

Alteration In The Cavity Size Adjacent To The Active Site of RB69 DNA Polymerase Changes Its Conformational Dynamics

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SUPPLEMENTARY MATERIALS

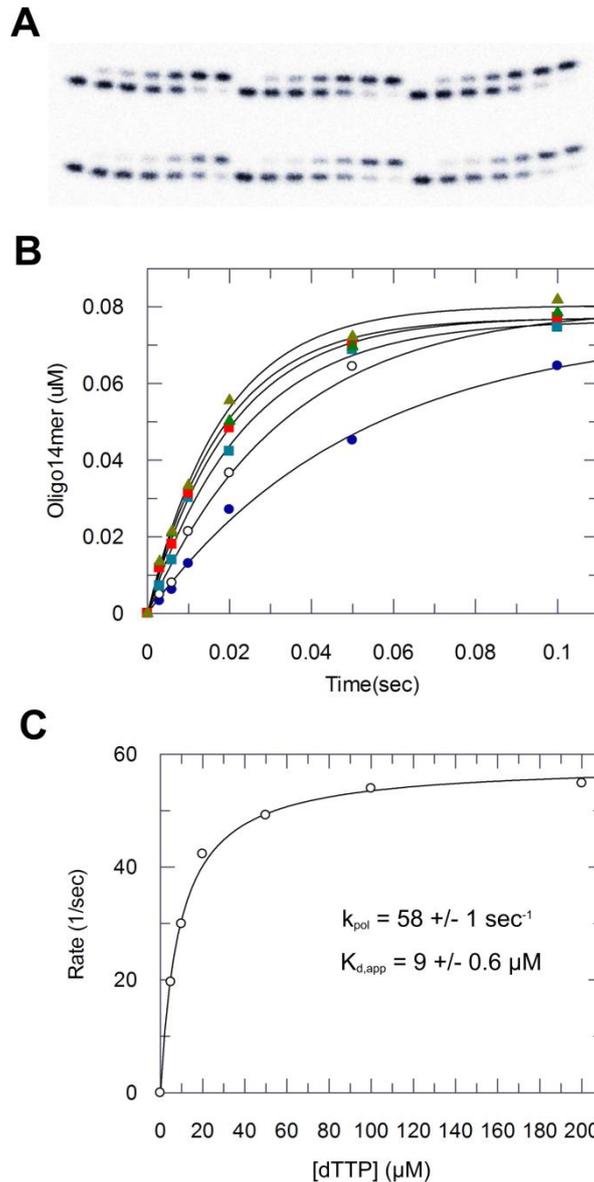


Figure S1. Raw data and plots for the insertion of dTMP opposite dA by the L415G mutant. (A) A denaturing PAGE gel showing the time course of dTMP incorporation with different [dTTP]: bottom lane (5 μM, 10 μM, 20 μM from left to right) and top lane (50 μM, 100 μM, 200 μM from left to right). (B) Progress curves at various [dTTP], 5, 10, 20, 50, 100, 200 μM (from bottom to top), fit to a single-exponential equation. (C) Plot of k_{obs} vs [dTTP] fit to a hyperbolic equation to yield k_{pol} and $K_{d,app}$.

