Supporting Information

Single-Molecule Spectroscopic Study of Dynamic Nanoscale DNA Bending Behavior of HIV-1 Nucleocapsid Protein

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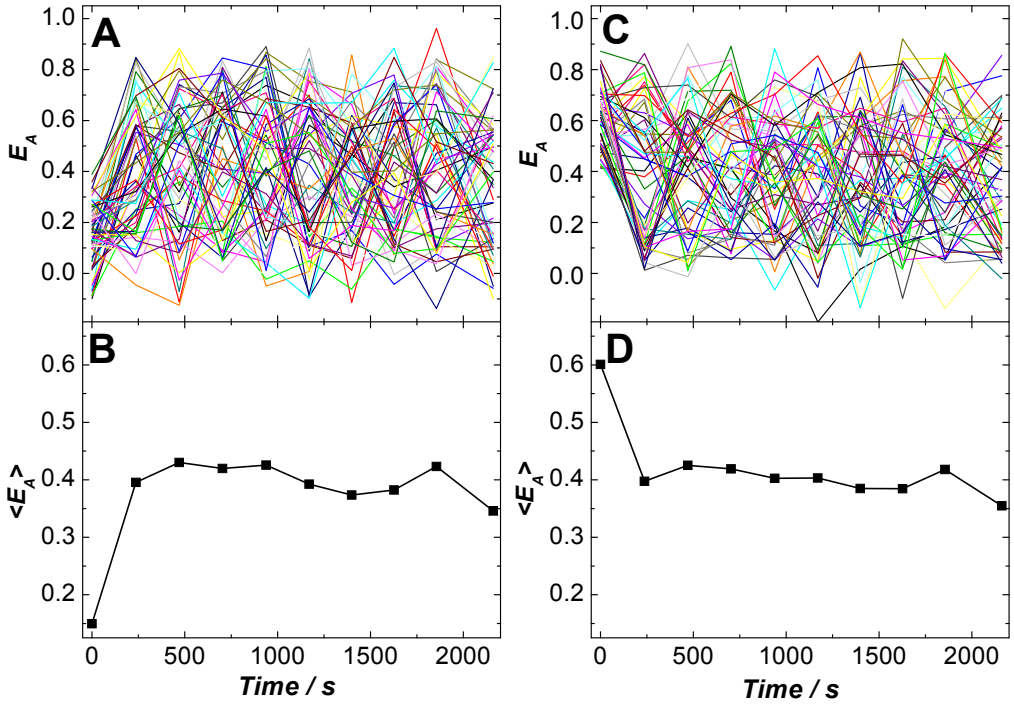

Figure S1. "Hole-burning" analysis of the FRET trajectories (obtained in image scanning mode) of 120 TAR-cTAR DNA duplex molecules in 2 μM NC. Panels A and C show the E_A trajectories of a sub-ensemble composed of 60 molecules with initial E_A values smaller than 0.4 and a sub-ensemble composed of 60 molecules with initial E_A values larger than 0.4, respectively. The corresponding molecular-averaged E_A trajectories of the two sub-ensembles are shown in Panels B and D.

