

Single-molecule conformational dynamics of a transcription factor reveals a continuum of binding modes controlling association and dissociation

Wei Chen¹, Wei Lu², Peter G. Wolynes² and Elizabeth A. Komives^{1,*}

¹Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, California, USA.

²Center for Theoretical Biological Physics, Departments of Chemistry, Physics, and Biosciences, Rice University, Houston, Texas, USA

Correspondence should be addressed to E.A.K. (ekomives@ucsd.edu).

Supplementary Information

Figure S1-S9

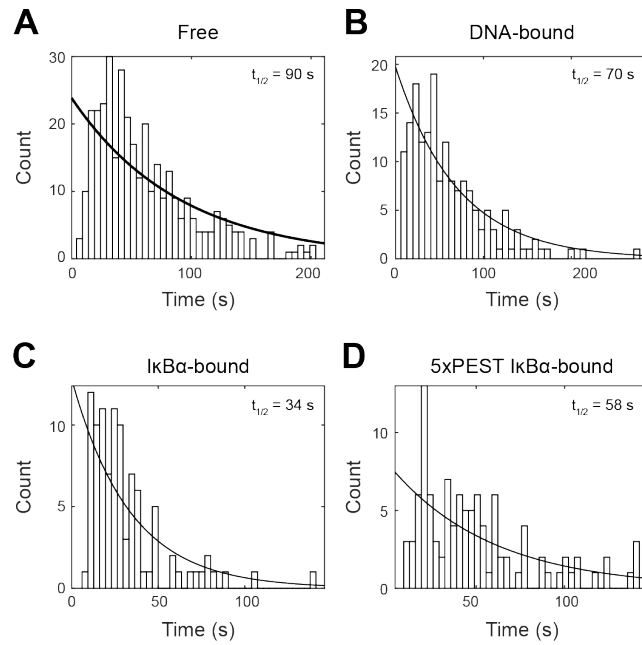


Figure S1. Distribution of photobleaching time for **(A)** free NF- κ B, **(B)** DNA-bound NF- κ B, **(C)** I κ B α -bound NF- κ B, and **(D)** 5xPEST I κ B α -bound NF κ B. The half-life ($t_{1/2}$) of the dye for each set of experiments was obtained from exponential fitting.

