CHEMPHYSCHEM

Supporting Information

Characterizing Single-Molecule FRET Dynamics with Probability Distribution Analysis

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cphc_201000129_sm_miscellaneous_information.pdf

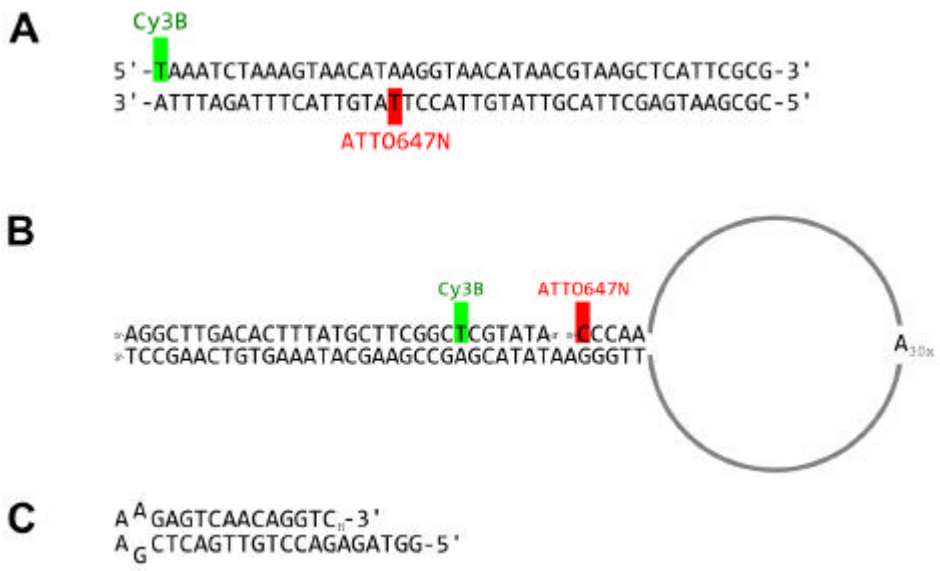

Figure S-1. List of DNA sequences. The position of the fluorophore labels are as highlighted (green for FRET donor and red for FRET acceptor). (A) $T_{1\text{-Cy3B}}$,$B_{18\text{-ATTO647N}}$ double stranded DNA. (B) A DNA-hairpin with 5 bp stem and a poly?A loop. (C) The unlabelled DNA-hairpin substrate for Pol I.

