Structural insights into telomerase elongation complex formation

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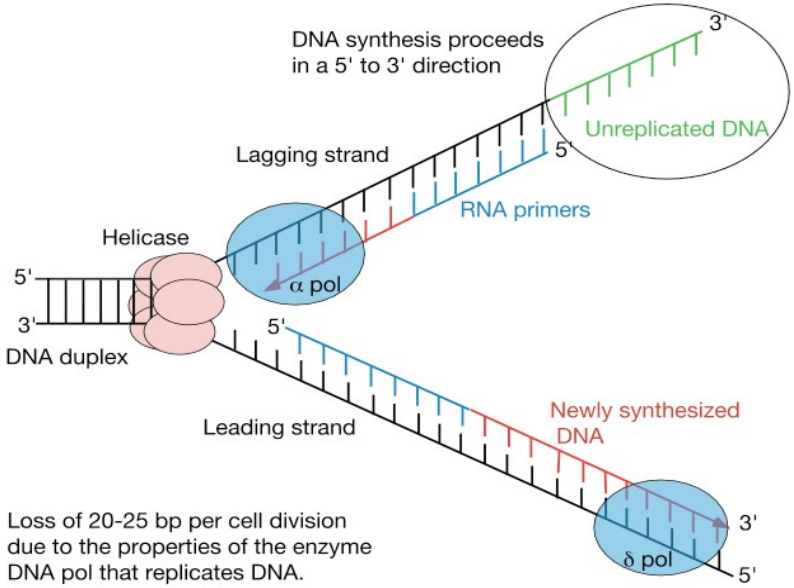
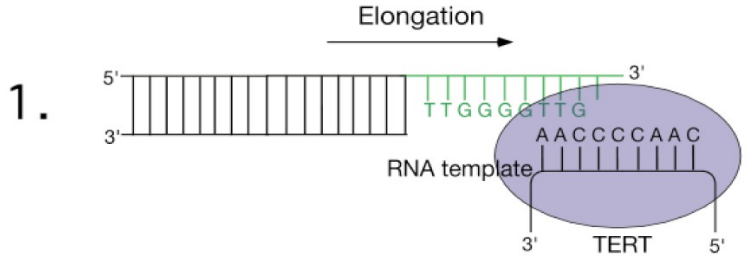
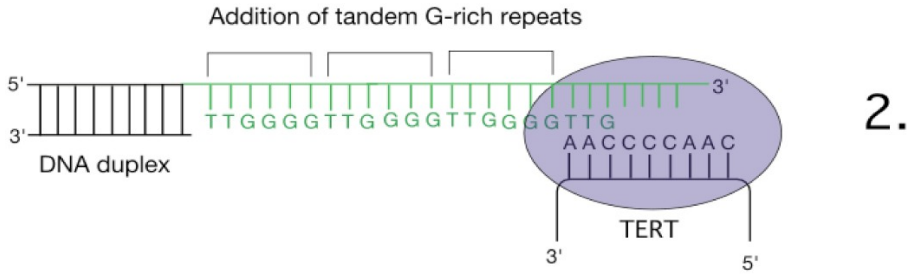
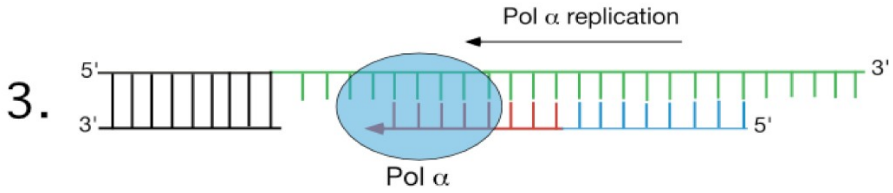
Telomerase Replicates the 3'-Ends of Linear Chromosomes

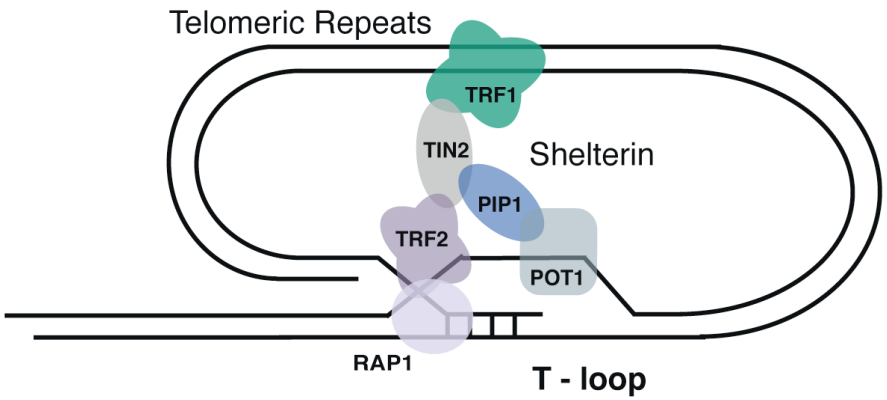


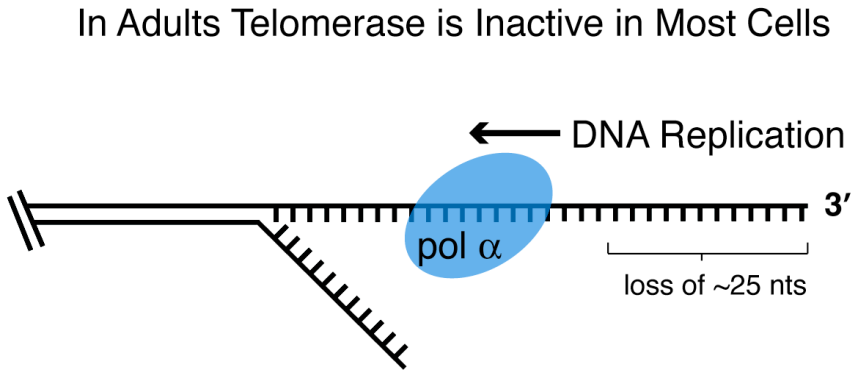






Telomere Function and the “Hayflick Limit”





Genomic Instability

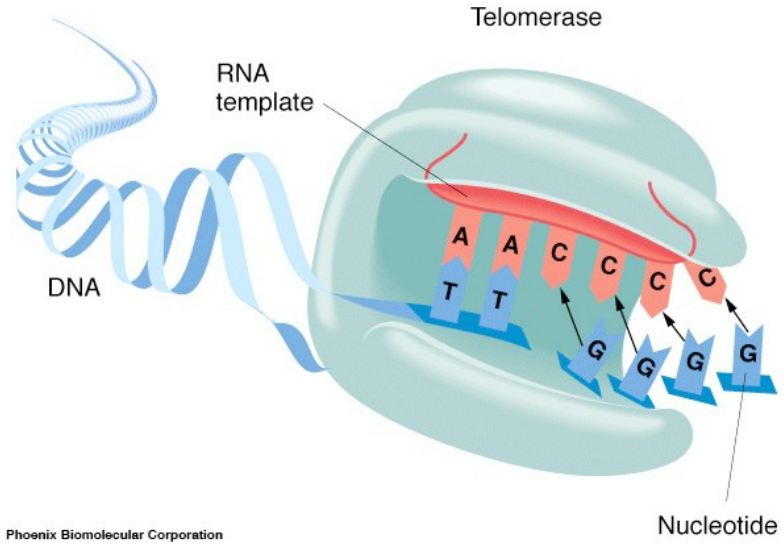
CANCER

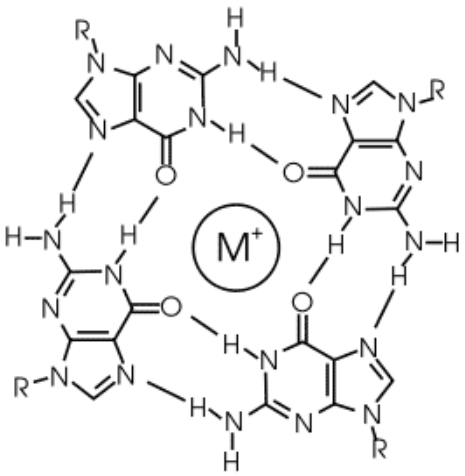
Telomere Shortening

AGING

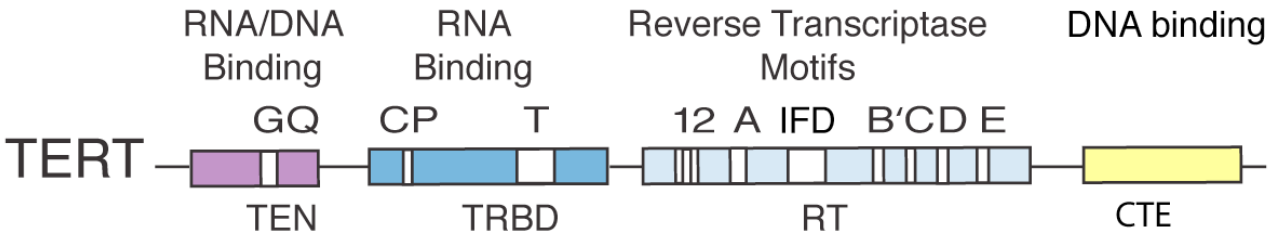
Telomerase, a Target for Cancer Therapies

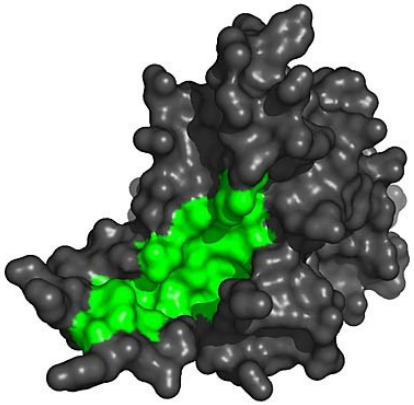
- **Telomerase**
  - **is active in embryonic cells**
  - **Inactive in most somatic cells during adulthood**
- **Telomerase commonly active in tumors**
- **Major cancer drug target**
  - **Small molecule inhibitors - e.g. HIV RT-related inhibitors**
  - **Ribozymes**
  - **Immunotherapies**
  - **Antisense Oligonucleotides**
  - **G-quadruplex stabilizers**