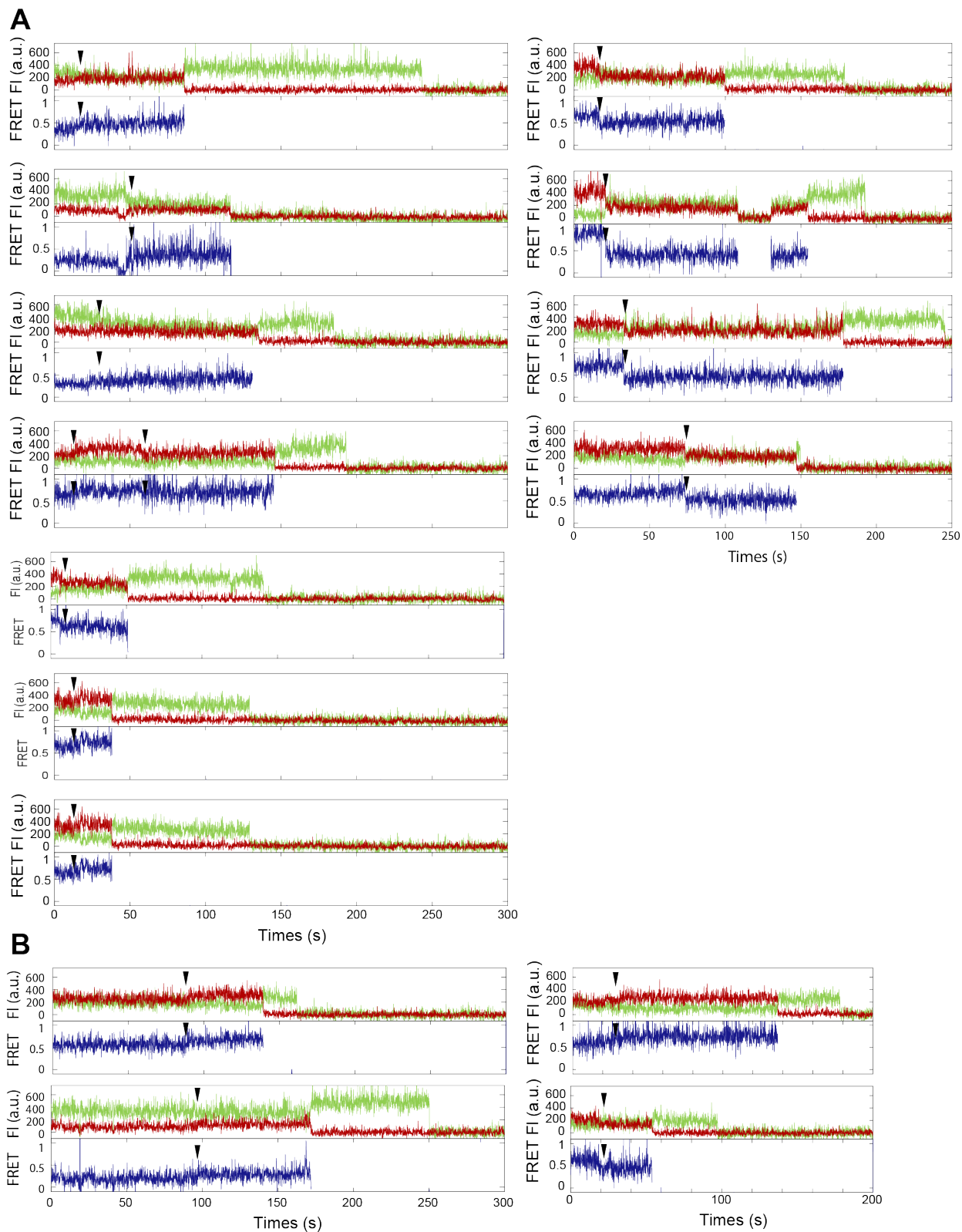


Figure S2. Traces with transitions (arrowheads) between long-lived states for (A) free NF-κB and (B) DNA-bound NF-κB.

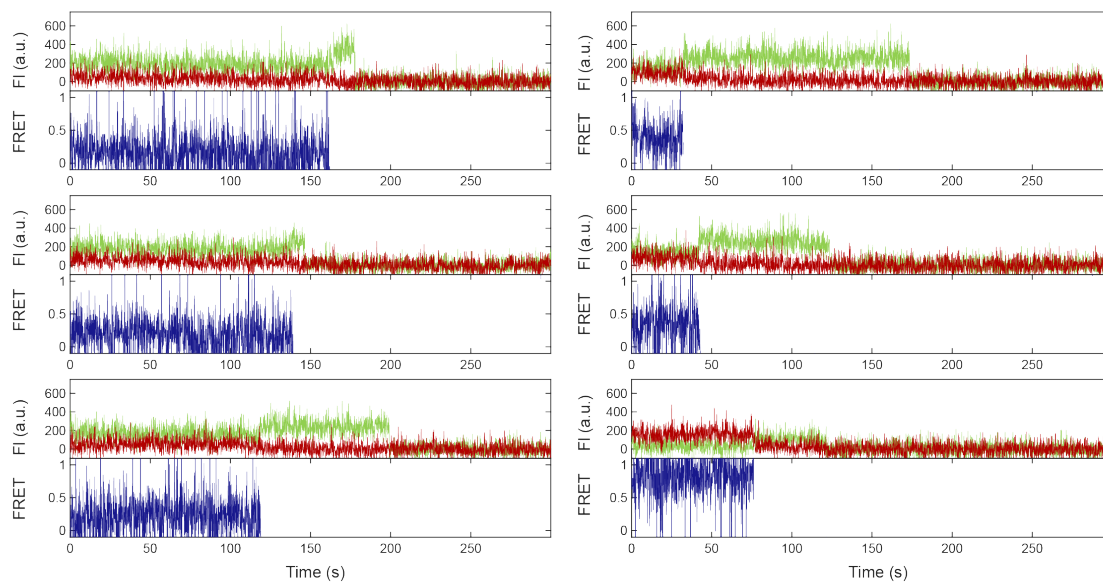


Figure S3. Representative traces for free NF- κ B with the addition of DTT. In the presence of 1 mM DTT, long-lived states with a broad range of FRET efficiencies from low to high were still observed, eliminating the possibility that conformational heterogeneity could be caused by disulfide bond formation.

