Supporting Information

Dynamics of site switching in DNA polymerase

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Supporting Experimental Procedures.

Fluorescence anisotropy titration. Aliquots of unlabeled wt KF were added to a solution of 25 nM A488-labeled primer/template (containing a terminal mismatch, 1 mm, Table 1, main text) in KF binding buffer (main text) and the fluorescence anisotropy of A488 was measured after each addition (excitation at 480 nm, emission at 518 nm), with correction for the instrumental G-factor. Aliquots were added until there was no further change in the anisotropy, signifying the end point of the titration. The resulting profile of anisotropy versus KF concentration was fitted to a 1:1 binding model, adjusting the K_d value and limiting anistropy for best fit, as described[1].

(1) Bailey, M. F.; Van der Schans; E. J. C. and Millar, D. P. *J. Mol. Biol*. **2004**, *336*, 673.

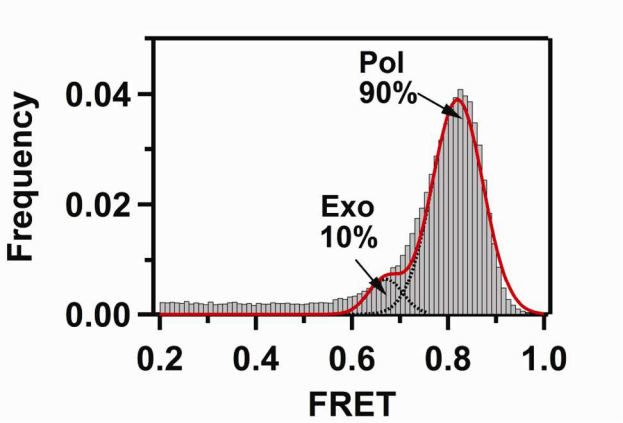

Figure S1. FRET efficiency histogram for wt KF interacting with fully matched primer/template. The complete DNA sequence is shown in Table 1 (0 mm) in the main text. The histogram was fitted with two Gaussian functions, with the FRET efficiency of the minor species constrained to the same value (0.67 efficiency) obtained in Fig. 2B (main text). The remaining parameters for each Gaussian peak were optimized for best fit. The fitted width of the low FRET peak is 0.05, while the fitted center and width of the high FRET peak are 0.82 and 0.075, respectively. The dashed lines show the individual Gaussian fits while the solid red line shows the composite fit. The fractional areas enclosed by the two peaks are indicated.

