Supplementary Information for

Structural and functional insight into mismatch extension by human DNA polymerase α

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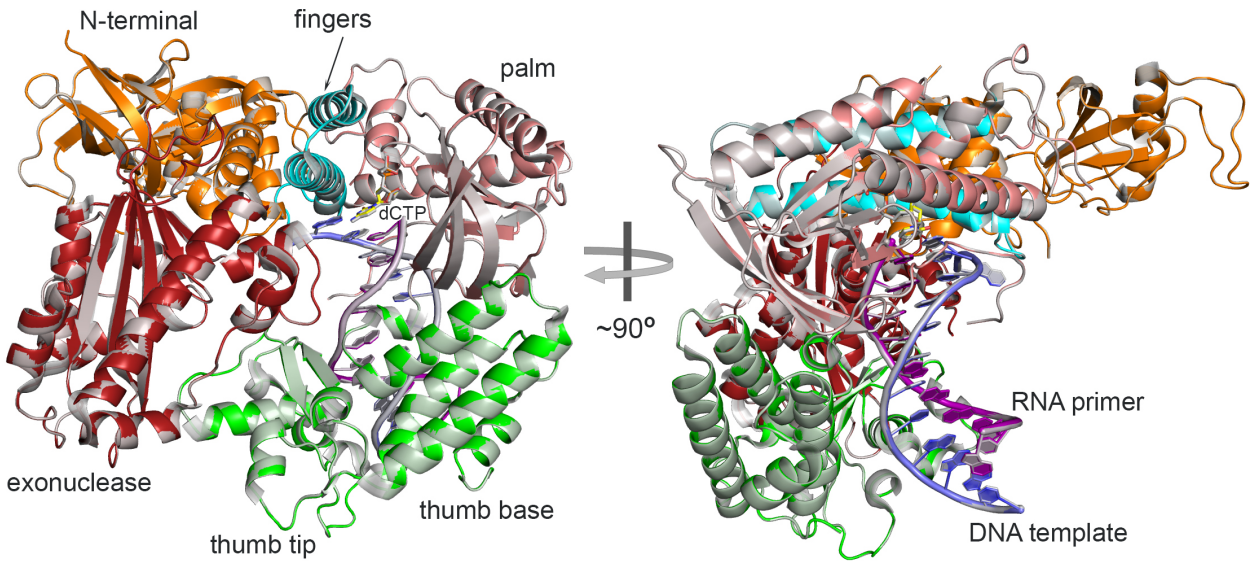

Fig. S1. Overall view of aligned human Polα_{CD} ternary complexes containing a matched and mismatched template:primer. In the complex with a mismatched template:primer, the carbons of dCTP, DNA template, and RNA primer are colored yellow, marine, and purple, respectively. Subdomains of Polα_{CD}: N-terminal, exonuclease, fingers, palm, and thumb, are colored orange, red, cyan, salmon, and green, respectively. In the complex containing a correct template:primer (PDB code 4QCL), all molecules are colored gray with 10% transparency. The complexes are aligned with RMSD of 0.075 Å for 850 Cα atoms.