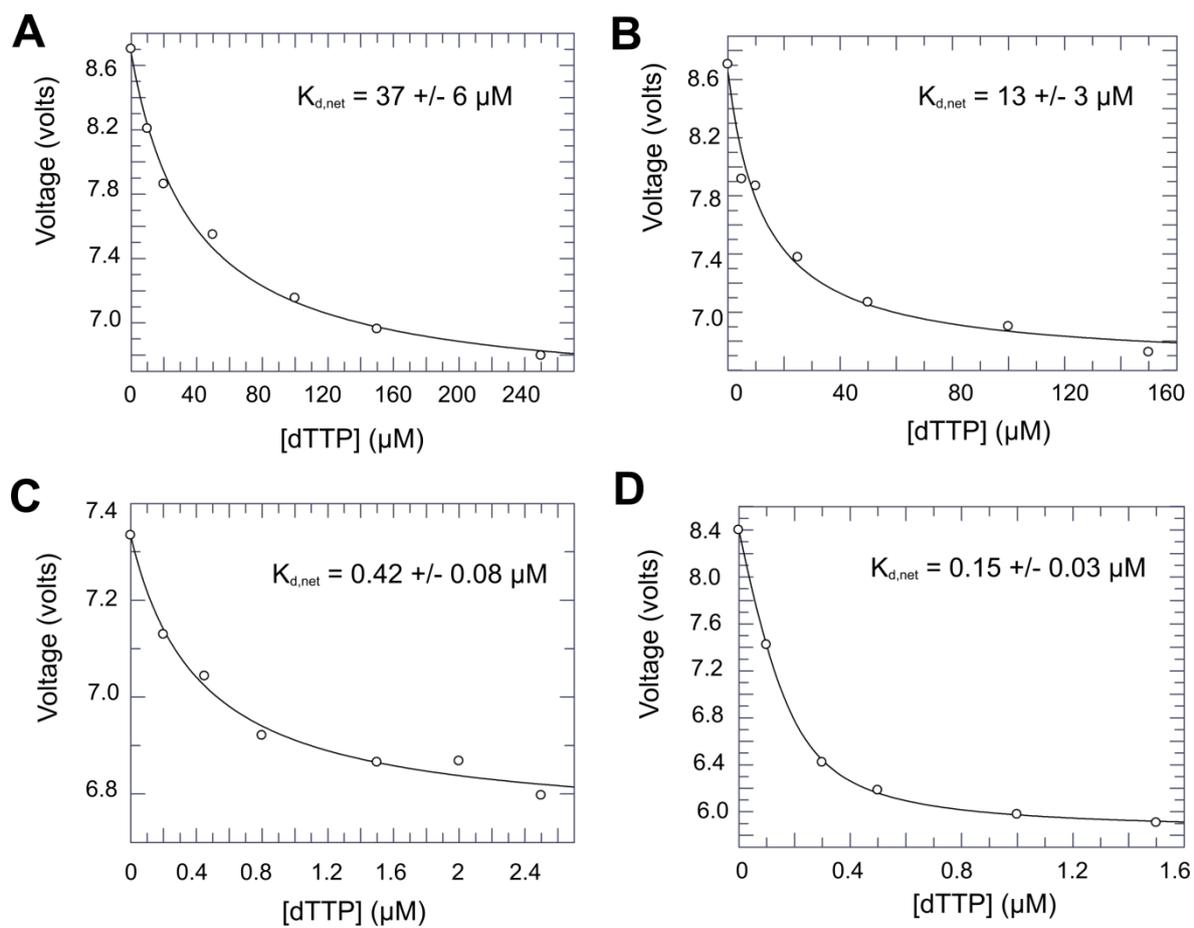


Figure S2. Plots of changes in amplitude obtained by stopped-flow fluorescence for dTTP opposite 2AP with; (A) wt RB69pol; (B) L415M; (C) L415G and L415A variants. Representative time courses of stopped-flow fluorescence for dTTP opposite 2AP with L415G mutant is shown in Figure S3.