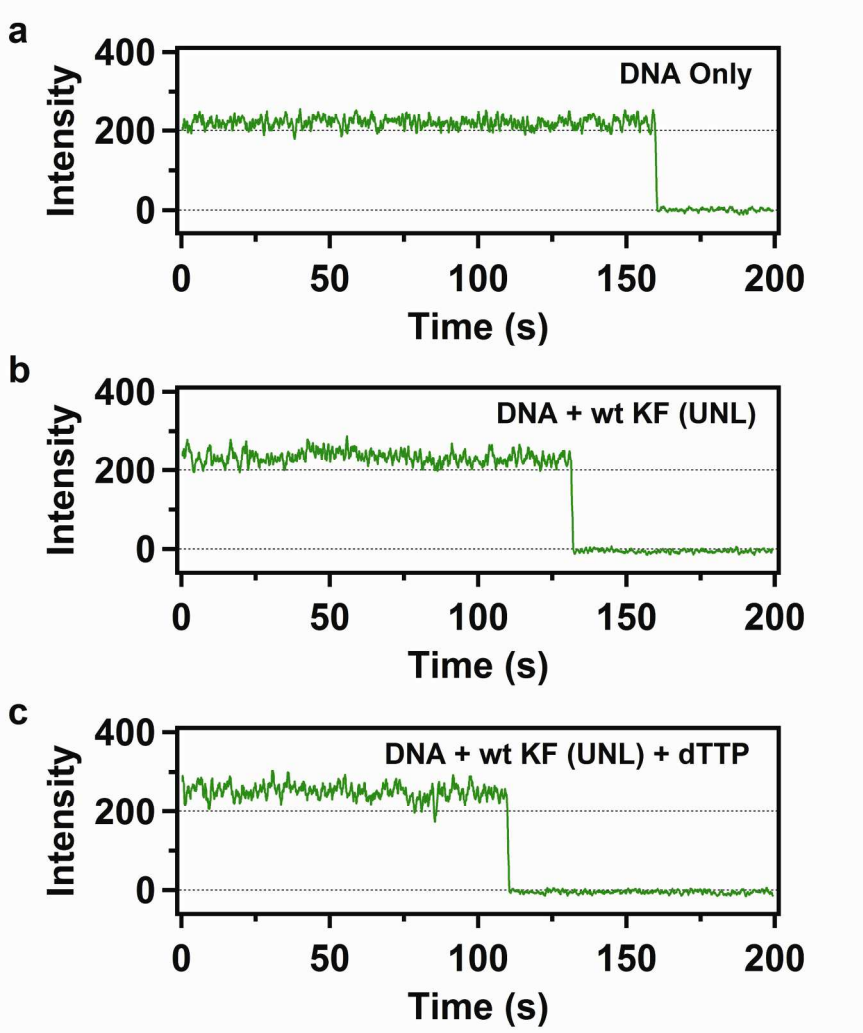

Figure S2. Intensity time traces of immobilized A488-labeled primer/template, containing a terminal mismatch. The complete DNA sequence is shown in Table 1 (1 mm) in the main text. Time traces were measured in the TIRF microscope under the same conditions as used in Fig. 2 in the main text. (a) A488-labeled primer/template alone. (b) A488-labeled primer/template in the presence of 5 nM unlabeled wt KF. (c) A488-labeled primer/template in the presence of 5 nM unlabeled wt KF and 1 mM dTTP.

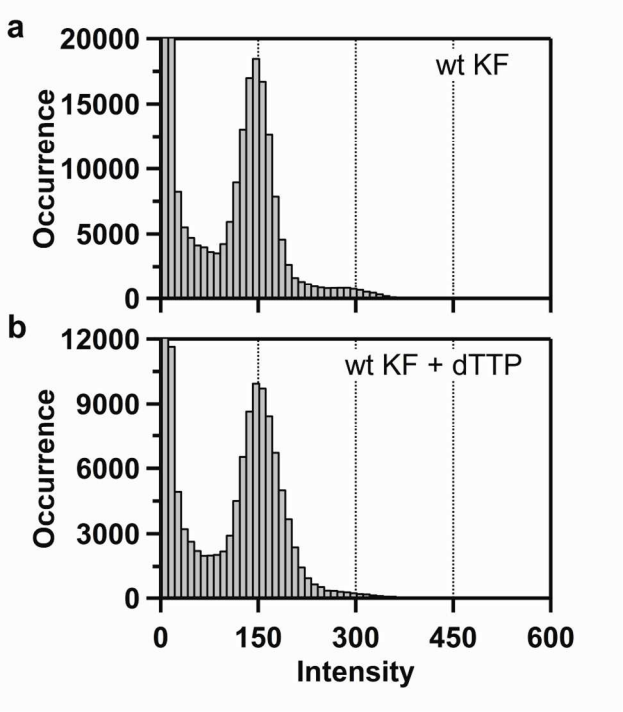

Figure S3. Intensity histograms of A594-labeled KF, measured by direct excitation of A594 at 532 nm. Histograms were compiled from time traces of A594 emission measured in the TIRF microscope. (a) Intensity histogram compiled from 120 time traces for KF (2 nM) interacting with unlabeled primer/template (containing a terminal mismatch). The large peak at zero intensity is due to the background, the peak around 150 camera counts is due to a single KF molecule bound to the primer/template, and the shoulder at ~ 300 camera counts is due to a small population (< 5%) of complexes containing two KF molecules. (b) As for part (a), but in the additional presence of 1 mM dTTP.