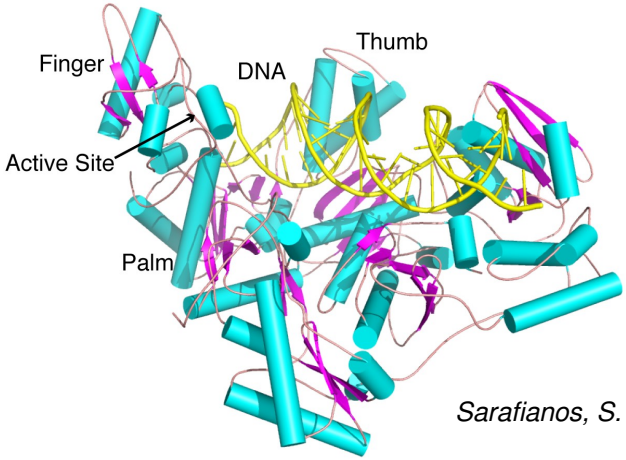


Telomerase is a Large Multi-Domain Protein





Jacobs, S. et al., NSMB 2006



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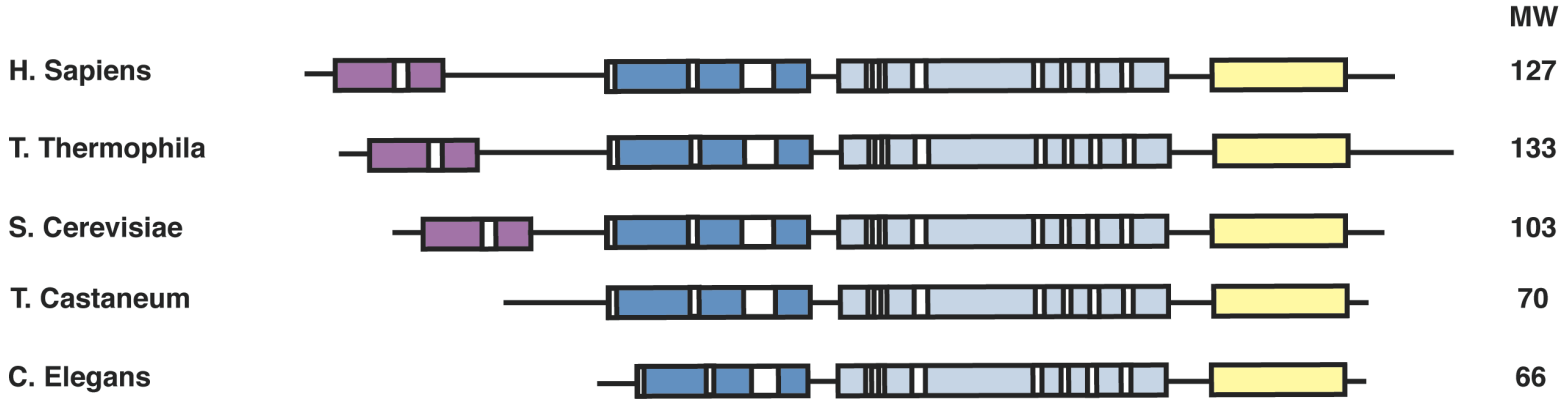
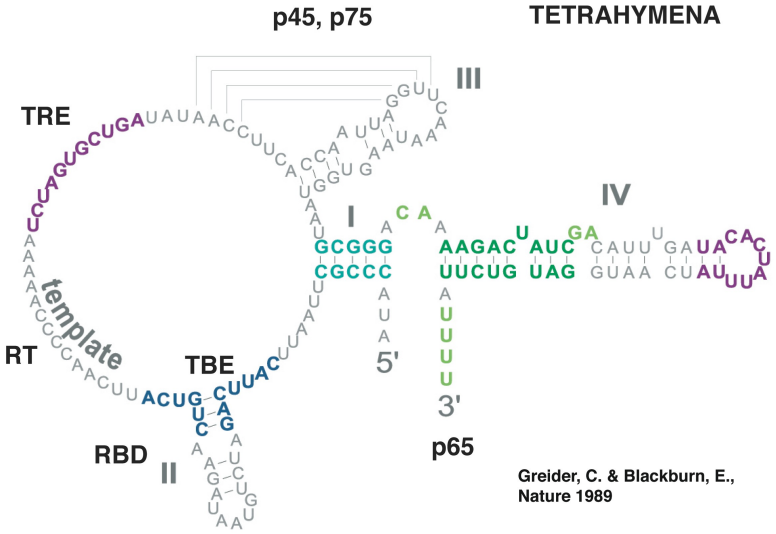
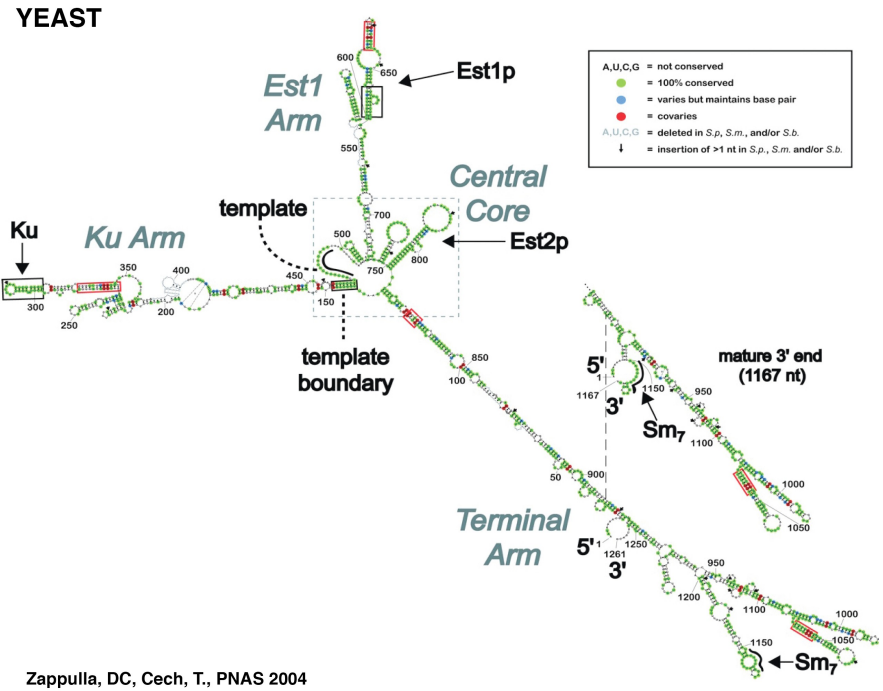


Telomerase RNA Component



Greider, C. & Blackburn, E., Nature 1989



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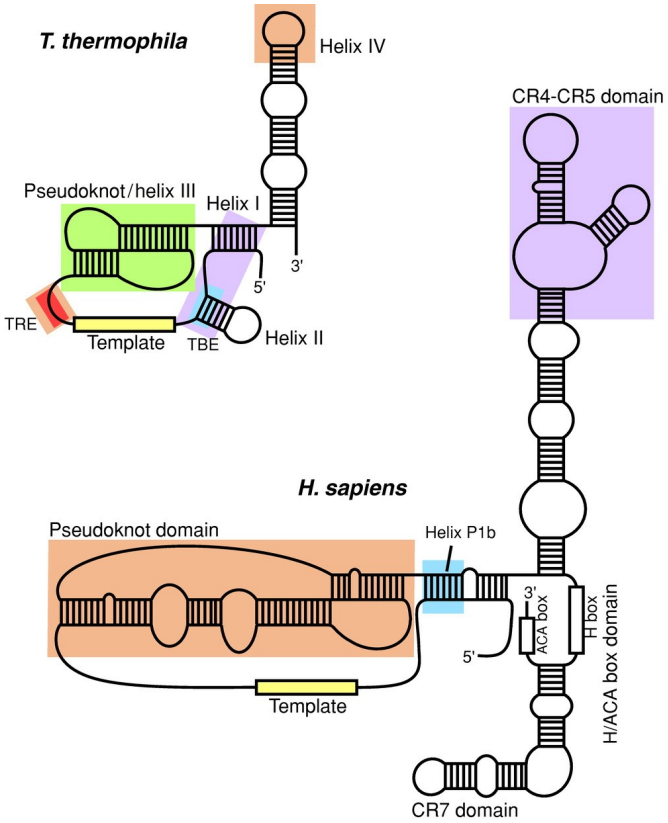

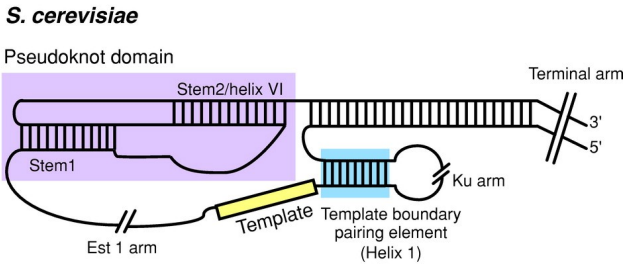

Autexier C, Lue NF. 2006.
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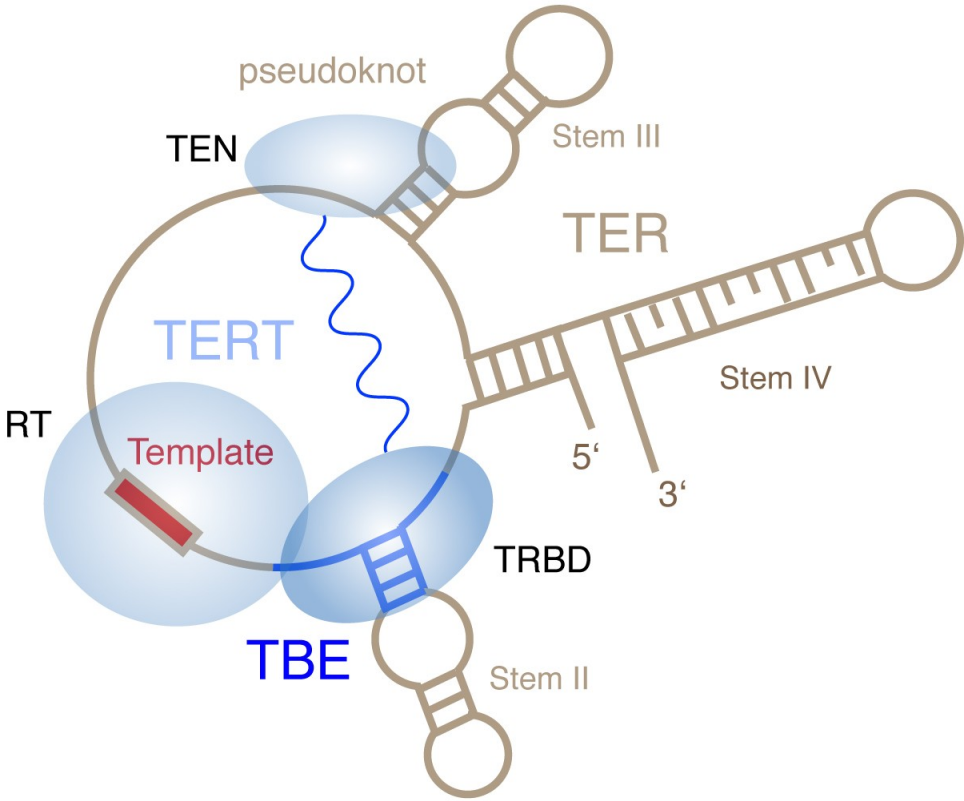
Telomerase Assembly

