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# Supplemental Data

# Structures of DNA Polymerase β with Active-Site Mismatches Suggest a Transient Abasic Site Intermediate during Misincorporation

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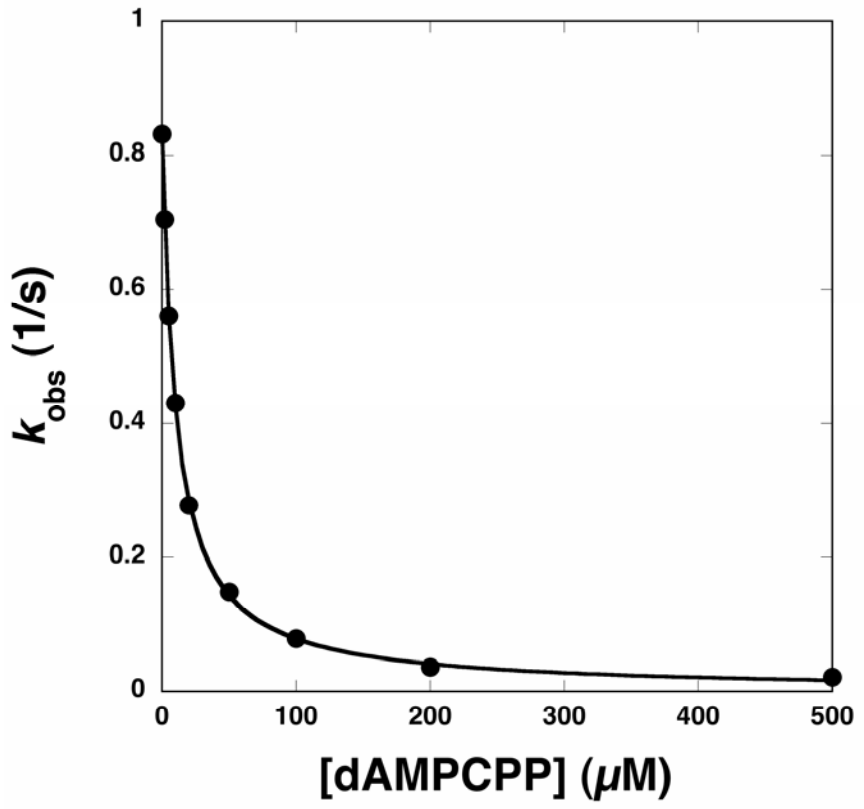


**Figure S1. Inhibition of dATP Incorporation by dAMPCPP**

DNA synthesis was assayed on a single-nucleotide gapped DNA substrate where the templating base in the gap was thymine. The reaction mixture contained 50 mM Tris-HCl, pH 7.4, 20 mM $MgCl_2$, 200 nM single-nucleotide gapped DNA, 10 μM dATP and various dAMPCPP concentrations. Reactions were initiated with 0.5 nM enzyme at room temperature and stopped with EDTA mixed with formamide dye. The substrates/products were separated on 15% denaturing polyacrylamide gels and quantified in the dried gels by phosphorimagery. Dixon analysis for competitive inhibition indicates that the $K_i$ for dAMPCPP is 2.3 μM (solid line).

