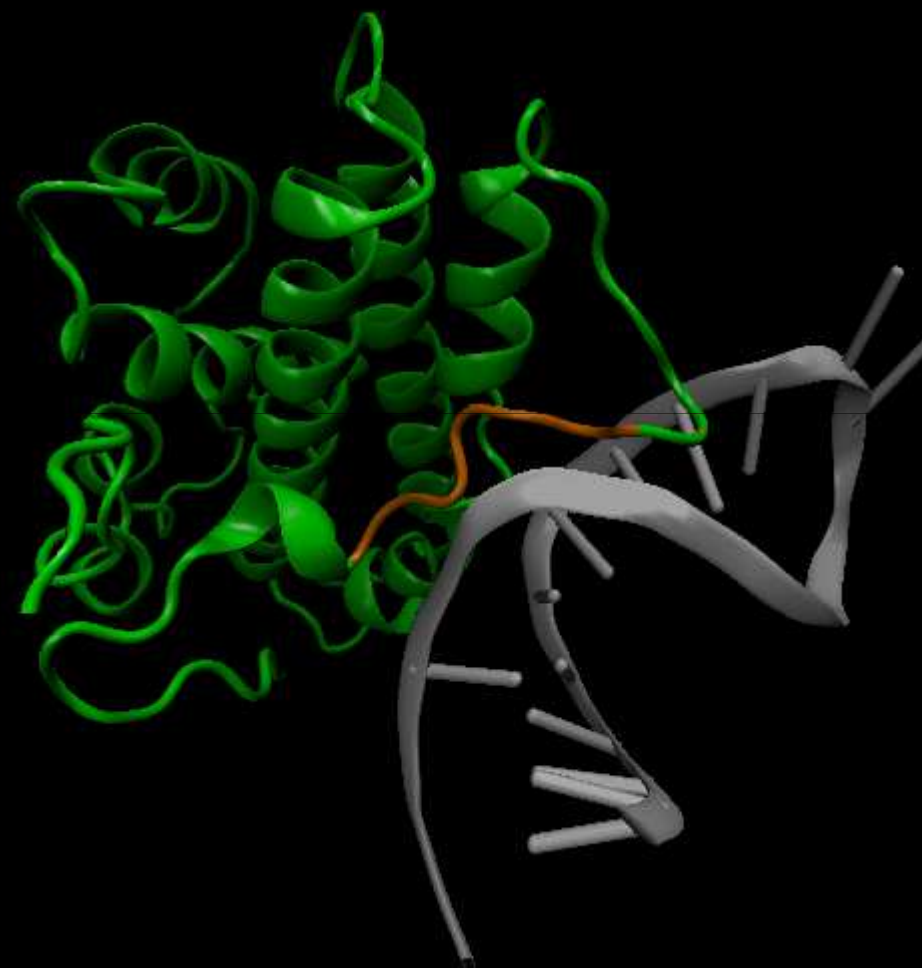
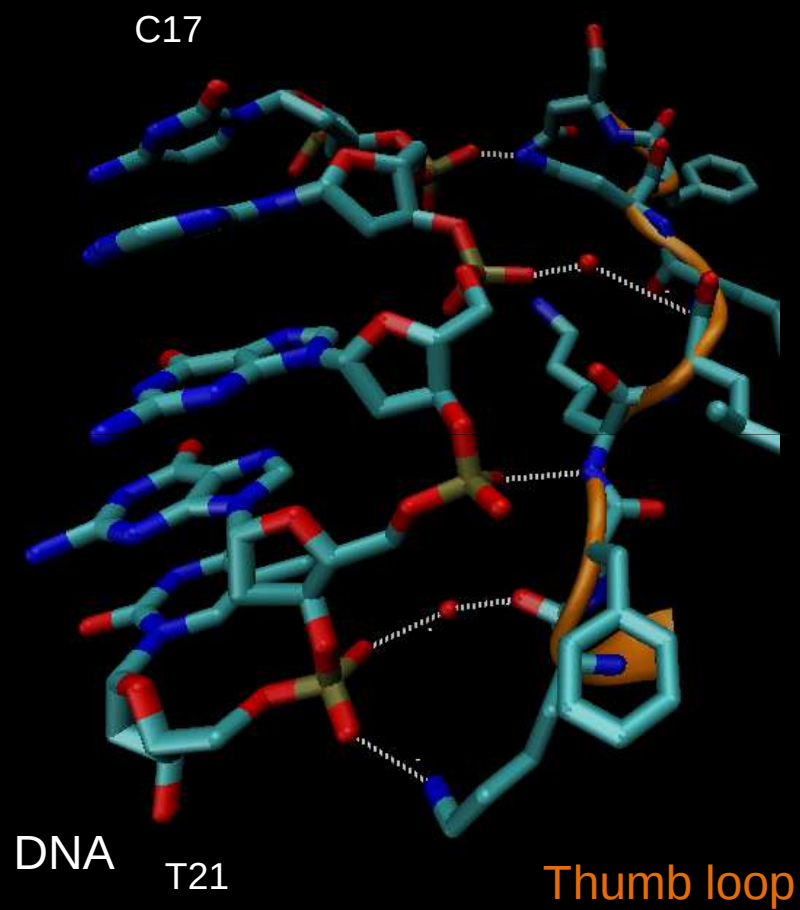
It's an elongated helical bundle → makes direct contact with the DNA

Important in template binding and in processive nucleotide addition

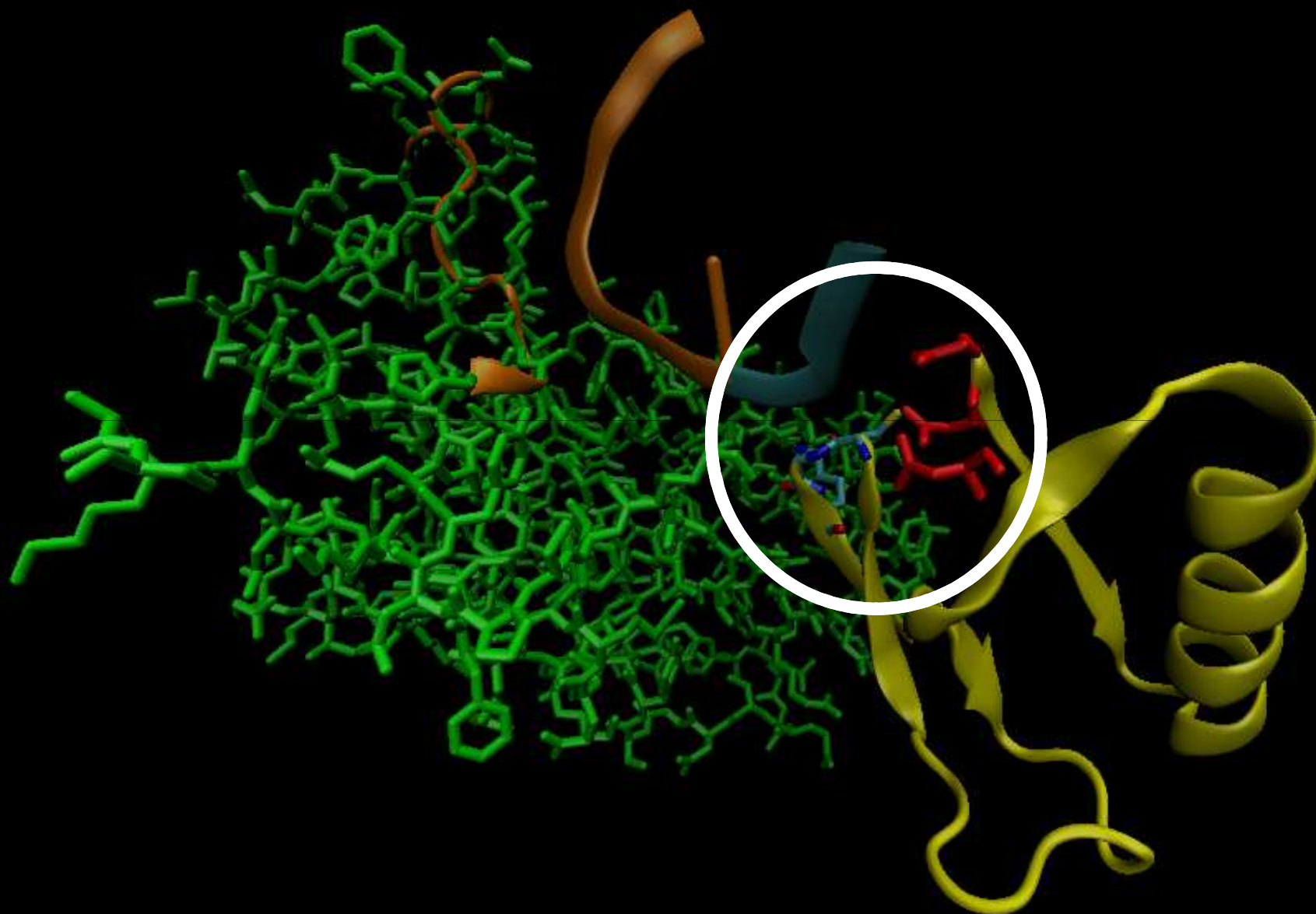
Sits in the minor groove of the RNA-DNA heteroduplex