- **Telomerase oligomeric state**
  - ***Tetrahymena* - Monomer**
  - **Human & Yeast - Dimer**

- **A functional telomerase holoenzyme requires the stable association of TERT with TER**

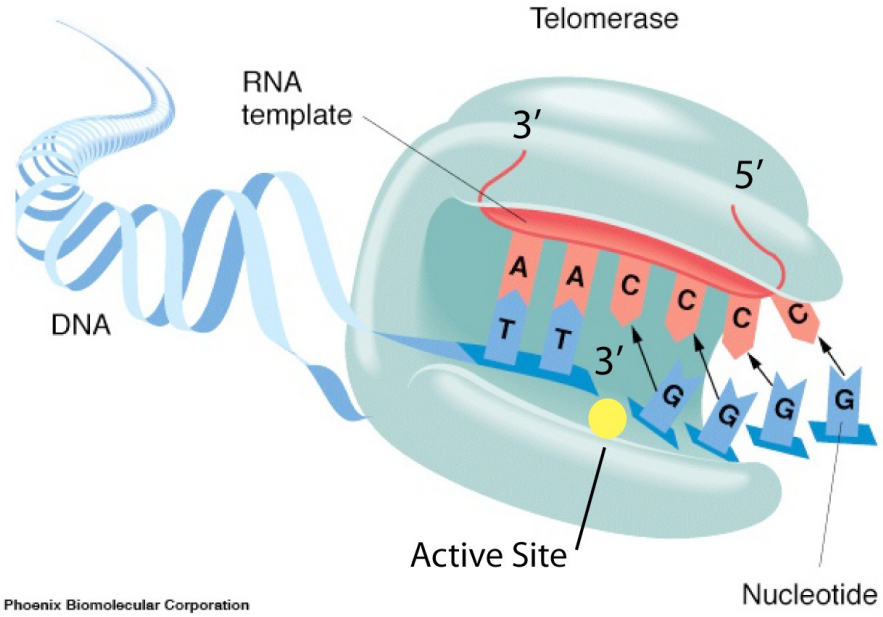
- **TERT-TER assembly is mediated in large part by the TRBD**

T. Thermophila Telomerase



Telomerase Function Overview

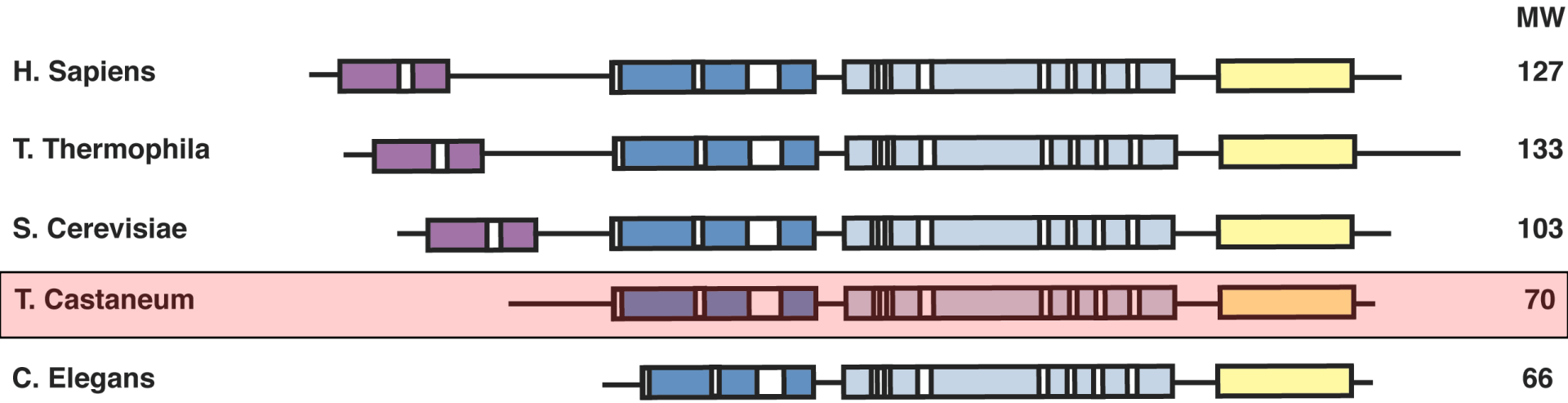
- **Initiation of telomere replication requires**
  - **Telomerase loading at the end of chromosomes**
  - **Pairing of Telomerase templating region with telomeric ssDNA**
  - **RNA/DNA pairing alone is not sufficient for a stable and active telomerase elongation complex**
  - **Placement of the 3'-end of the DNA substrate at the active site**



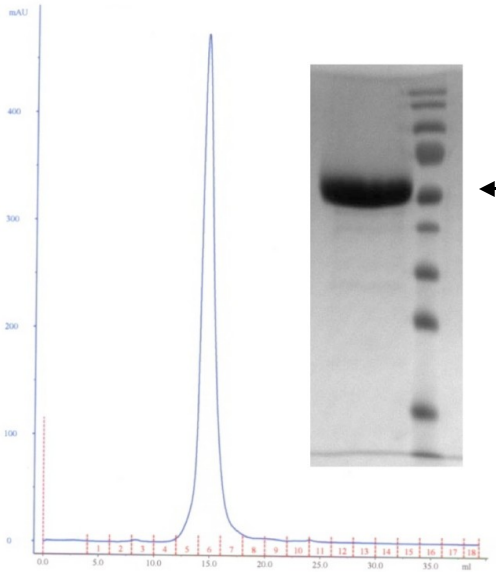
Questions we are Looking to Address

- **How does TERT assemble with TER to form a functional enzyme?**
- **How does telomerase interact with DNA at the end of the chromosomes?**
- **What is the catalytic mechanism of nucleotide addition?**

In Search for the "Right" TERT Gene for Structural Studies



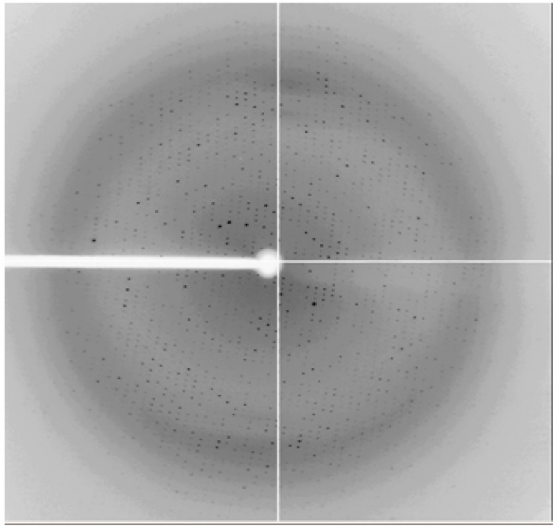
Protein Purification



← *T. Castaneum* TERT

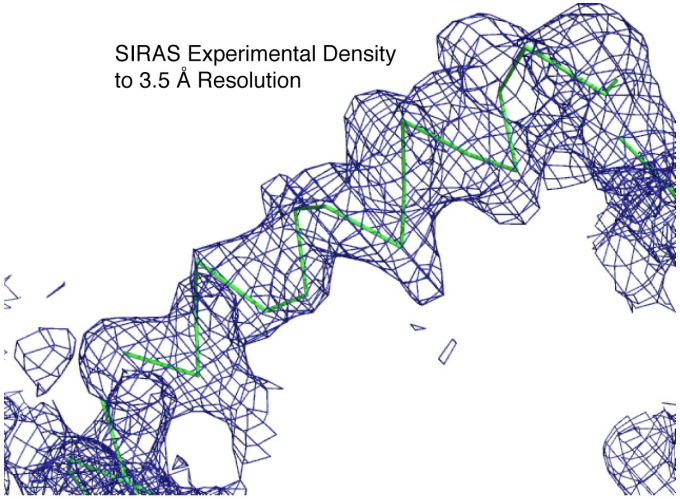
T. Castaneum TERT Structure Solved by the X-ray Method to 2.7 Å Resolution