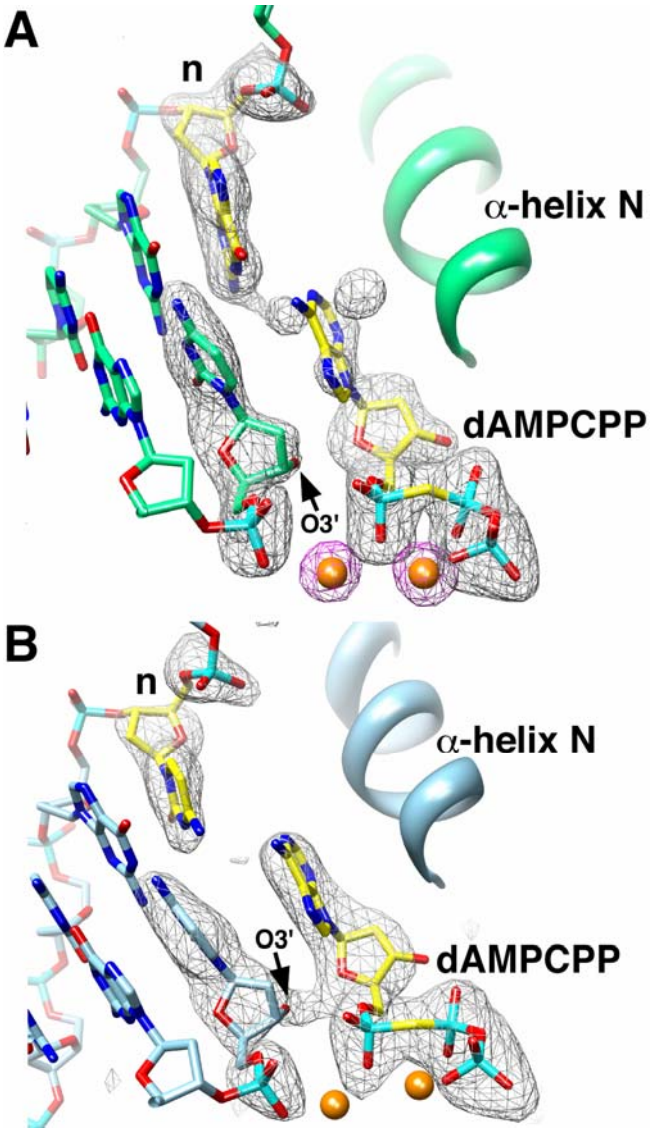


**Figure S2. Nascent Mismatch Omit Maps**

(A) A $F_o$-$F_c$ simulated annealing electron density omit map (gray) contoured at 3.0σ showing electron density corresponding to the dG—dAMPCPP mismatch (yellow) and primer terminus. The O3' position of the terminal primer nucleotide is indicated. Additionally, an anomalous difference density map contoured at 6σ (magenta) is shown indicating the position of the active site $Mn^{2+}$ atoms (orange spheres).

(B) A $F_o$-$F_c$ simulated annealing electron density omit map (gray) contoured at 3.0σ showing electron density corresponding to the dC—dAMPCPP mismatch (yellow) and primer terminus. The O3’ position of the terminal primer nucleotide is indicated and the active site $Mn^{2+}$ atoms are represented as orange spheres.