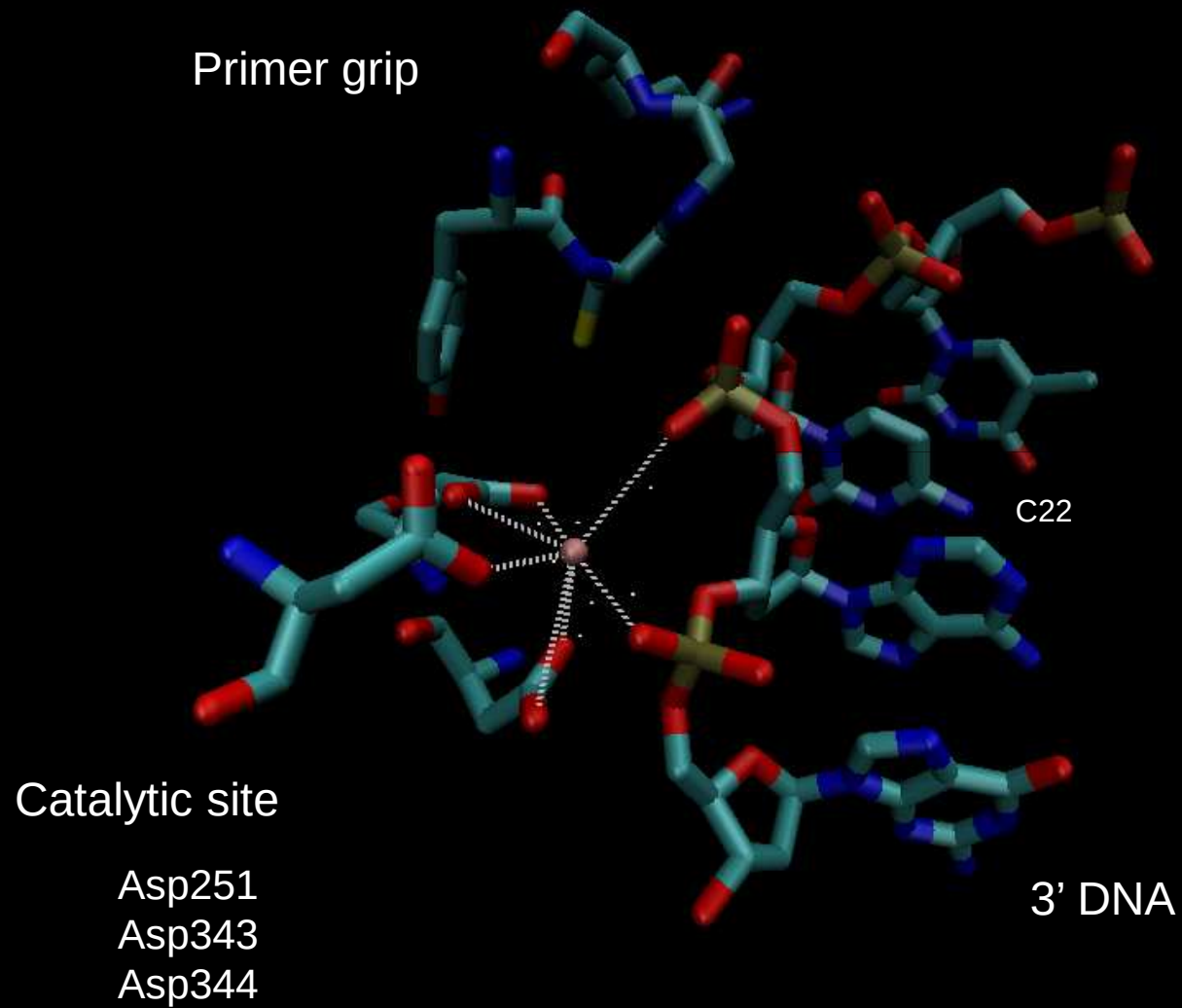
Thumb loop – DNA Interactions



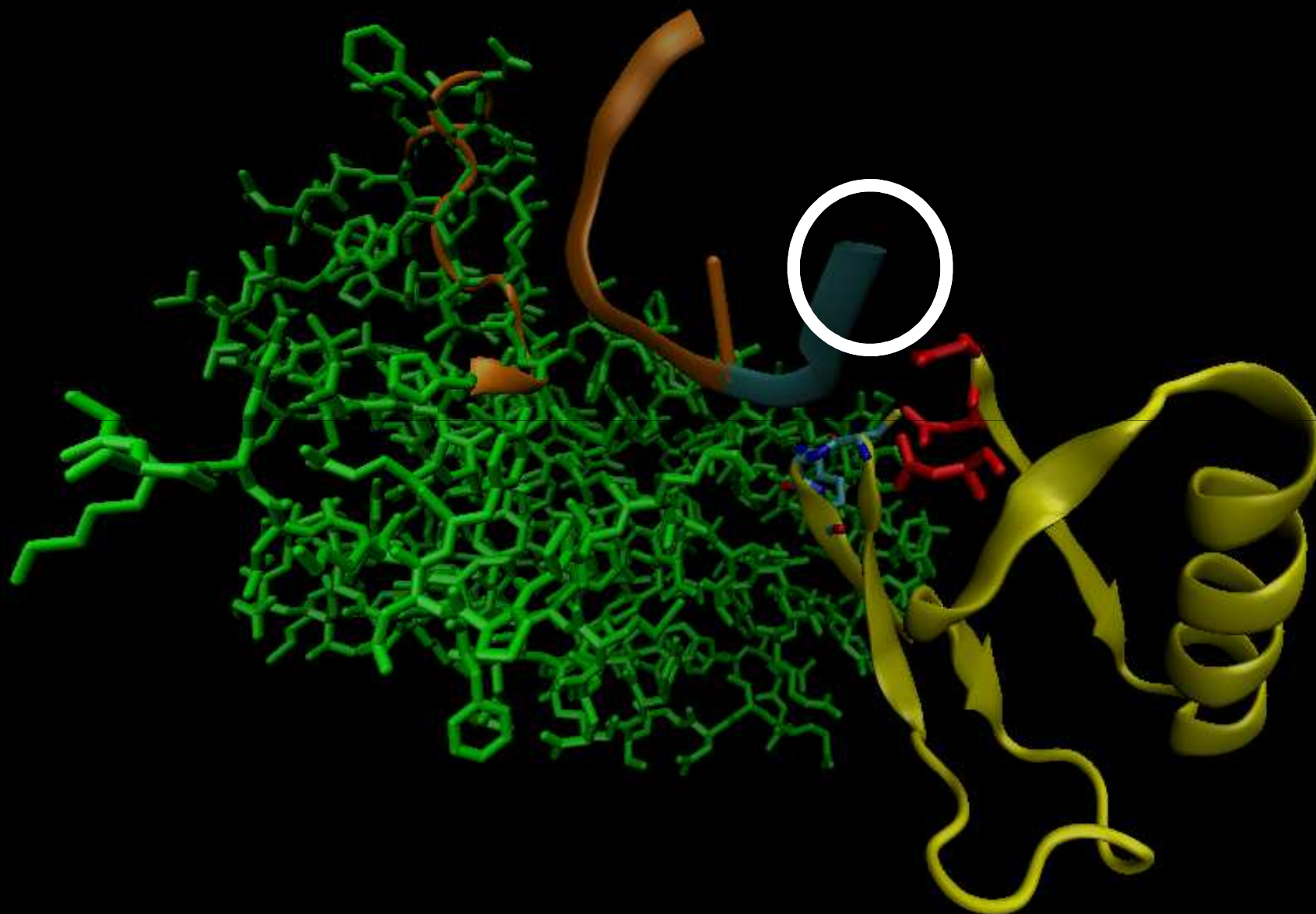
Thumb loop – DNA Interactions



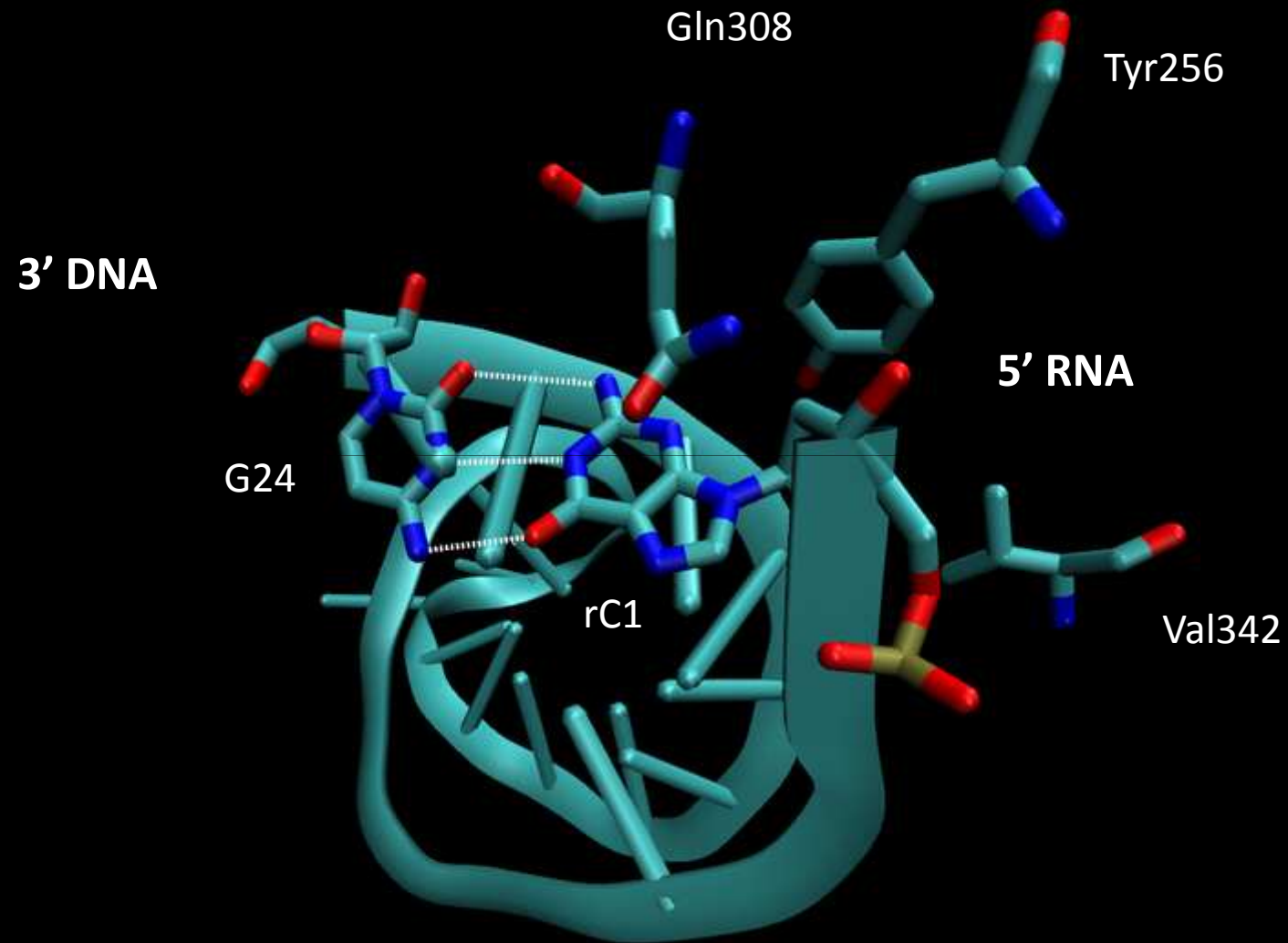
Thumb loop – DNA Interactions



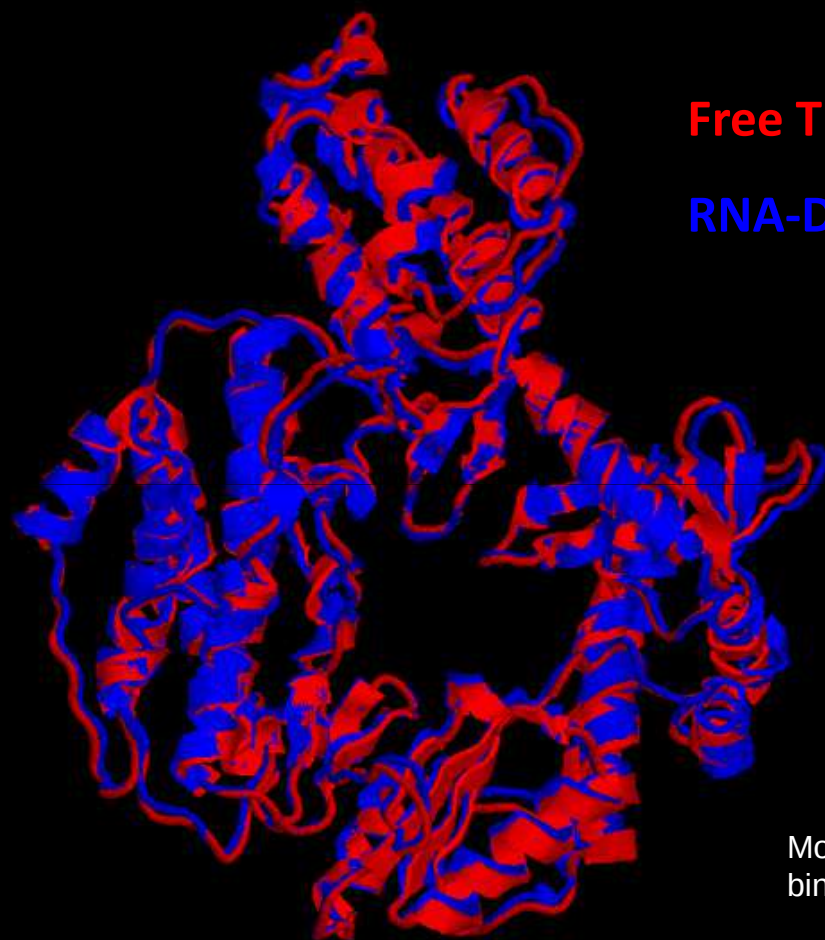
Thumb loop – DNA Interactions



DNA – RNA Interactions



Free TERT and RNA-DNA bounded TERT superimposition



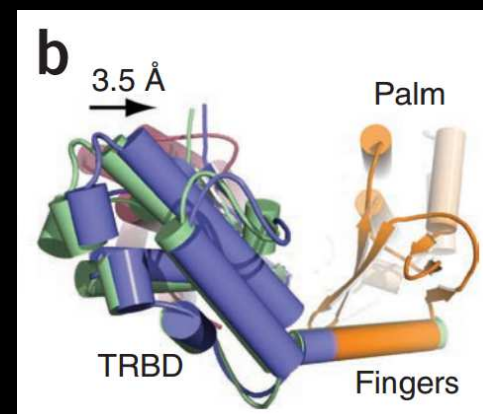
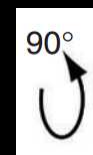
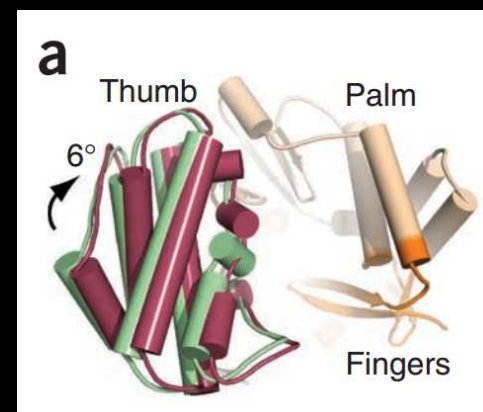
Free TERT

RNA-DNA TERT

RMSD : 1.30

Score : 8.92

More narrow RNA
binding pocket



RNA subunit of telomerase, **TER**

Telomerase RNA, **TR/TER**

Essential for telomerase function

Conservation of secondary structure (despite divergence in length and primary sequence)

2 conserved and potentially universal structures (required for enzymatic activity) → Template/pseudoknot domain

CR4/5 domain

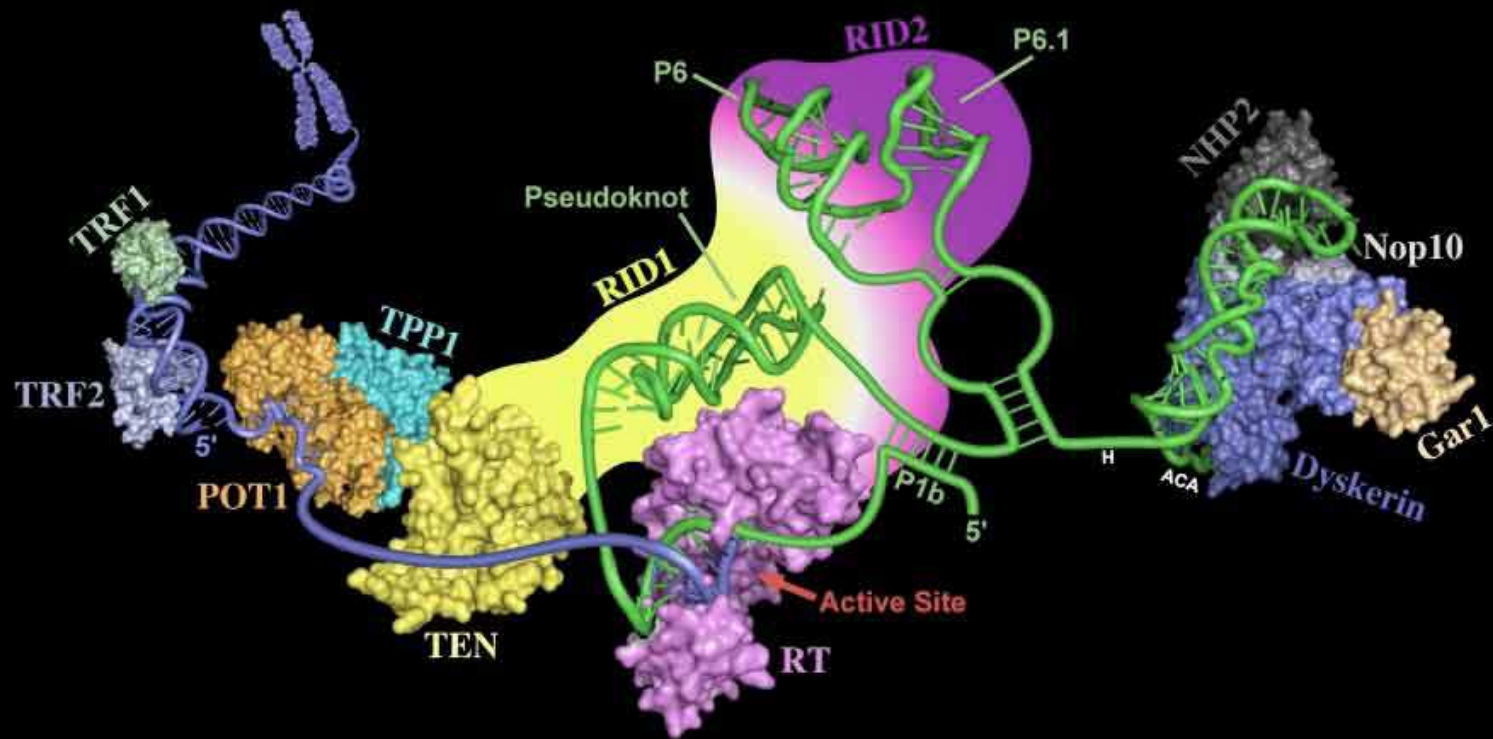
H/ACA domain in vertebrates → Homology to small nucleolar RNA and small Cajal-body-specific RNA



CR7 domain

2 stem-loops separated by
box H and box ACA

Tribolium castaneum catalytic subunit of telomerase, **TERT**

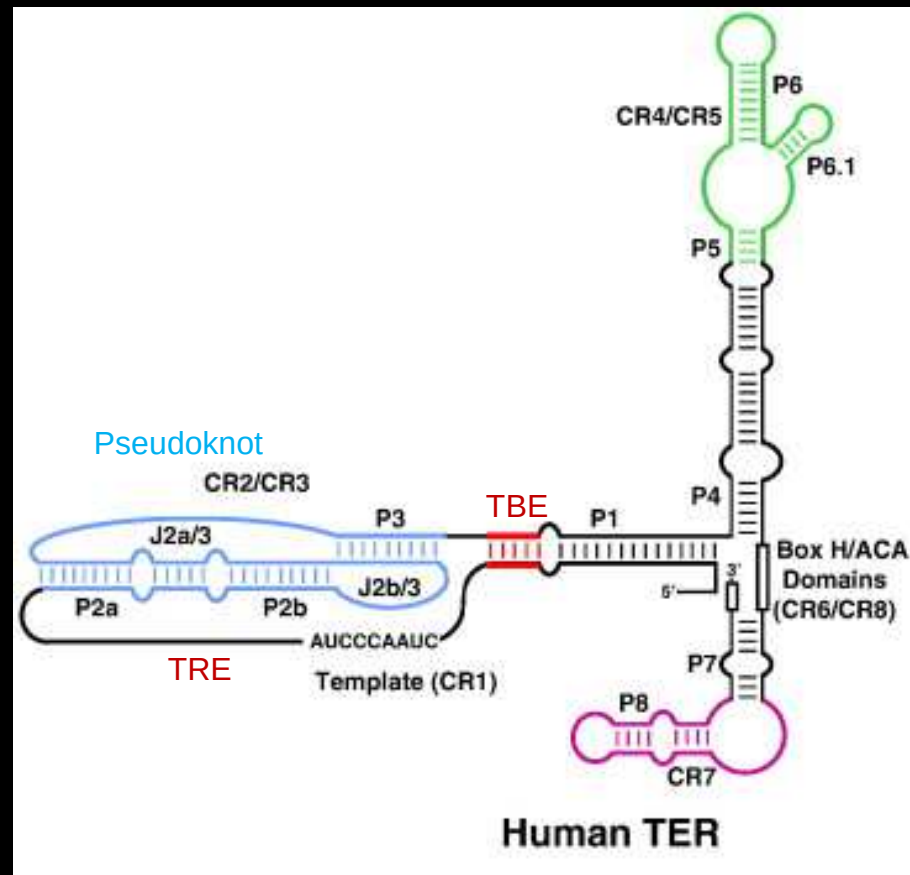


Telomerase RNA, **TR/TER**

nt 33–147
nt 163–330

hTERT-binding sites
(critical for
telomerase activity)

