Supplemental Information

Investigation of the Intradomain Motions of a Y-Family DNA Polymerase during Substrate Binding and Catalysis

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Figures S1-S4

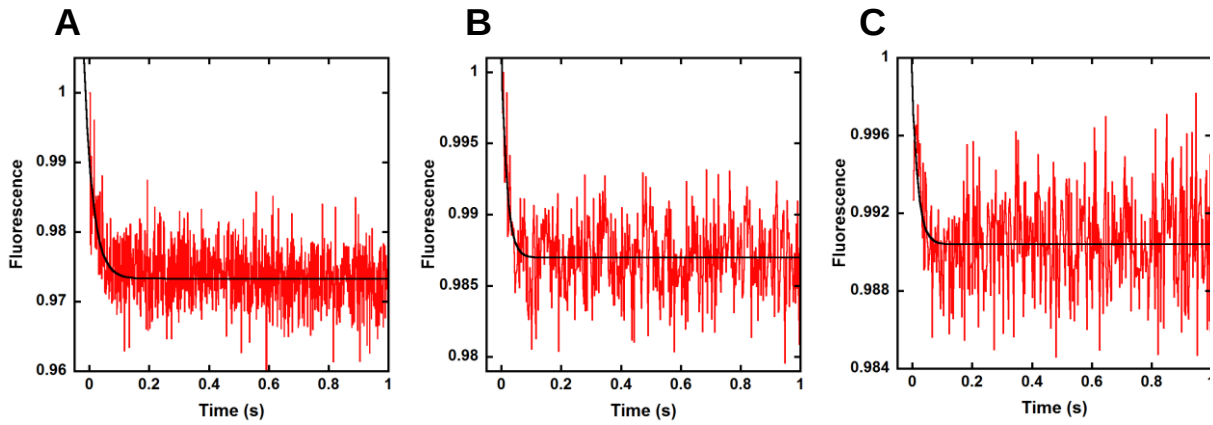

Figure S1. Conformational changes upon DNA binding for select intradomain Dpo4 FRET constructs. 100 nM DNA^{OH} was rapidly mixed with 100 nM of (A) Y118W-$K137C^{CPM}$, (B) Y224W-$K172C^{CPM}$, and (C) Y224W-$K212C^{CPM}$ and CPM fluorescence was recorded upon excitation at 290 nm. The black lines are single exponential fits to the data to yield observed association rates of 80 ± 20 s^{-1}, 50 ± 10 s^{-1}, and 30 ± 10 s^{-1}. Errors are standard deviations calculated from ≥ 5 replicate experiments.