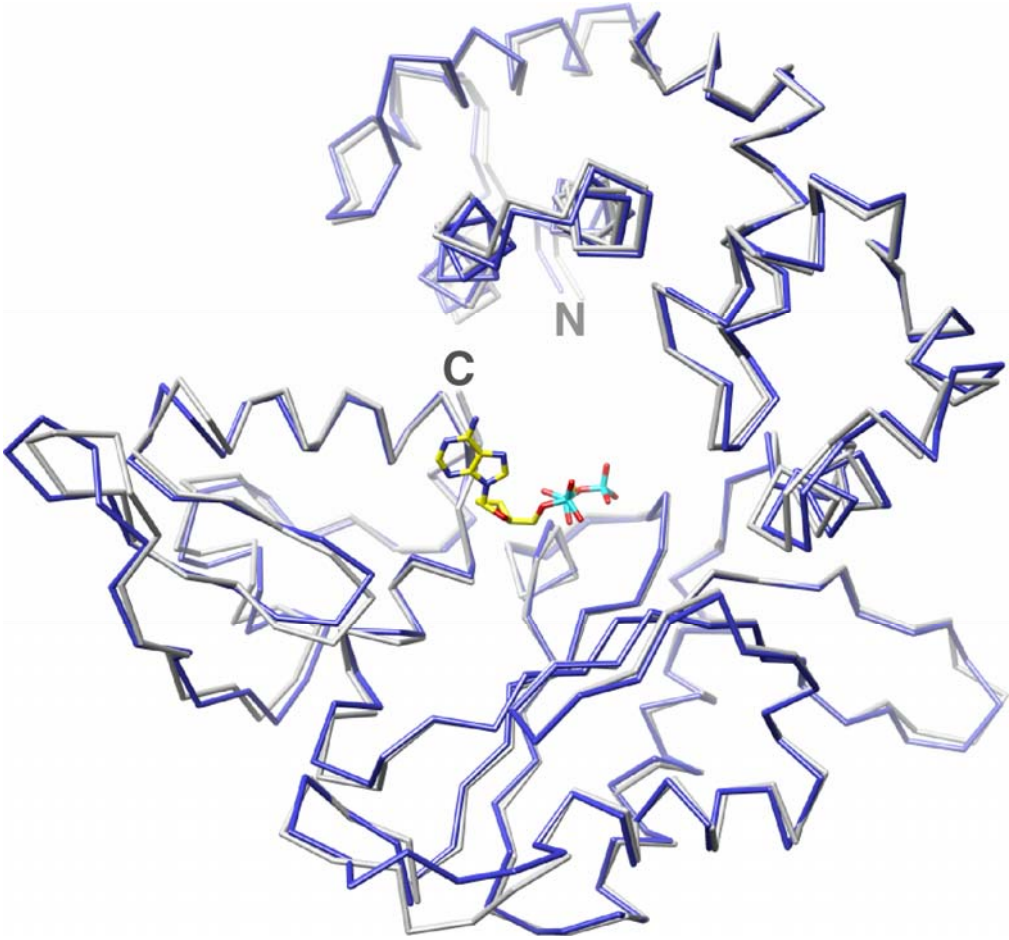


**Figure S3. Closed Conformation of the Ternary Substrate Complex with an Active Site Mismatched Nascent Base Pair**

Stick representation of the Pol β backbone (Cα) of the ternary substrate complex with correct (gray) or incorrect (blue) incoming nucleotides. The polymerase domain with a correct (dA—dUMPNPP; PDB ID 2FMS) and incorrect (dC—dAMPCPP; PDB ID 3C2L) nascent base pair were superimposed with an rmsd of 0.70 Å (314 Cα). The incoming dAMPCPP of the mismatch structure is shown (yellow carbons), but the DNA is omitted for clarity. The amino– and carboxyl–termini (N and C, respectively) are indicated.

