Supporting information for the manuscript:

Sensing DNA opening in transcription using quenchable FRET

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ADDITIONAL EXPERIMENTAL RESULTS AND SUPPLEMENTAL FIGURES

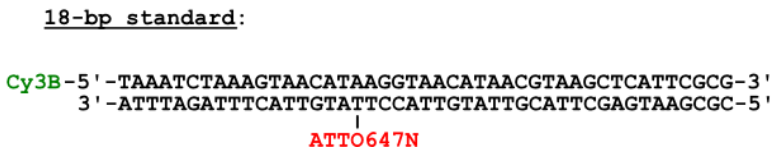

Figure S1. Oligonucleotide sequence of dsDNA with 18-bp separation for dye pair Cy3B/ATTO647N.

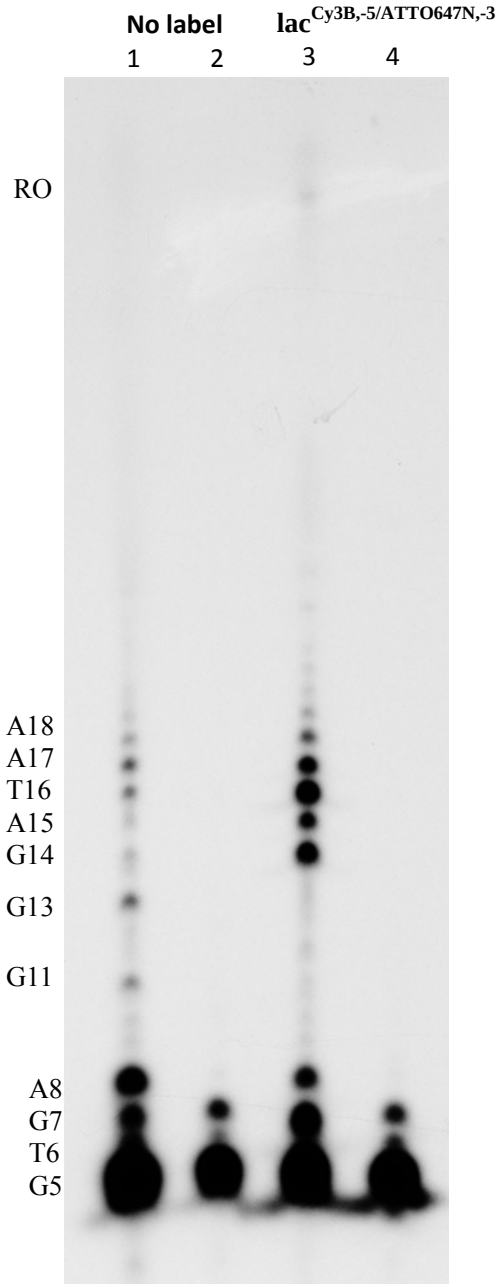

Figure S2. Functional RNAP assay using radioactivity: Comparison between *in vitro* transcription product profiles when using an unlabeled (lanes 1 and 2) and labeled (lanes 3 and 4) dsDNA template ($lac^{Cy3B,-5/ATTO647N,-3}$) in the presence of

0.3μCi/μl α-32P GTP: lanes 1&3 – full transcript, lanes 2&4 – 7 nt-long transcript corresponding to the $RP_{itc,7}$ reaction. In designating the mRNA products, the letter corresponds to the 3'-most base of the transcript (G, A, U), while the number indicates its length (5-8, 11, 13, 14-18). RO stands for run-off.

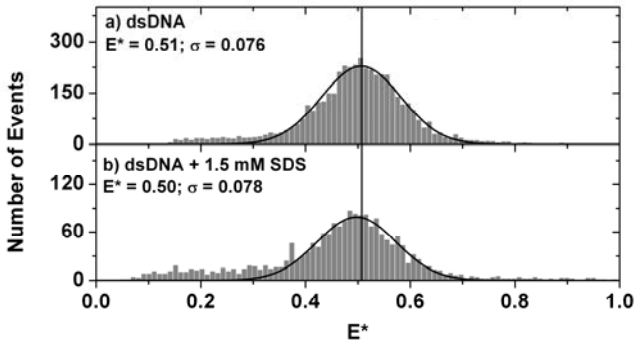

Figure S3. Photophysical control experiments for Cy3B/ATTO647N on an 18bp standard DNA: ALEX-based *E** histograms of single diffusing molecules (Cy3B/ATTO647N fluorophore pair on dsDNA with 18-bp separation between the two dyes) with an apparent FRET of E* ≈ 0.5. The DNA sequence is found in Figure S1. Measurement time was 20 min for each panel.