X-ray Diffraction 2.7 Å

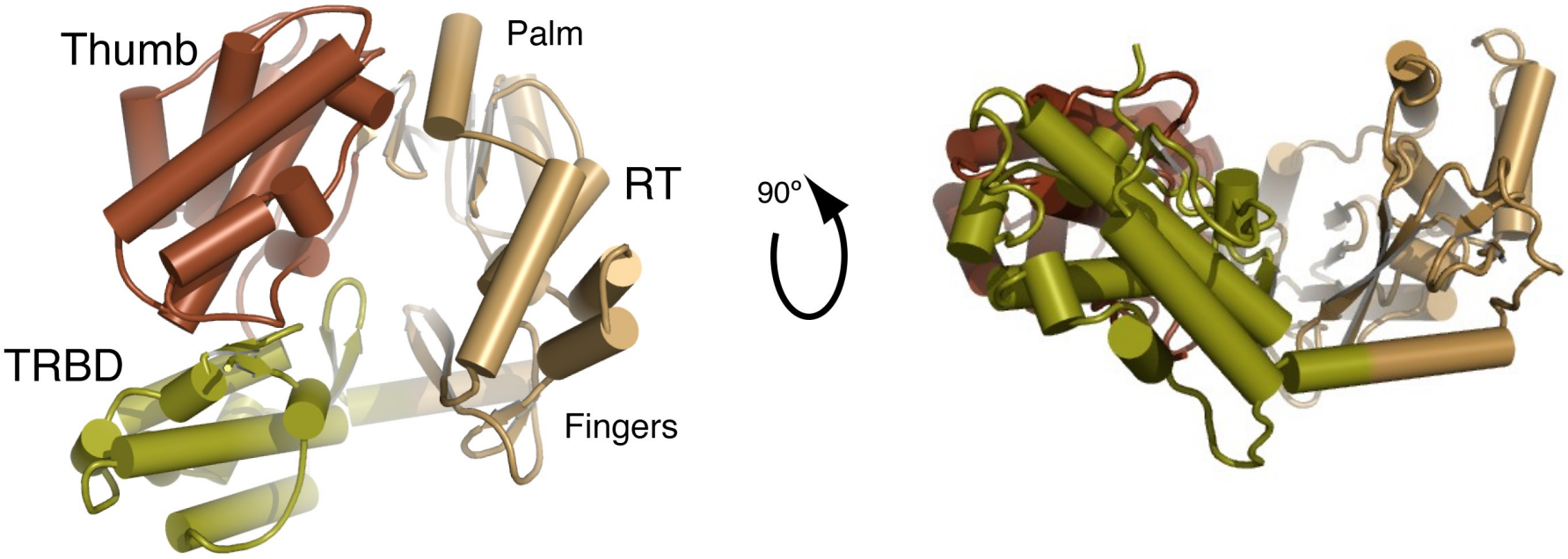


- **Co-crystallization with the telomeric DNA**
- **Space group $P2_12_12_1$; one dimer in the AU**
- **Experimental phases obtained using a Hg derivative to 3.5 Å.**
- **Phase extension to 2.7 Å in DM with 2-fold NCS**
- **Refinement statistics: R_{free}=27.5, R_{work}=23.4.**

SIRAS Experimental Density
to 3.5 Å Resolution

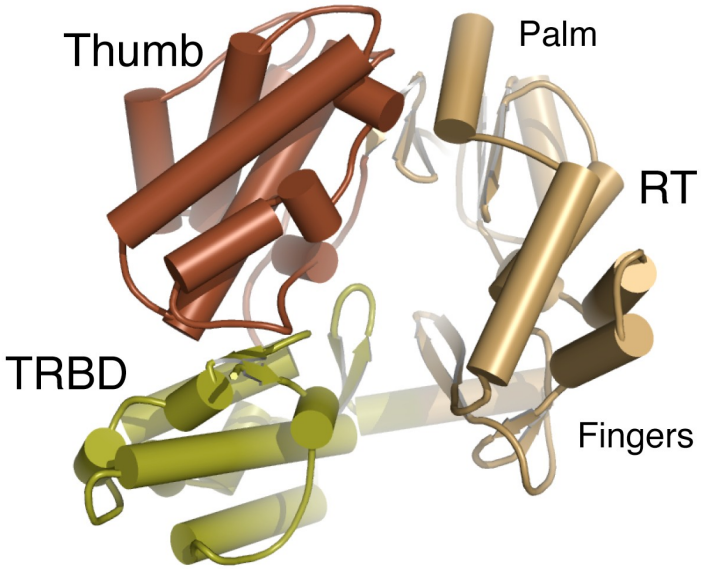


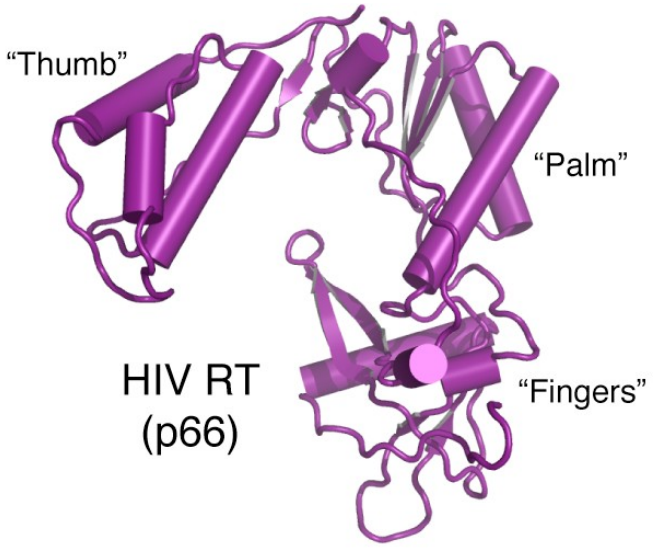
Structure of the Catalytic Subunit of Telomerase, TERT

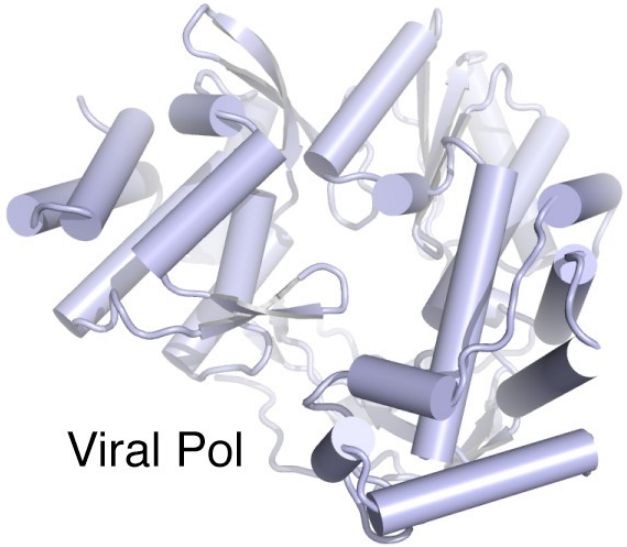


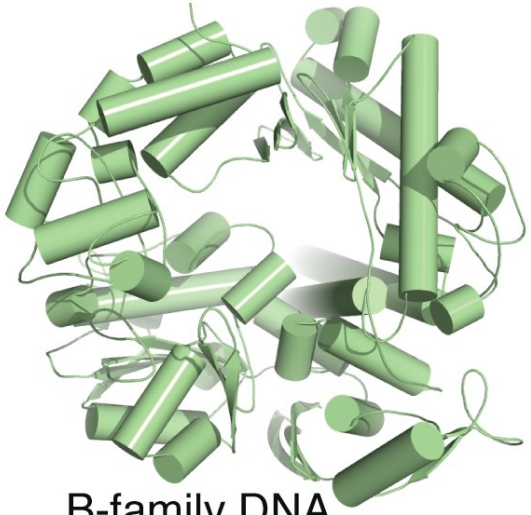
Gillis, Schuller &
Skordalakes, Nature
455:633-637, Sep (2008)