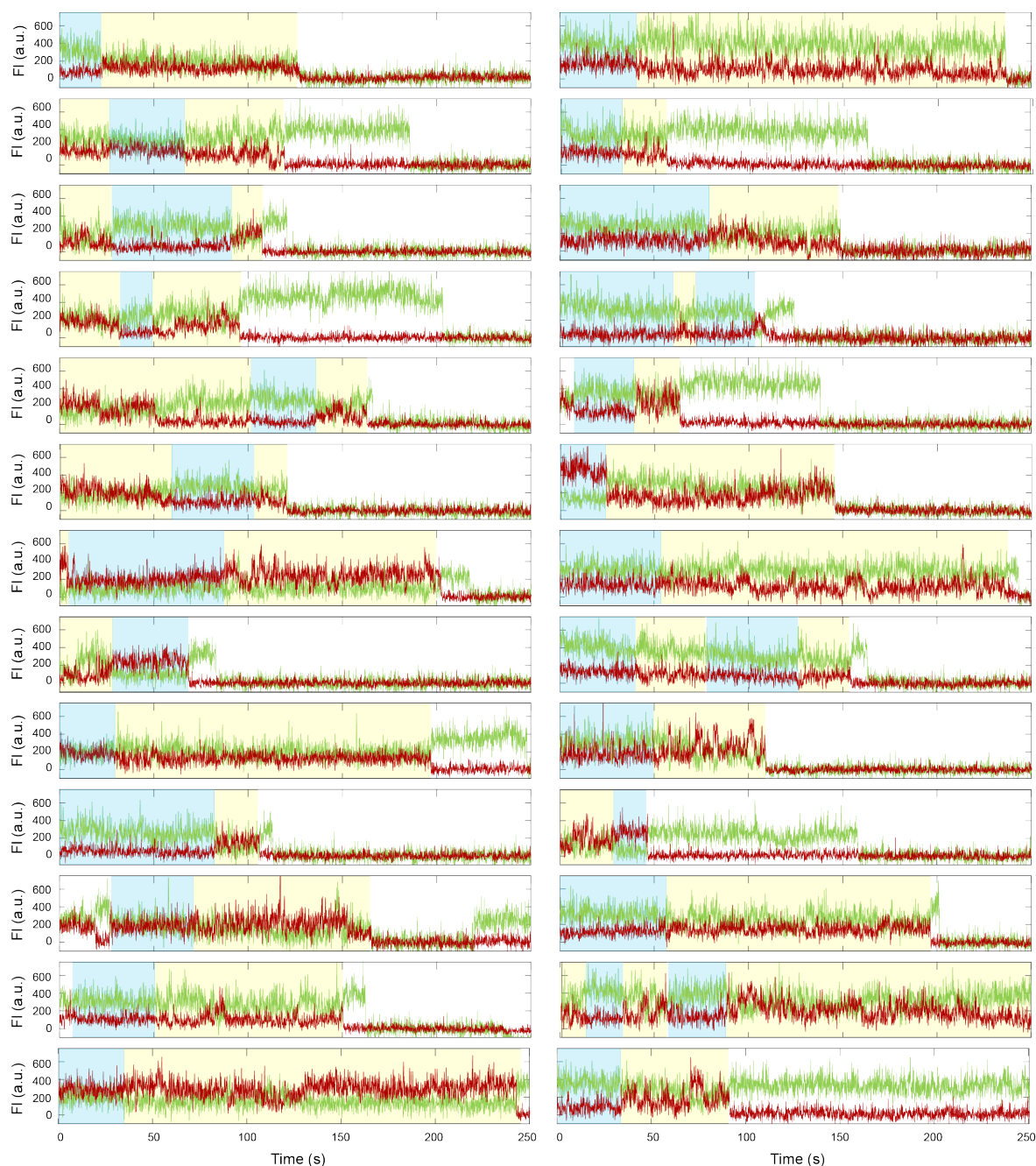


Figure S4. Traces of free NF- κ B showing transitions between long-lived FRET states (light blue) and periods of fluctuating FRET (light yellow). Traces are truncated to 250 seconds but all molecules did show both donor and acceptor single photobleaching events. Out of 426 traces of free NF- κ B, there were 26 transitioning traces (6.1%). There were 26 transitions from long-lived states to periods of fluctuating FRET and 11 transitions the other way around. Of the 26 transitioning traces, 19 traces only captured one transition before photobleaching. We observed 12 long-lived states that were preceded and followed by periods of fluctuating FRET. These 12 long-lived states averaged 36 ± 17 seconds. We also observed 4 periods of fluctuating FRET preceded and followed by long-lived states. The dwell-times for these periods of fluctuating FRET were 4.2, 39.3, 31.6, and 95.6 seconds.