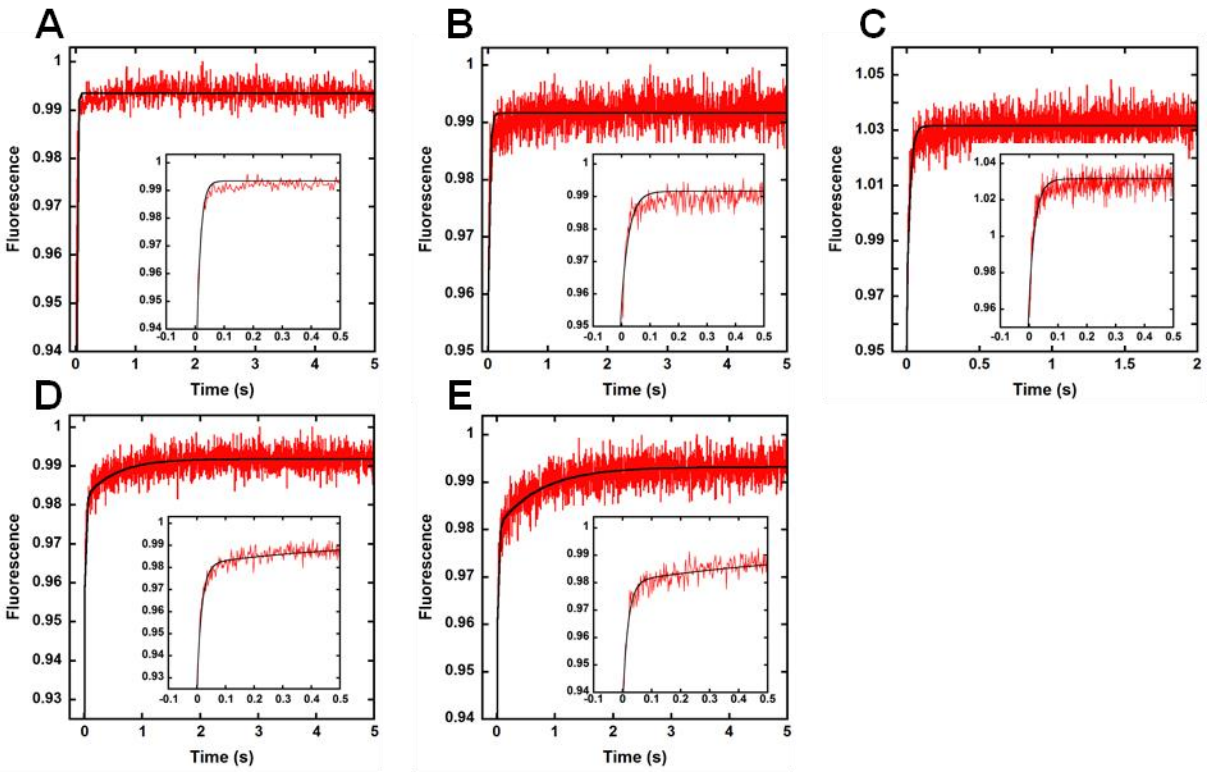

Figure S2. Conformational Dynamics of Select Intradomain Dpo4 FRET Constructs during DNA Binding. 100 nM DNA^{OH} was rapidly mixed with 100 nM of LF mutants (A) Y274W-R267C^{CPM}, (B) Y274W-E291C^{CPM}, and (C) Y274W-K329C^{CPM} as well as Finger mutants (D) S22W-K56C^{CPM} and (E) S22W-V62C^{CPM} and CPM fluorescence was recorded upon excitation at 290 nm. For traces A-C, the black line depicts single exponential fits to the data yielding observed rates of association of 75 ± 4 s^{-1}, 55 ± 3 s^{-1}, 86 ± 12 s^{-1}, respectively. For traces D and E, the black line depicts double exponential fits to the data yielding the following kinetic parameters: k_1 = 57 ± 8 s^{-1}, A_1 = 0.83 ± 0.01, k_2 = 2.0 ± 0.3 s^{-1}, and A_2 = 0.17 ± 0.01 for trace D; k_1 = 55 ± 11 s^{-1}, A_1 = 0.76 ± 0.01, k_2 = 1.6 ± 0.4 s^{-1}, and A_2 = 0.24 ± 0.01 for trace E. The inset figures feature the fast time points for clarity.