TERT Structural Homologues

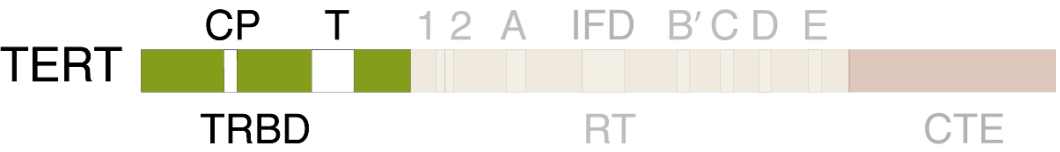


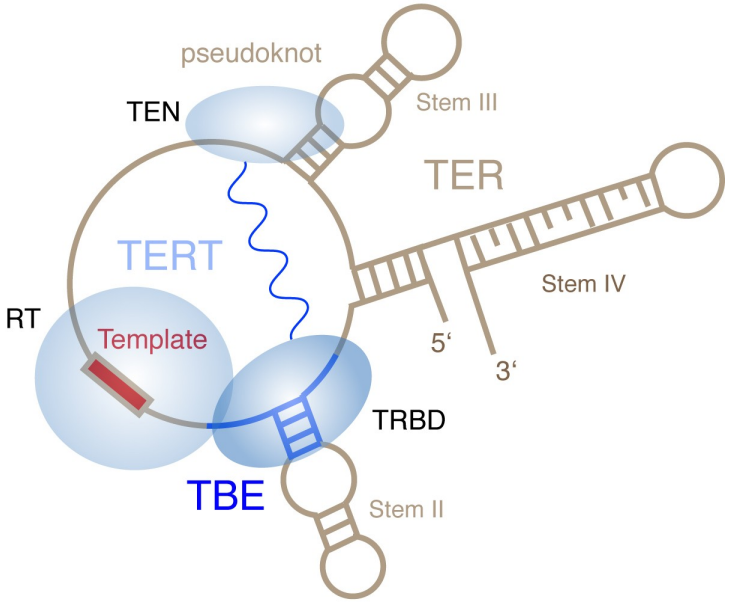


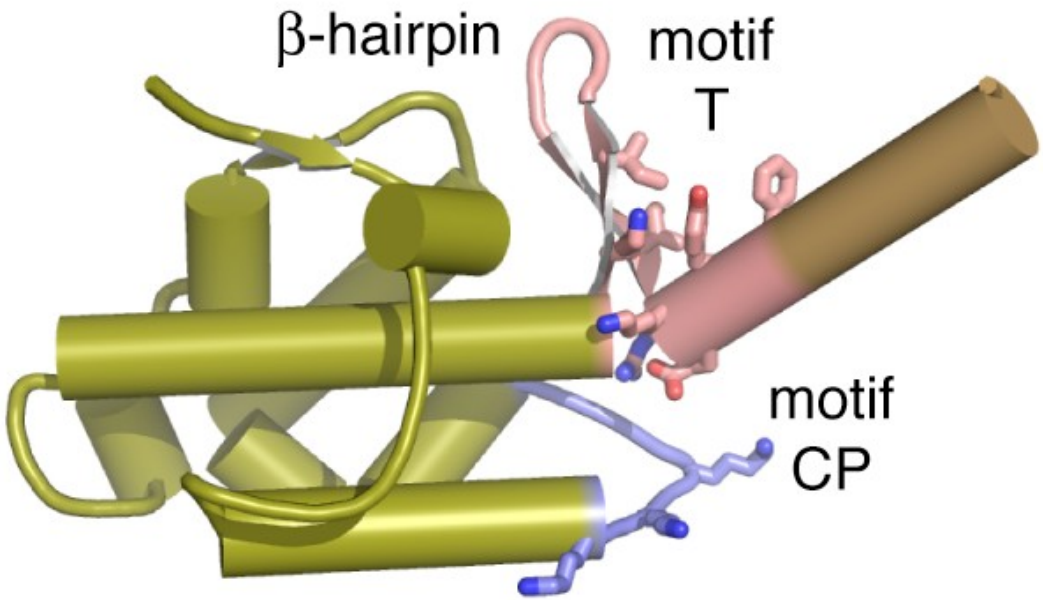


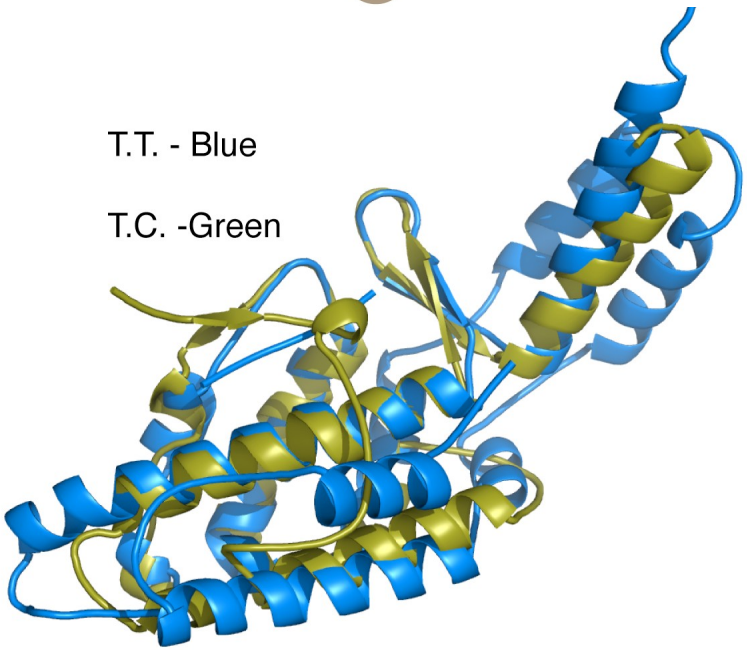


TRBD Fold Conserved Among Telomerases

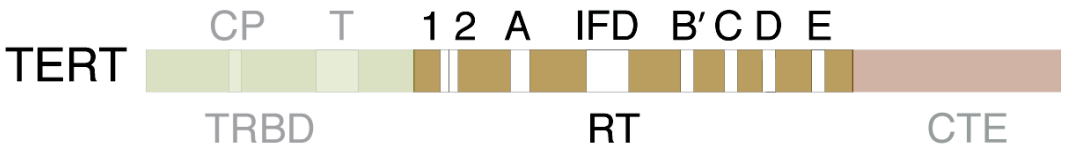


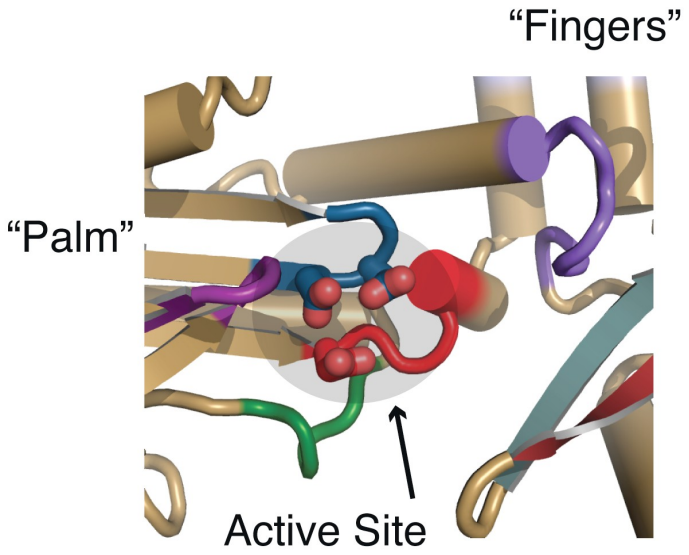


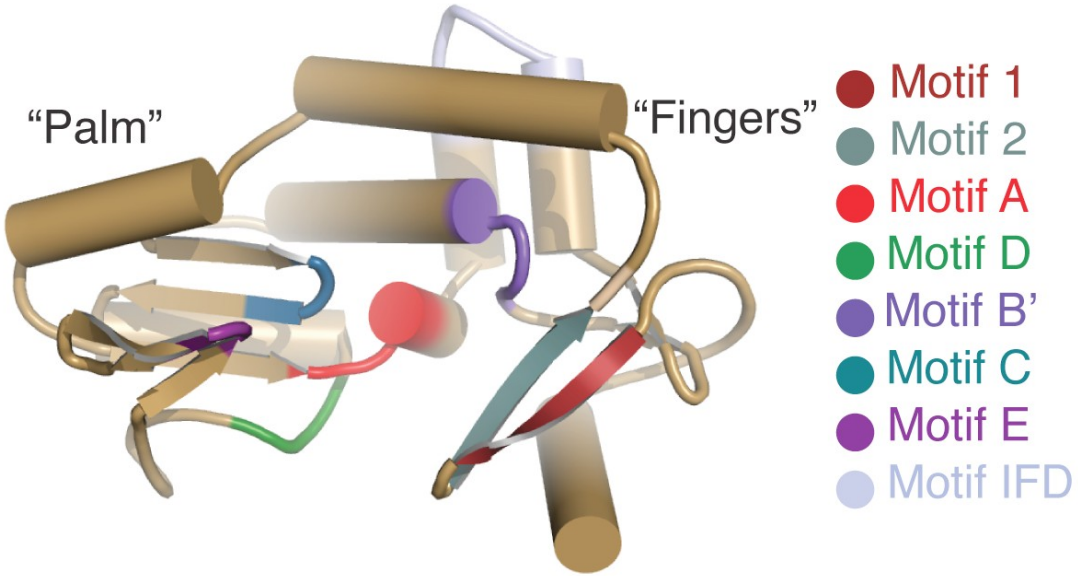


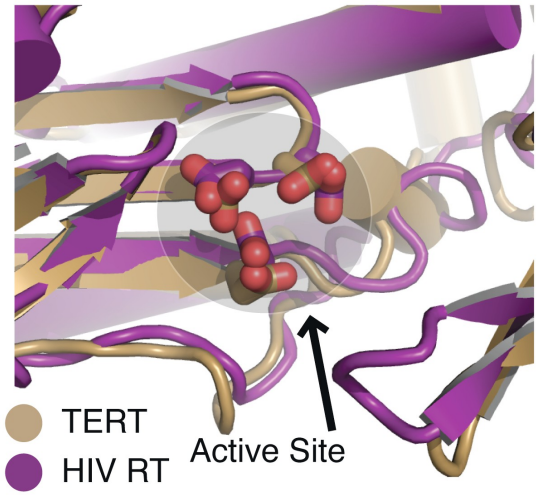


Telomerase's Reverse Transcriptase Domain

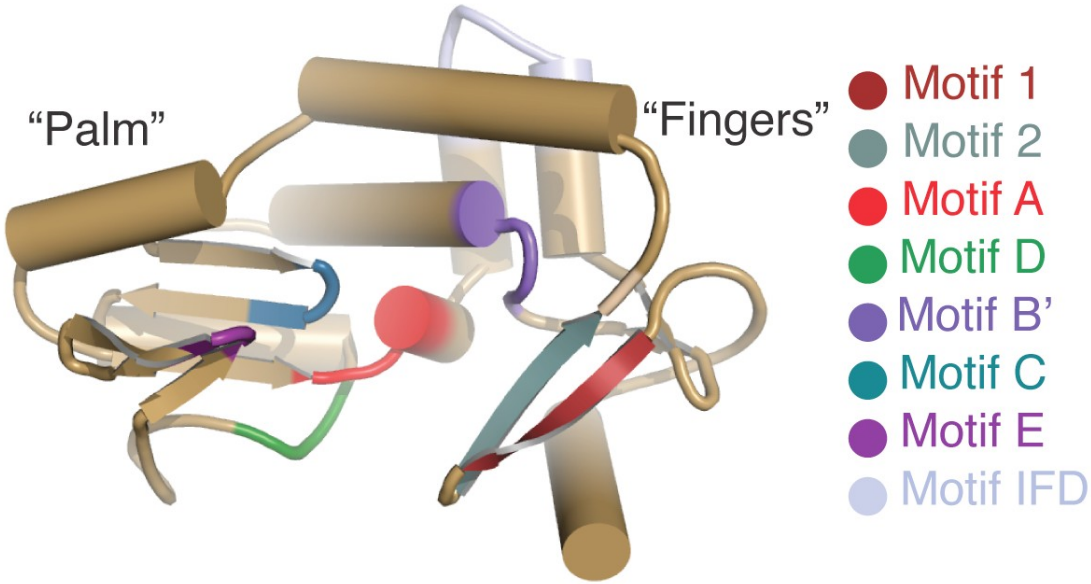




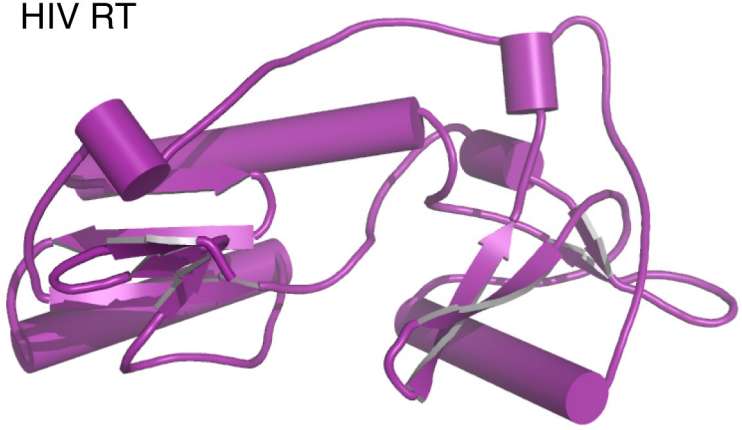