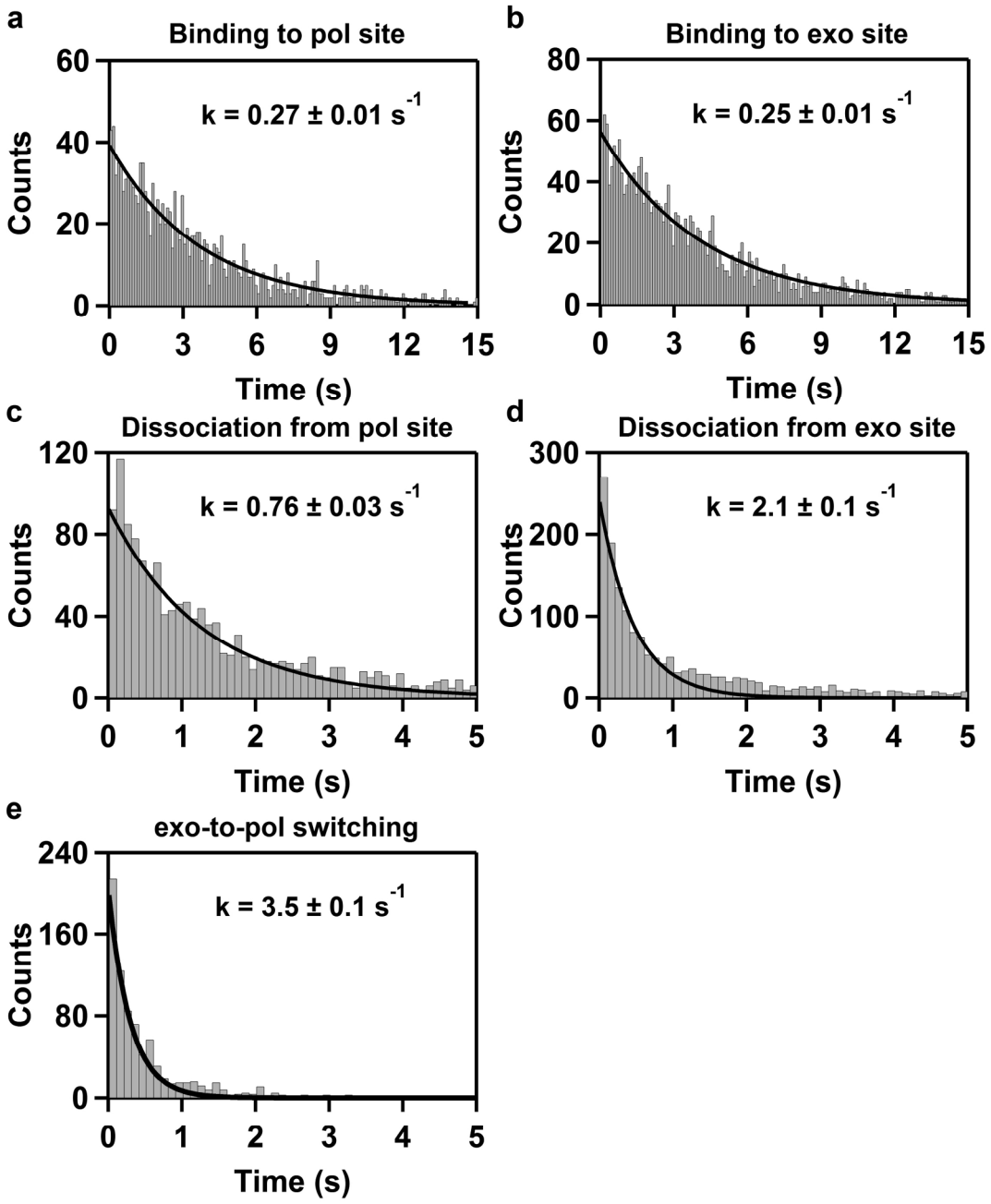

Figure S4. Dwell time histograms for wt KF (5 nM) interacting with primer/template containing a terminal mismatch. A set of 230 FRET trajectories were analyzed by Hidden Markov modeling, as described in the main text. (a) Dwell times prior to binding to the pol site, compiled from 1470 transitions. (b) Dwell times prior to binding to the exo site, compiled from 2307 transitions. (c) Dwell times spent in the pol site prior to

dissociation, compiled from 1501 transitions. (d) Dwell times spent in the exo site prior to dissociation, compiled from 1761 transitions. (e) Dwell times in the exo site prior to switching to the exo site, compiled from 822 transitions. In all cases, the solid lines are single exponential fits, with the rate constants indicated.