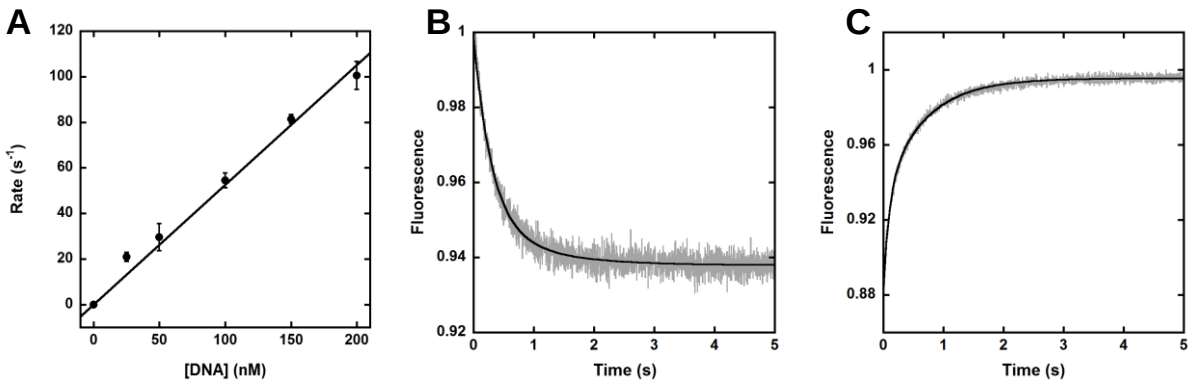

Figure S3. Conformational dynamics of Y274W-E291C^{CPM} during DNA association and dissociation. (A) Observed rates collected for Y274W-E291C^{CPM} binding to varying concentrations of DNA^{OH} (25-200 nM) plotted against DNA concentration. From the slope of the best fit line, the second-order, bimolecular association rate constant (k_{on}) was calculated as 5.3x10^{8} M^{-1} s^{-1}. (B) CPM fluorescence of Y274W-E291C^{CPM} (100 nM) pre-incubated with DNA^{OH} (100 nM) upon excitation at 290 nm following rapid mixing with wt Dpo4 trap (2 μM). The data were fit to a double exponential equation (black line) to yield fast and slow phase rates of 3.5 ± 0.2 s^{-1} (A_1 = 0.7 ± 0.1) and 1.3 ± 0.4 s^{-1} (A_2 = 0.3 ± 0.1), respectively. (C) CPM fluorescence of Y274W-E291C^{CPM} (2 μM) upon excitation at 290 nm following rapid mixing with a pre-incubated solution of wt Dpo4 (100 nM) and DNA^{OH} (100 nM). The data were fit to a triple exponential equation (black line) to yield the following kinetic parameters: k_1 = 80 ± 13 s^{-1}, A_1 = 0.31 ± 0.01, k_2 = 5 ± 1 s^{-1}, A_2 = 0.37 ± 0.04, k_3 = 1.2 ± 0.2, and A_3 = 0.32 ± 0.05.

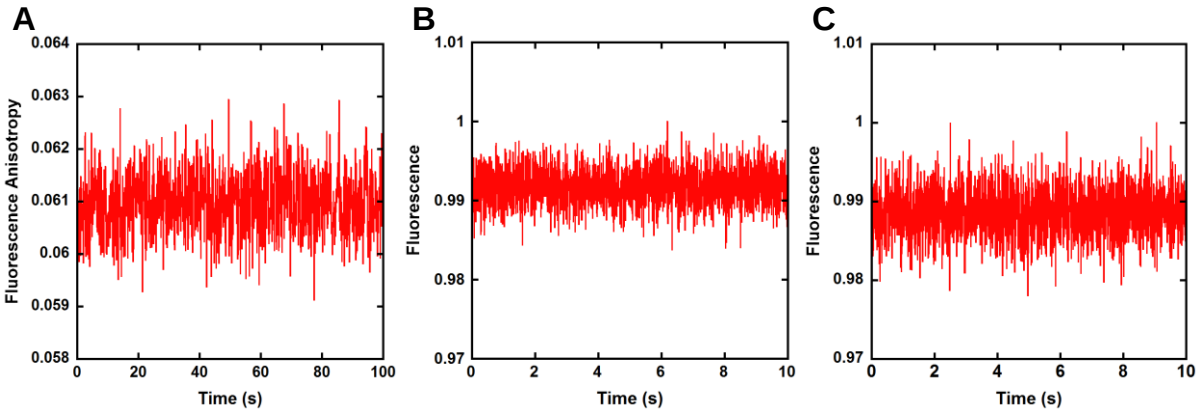

Figure. S4. Control stopped-flow experiments. (A) Fluorescence anisotropy recorded upon mixing Alexa488-labeled DNA^{OH} (500 nM) with buffer. Anisotropy did not vary over time indicating that the changes in anisotropy displayed in Fig. 4B occurred as a result of Dpo4 binding. (B) A pre-incubated solution of Dpo4 intradomain mutant Y274W-K329C (200 nM) and DNA^{OH} (300 nM) was rapidly mixed with dTTP (1 mM) and the Trp fluorescence was monitored upon excitation at 290 nm. No detectable change in fluorescence was observed suggesting that FRET changes observed in Fig. 5 were due to distance changes between the donor and acceptor probes rather than variations in the local environment of the Trp probe. (C) $Y274W\text{-}K329C^{CPM}$ (200 nM) was rapidly mixed with dTTP (1 mM) and CPM-fluorescence was monitored upon excitation at 290 nm. In the absence of DNA, no detectable change in fluorescence was observed, thereby demonstrating that inner filtering, caused by the large concentration of dTTP, occurred within the dead time of the instrument (~1 ms). Accordingly, the changes in CPM-fluorescence observed in Fig. 5 serve as proxies for relative distance changes between the donor and acceptor probes.