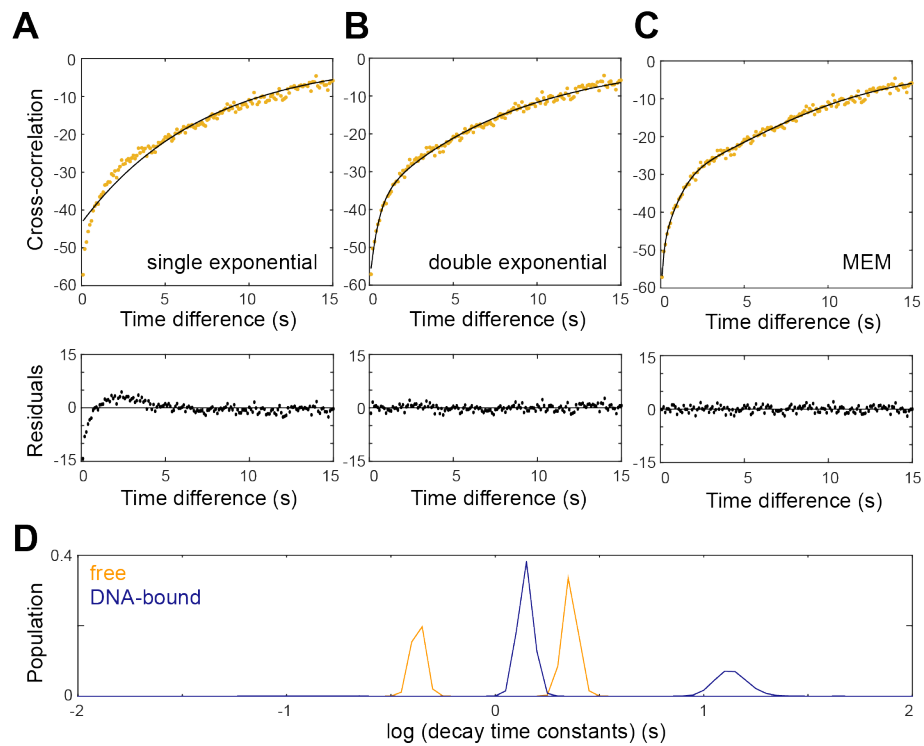


Figure S5. Analyses to determine the best model for cross-correlation fitting. **(A)** Single exponential fitting of the free NF-κB data. **(B)** Double exponential fitting of the free NF-κB data with decaying time constants of 0.50 ± 0.08 s and 8.4 ± 0.7 s and corresponding amplitudes -22 ± 2 and -38 ± 1 . **(C)** Fitting of the free NF-κB data with the maximum entropy method (MEM). **(D)** Distribution of decay time constants from MEM fitting gave 2 time constants for both free and DNA-bound NF-κB, consistent with the double exponential fitting. The residual plots from **A-C** show that two decay time constants best described the cross-correlation regardless of fitting with double exponential or MEM.