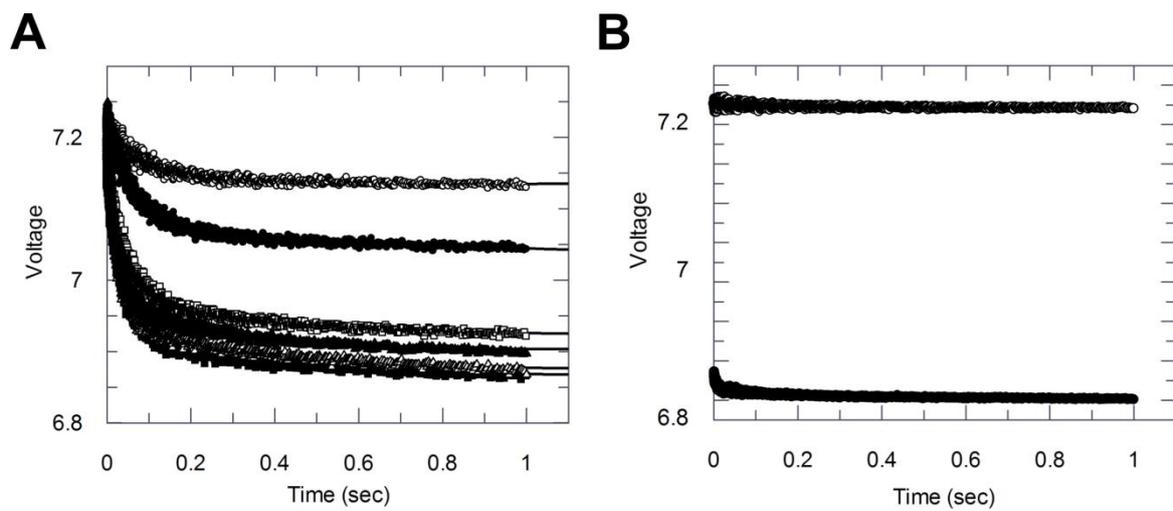


Figure S3. Stopped-flow fluorescence with dTTP opposite 2AP with L415G mutant. (A) Time courses of fluorescence quenching with different [dTTP], 0.2, 0.45, 0.8, 1.5, 2, 2.5 μM (from top bottom), fit to a double-exponential equation. (B) Time courses of fluorescence quenching in the absence of dTTP (top) and in the presence of 100 μM dTTP (bottom).