Pseudoknot
Template
domain



CR4/5 domain

H/ACA box

CR7 domain

TER pseudoknot/template domain

Pseudoknot domain

Compact well-ordered structure with extensive tertiary contacts

Stabilized by triple helix

Pseudoknot and template located adjacent in secondary structure
(but not in primary structure)

Template sequence

Binds to telomeric DNA and codes telomeric repeats

5' region → encodes telomeric DNA repeats

3' region → anneals to DNA after template translocation

TER pseudoknot/template domain

Template recognition element -TRE(ssRNA)

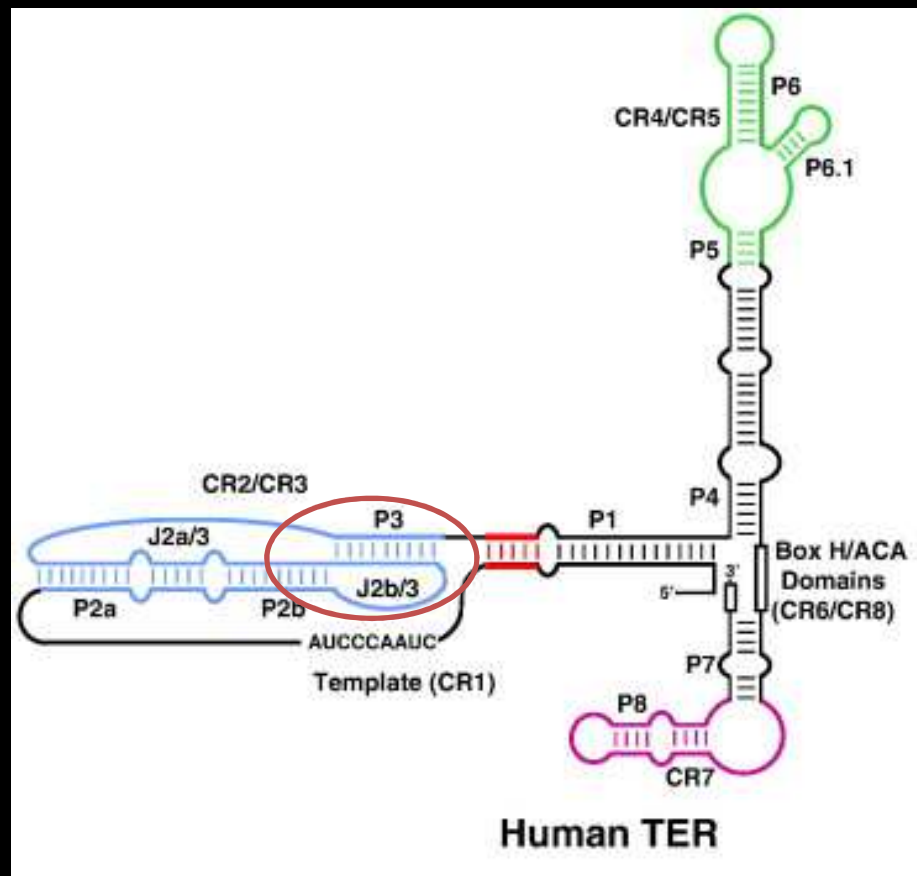
- Nt TERT-binding site
- Facilitates 3' template positioning in the active site
- Stimulates telomerase activity and repeat addition processivity



Template boundary region-TBE(dsRNA)

- TERT-binding site
- Regulates nucleotides addition during RT

TER pseudoknot domain

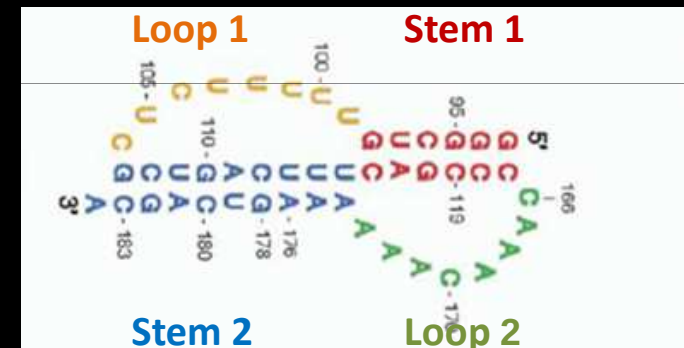


J2b/3
8nt
U rich

P2b
6pb
G-C rich

Loop 1

Stem 1



Stem 2

Loop 2

P3
9pb
A-U rich

J2a/3
8nt
A rich

hTER **pseudoknot** domain

Stem 1

Stem 2

Sequence logo (5' to 3'):

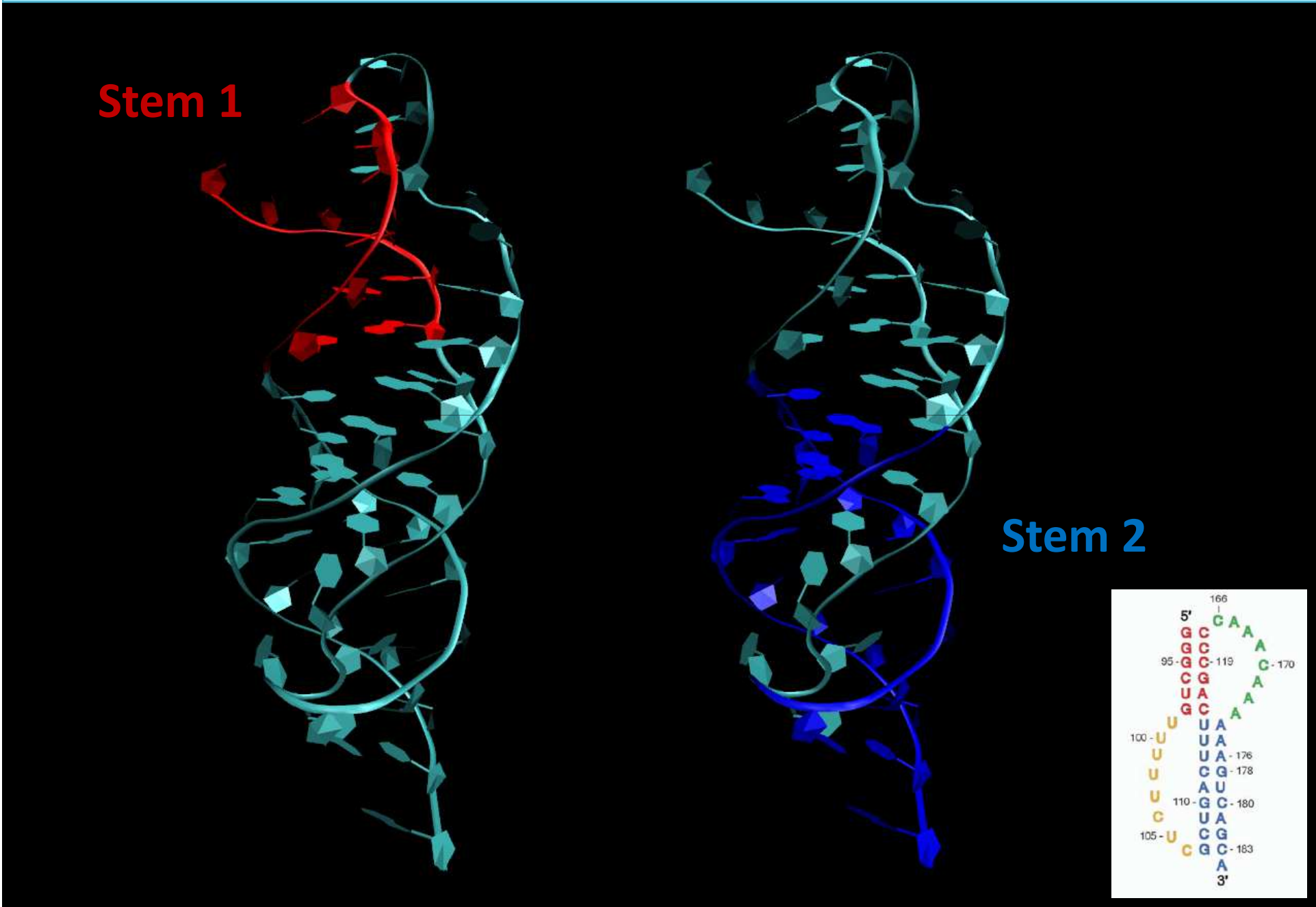
Position	Sequence
166	C
165	G
164	C
163	C
162	G
161	G
160	C
159	C
158	G
157	C
156	U
155	A
154	C
153	U
152	A
151	A
150	A
149	A
148	A
147	A
146	A
145	A
144	A
143	A
142	A
141	A
140	A
139	A
138	A
137	A
136	A
135	A
134	A
133	A
132	A
131	A
130	A
129	A
128	A
127	A
126	A
125	A
124	A
123	A
122	A
121	A
120	A
119	A
118	A
117	A
116	A
115	A
114	A
113	A
112	A
111	A
110	A
109	A
108	A
107	A
106	A
105	A
104	A
103	A
102	A
101	A
100	A
99	A
98	A
97	A
96	A
95	A
94	A
93	A
92	A
91	A
90	A
89	A
88	A
87	A
86	A
85	A
84	A
83	A
82	A
81	A
80	A
79	A
78	A
77	A
76	A
75	A
74	A
73	A
72	A
71	A
70	A
69	A
68	A
67	A
66	A
65	A
64	A
63	A
62	A
61	A
60	A
59	A
58	A
57	A
56	A
55	A
54	A
53	A
52	A
51	A
50	A
49	A
48	A
47	A
46	A
45	A
44	A
43	A
42	A
41	A
40	A
39	A
38	A
37	A
36	A
35	A
34	A
33	A
32	A
31	A
30	A
29	A
28	A
27	A
26	A
25	A
24	A
23	A
22	A
21	A
20	A
19	A
18	A
17	A
16	A
15	A
14	A
13	A
12	A
11	A
10	A
9	A
8	A
7	A
6	A
5	A
4	A
3	A
2	A
1	A

Stem 1

Stem 2

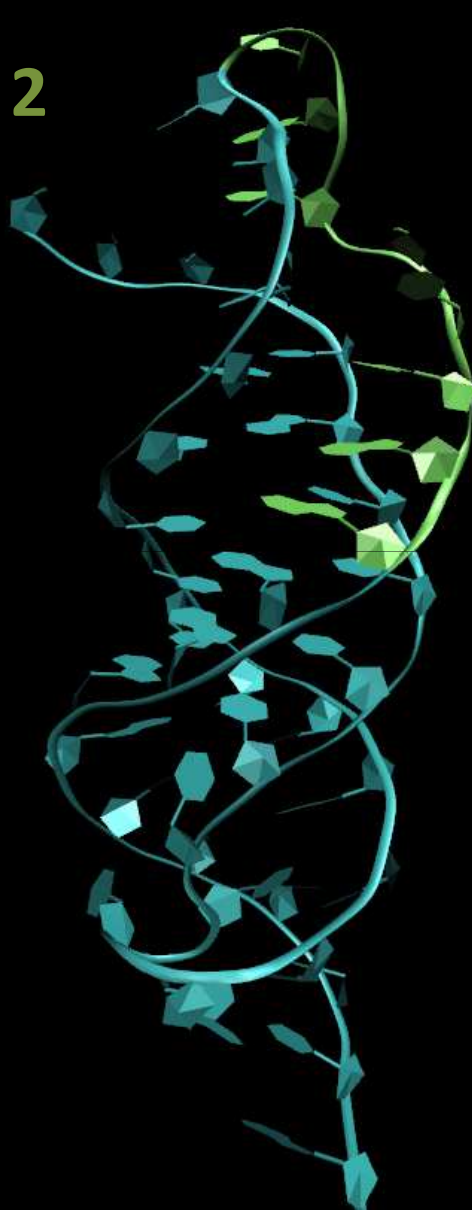
Sequence logo (5' to 3'):

Position	Sequence
5'	G C C G C G U A C
95	G C C G C G U A C
100	U U U U C
105	U C
110	U A U C G C A
115	U A U C G C A
120	U A U C G C A
125	U A U C G C A
130	U A U C G C A
135	U A U C G C A
140	U A U C G C A
145	U A U C G C A
150	U A U C G C A
155	U A U C G C A
160	U A U C G C A
165	U A U C G C A
170	U A U C G C A
175	U A U C G C A
180	U A U C G C A
183	U A U C G C A
3'	A

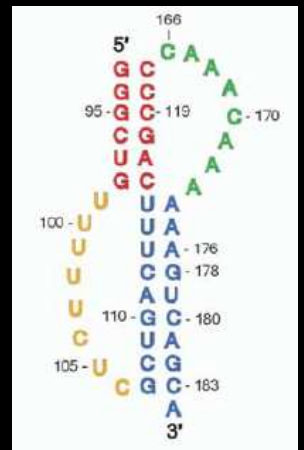
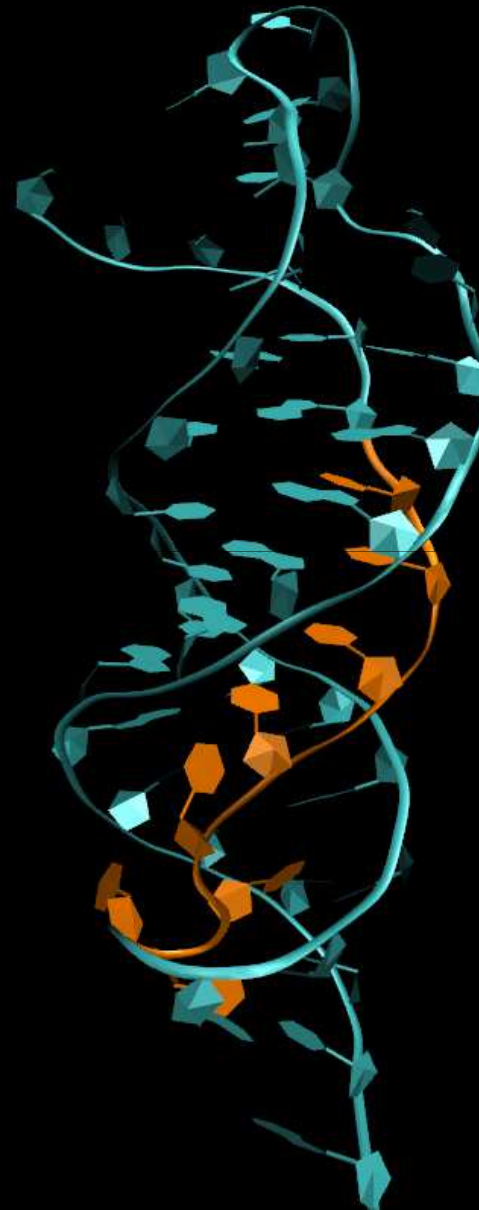


hTER **pseudoknot** domain

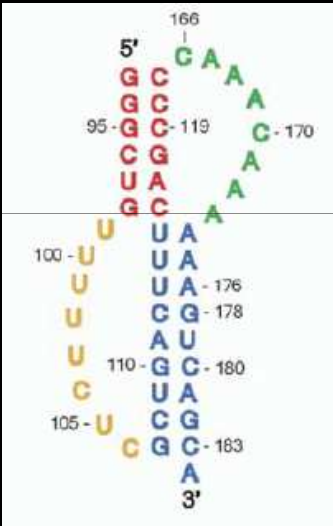
Loop 2



Loop 1



hTER **pseudoknot** domain



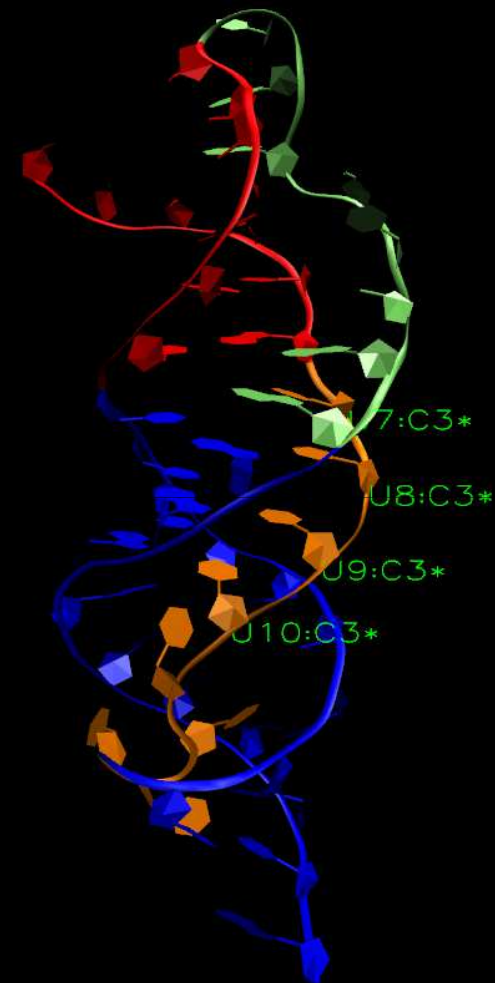
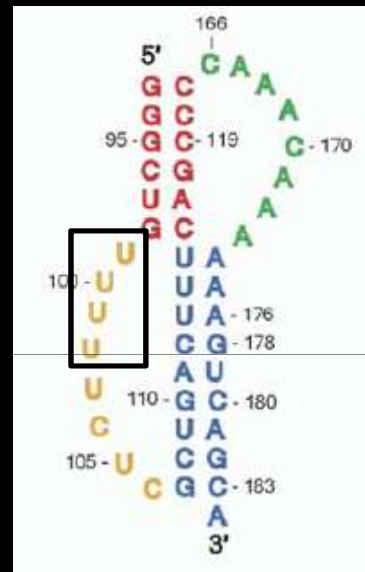
Loop 2