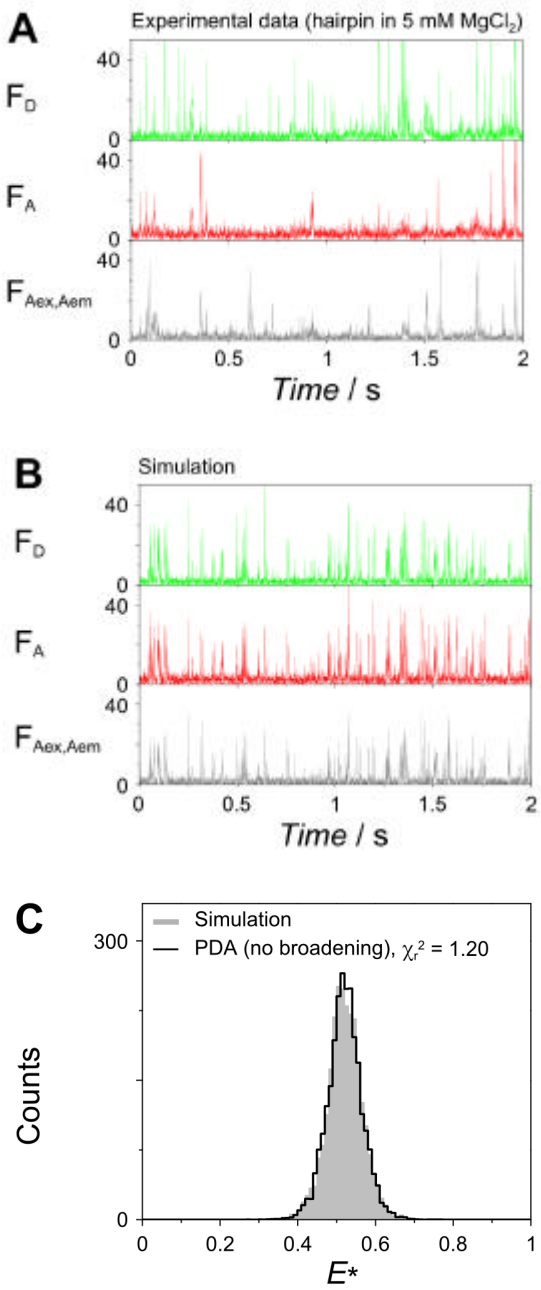

Figure S-2. Contributions of background photons can be safely ignored in the analysis as long as the counts are low (< 6kHz in each channel). (A) Time traces of experimental data (hairpin in 5 mM $MgCl_2$) binned at 0.5-ms resolution. Green: F_D (photon counts in the donor channel due to donor excitation); red: F_A (photon counts in the acceptor channel due to donor excitation); grey: $F_{Aex,Aem}$ (photon counts in the acceptor channel due to acceptor excitation). (B) Time traces of

Monte-Carlo simulation with similar background counts as in A. (C) The simulated data in B can be fitted very well to a single-state PDA model with no broadening ($?_r^2$=1.20), even without accounting for the presence of the background counts. The absolute value of E^* is shifted due to the differences in average background counts between the F_D and F_A channels, however this is likely to be a problem as most smFRET experiments are concerned only with relative FRET changes (see main text).

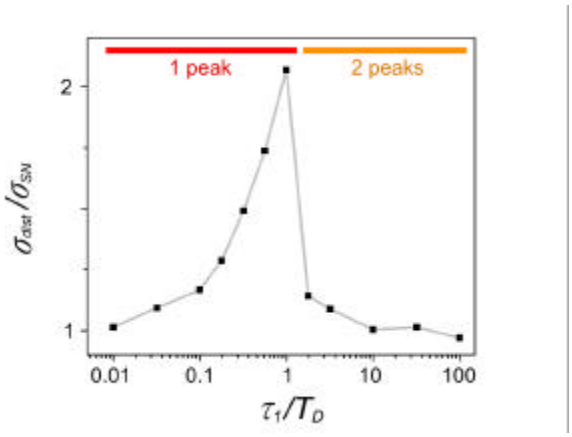

Figure S-3. The sensitivity of PDA is assessed by comparing the width of E^* distributions (s_{dist}) to the width of a simple one-state shot-noise limited distribution (s_{SN}). The closer the E^* distribution to the shot-noise limited distribution (s_{dist}/s_{SN}=1), the less sensitive PDA in detecting the conformational dynamics. We note that PDA is most sensitive when 0.01 = (t_1/T_D) = 10. s_{dist} was obtained by simulating a two-state FRET system using the following parameters: E_1=0.4, E_2=0.6, s_1=s_2=0, t_1=t_2, T_D=1ms, F=100 photons. When t_1/T_D ~ 2, the FRET distribution splits into two distinct peaks (see figure 1B in main text); the s_{dist} width reported for this range (t_1/T_D=2) is calculated from one peak only.

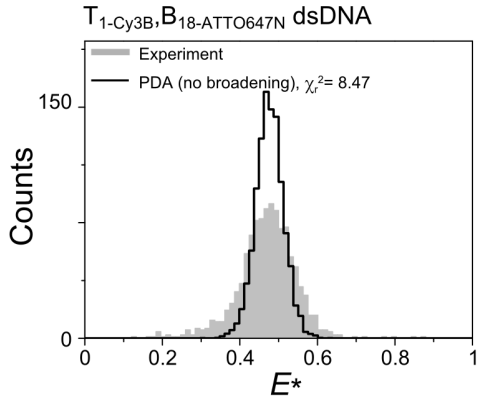

Figure S-4. Experimental FRET histogram of double stranded DNA ($T_{1\text{-Cy3B}}$,$B_{18\text{-ATTO647N}}$; grey histogram) is approximately 1.8 times wider than the histogram due to shot noise alone (PDA prediction with no broadening; black line). The experimental histogram can be fitted to a Gaussian distribution with a standard deviation of $s_{experimental} = 0.061$. Meanwhile, PDA prediction due to shot-noise alone has a width of $s_{ShotNoise} = 0.034$.

