SUPPORTING INFORMATION

The Reopening Rate of the Fingers Domain is a Determinant of Base Selectivity for RB69 DNA Polymerase

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This file includes:

Explanation, scheme and estimated kinetic parameters for the Sequenase:dP/T^+ trapping experiment.

Supplemental Figs. 1-2

Supplemental Material

As mentioned in the main text of the paper, the rate of dAP fluorescence enhancement in the Sequenase:**d**P/T^{+} trapping experiment was biphasic with a rapid rate of 50s^{-1} and a slower rate of 4s^{-1}. The amplitudes of the fast and slow rates were equal. There are several possible explanations for the biphasic kinetics of the dTTP release rate, assuming that the off rate (k_{-3}) is essentially irreversible in the Sq:**d**P/T^{+} trapping experiment. The justification for this assumption is based on the high affinity of the Sq:**d**P/T for dNTPs (0.8nM)(Supp. Fig. 2). The most likely possibilities are **(i)** Parallel pathways for dTTP release from *FDN* where the following scheme would apply:

$$FDN^{C1} \underset{>1000s^{-1}}{\overset{4s^{-1}}{\rightleftharpoons}} ED*N \xrightarrow{>>1000} ED*+N$$

$$FDN^{C2} \underset{>1000s^{-1}}{\overset{50s^{-1}}{\rightleftharpoons}} ED*N \xrightarrow{>>1000} ED*+N$$

$$FDN^{C1} \overset{K=1}{\rightleftharpoons} FDN^{C2}$$

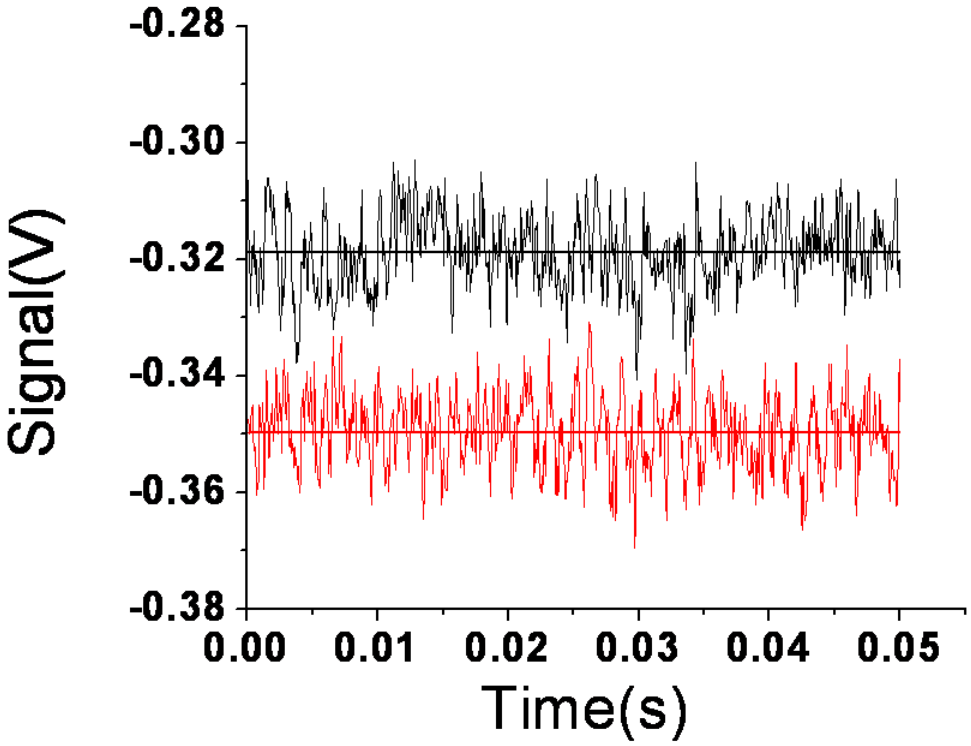
Where the equilibrium constant is ~1 but the rate of interconversion is slow, and where FDN^{C1} converts to *ED*N* at 4s^{-1} while FDN^{C2} converts to *ED*N* at 50s^{-1}. **(ii)** A sequential release of dTTP from two low *FDN* fluorescence states: FDN^{C1} and $FD*N^{C2}$. In this situation, the following scheme would apply:

$$FDN^{C1} \underset{>1000}{\overset{50s^{-1}}{\rightleftharpoons}} FD*N^{C2} \underset{>1000}{\overset{4s^{-1}}{\rightleftharpoons}} ED**N \xrightarrow{>>1000} ED**+N$$

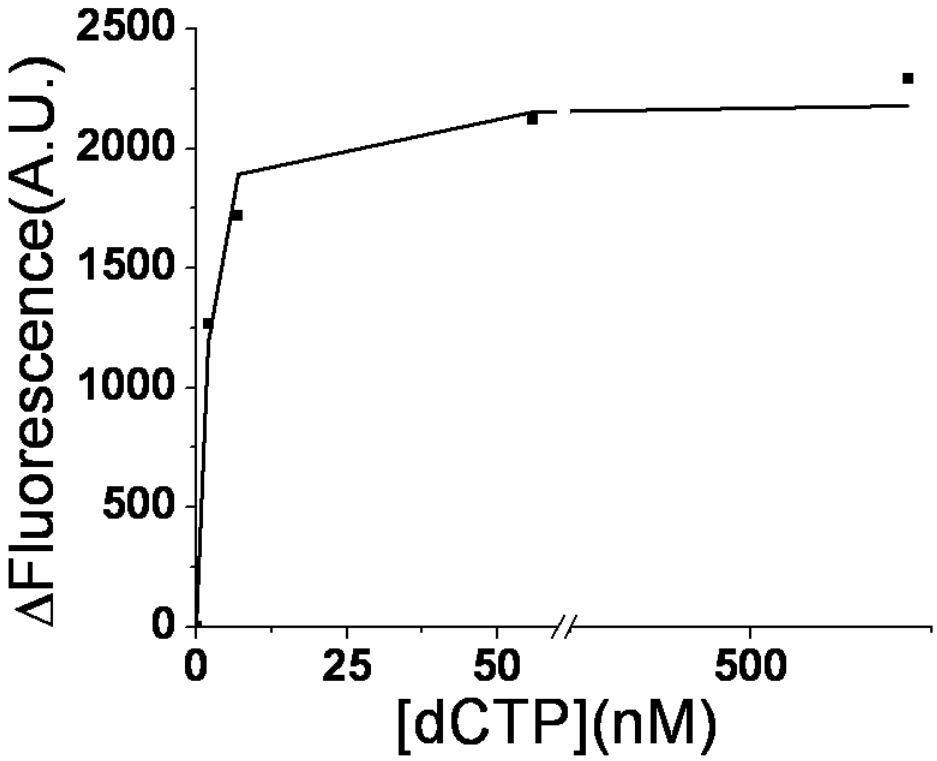
Figure Legends

Supplemental Figure 1. Stopped-flow fluorescence scans of dCTP in 50mM MOPS pH 7 and 2mM $CaCl_2$. 0mM dCTP is in black and 1mM dCTP is in red. This suggests that dCTP alone contributes a small amount to the fluorescence quenching in mismatched dCTP binding experiments (0.03V/mM).

Supplemental Figure 2. Equilibrium fluorescence titrations of 200nM Sequenase and 2nM cy3 labeled dP/T. The change in Cy3 fluorescence versus [dCTP] fit best to a quadratic equation with a K_{dg}^{app} of 0.83 (± 0.29) nM. The fluorescence change (**ΔF**) with increasing [dCTP] indicates the amount of fluorescence quenching for the ternary Sq:**d**P/T:dCTP complex compared with the binary Sq:**d**P/T complex.



Supplemental Fig. 1



Supplemental Fig. 2