Loop 1

Stem 2

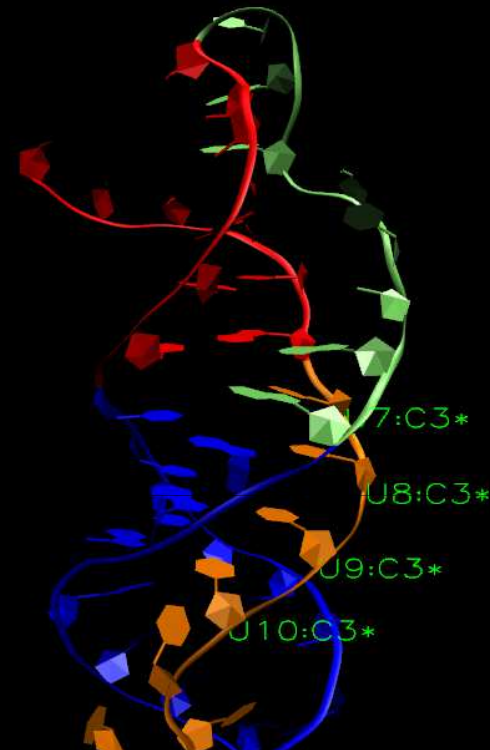
hTER pseudoknot domain

The first 4Us of loop1 are 100% conserved among vertebrates



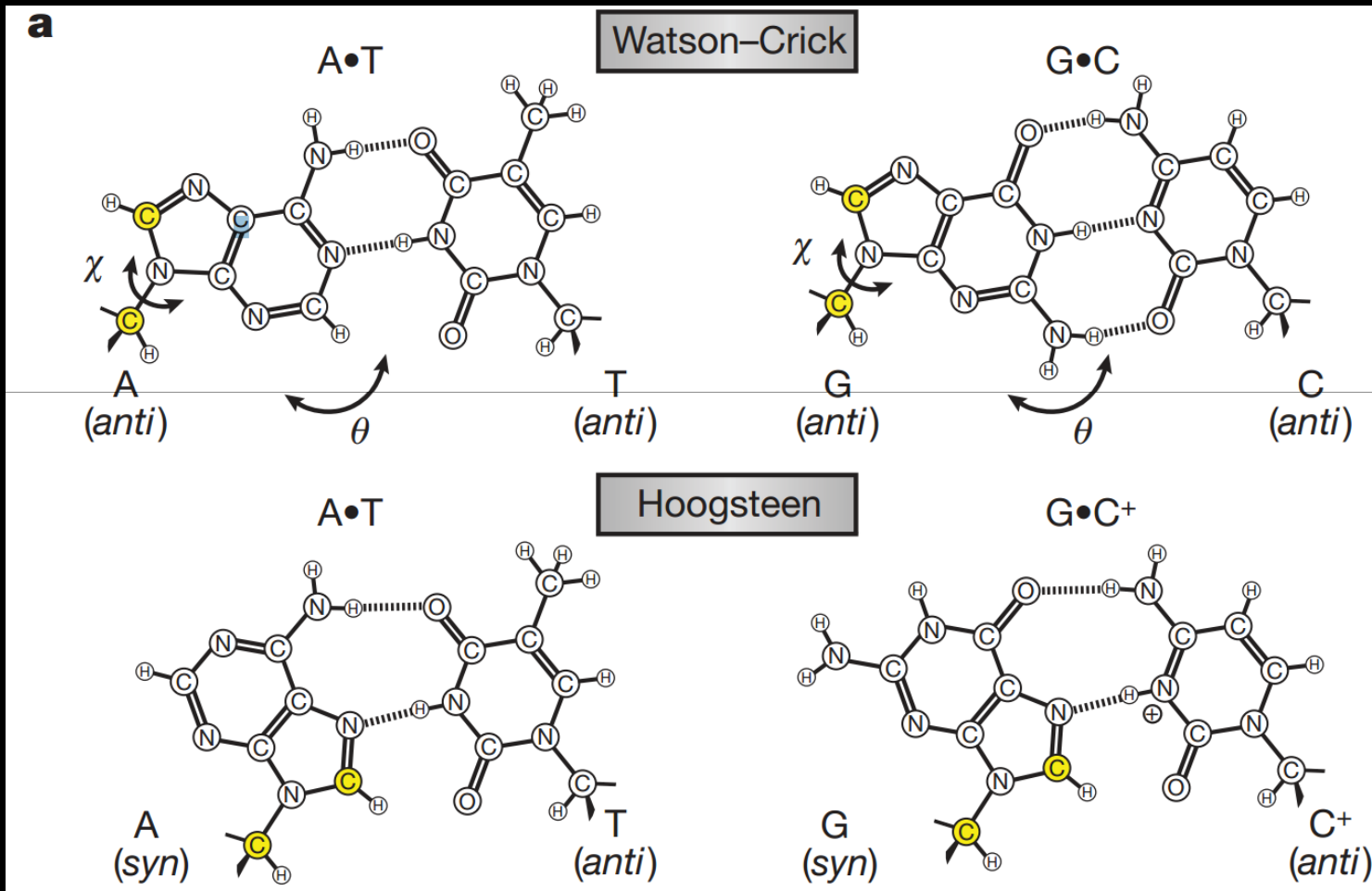
hTER **pseudoknot** domain

The first 4Us of loop1 are 100% conserved among vertebrates



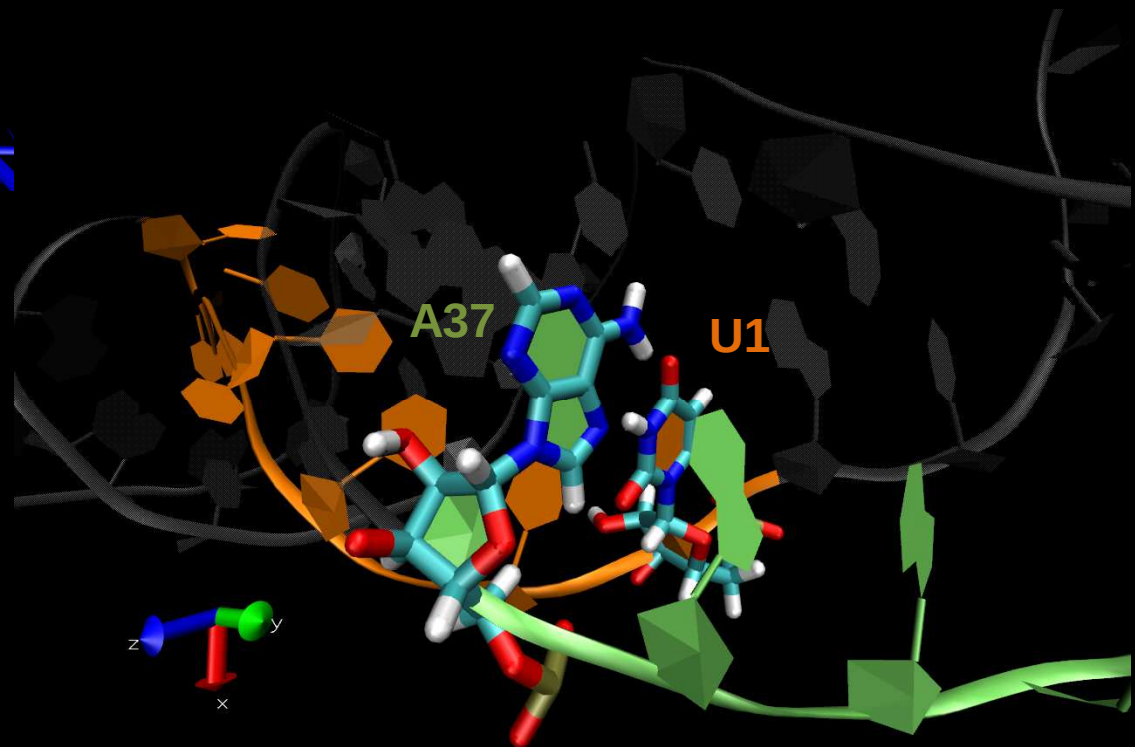
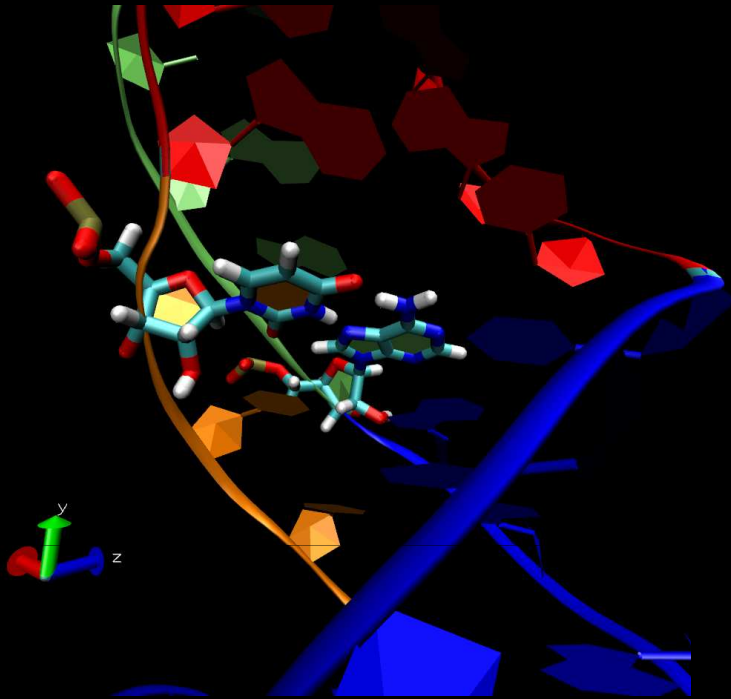
	Pseudoknot-CR2	P2a.1
Homo_sapiens	CUUUUGCUC-C-CC-----GCGCGCUCUUUUJCUCGCUGACUUUCAGCGGGCG-GA--AAAGCCUCGGCC---U	139
Rabbit	CUUUUGUUUCC-CC-----GCGCGCUCUUUUJCUCGCUGACUUUCAGCGCGUGGGA--AAAGCCUUGGCC---U	236
Hamster	CUUUCGUC--U-CC-----GCCCGCUCUUUUJCUCGCUGACUUCAGCGGGCG-GG--AAAGUCCAGACC---U	190
Cat	CUUUUGUUU-C-CC-----GCACGCUCUUUUJUCGCUGACUUUCAGCGGGCG-GA--AAAGCCUCGGCC---U	237
Pig	CUUUUGCUII-C-AC-----GCGCGCUCUUUUJCUCGCUGACUUUCAGCGGGCG-GA--AAAGCCUCGGCC---U	239
Horse	CUUUUGCUC-C-CC-----GCGCGCUCUUUUJCUCGCUGACUUUCAGCGGGCG-GA--AAAGCCUCGGUC---U	236
Bullfrog	UGCU-CUU-C-AU-----GUCGUUCUUUUJCUCGCUGACUUUCAGCGGGCG-AA--AGAGCAAUGGAA---G	215
Xenopus	UCUUCGCGG-CGGU-----GCCUGUUCUUUUACUUGCUGACUUUCAGCGGGCACGG--AGAGCAAGCGUAGACG	277
Shark	CA-CUGCCU-C-CCGUGCGUGAAACUCGCUCUUUUJUUGCUAACUUUCAGCGGAGGU-GAGGCAAG--GCGGCA---A	259
	* * * * *	**