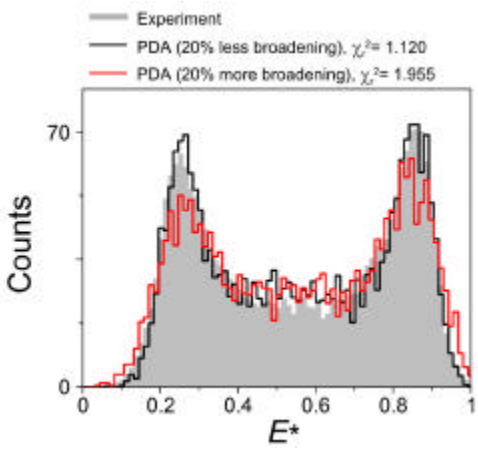

Figure S-5. Experimental FRET histogram of the hairpin DNA in buffer containing 5 mM $MgCl_2$ (grey histogram) and the corresponding PDA predictions given 20% less broadening (black line, s_1=s_2=0.04) and 20% more broadening (red line, s_1=s_2=0.06) as compared to the broadening of dsDNA (s=0.05). Even with 20% error in the width parameters, the PDA predictions still produced good fit to the experimental data (see $?_r^2$ values in the figure legend).

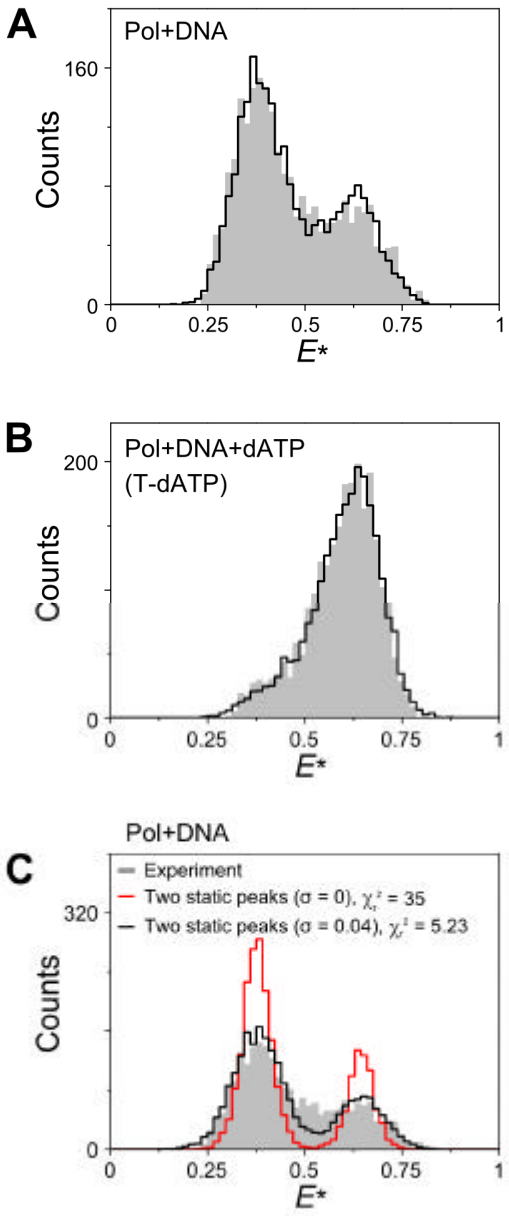

Figure S-6. Using PDA to uncover kinetic parameters of the Pol-DNA binary and Pol-DNA-dATP ternary complexes. (A) The *E** histogram of Pol-DNA binary complex can be fitted using a two-state system with the following parameters: E_{open}=0.5, s_{open}=0.035, E_{closed}=0.715, s_{closed}=0.04, $k_{open\ to\ closed}$=59 (±5) s^{-1}, $k_{closed\ to\ open}$=111 (±13) s^{-1}, $?_r^2$=2.02. (B) The *E** histogram of Pol-DNA-dATP ternary complex can be fitted using a two-state system with the following parameters: E_{open}, s_{open}, E_{closed}, and s_{closed} are the same as in A, $k_{open\ to\ closed}$=498

(±53) s^{-1}, $k_{closed\ to\ open}$=101 (±13) s^{-1}, $?_r^2$=1.95. (C) On the other hand, PDA predictions using two non-interconverting FRET peaks can not account for the experimental histogram of the Pol-DNA binary complex. The PDA prediction using two static peaks with no broadening (red line; s=0) differs considerably from the experimental histogram ($?_r^2$=35), while the prediction with additional broadening (black line; s=0.04) matches the experimental data better ($?_r^2$=5.23), even though it still underestimates the number of events between the two peaks.