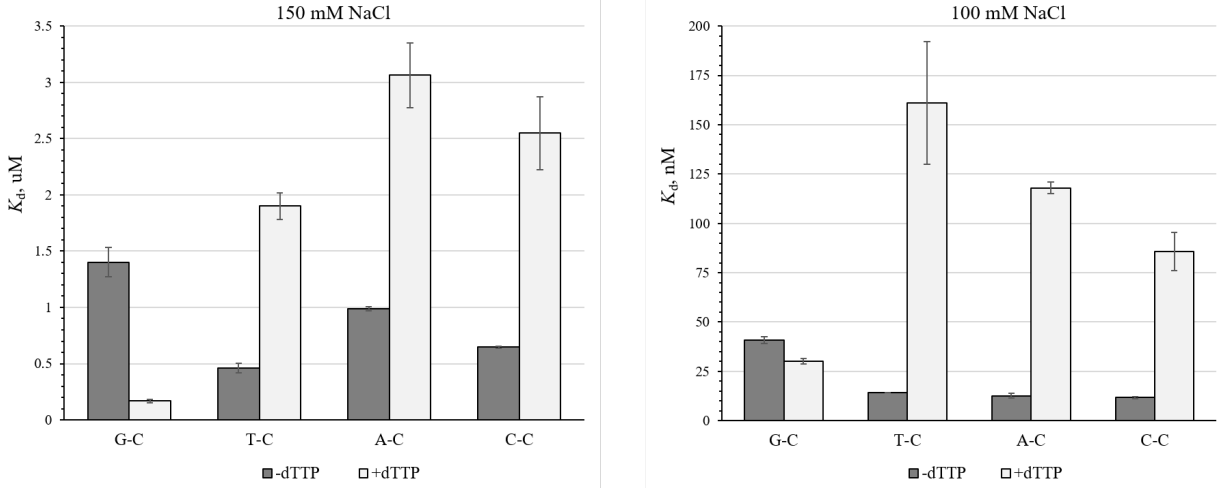

Fig. S2. Effect of salt, incoming dNTP, and a 3'-terminal mismatch on human Polα affinity to DNA. Data are presented as a bar graph showing the mean ±SD. The 3'-terminal base-pair or mispair is indicated below the bar.

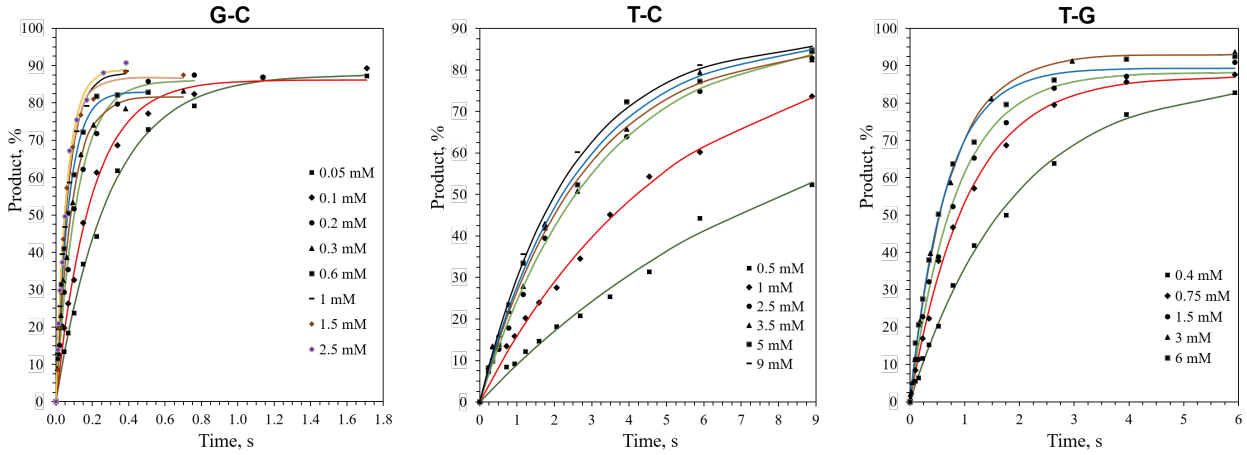

Fig. S3. Pre-steady-state kinetics of cognate and mismatched primer extension at different dTTP concentration. Percent of extended primer was plotted against time and the data were fit to a single-exponential equation. The 3'-terminal base-pair or mispair is indicated above the graphs.