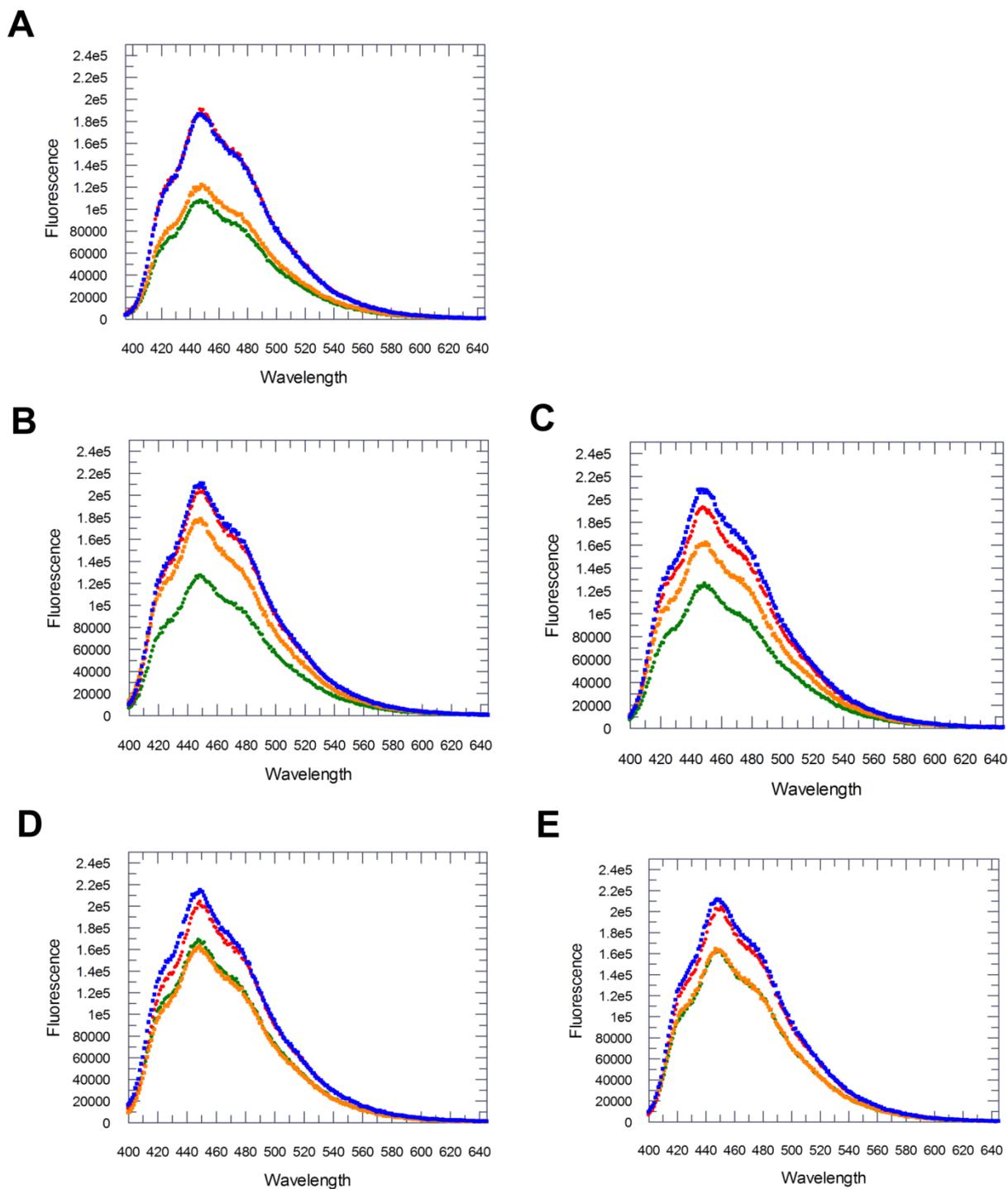


Figure S4. Fluorescence spectra of various tC^o -containing P/Ts in the absence and presence of pol: (A) P/Ts alone; (B) P/Ts with wt RB69pol; (C) P/Ts with L415M mutant; (D) P/Ts with L415A mutant and (E) P/Ts with L415G mutant. Sequences of P/Ts used are shown in Figure 2C. Red dotted line is the spectrum with Con-P/T; green dotted line is the spectrum with Con-P/T- tC_{nitro} ; blue dotted line is the spectrum with Mis-P/T; and orange dotted line is the spectrum with Mis-P/T- tC_{nitro} .