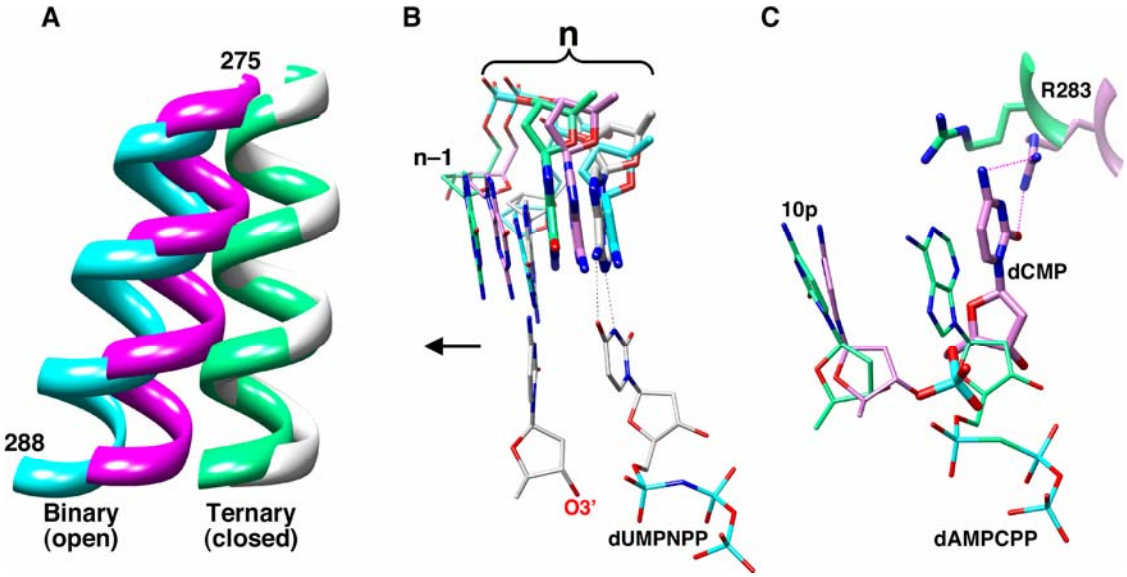


**Figure S4. Comparison of Ternary Substrate and Binary Product Complexes with Active-Site Mismatches**

(A) Relative position of α-helix N (residues 275–288) from superimposed structures of DNA binary product complexes with a matched (dC—dG, PDB ID 1BPZ, light blue) or mismatched (dA—dC; PDB ID 1TV9, magenta) base pair in the active site with ternary substrate complexes with a matched (dA—dUMPNPP, PDB ID 2FMS, gray) or mismatched (dC—dAMPCPP, PDB ID 3C2L, green) base pair in the active site. The open or closed state of the N–subdomain of Pol β is deduced from the amino-terminal position (residue 288) of α-helix N. The nicked DNA product complex with a correct base pair at the primer terminus (i.e., situated in the nascent base pair binding pocket) is in the open state, whereas the mismatched product complex approaches the fully open state (Krahn et al., 2004). We have previously referred to this state as an intermediate conformation. This intermediate conformation probably results from α-helix N side chain interactions with the primer terminus of the mismatch that are not observed when a correct base pair is situated in the nascent base pair binding pocket as product nicked

DNA. In contrast, α-helix N of the ternary substrate complexes (matched and mismatched) is in a closed conformation.

(B) Ternary complex structures of Pol β with a Watson-Crick base pair (gray, 2FMS) and dG—dAMPCPP mismatch (green, 3C2M) where superimposed with nicked DNA product binary complexes with a matched (light blue) or mismatched (magenta) base pair at the primer terminus. The templating base (n) in each case is shown in a thick stick representation and the template base pair upstream (n–1) is illustrated as thin sticks. The arrow indicates the upstream duplex. The correct incoming nucleotide of the ternary substrate complex (dUMPNPP) is also shown base pairing (black dashed lines) with the templating adenine. The primer terminus of this ternary substrate complex is also illustrated.

(C) Superimposed ternary substrate (dC—dAMPCPP, green) and binary product (dA—dC, magenta) complexes with mismatched base pairs in the nascent base pair suggest that opening of the N–subdomain results in repositioning of the 3'-terminal nucleotide of the mismatch and the nucleotide originally serving as the primer terminus (i.e., prior to misinsertion, 10p). In the closed position of α-helix N of the ternary mismatch structure, Arg283 does not interact with the incoming nucleotide; however in the open product complex, it forms two hydrogen bonds. It remains to be determined if this might partially be due to the different identities of the nucleotide in the binding pocket providing different hydrogen bonding capacities.

**Table S1. Effect of Metals on Single-Turnover Kinetics of Correct Nucleotide Insertion**[a]

| dX—dNTP[b] | $k_{pol}$ | $K_{d,dNTP}$ | $k_{pol}/K_d$ |
|---|---|---|---|
| | $s^{-1}$ | $\mu M$ | $\mu M^{-1}\ s^{-1}$ |
| $Mg^{2+}$ | | | |
| dG—dCTP | 0.86 (0.06) | 2.8 (0.8) | 0.31 (0.09) |
| dC—dGTP | 0.59 (0.02) | 2.5 (0.3) | 0.24 (0.03) |
| $Mn^{2+}$ | | | |
| dG—dCTP | 0.52 (0.01) | 0.15 (0.02) | 3.47 (0.47) |
| dC—dGTP | 0.92 (0.01) | 0.33 (0.01) | 2.79 (0.05) |

[a] Values represent the best-fit kinetic parameters (standard error).
[b] Identity of templating base (dX) and incoming nucleotide (dNTP)

## Supplemental References

Krahn, J. M., Beard, W. A., and Wilson, S. H. (2004). Structural insights into DNA polymerase deterrents for misincorportion support an induced-fit mechanism for fidelity. Structure (Camb) *12*, 1823-1832.