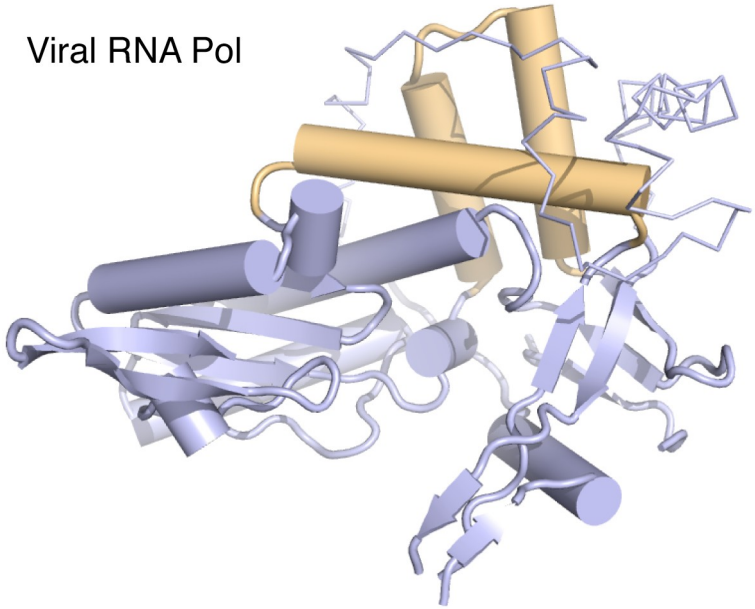
The IFD Motif of Telomerase



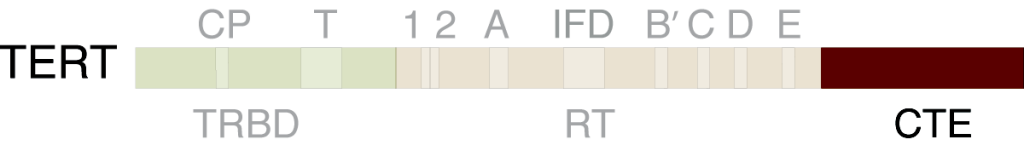
HIV RT

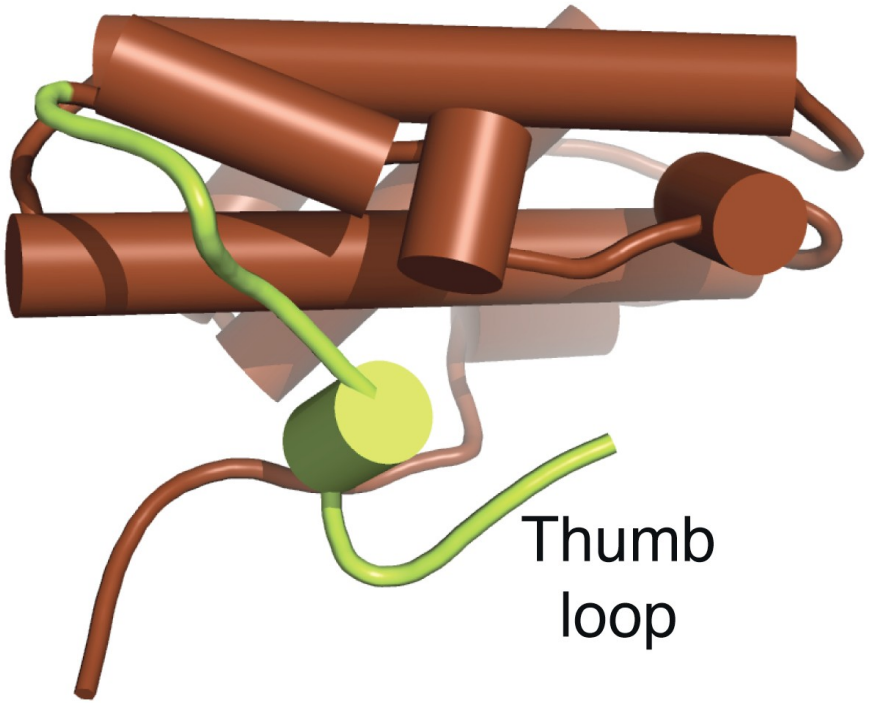


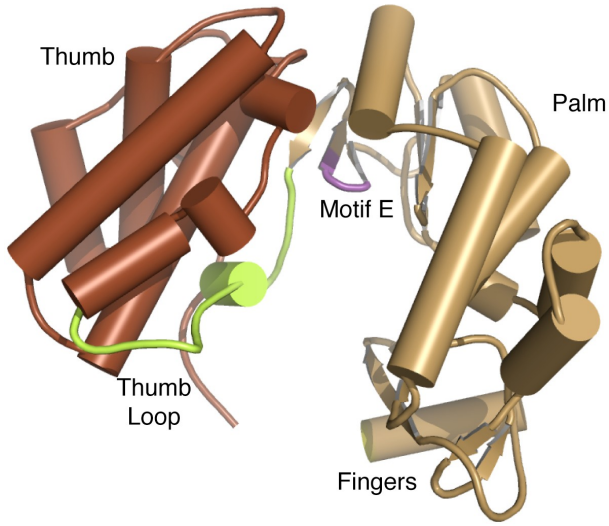
Viral RNA Pol

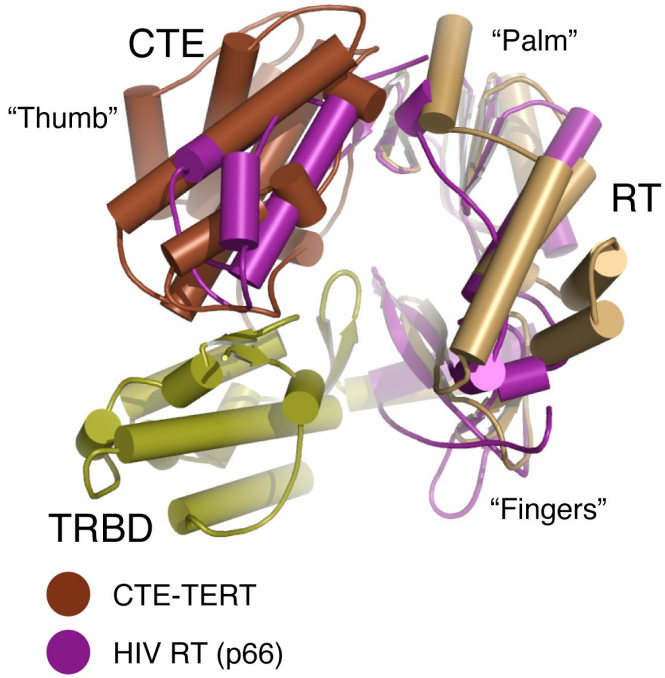


The Thumb domain of Telomerase

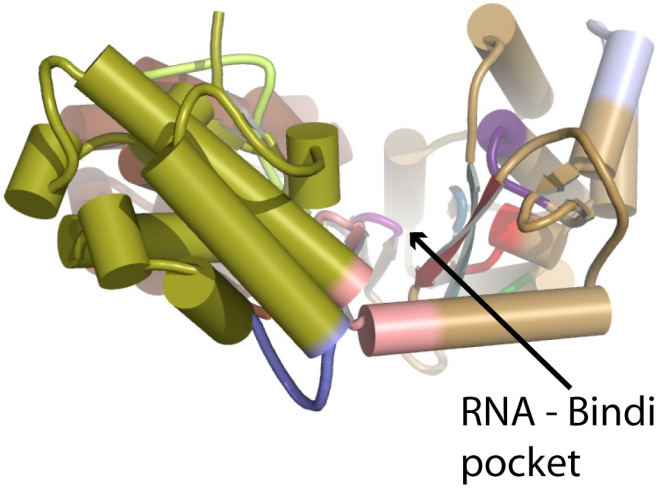


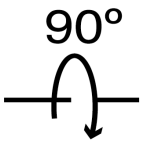


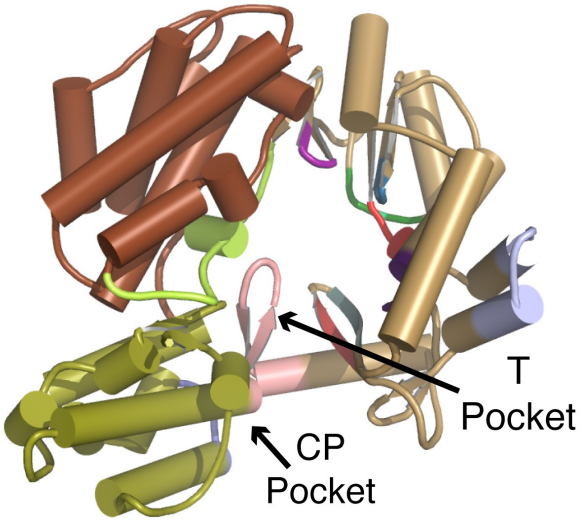


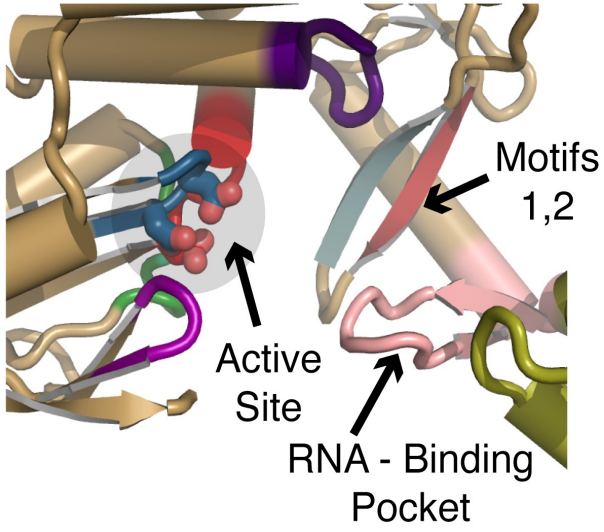


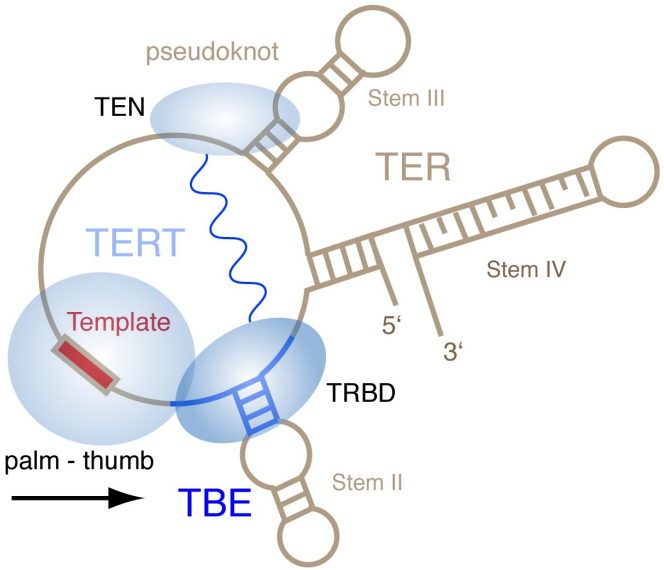
TRBD Facilitates Positioning of the Template at the Active Site of TERT