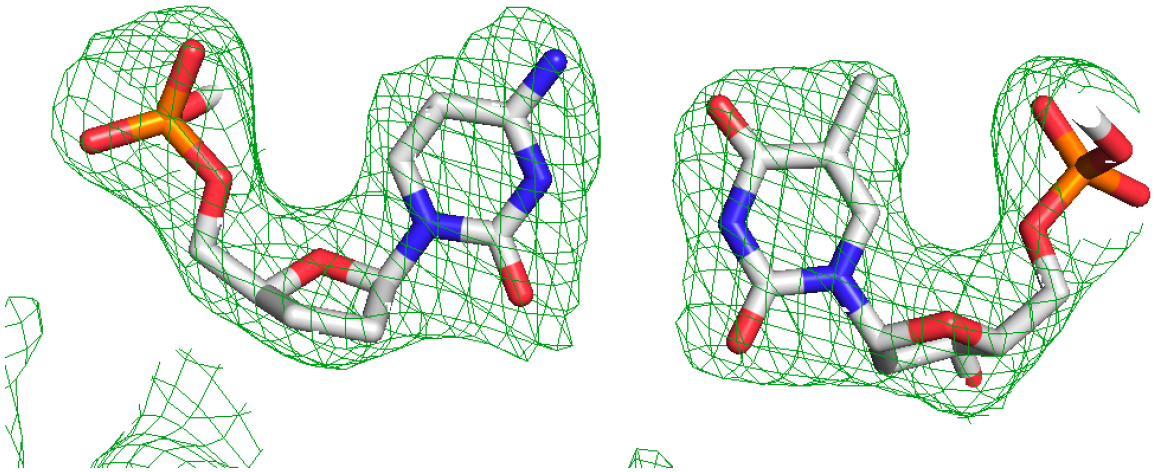

Fig. S4. The $2F_o - F_c$ Fourier map for the T-C mismatch at contour level of 1.5 σ.

Table S1. Summary of data collection and refinement statistics

Data collection	
Space group	$P\,3_221$
Cell dimensions: *a*, *c* (Å)	140.285, 181.066
Resolution (Å)	50 – 2.9 (2.95 – 2.9)[a]
R_{merge}	0.079 (0.437)
$I/\sigma I$	29.3 (3.2)
Completeness (%)	98.7 (97.7)
Unique reflections	45582 (2211)
Redundancy	5.5 (3.7)
Refinement	
Resolution (Å)	44.51 – 2.9 (3.08 – 2.9)
No. reflections	45573 (6973)
R_{work}/R_{free}	0.195/0.228 (0.316/ 0.324)
No. atoms	
Protein	6948
DNA/RNA	494
Ligand/ion	55
Water	110
Mean B-factors (Å^2)	51.1
R.m.s deviations	
Bond lengths (Å)	0.008
Bond angles (°)	1.4
Ramachandran plot (%)	
Core	87.5
Allowed	11.1
Generously allowed	1.3
Disallowed	0.1

[a] Numbers in parentheses refer to the highest-resolution shell.

Table S2. Oligonucleotides used in this study

Sequence	Description	Application	Length
5'-AATGTTTCTAGGCAGCTCGGAGTCC [a]	Template	kinetic studies	25
5'-AATGTTTCTATGCAGCTCGGAGTCC	Template (T-C, T-G mismatch)		25
5'-/Cy3/GGACTCCGAGCTGCC	Primer		15
5'-/Cy5/GGACTCCGAGCTGCG	Primer (T-G mismatch)		15
5'-/BiotinTEG/AATACATAAGCGCTCCAGGCAAT	Template	binding studies	23
5'-/BiotinTEG/AATACATAATCGCTCCAGGCAAT	Template (T-C mismatch)		23
5'-/BiotinTEG/AATACATAACCGCTCCAGGCAAT	Template (C-C mismatch)		23
5'-/BiotinTEG/AATACATAAACGCTCCAGGCAAT	Template (A-C mismatch)		23
5'-GCCTGGAGCG/ddC/	primer		11
5'-ATAGTCGCTCCAGGC	template	crystallization	15
5'-r(GCCUGGAGCG)/ddC/	primer		11

[a] The region complementary to a primer is underlined.