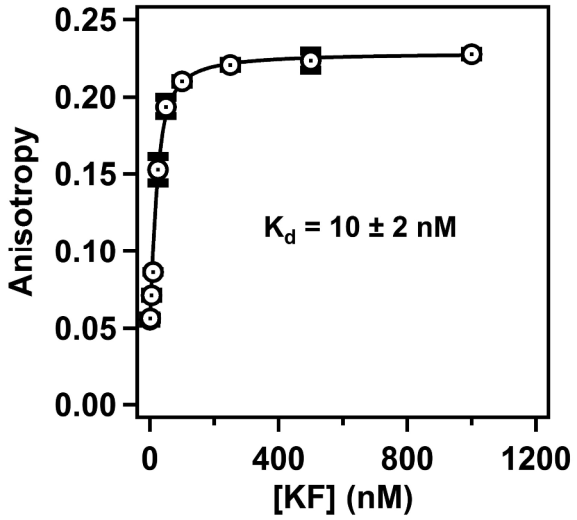

Figure S5. Steady-state fluorescence anisotropy titration of primer/template containing a terminal mismatch with unlabeled wt KF. The solid line is the best fit to a 1:1 binding model, with the indicated K_d value.

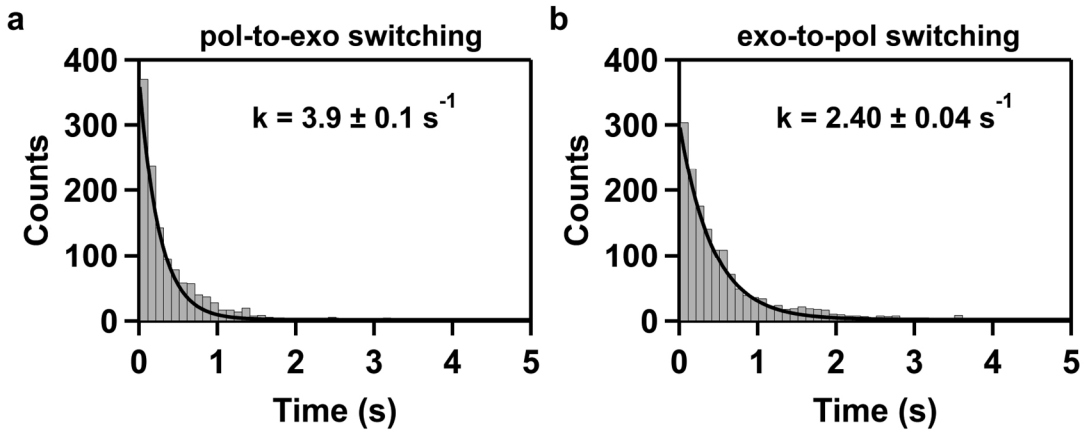

Figure S6. Dwell time histograms for wt KF interacting with primer/template containing an internal mismatch. The complete DNA sequence (+1 mm) is shown in Table 1 in the main text. (a) Dwell time spent in the pol site prior to switching to the exo site, compiled from 1295 transitions. (b) Dwell time spent in the exo site prior to switching to the pol site, compiled from 1598 transitions. In both cases, the solid lines are single exponential fits, with the rate constants indicated.