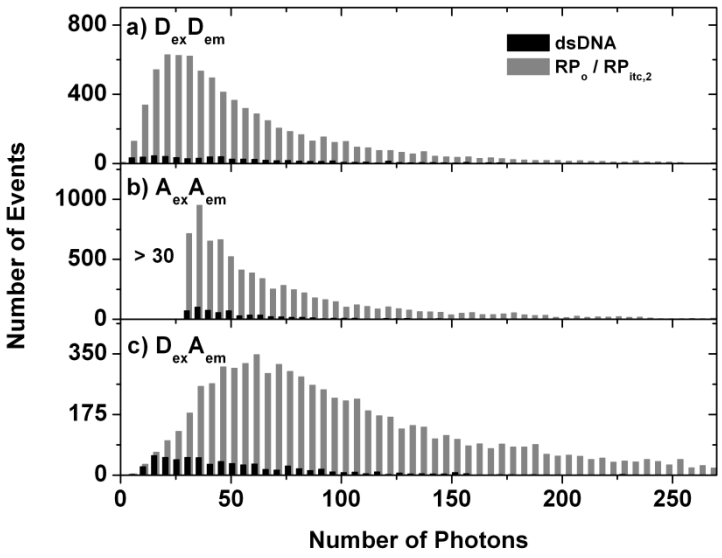

Figure S4. PCH analyzing quFRET: Photon counting histograms for different states of dsDNA ($lac^{Cy3B,-5/ATTO647N,-3}$) for (a) donor-excitation donor-detection $D_{ex}D_{em}$, (b) acceptor-excitation acceptor-detection $A_{ex}A_{em}$, and (c) donor-excitation acceptor-detection $D_{ex}A_{em}$. Please note that the histograms only incorporate bursts with an acceptor present and have an additional per-bin threshold of $A_{ex}A_{em} > 30$.