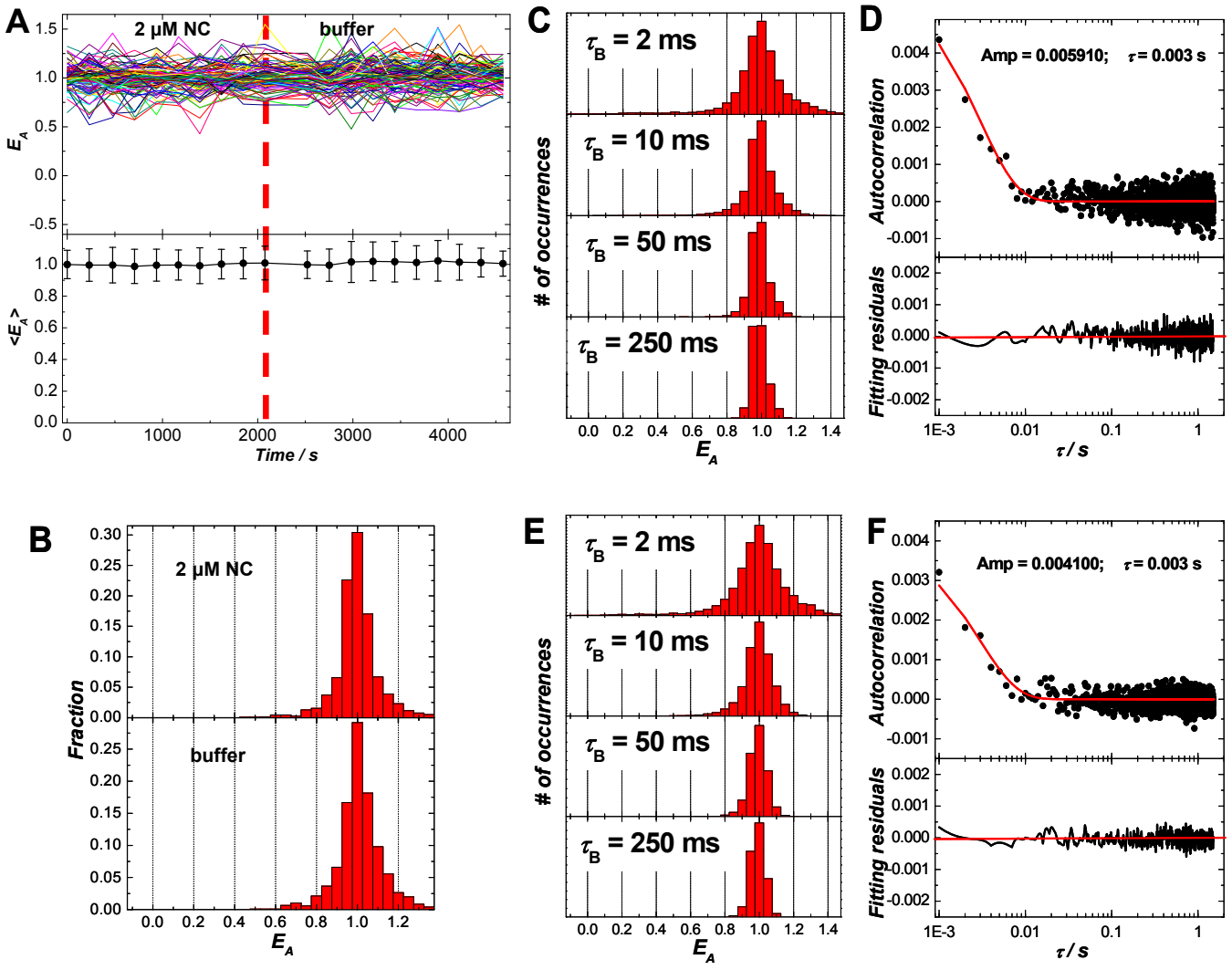

Figure S2. FRET fluctuations of fully duplexed TAR-cTAR with Cy3 and Cy5 at the same end of the DNA duplex. (A) Single-molecule FRET trajectories (obtained in the image scanning mode) of 157 molecules found in a 30 μm X 30 μm region and the corresponding molecularly-averaged FRET trajectory. The dual dye-labeled DNA duplexes were formed through NC-chaperoned annealing between the Cy3-TAR and 3'-Cy5-cTAR DNA oligonucleotides. The duplexed DNA molecules were immobilized on a coverslip and were exposed to 2μM NC and buffer during time periods I and II, respectively. (B) FRET histograms of all spectroscopic occurrences of the DNA duplex molecules in 2μM NC (*upper panel*) and buffer (*lower panel*). (C) The ensemble FRET histograms (obtained in the individual trajectory mode) constructed from FRET trajectories of 50 molecules in 2μM NC with a bin time τ_B of 2 ms, 10 ms, 50 ms, and 250 ms. (D) The ensemble FRET autocorrelation obtained from FRET trajectories of the 50 molecules in 2μM NC. (E) The ensemble FRET histograms obtained from FRET trajectories of 50 molecules in buffer with a bin time τ_B of 2 ms, 10 ms, 50 ms, and 250 ms. (F) The ensemble FRET autocorrelation obtained from FRET trajectories of the 50 molecules in buffer.