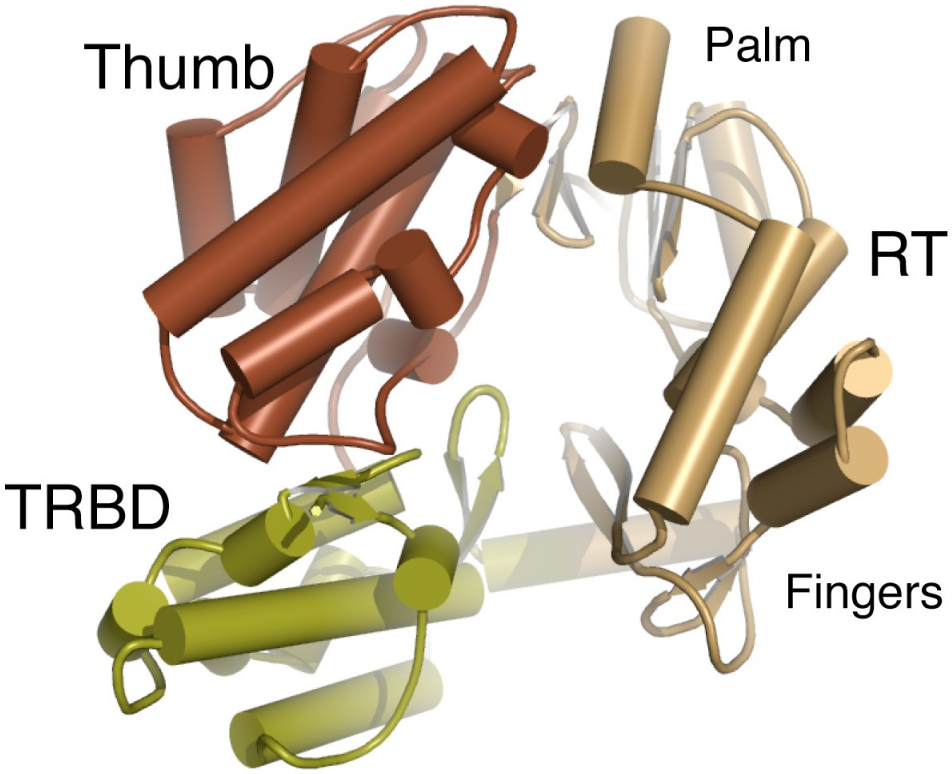


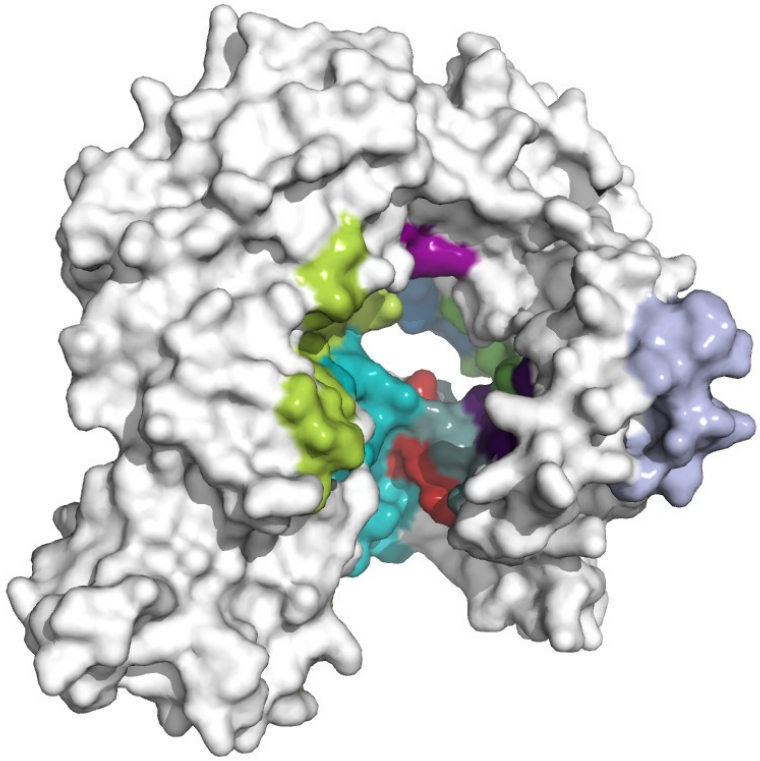




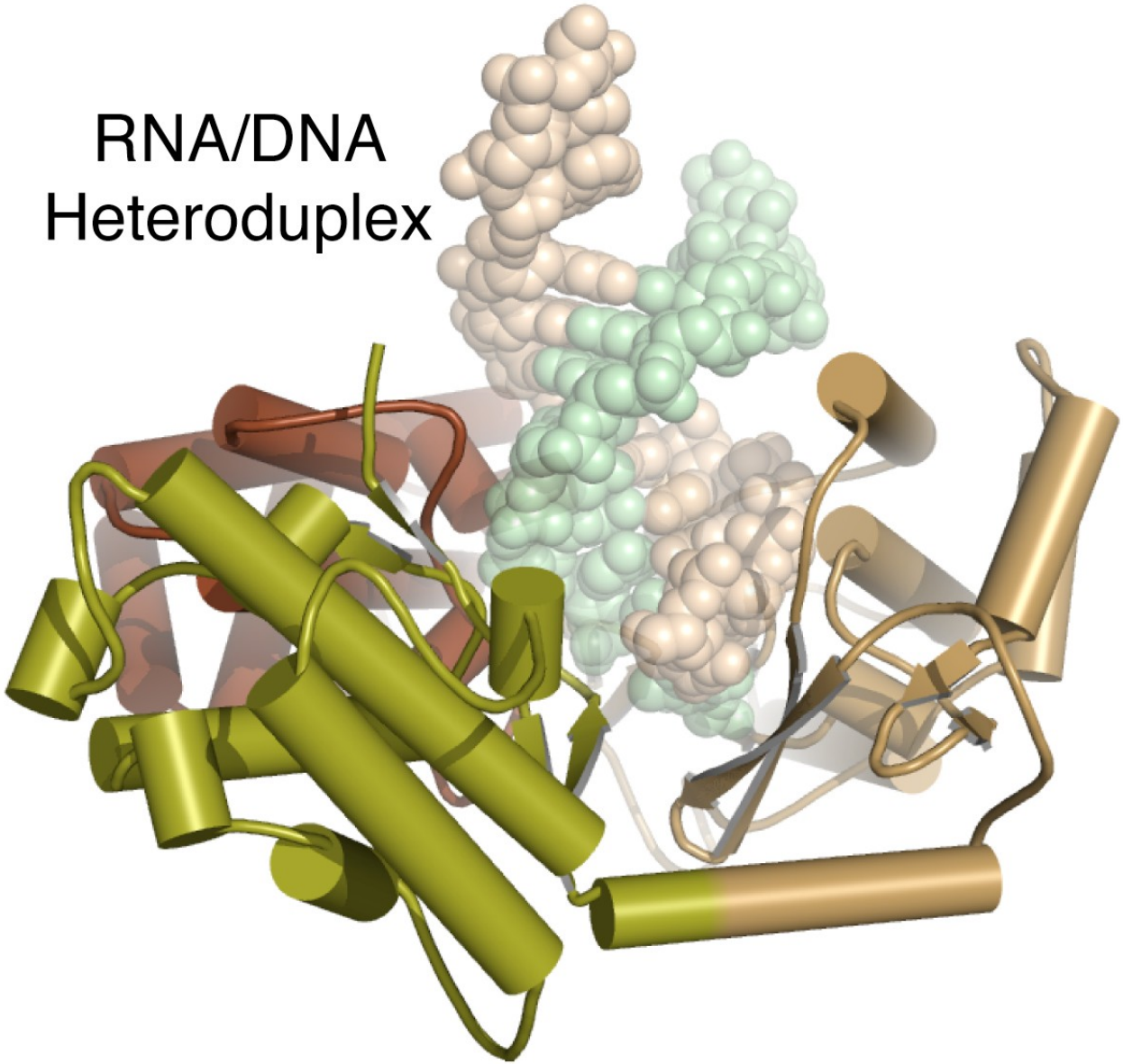


The Interior Cavity of the TERT Ring Binds Double Stranded Nucleic Acid

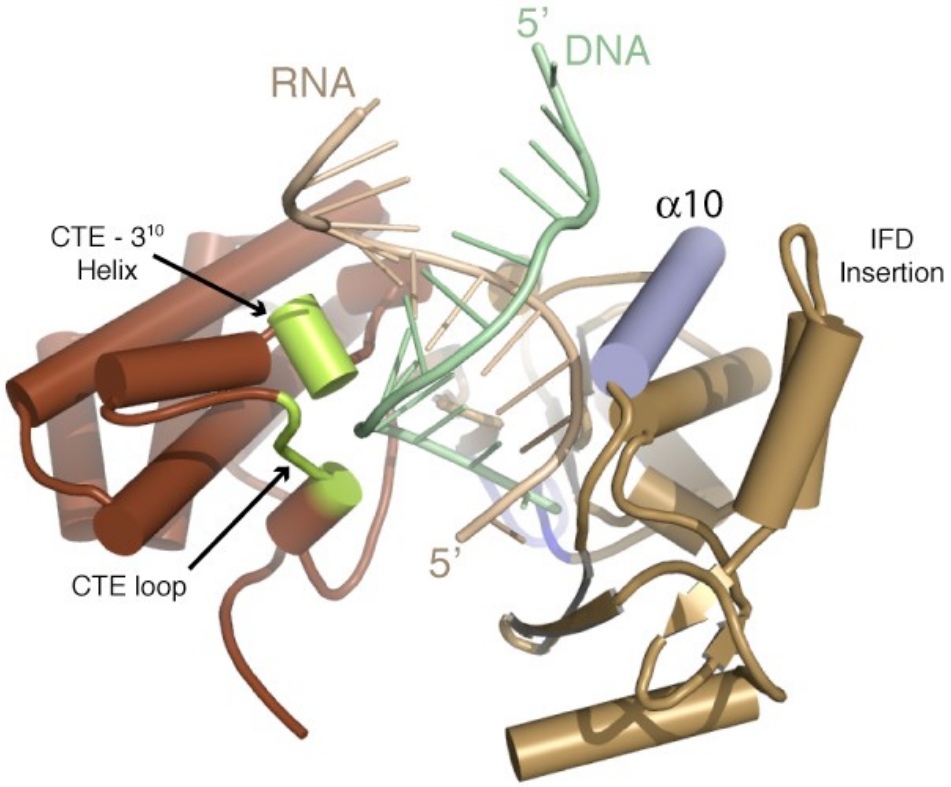


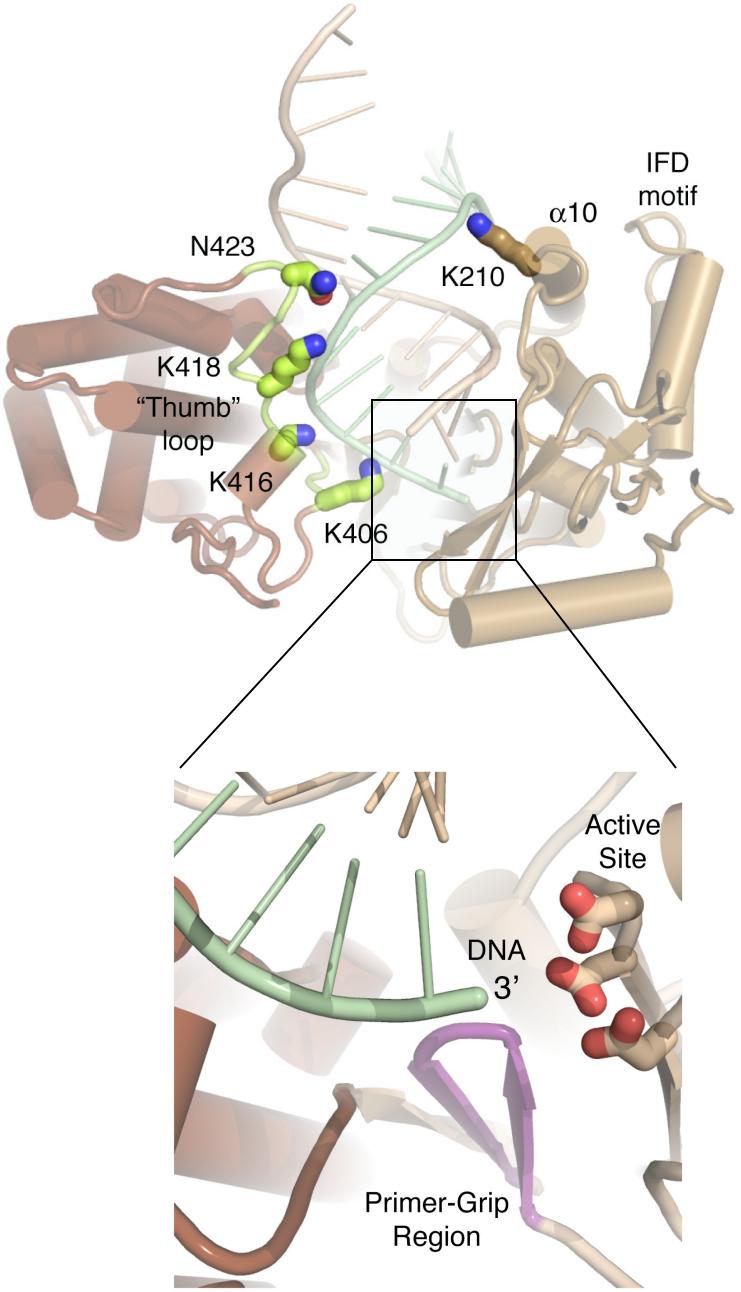


TERT-RNA-DNA Model Based on the HIV RT Structure

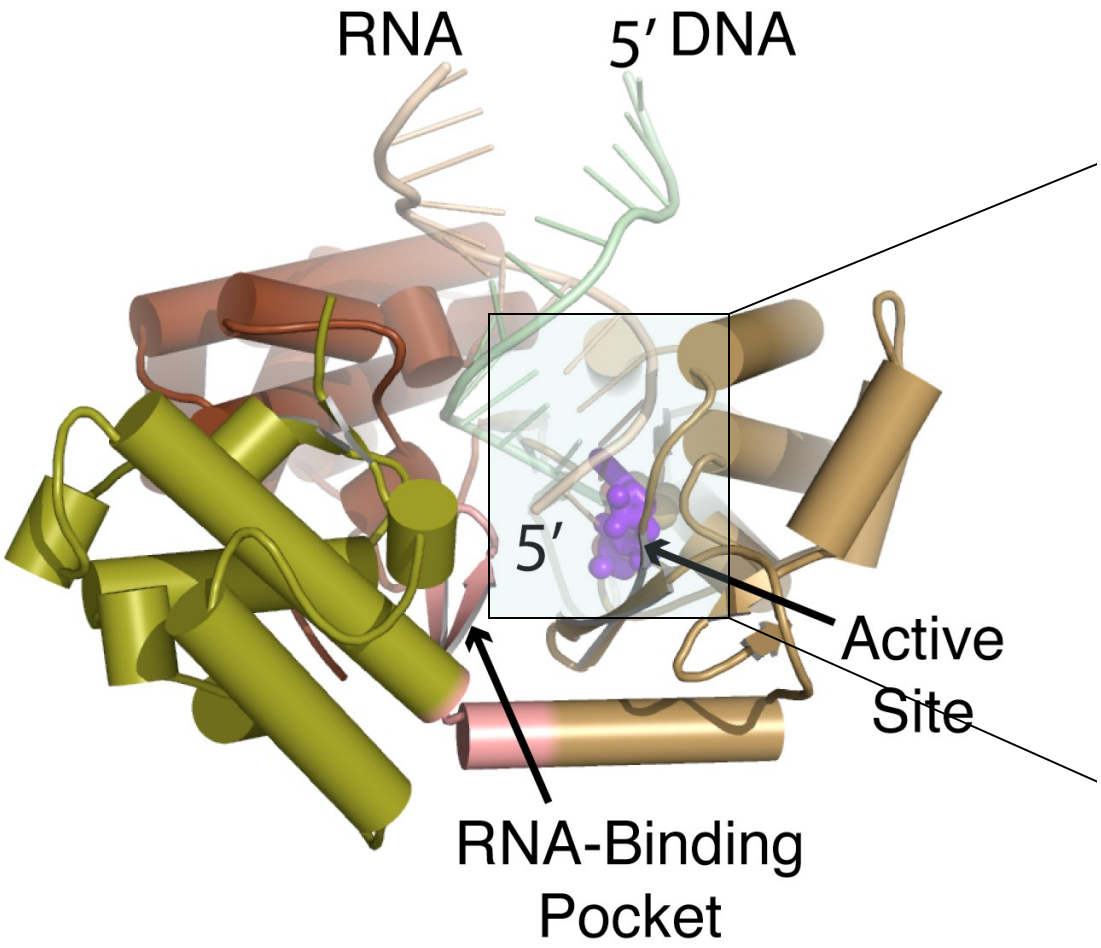


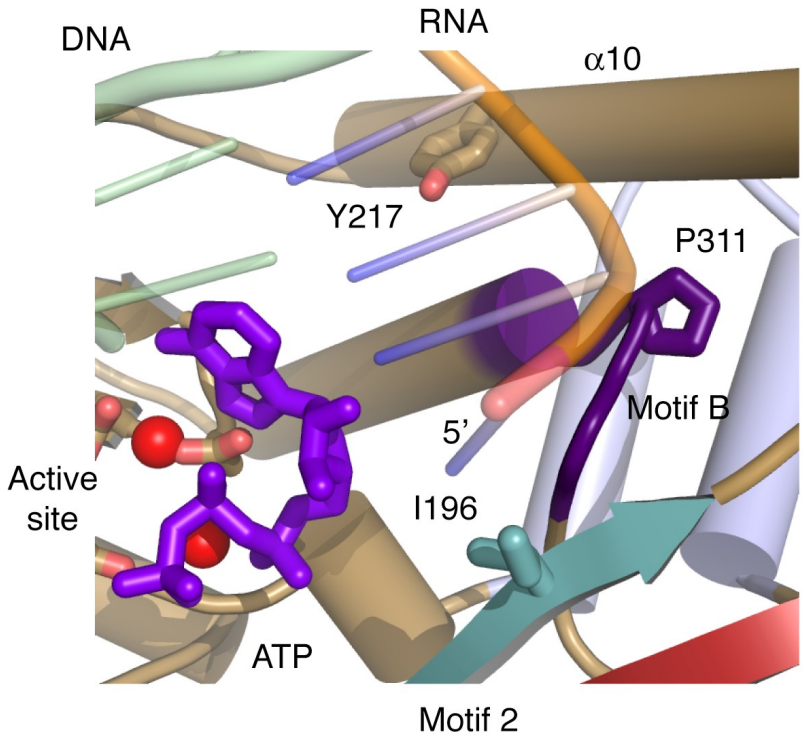
TERT Association with the DNA Primer to be Extended





TERT RNA-Template Binding and Nucleotide Selectivity





Conclusions

- TERT domains are organized into a ring configuration similar to HIV RTs, RNA pols and B-family DNA pols
- The interior cavity of the TERT ring can accommodate RNA-DNA hybrids ~seven bases long
- TRBD is important for telomerase assembly, repeat addition processivity and indirectly nucleotide selectivity
- The fingers domain is implicated in RNA and nucleotide binding
- The palm domain is involved in RNA- and DNA-binding and contains the active site of the enzyme
- The thumb domain promotes the formation of a stable telomerase elongation complex
- Overall the mechanism of telomerase elongation complex formation is similar to HIV RTs and viral RNA polymerases

Acknowledgements

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