hTER **pseudoknot** domain

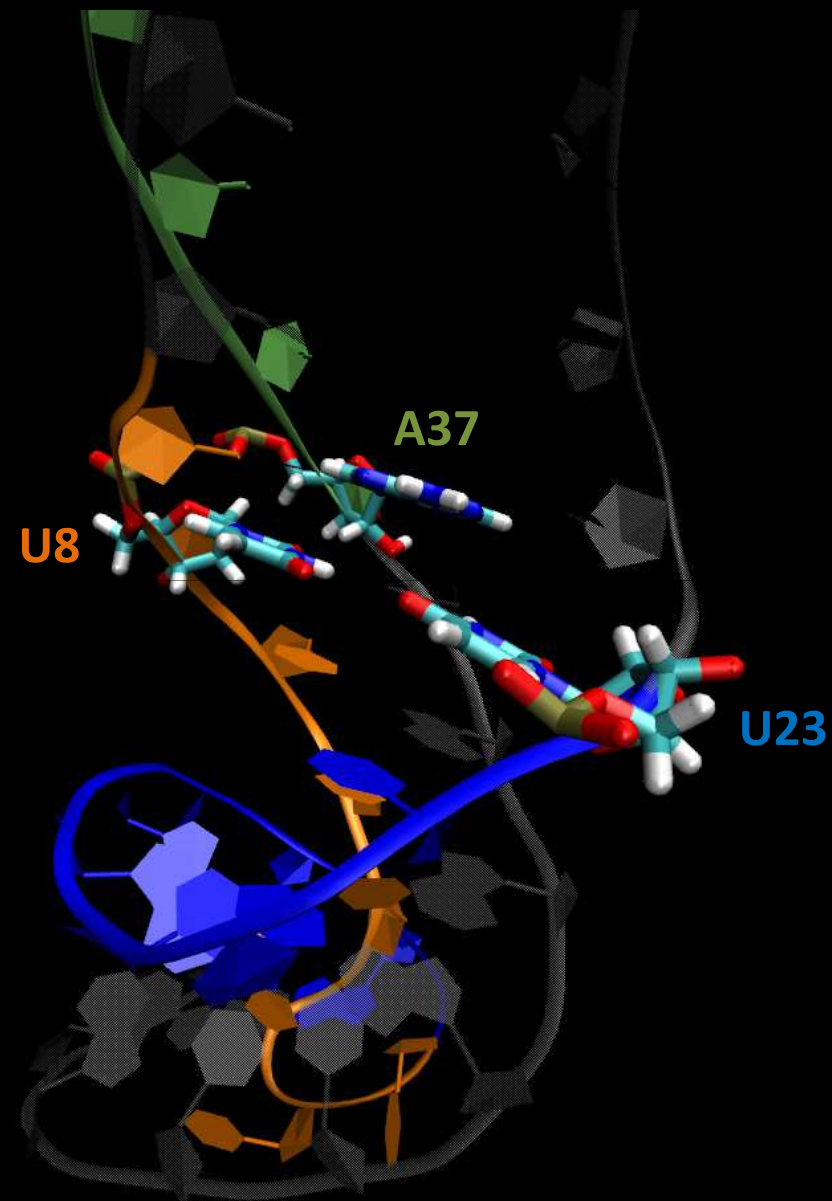
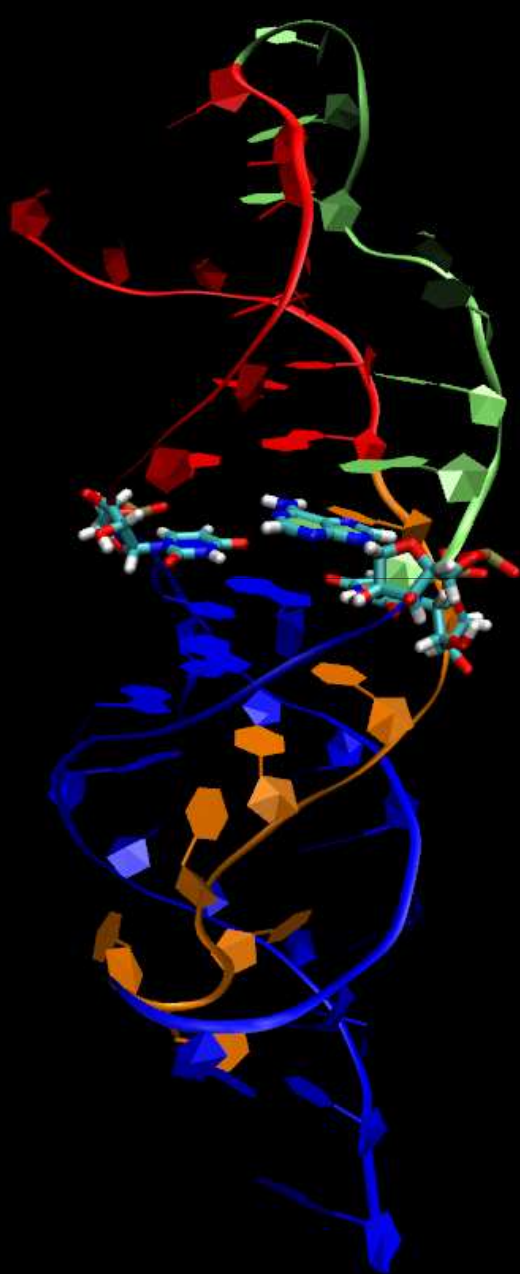


hTER **pseudoknot** domain

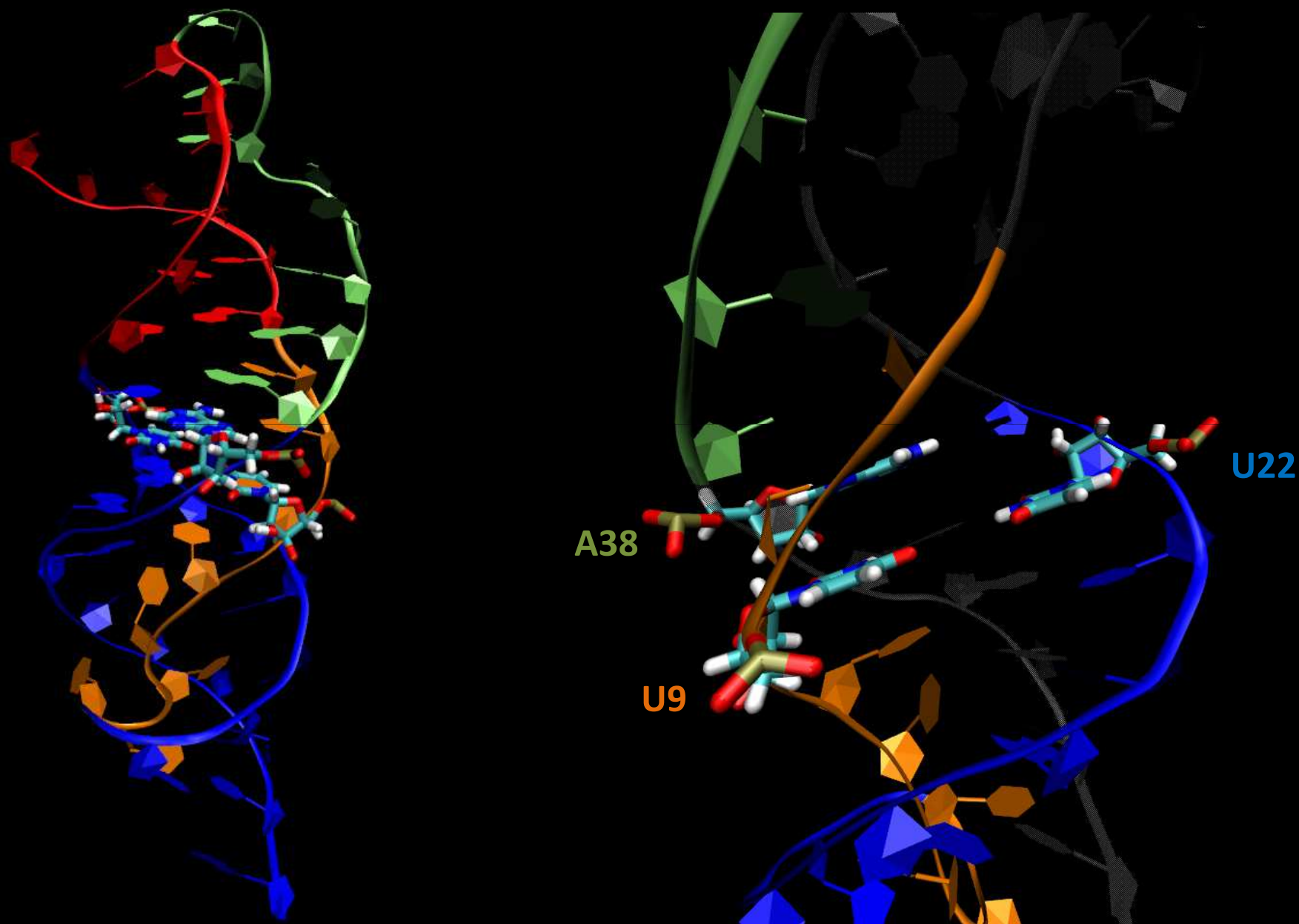
First nt of loop1 and last nt of loop2
interact to form a Hoogsteen A·U bp



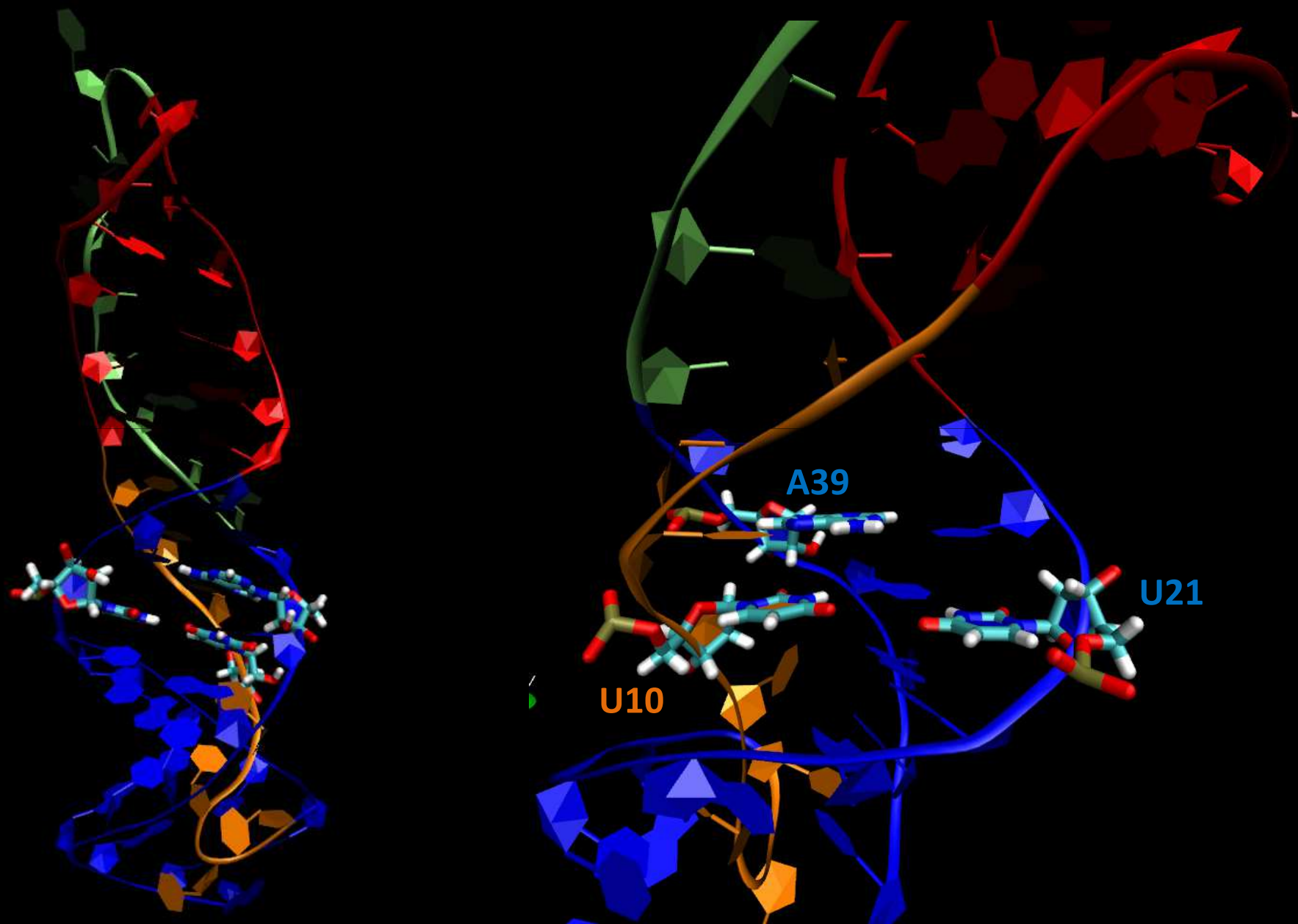
hTER **pseudoknot** domain: Hoogsteen base triples