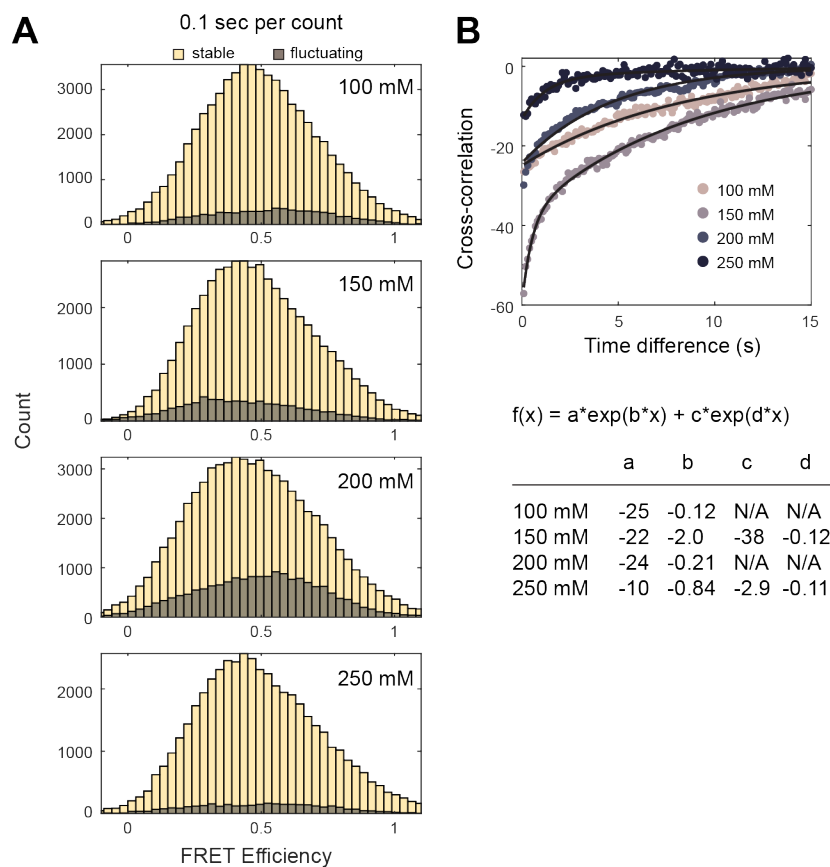


Figure S6. The effect of ionic strength on the conformational dynamics of free NF- κ B. **(A)** FRET histograms for free NF- κ B at different NaCl concentrations from 100 mM to 250 mM. The broad distribution of stable traces was independent of ionic strength. The relative population and the shape of the distribution of fluctuating traces are dependent on ionic strength but not with a simple monotonic trend. **(B)** Cross-correlation analyses on the fluctuating traces and fitting with single or bi-exponential functions showing the fluctuation amplitudes and rates are ionic strength dependent but not with a monotonic trend.

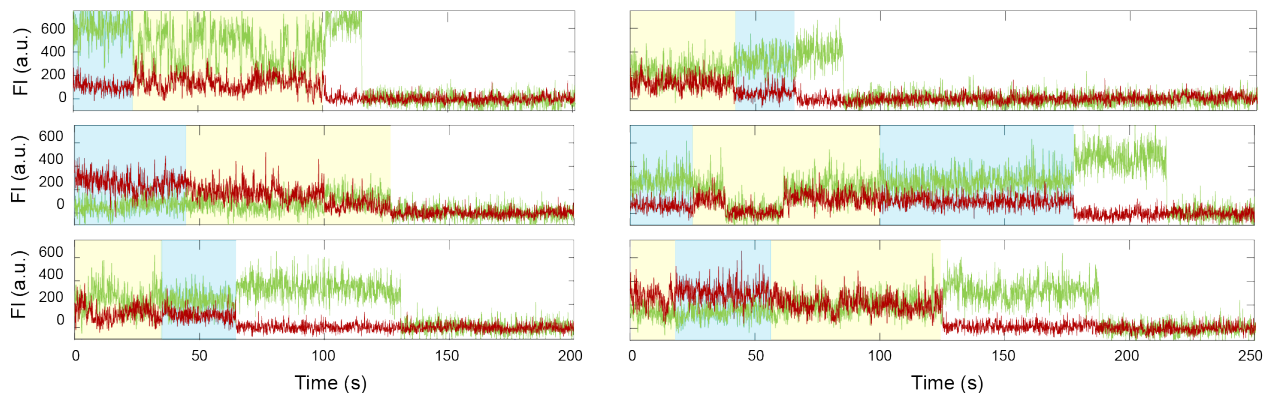


Figure S7. Traces of DNA-bound NF- κ B showing transitions between long-lived FRET states (light blue) and periods with fluctuating FRET (light yellow). Traces are truncated to 200 and 250 seconds. Out of 199 traces of DNA-bound NF- κ B, there were 6 traces (3.0%) showing transitions between long-lived states and periods with fluctuating FRET, 2 of which captured more than one transition. Only one trace showed a period with fluctuating FRET that was preceded and followed by long-lived FRET states, and this state had a dwell time of 73.6 seconds. Similarly, there was only one trace with a long-lived FRET state that was preceded and followed by periods with fluctuating FRET and the dwell time was 32.3 seconds.