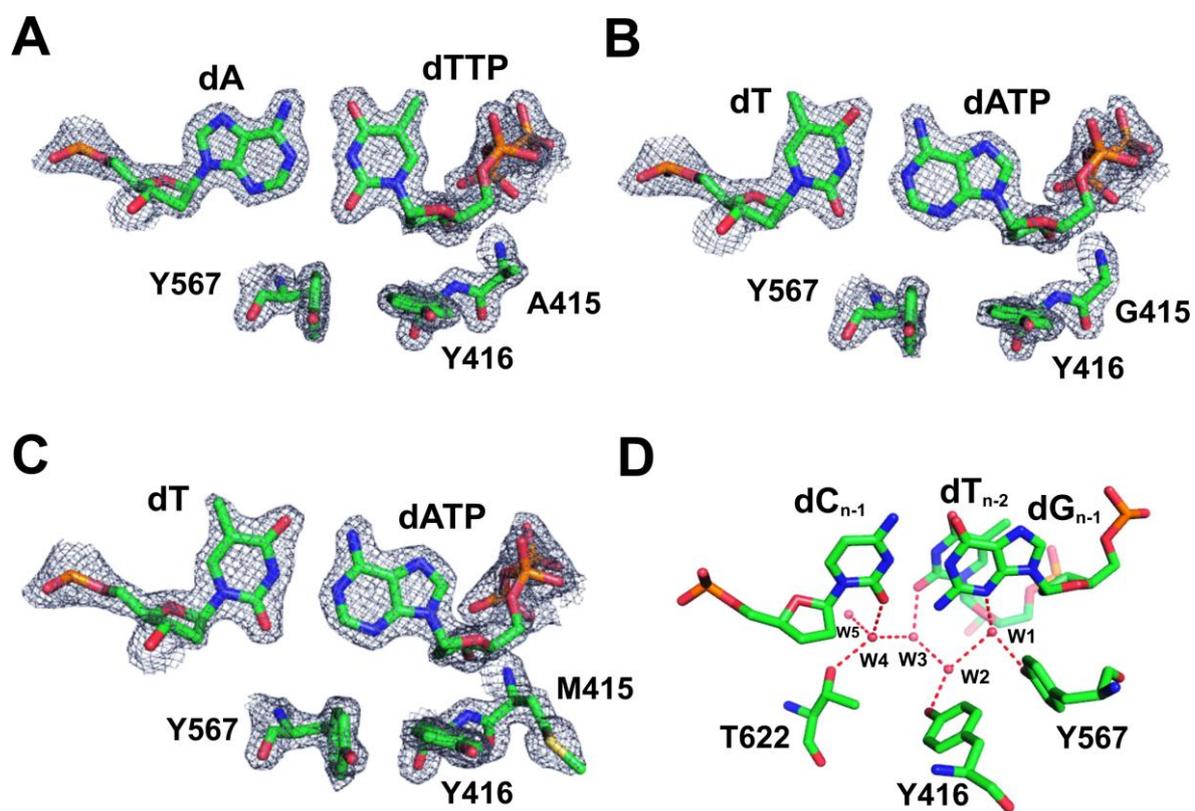


Figure S5. Structures of NBP of L415 variants. (A) Final $2F_o - F_c$ electron density map at 1.80 Å resolution for the dTTP/dA-containing L415A complex contoured at 1.8 σ . (B) Final $2F_o - F_c$ electron density map at 2.04 Å resolution for the dATP/dT-containing L415G complex contoured at 1.8 σ . (C) Final $2F_o - F_c$ electron density map at 2.02 Å resolution for the dATP/dT-containing L415M complex contoured at 1.8 σ . (D) The hydrogen-bonding network at the minor groove of the P/T junction of L415A complex.