hTER **pseudoknot** domain: Hoogsteen base triples

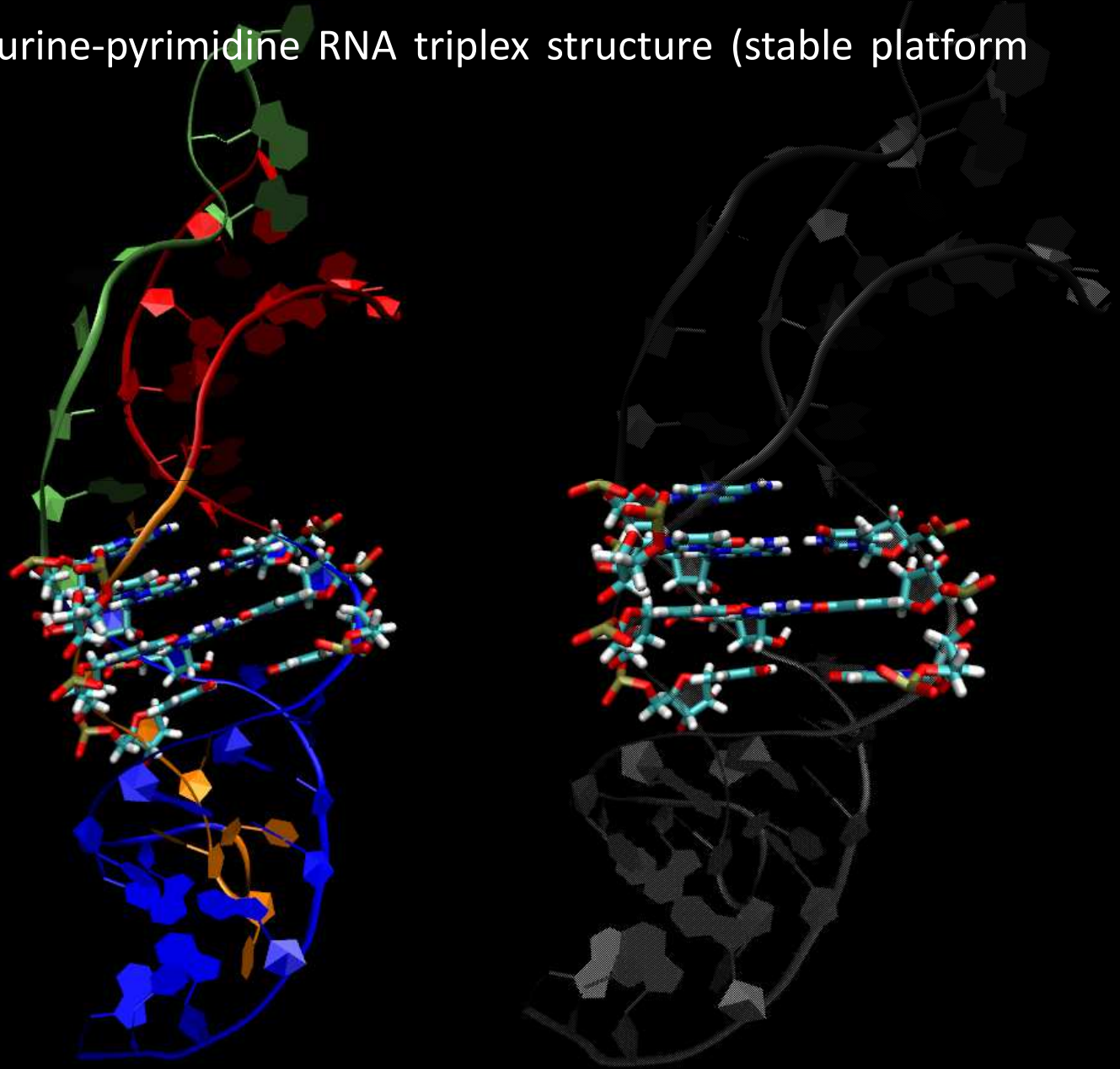


hTER **pseudoknot** domain: Hoogsteen base triples

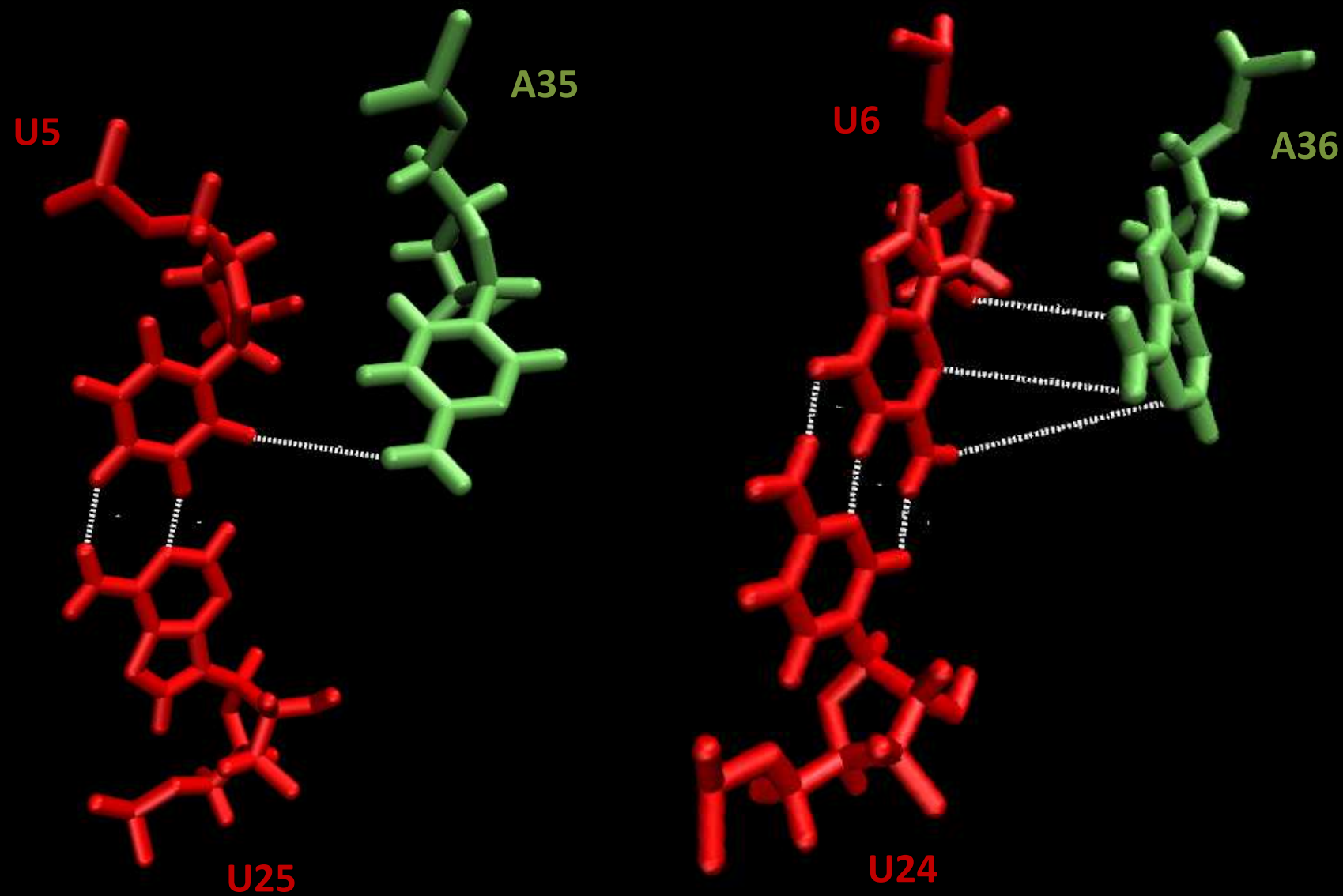


hTER **pseudoknot** domain: Hoogsteen base triples

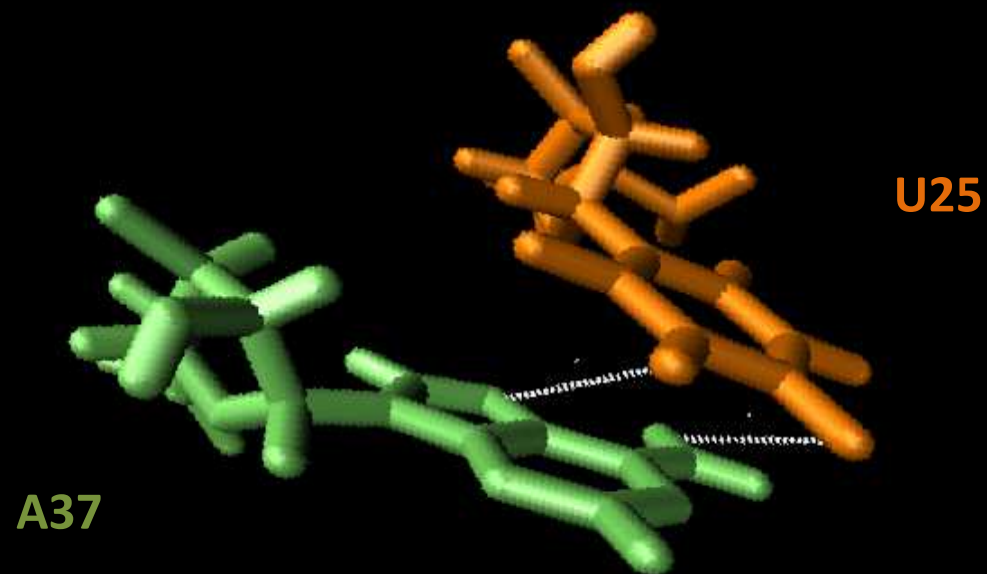
Short pyrimidine-purine-pyrimidine RNA triplex structure (stable platform for interactions)



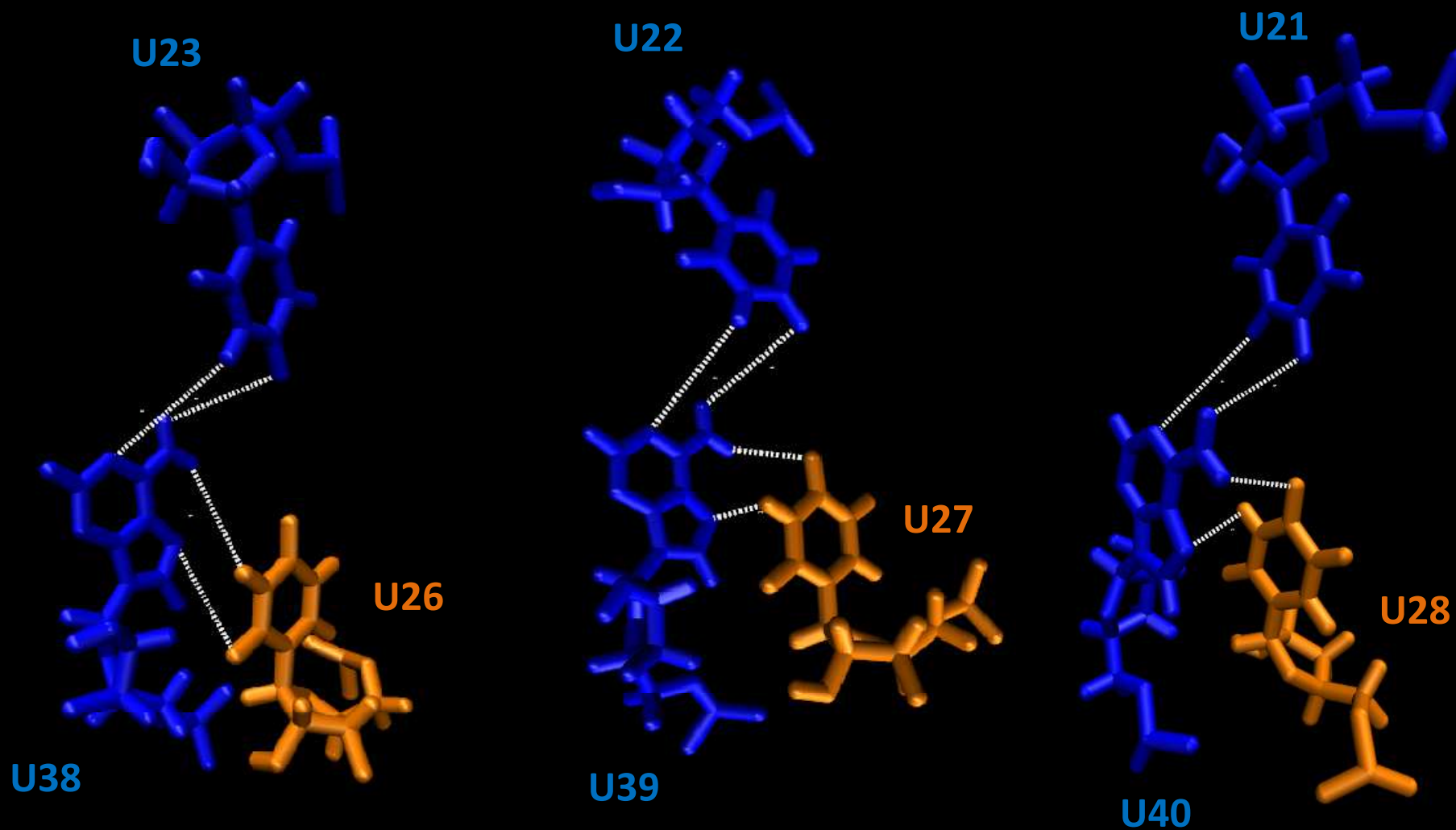
hTER **pseudoknot** domain: Hydrogen bonds (**Stem1-Loop2**)



hTER **pseudoknot** domain: Hydrogen bonds (**Loop1-Loop2**)



hTER **pseudoknot** domain: Hydrogen bonds (**Stem2-Loop1**)



TER **CR4/5** domain

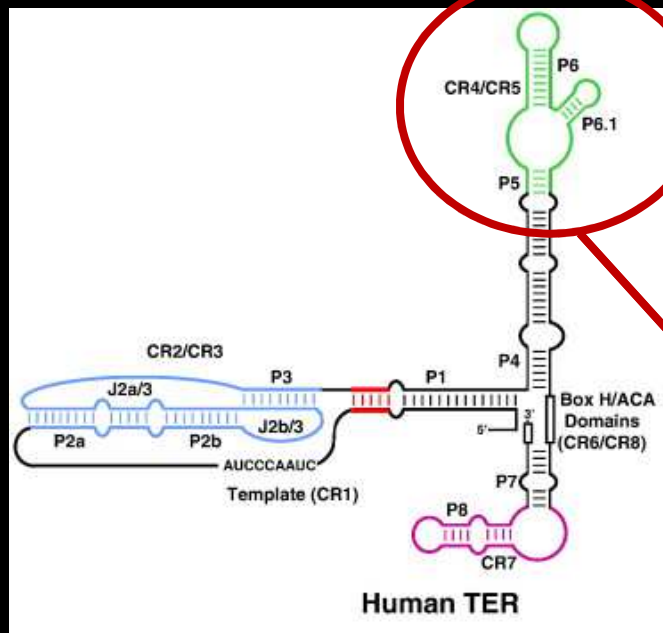
CR4/5 domain (trans-activating domain)

Distal to template/pseudoknot domain

3-way junction of helices (P5, P6 and P6.1 in vertebrates)

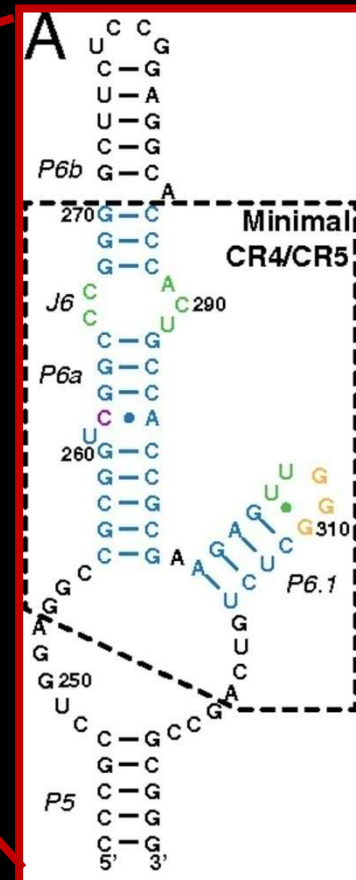
Contains a high-affinity site for TERT interaction

Contributes to correct folding of pseudoknot, nucleotide addition and repeat processivity.



P6b

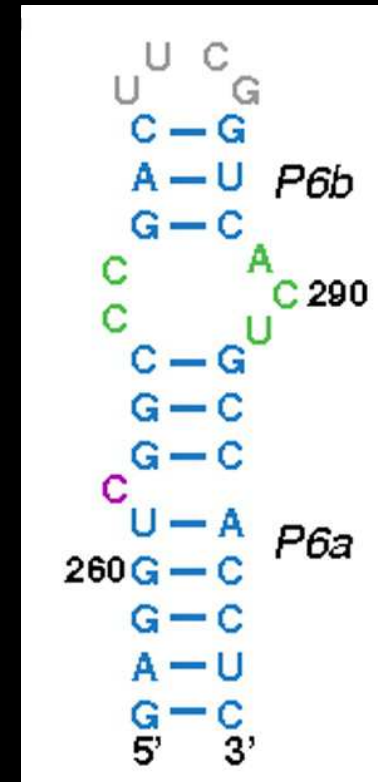
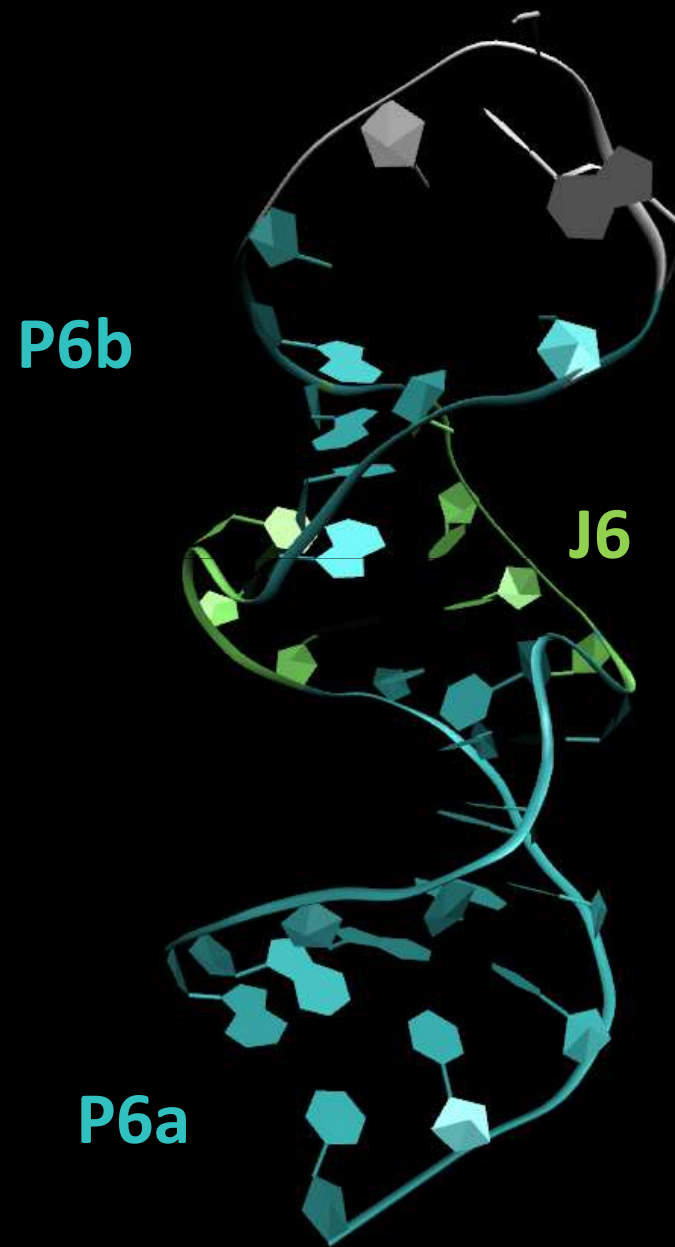
P6a