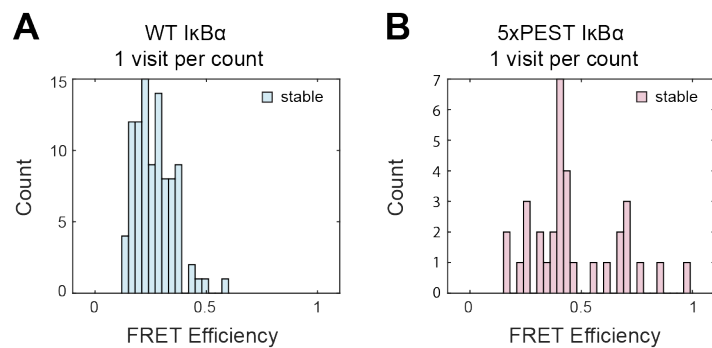


Figure S8. FRET histograms obtained by counting visits to long-lived states for IκBα-bound NF-κB. **(A)** NF-κB bound to wildtype IκBα adopted a narrow conformational distribution with low-FRET efficiencies. **(B)** NF-κB bound to the stripping impaired 5xPEST mutant IκBα adopted a broad distribution of long-lived states.

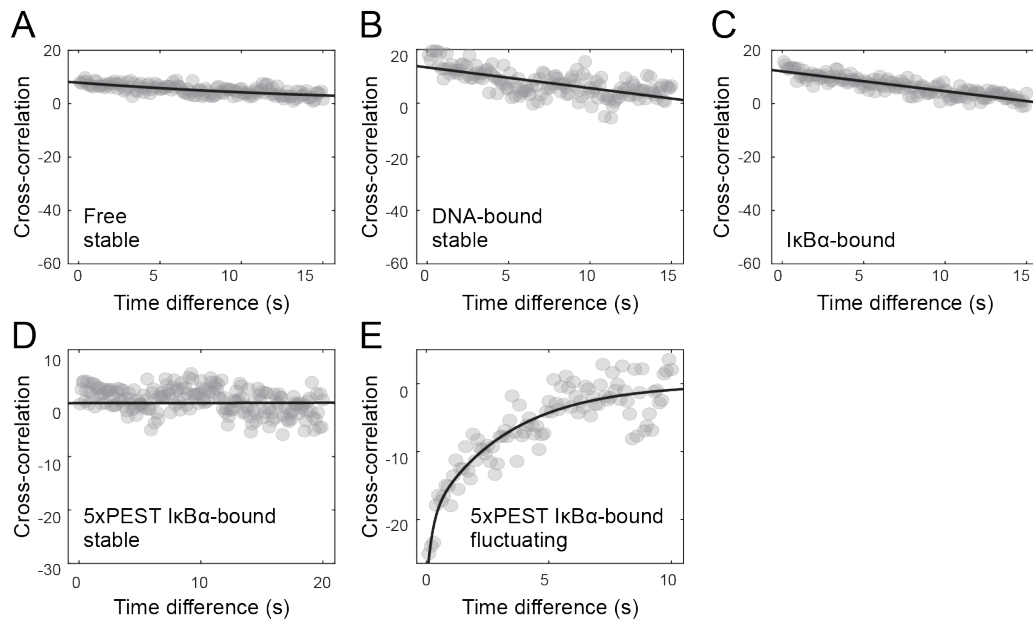


Figure S9. Cross-correlation analyses of smFRET traces. (A) Long-lived state of free NF- κ B, (B) long-lived states of DNA-bound NF- κ B, (C) all traces of I κ B α -bound NF- κ B, and (D) long-lived states of 5xPEST I κ B α -bound NF- κ B showed no anti-correlation. (E) Anti-correlation was observed for the fluctuating traces of 5xPEST I κ B α -bound NF- κ B.