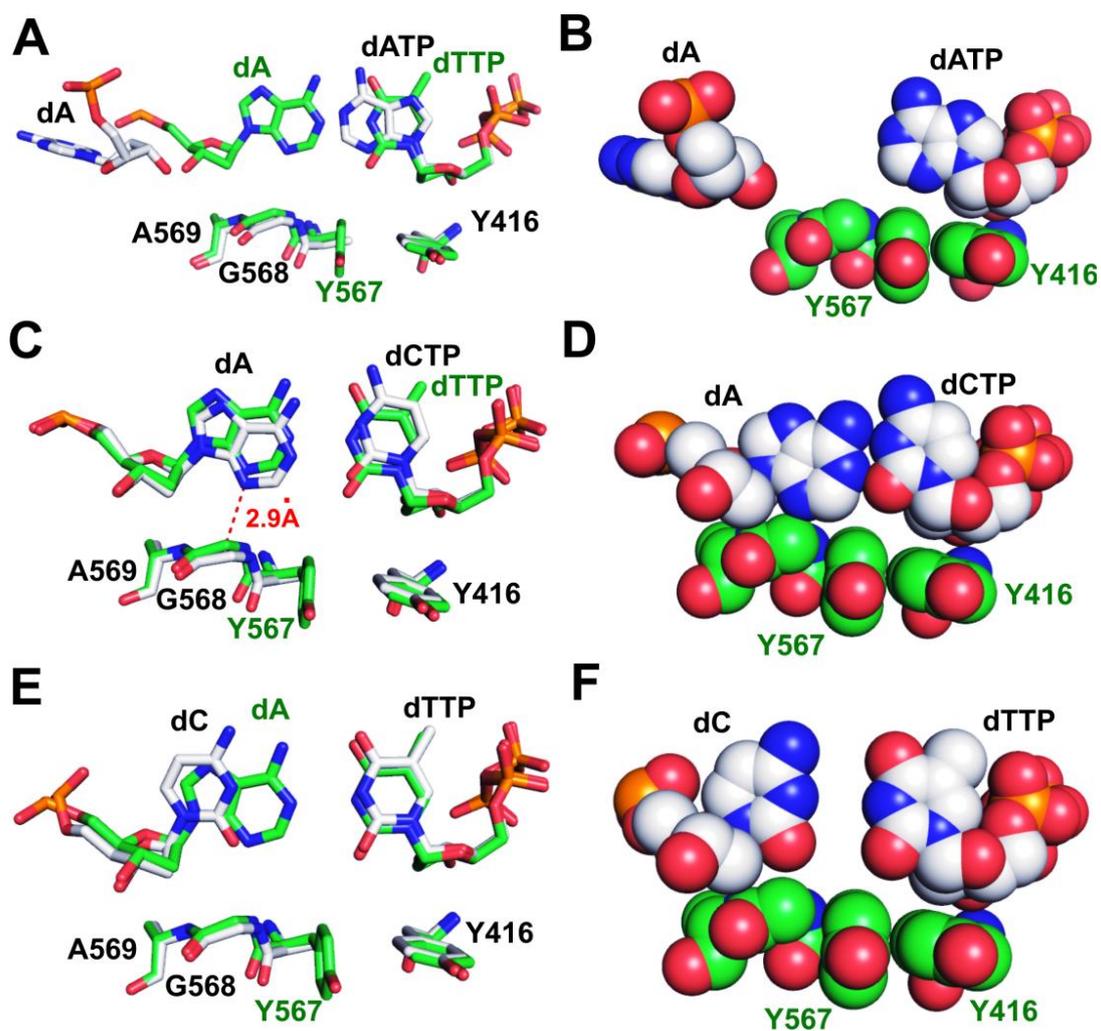


Figure S6. Superposition of mismatches-containing structures of the qm RB69pol onto the dTTP/dA-containing L415A. The structure with a mismatch is shown in gray, and the L415A structure is shown in green. (A) dATP/dA; (C) dCTP/dA and (E) dTTP/dC. (B), (D) and (F) are space-filling modes of (A), (C) and (E) respectively.

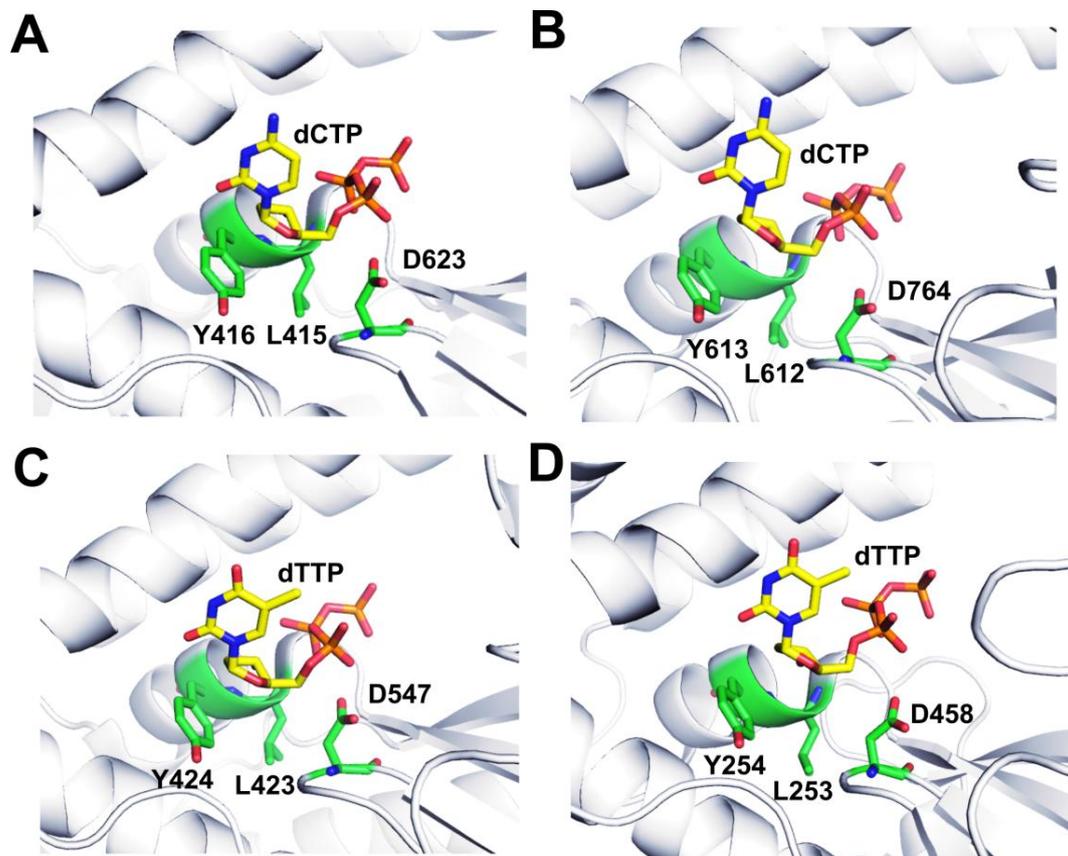


Figure S7. Conserved NBP residues in (A) RB69pol, (B) pol δ , (C) polIII, and (D) Phi29 DNA pol.