P6.1

P5

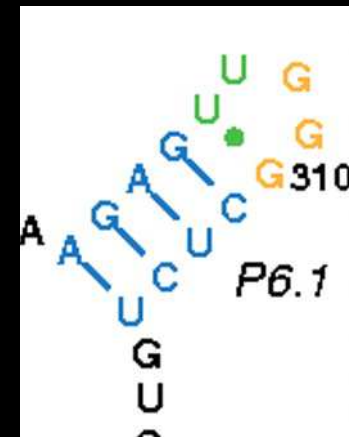
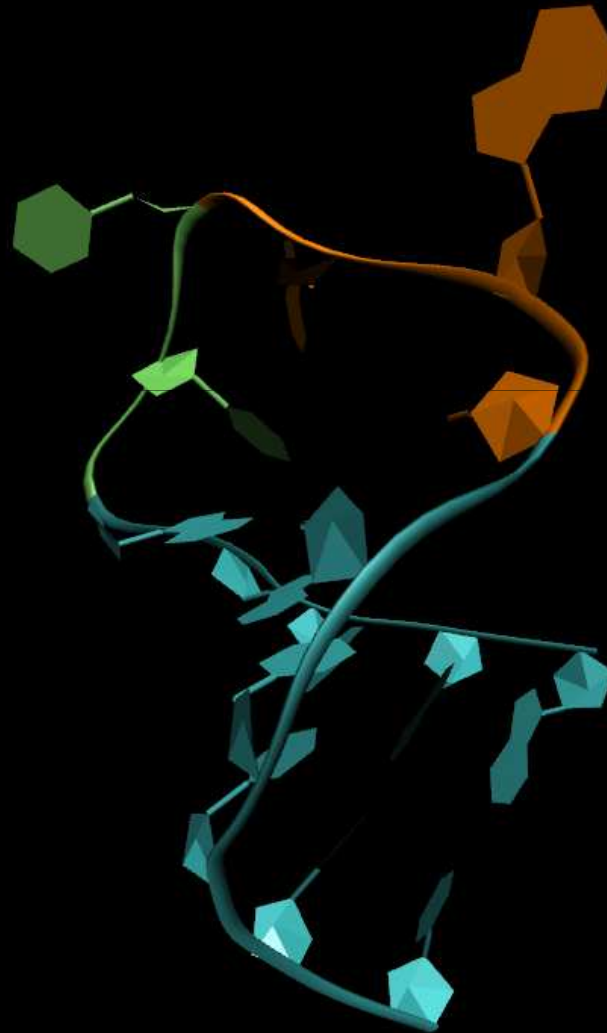
hTER CR4/5 domain



P6a stem forms a continuous 9bp helix with a single nucleotide bulge

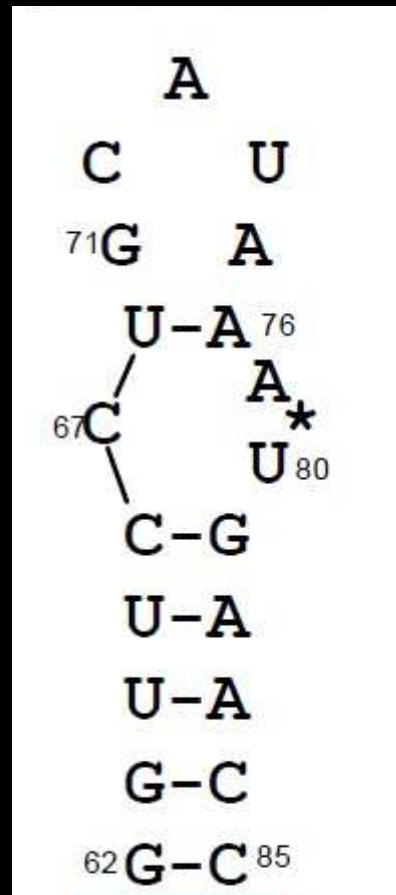
hTER **CR4/5** domain

P6.1 helix (GA bulge in *T. thermophila*) is critical for telomerase activity
→ Participates in long range interactions with template region

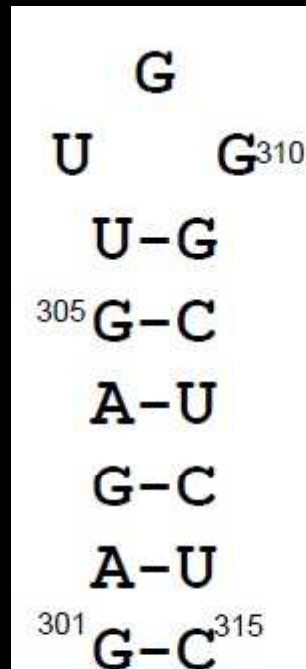


hTER CR4/5 domain

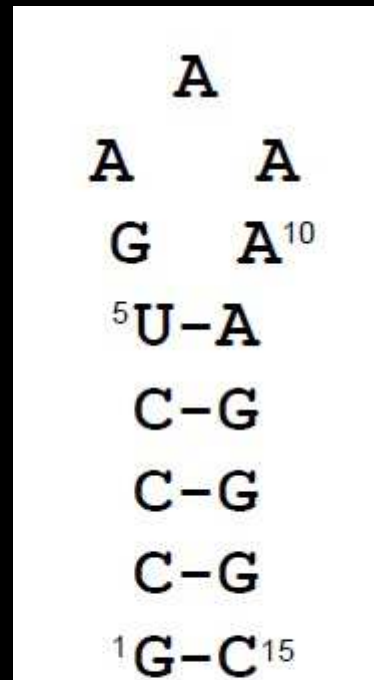
P6.1 helix (GA bulge in *T. thermophila*)



U6snRNP
(1LC6)



P6.1
(10Q0)



boxB
bacteriophage
(1QFQ)

CLUSTAL W(1.60) multiple sequence alignment

```

1LC6_A|PDB      GGTTCCTCCCTGCATAAGGATGAACC
1QFQ_A|PDB      ----GCCCTGAAAAAGGGC-----
10Q0_A|PDB      -----GAGAGTTGGGCTCTC-
                  *   **
    
```

hTER CR4/5 domain

P6.1 helix (GA bulge in *T. thermophila*)

```
      1      2      3
1      2.07 2.35
2 0.00      2.77
3 0.00 0.00

Backbone atoms are      :  N      CA      C      P      O5'      C5'      C4'

For molecule      1
residues considered      :      6.. 19

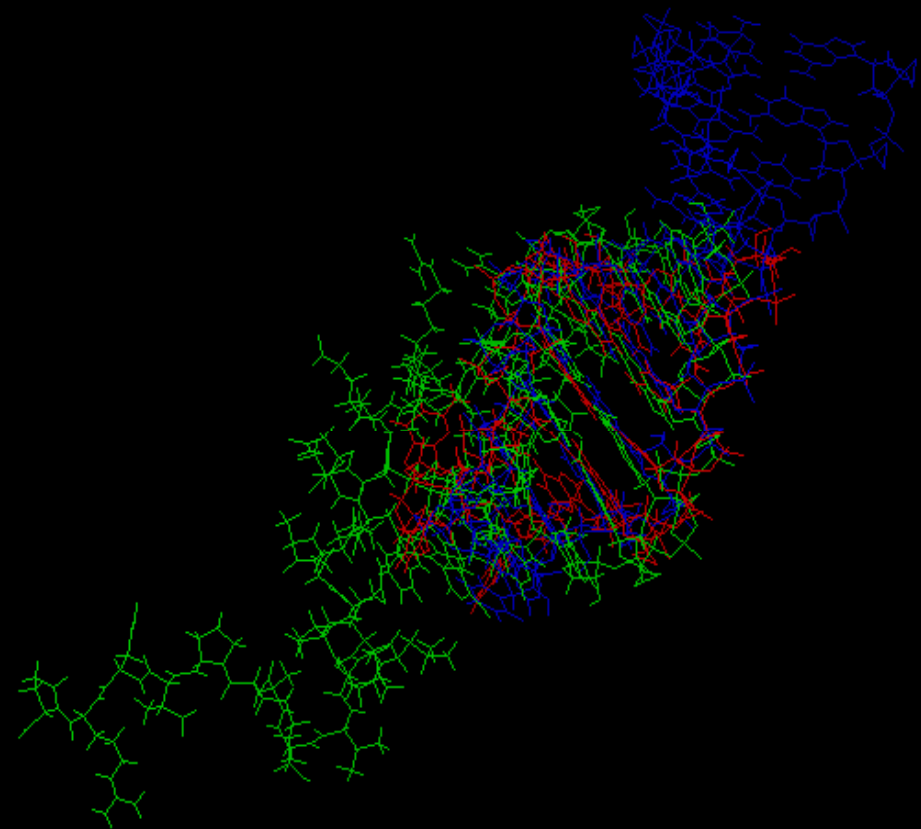
For molecule      2
residues considered      :     37.. 50

For molecule      3
residues considered      :      2.. 15

num. of residues considered:     14  ( 58%)

mean global backbone RMSD :  2.40 +/- 0.29 A  (2.07..2.77 A)
mean global heavy atom RMSD: 0.00 +/- 0.00 A  (0.00..0.00 A)

average rmsd of each struct. to the rest:
Structure 1 (BB): 2.21 +/- 0.14 A  (2.07..2.35 A)
              (H):  0.00 +/- 0.00 A  (0.00..0.00 A)
Structure 2 (BB): 2.42 +/- 0.35 A  (2.07..2.77 A)
              (H):  0.00 +/- 0.00 A  (0.00..0.00 A)
Structure 3 (BB): 2.56 +/- 0.21 A  (2.35..2.77 A)
              (H):  0.00 +/- 0.00 A  (0.00..0.00 A)
===== BESTFT =====
```



U6snRNP

boxB bacteriophage

P6.1

TER **H/ACA** domain

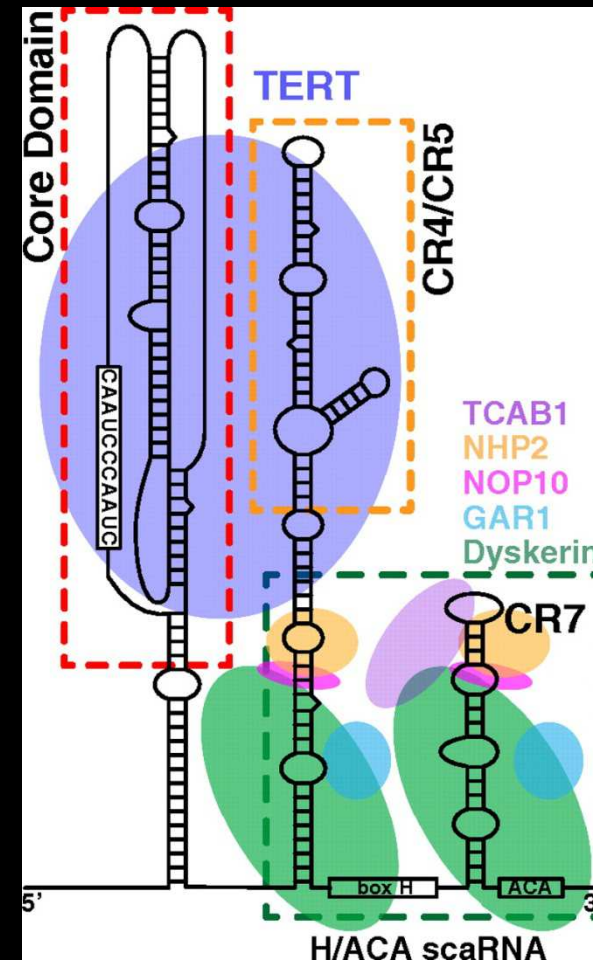
H/ACA domain (vertebrates)

3' end

Contains 2 stem-loops separated by the box H/ACA moieties

→ Binding sites for –

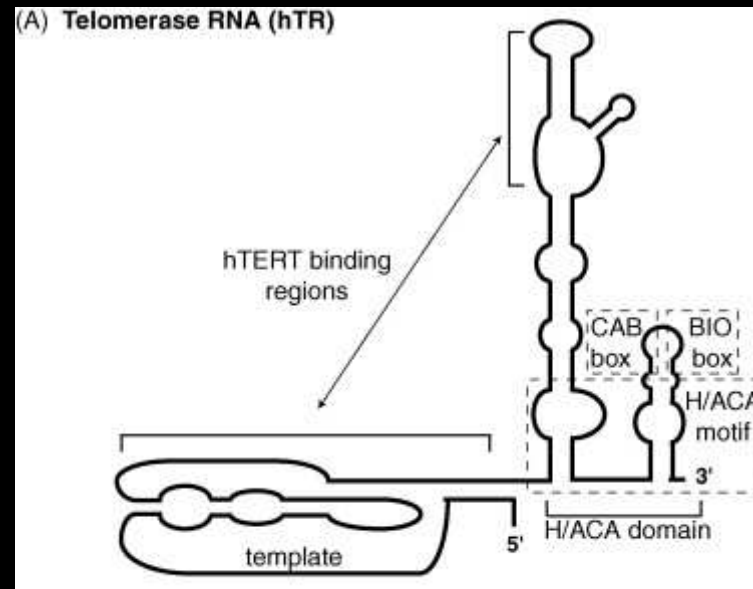
Dyskerin
NOP10
NHP2
GAR1



TER H/ACA domain

CAB box in 3' stem-loop of H/ACA

→ Binding site for TCAB1 (telomerase Cajal body protein1)



CR7 domain

Participates in nucleolar localization of telomerase RNA

Mutations

destabilize the RNA component in vivo and disrupt its localization to the nucleolus.