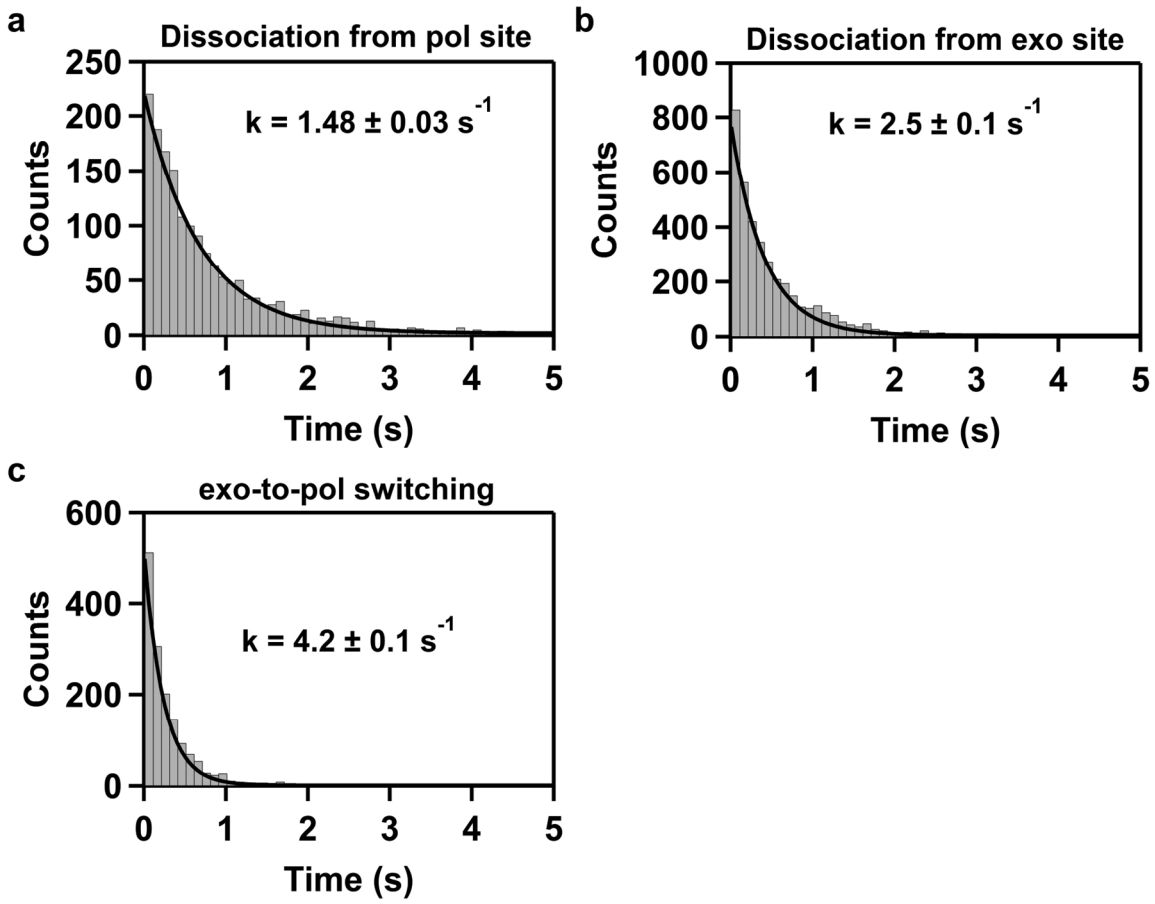

Figure S7. Dwell time histograms for wt KF (5 nM) interacting with primer/template containing a terminal mismatch in the presence of 1 mM dTTP. (a) Dwell time spent in the pol site prior to dissociation, compiled from 1747 transitions. (b) Dwell times spent in the exo site prior to dissociation, compiled from 3949 transitions. (c) Dwell time spent in the exo site prior to switching to the pol site, compiled from 1552 transitions. In all cases, the solid lines are single exponential fits, with the rate constants indicated.

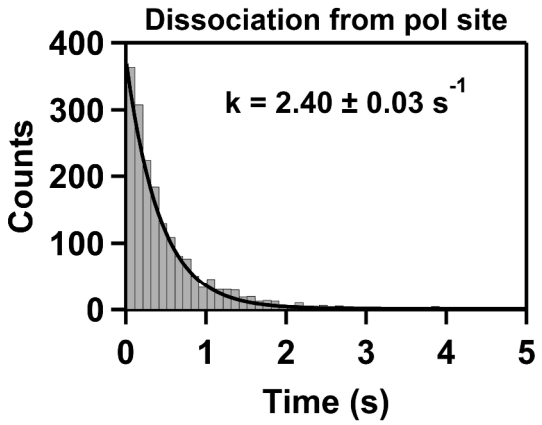

Figure S8. Dwell time histogram for wt KF (5 nM) interacting with primer/template containing a terminal mismatch in the presence of 1 mM dATP. Shown is the histogram of dwell times spent in the pol site prior to dissociation, compiled from 1860 transitions. The solid line is the best fit to a single exponential function, with the rate constant indicated.