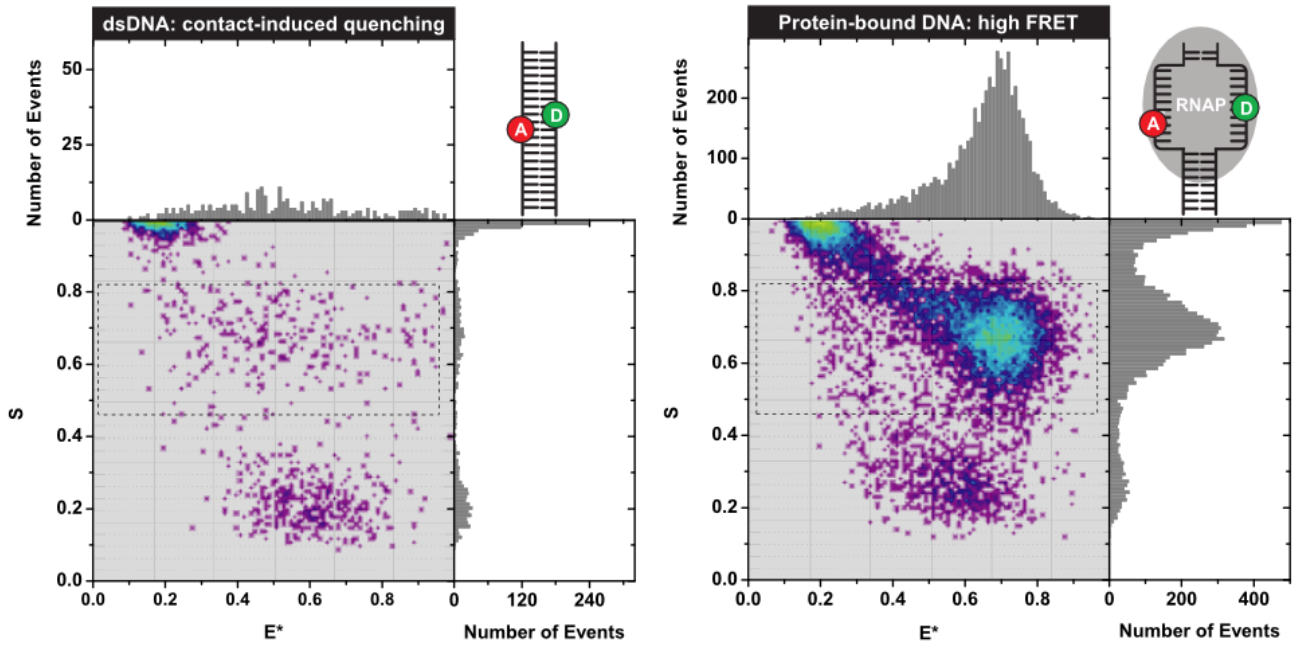

Figure S5. Analysis of the quFRET principle including all photons: The figure shows the same experimental results from ALEX-based smFRET as in Figure 1 (main text). One-dimensional FRET histograms were obtained by selecting all molecules in the lower panels with intermediate S ($0.45 < S < 0.8$). Both experiments were conducted for 20 minutes at 37°C at a concentration of 50-100 pM. Bursts corresponding to a single-molecule were identified using parameters M = 12, T = 500 µs and L = 30 and an additional per-bin threshold for all photons > 100.

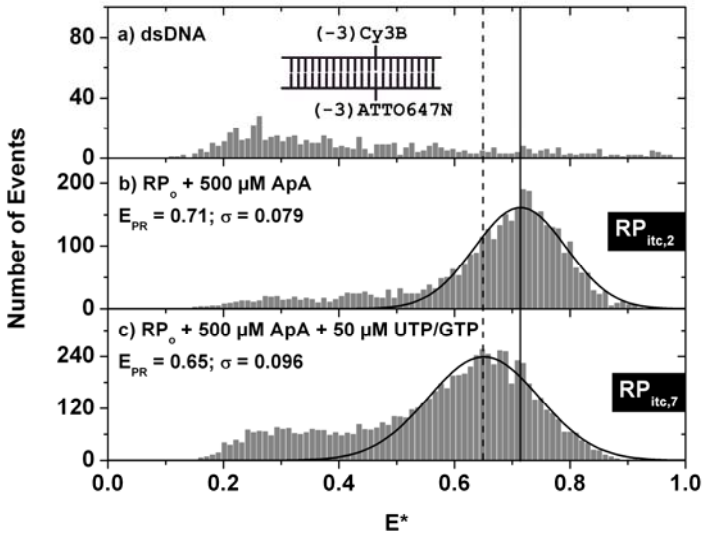

Figure S6. quFRET with lac$^{Cy3B,-3/ATTO647N,-3}$: ALEX-based *E** histograms of single diffusing molecules of transcription complexes formed using lac$^{Cy3B,-3/ATTO647N,-3}$ DNA at 37° C and at a concentration of ≈50-100 pM of DNA. The figure

shows the number of events of the apparent FRET E* for different buffer conditions (100 bins). Presence of different nucleotides in the buffer is indicated in the different panels. Measurement times were ≈60 min for each panel.

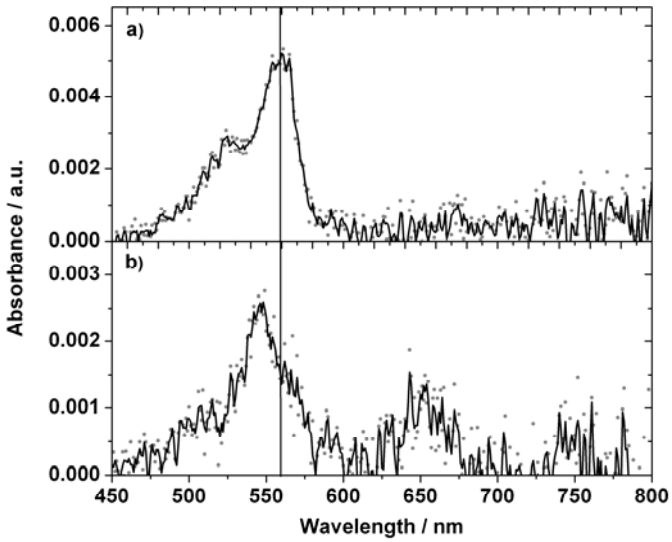

Figure S7. Absorption spectra of Cy3B and lac$^{Cy3B,-3/ATTO647N,-3}$: a) Absorption spectra of free Cy3B: data points in grey, smoothed data as a black line. b) Absorption spectra of Cy3B in lac$^{Cy3B,-3/ATTO647N,-3}$: data points in grey, smoothed data as a black line. Note that the absorption of ATTO647N (maximum ≈650) in lac$^{Cy3B,-3/ATTO647N,-3}$ is not easily observable due to the low concentrations of the sample and due to possible changes in the absorbance spectrum of ATTO647N when participating in quFRET.