Vertebrate TER comparison

```
Homo_sapiens -GGCCUGG--GAGGG-----UGGUGGC AUUUUUU--GUUAACCUA-ACUGAGA--AGGCGG-UA-CCG--CCUG 78
Rabbit      -GGCUCGG--GAGGG-----C-CCGUAUUCUC--AUCUAACCUA-ACUGAGC--AGGCGG-UA-CCG--CCGCG 173
Hamster     -A-CCUG--GCG--CG-----CGGCGG-----A-GUUAACCUA-ACUGAGA--GAGCUG-UG-CCA--CUGUG 130
Cat         -GGCCUGG--GAGGG-----AAGCGGAGUUUUU--GUUAACCUA-ACUGAGA--AGGCGG-UA-CCG--CCGCG 176
Pig         -GGCCAG--GAGGG-----UGGCGG AUUUUUUAAGUUAACCUA-ACUGAAA--GAGCGG-UA-CCG--CUGCG 178
Horse       -GGUCUGG--GCGGG-----CGGCGGACGUUUU--GUUAACCUA-ACUGAGC--UGGCGG-CA-CCG--CCGCG 175
Bullfrog    -G-CCCUC--CAGCGUAUAGGAGAGUUC--UAUCC--UUUAACCUA-AUGCA-----CA-ACC--CCUG 155
Xenopus     AAGCUCAA--UGUGA-----CGAGGUUCUGUUU--CGUAACCUA-AUACACU--GGCUC--AG-CCG--AUGCG 211
Shark       -A-CAUUGCUGCGG-----UGGAGG--GUUUUU--AAUAACCUA-AUCAGAGUAAGUGGUGG-CCUCCUCA 188

          CR1          P2a.1 **
          * * * * *
          Pseudoknot-CR2
          * * * * *
          P2a.1
          * * * * *

Homo_sapiens CUUUUGCUC-C-CC-----CGCGCUUUUUUCUGGUGACUUUCAGCGGGCG-GA--AAAGCUCGCGCC--U 139
Rabbit      CUUUUGUUUC-C-CC-----CGCGCUUUUUUCUGGUGACUUUCAGCGGUGGA--AAAGCUCGCGCC--U 236
Hamster     CUUUUGCUC-U-CC-----CGCGCUUUUUUCUGGUGACUUUCAGCGGGCG-GG--AAAGCUCAGACC--U 198
Cat         CUUUUGUUU-C-CC-----CGACGCUUUUUUCUGGUGACUUUCAGCGGGCG-GA--AAAGCUCGCGCC--U 237
Pig         CUUUUGCUU-C-AC-----CGCGCGUUUUUCUGGUGACUUUCAGCGGGCG-GA--AAAGCUCGCGCC--U 239
Horse       CUUUUGCUC-C-CC-----CGCGCGUUUUUCUGGUGACUUUCAGCGGGCG-GA--AAAGCUCGCGCC--U 236
Bullfrog    CUGCU-CUU-C-AU-----CGUGGUUUUUUCUGGUGACUUUCAGCGGGCG-AA--AGAGCAAGUGAA--G 215
Xenopus     UCUCUGCGG-CGU-----CGUGGUUUUUUCUGGUGACUUUCAGCGGGCACGG--AGAGCAAGUGAGCG 277
Shark       CA-CUGCCU-C-CCGUGCGUGAAACUUCGUGUUUCUGGUGACUUUCAGCGGAGU-GAGGCAG--CGCGCA--A 259

          Pseudoknot-CR3
          * * * * *

Homo_sapiens GCGGCUUC--CACGUUCAUUCUAGGCAACAAAAAUGUCAGUGUCGCCGUUCGCCCU--CCCGG----- 207
Rabbit      ACCGCGUC--CACGUUCAUUCUGGAGUAAACAAAAAUGUCAGUGUCGCCGUUCGCCCU--CCCGG----- 304
Hamster     CCGCGGCG--CAUCGCGGUUUUGGAGCAACA-AAAAAUGUCAGUGUCGCCGUUCGCCCU--CCCGG----- 252
Cat         ACCGCGUC--CACGUACAGUUUGGAGCAACA-AAAAAUGUCAGUGUCGCCGUUCGCCCU--CCCGG----- 305
Pig         ACCGCGAUC--CACCAUCCAGUCUGAGAAACA-AAAAAUGUCAGUGUCGCCGUUCGCCCU--CCCGG----- 307
Horse       ACCGCGACU--UACCAUCCAGUCUGAGAAACA-AAAAAUGUCAGUGUCGCCGUUCGCCCU--CCCGG----- 304
Bullfrog    CUGA-----CUAAAAACUUCAGUGAGUAGGUUUUCU--A--GCGC----- 258
Xenopus     ACAGUAAA--AAACGU-CAGCUGGAGAC-UCCUCCGUUCGACAGCGCCACCCUGUCUUGCU--CCAG-- 343
Shark       AAAAAAGUUGGAGCA-GCAACGGCA-GCAAAAAAAGAGUUCACCGAGGCU-CCUACCAACGCGCGUGGUCUGAG 337

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Homo_sapiens -----GGACUUECG-----GGCGGUG--GCCUGCC-CAG 233
Rabbit      -----GGACUUECG-----GUGGUGU--GCCCGCC-CGG 330
Hamster     -----AGCCUUECG-----GC-----GGCCGCC-CAG 274
Cat         -----GACCUUECG-----GUGGUGU--GCCUCCU-UAG 331
Pig         -----GAACUUECG-----GUGGUGU--GCCCGCC-CAG 333
Horse       -----GACCUUECG-----ACGGGU--GCCCGCC-CAG 330
Bullfrog    -----AGCCUUECG-----UGUCAUGU-----GCCCGGCCUUGCUGGCAUUG 298
Xenopus     -----AGCCUUECG-----UUUUU-----UGUGGAA-UGU 369
Shark       GGUAACAUGCCUUCGGGAGAGAGCCAAACGGGGGGGCCCGGUCGGCUAACAGCGCAGGAGGU--GGCCCCAAUUG 416

          CR4          CR5
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Homo_sapiens CCCCCGAACCCCGC--UGGAGCGCGGUGGCGCGCGGCGUUCU--CGGAGGCACACUCCACCGCAAGAUUG 309
Rabbit      CCCCCGUGCCCCCGC--U-GAGCGCGGUGGCGCGCGGCGUUCU--CGGAGGUGCCAAUCCCGCGCAAGAUUG 405
Hamster     CC-CGCGAC-CCCGC--U-GAGCGCGGUGGCGCGGCGUUCU--GGGCUCCGUC--CCCGCAAGAUUG 342
Cat         CCCCCGUGCCCCCGC--UAGAGCGCGGUGGCGCGGCGUUCU--CGGAGGCACACUCCACCGCAAGAUUG 407
Pig         CCCCCGUGCCCCCGC--U-GAGCGCGGUGGCGCGGCGUUCU--CGGAGGCGCCAAUCCCGCGCAAGAUUG 408
Horse       CCCCCGUGCCCCCGC--UGGAGCGCGGUGGCGCGGCGUUCU--CGGAGGCGCCAAUCCCGCGCAAGAUUG 406
Bullfrog    CCGCAGCGCGUUCU--AUGAGCGCGGUGGCGCGGCGUUCU--AAUACCGCGCAUCCCGCAAGAUUG 359
Xenopus     AUCAGCGCGC--UCC-C-C--CGU--CUGGUGUGGCGG--CGC--GGUCGGCAUCCUCCCGCAAGAUUG 436
Shark       CC-UCAGCCCAACACUCUCGCGCGGCGCGGCGUUCUCCUCCUUGGCGCCAAUCCCGCGCAAGAUUG 495

          CR5
          * * * * *
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Homo_sapiens GCUUCUGGCGCG--C-GCG-UGCCU-CGCGG--GCC-----AAG-GG-----CGAGGCG--AGGCC--GU 359
Rabbit      GCUUCUGGCGCG--C-GCG-UGCCU-CGCGG--GCC-----AAG-GG-----CGAGGCG--AGGCC--GU 455
Hamster     ACUCUGGCGCG--C-GCG-UG-CA-CCG--GCU-----GG-GG-----CGAGGCG--CGG-----C 386
Cat         GCUUCUGGCGCG--C-GCG-UGCCU-UGGGGGGCC-----GAG-GG-----CGAGGCG--CGG-----C 451
Pig         GCUUCUGGCGCG--C-GCG-UGCCU-CGCGG--GCC-----AA-GG-----CGAGGCG--CGG-----C 449
Horse       GCUUCUGGCGCG--C-GCG-UGCCU-CGCGG--GCC-----AGG-GA-----CGAGGCG--CGG-----U 448
Bullfrog    UCUCUGGCGCG--U-UGG-CGCGUG-CGCGG--GAG-----UG-GG-----CGAGGCG--CU-----G 404
Xenopus     UCUCUGGCGCG--U-UGGCGGCGC-UGG-U--CGG-----AGUG-----AGAGGCG--CGG-----U 483
Shark       GCUUCUGGCGCGGCGCG--UGCGGUGGCG--CGCGGACAGAG--GACACACCGCGCGCGCGCGGAGUAGUA 571

          CR6          CR7
          * * * * *
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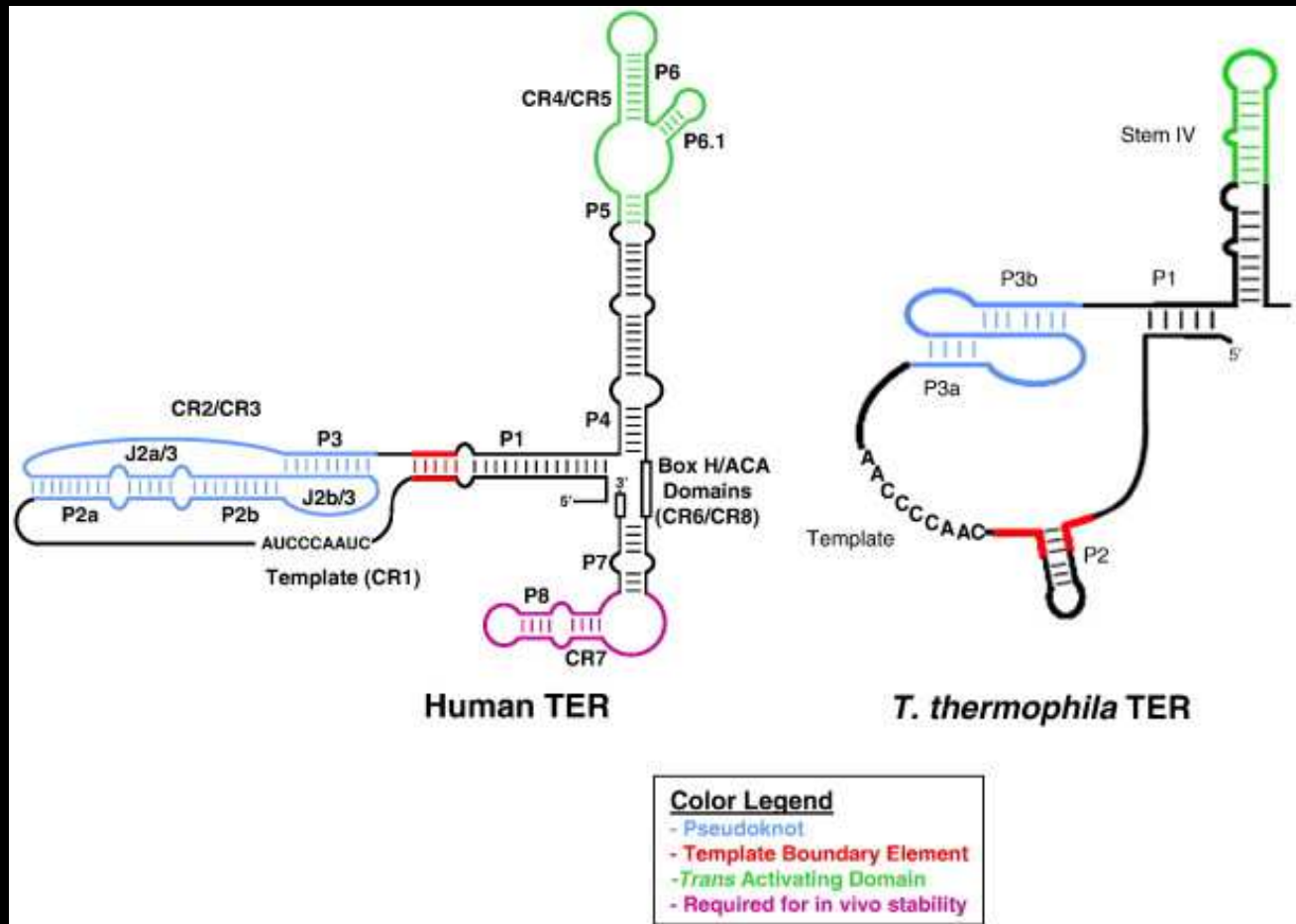
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Homo_sapiens CAGGC-CGAGGAGAG--GAC-----CAGCGAGUCCCGCGCGCGG-CGGA-U-UCCUGAGCUGGCGAGC 424
Rabbit      CUGGC-CGAGGAGAG--GAC-----CAGCGGUGCCCGCGCGGUGU-GCGC-U-UCCUGAGCUGGCGAGC 528
Hamster     AGCGC-CGAGGAGAG--GAC-----CAGCGGUGCCCGCGCGGUGU-GCGC-U-UCCUGAGCUGGCGAGC 449
Cat         CUGAC-CGAGGAGAG--GAC-----CAGCGGUGCCCGCGCGGUGU-GCGC-U-UCCUGAGCUGGCGAGC 517
Pig         CUGAC-CGAGGAGAG--GAC-----CAGCGGUGCCCGCGCGGUGU-GCGC-U-UCCUGAGCUGGCGAGC 514
Horse       CUGGC-CGAGGAGAG--GAC-----CAGCGGUGCCCGCGCGGUGU-GCGC-U-UCCUGAGCUGGCGAGC 513
Bullfrog    AUGGC-ACGUGAGAU--AA-C-----ACGCGAGUGGCGCGGUGUUGUCC-UGCGUGGUGGUGGUGU 471
Xenopus     CUGGGGUGGCGGAGCAAAAG--GGGCGCGGUGGUGGUGGUGGUGGUGGUGGUGGUGGUGGUGGUGGUGG 557
Shark       CCGCC-CGAGGAGAG--UAAAUAGUCC--UGACAGUGGCGCGGUGGUGU-GCGU-C-UCCUGAGCUGGCGAGC 641

          ACA BOX-CR8
          * * * * *
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Homo_sapiens UGC-ACCAGGAGUCCGCGAGCAGUCC 451
Rabbit      UGC-ACCAGGAGUCCGCGAGCAGUCC 547
Hamster     UGC-ACCAGGAGUCCGCGAGCAGUCC 476
Cat         UGC-ACCAGGAGUCCGCGAGCAGUCC 544
Pig         UGC-ACCAGGAGUCCGCGAGCAGUCC 541
Horse       UGC-ACCAGGAGUCCGCGAGCAGUCC 540
Bullfrog    GUGAGUUGCAGAGUCCGCGAGCAGU 499
Xenopus     UGC-GUGUACAGCAGUCCGCGAGUCC 584
Shark       CCG-AGG-CUGCGCUGGCGAGCAGUCC 667

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Homo sapiens telomerase RNA, **TER**



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ONLINE RESOURCES

<http://sgu.bioinfo.cipf.es/services/SARA/>

<http://rna.informatik.uni-freiburg.de:8080/>

<http://www.bio8.cs.hku.hk/RNASAlign/latest/id=1c1e1ed627ddb18f44be1680746a8139&prg=autoDetect>

<http://telomerase.asu.edu>

PEM

1. About TRBD domain of telomerase, mark the correct answer:

- a) It contains two conserved motifs
- b) It interacts with TBE from RNA telomerase
- c) The two answers before are correct
- d) It contains the active site
- e) All answers before are correct

2. Mark the correct answers about TRBD motifs:

- CP is formed by helix $\alpha 3$ and the following loop
- Y477 and W496 together form an hydrophobic pincer
- In contrast to T motif, which forms a narrow pocket, CP motif forms a wider pocket
- There are no interactions between T and CP motif

- a) 1,2,3
- b) 1,3
- c) 2,4
- d) 4
- e) 1,2,3,4

3. Mark the correct answer about TER:

- a) H/ACA box is a very high phylogenetic conserved domain
- b) CR4/5 and pseudoknot/template domains both have TERT binding sites
- c) Pseudoknot forms a triple helix with two stems loops
- d) b and c answers are correct
- e) None of them are correct

4. The telomerase reaction takes place in:

- a) **Cajal bodies**
- b) Ribosomes
- c) Nucleosomes
- d) Golgi
- e) Membrane

5. H/ACA domain of TER:

- a) **Is conserved among vertebrates**
- b) Is conserved among all species
- c) Is only present in human
- d) Is not present in human
- e) Is not conserved

6. The Template boundary region (TBE) of pseudoknot domain:

- a) **Is a TERT-binding site**
- b) Is at 3' from template
- c) Provides the template for telomerase
- d) Is distal from the template
- e) None of them is correct

7. Fingers and palm subdomains of RT are similar to:

- a) HIV retro transcriptase
- b) RNA-Pol
- c) B-family of DNA-Pol
- d) All the answers before are correct
- e) None of them is correct

8. Which of the following statements about thumb domain are true :

- a) It is an elongated helical bundle
- b) Thumb loop runs almost paralel to the curvature of the DNA primer
- c) Two answers before are correct
- d) TERT-DNA interactions are not mediated by this domain.
- e) All of them are correct

PEM

9. Mark the correct answers :

- RT domain is characterized for having D251, D343 and D344 in the active site.
 - The Motif T and CP are also involved in the catalytic site of this domain.
 - The RNA-template binding to TERT is mediated by the conserved motifs 2 and B' of the fingers and palm domain.
 - The RT domain is not conserved in all species.
- a) 1,2,3
 - b) 1,3**
 - c) 2,4
 - d) 1,2,3,4
 - e) All the statments are true

10. Which of the following statements are true about N terminal domain?

- Castaneum has the N terminal domain
 - There are some interactions that build up the TEN conformation: 2 hydrophobic interaction and a hydrogen bounding interaction.
 - The deep groove is formed by some aminoacids which are not very well conservated.
 - There are two glycins (gly144 and gly171) that are a really good conserved residus and help to form the twist of the $\alpha 5$ and $\alpha 6$, and, the $\beta 6$ and $\alpha 7$.
- a) 1,2,3
 - b) 2,4**
 - c) 1,3
 - d) 1,2,3,4
 - e) All the statments are false

THANK YOU FOR YOUR ATTENTION !