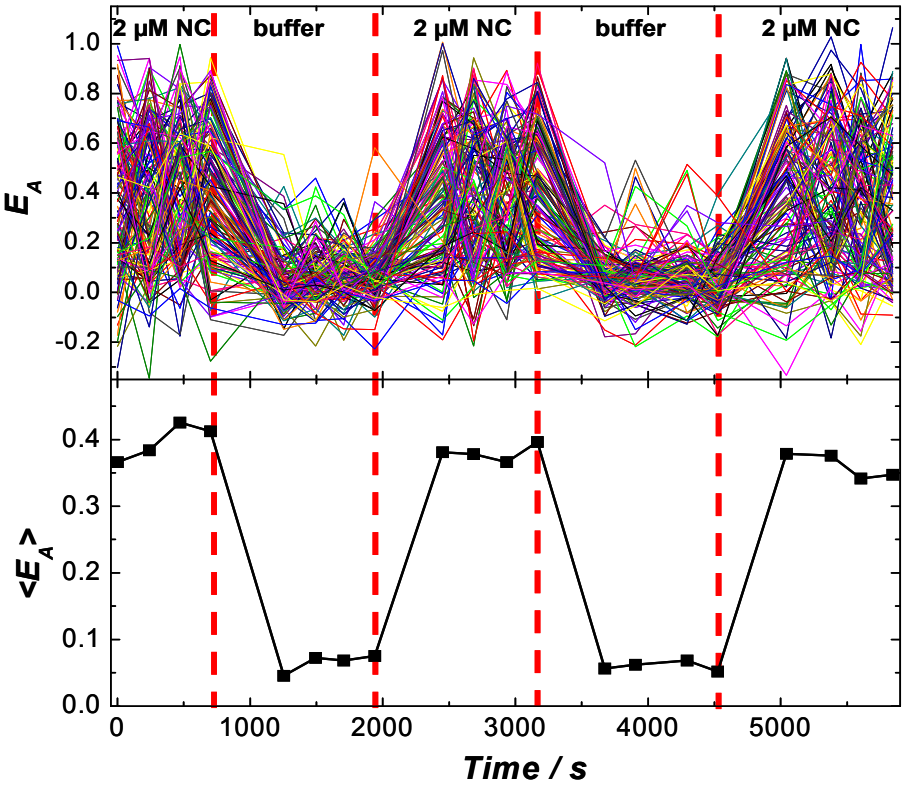

Figure S3. Conformational dynamics of TAR-cTAR DNA duplexes during multiple cycles of switching between 2 µM NC and buffer. Single-molecule FRET trajectories (obtained in the image scanning mode) of 142 molecules found in a 30 µm X 30 µm region (*upper panel*) and the corresponding molecularly-averaged FRET trajectory (*lower panel*).

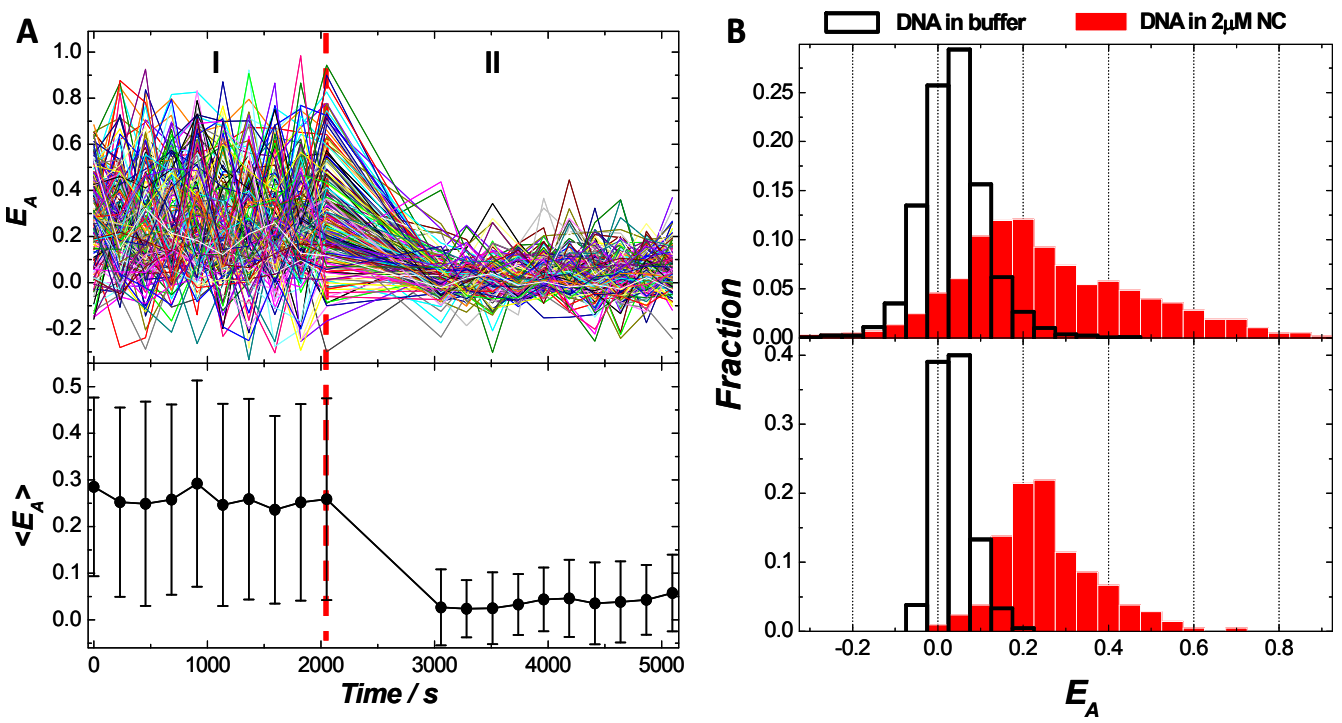

Figure S4. Conformational dynamics of a 59-bp fully duplexed DNA segment corresponding to the transcripts of the SL3-SL4 ψ recognition element region of HIV-1 genome in the presence and absence of NC on time scale of minutes. (A) Single-molecule FRET trajectories (obtained in the image scanning mode) of 210 molecules found in a 30 μm X 30 μm region and the corresponding molecularly-averaged FRET trajectory. The dual dye-labeled DNA duplexes were immobilized on a coverslip. During time periods I and II, 2μM NC and buffer were flowed into the reaction chamber, respectively. (B) FRET histograms of all spectroscopic occurrences (*upper panel*) and time-averaged FRET (*lower panel*) of the DNA duplex molecules in 2μM NC and buffer.

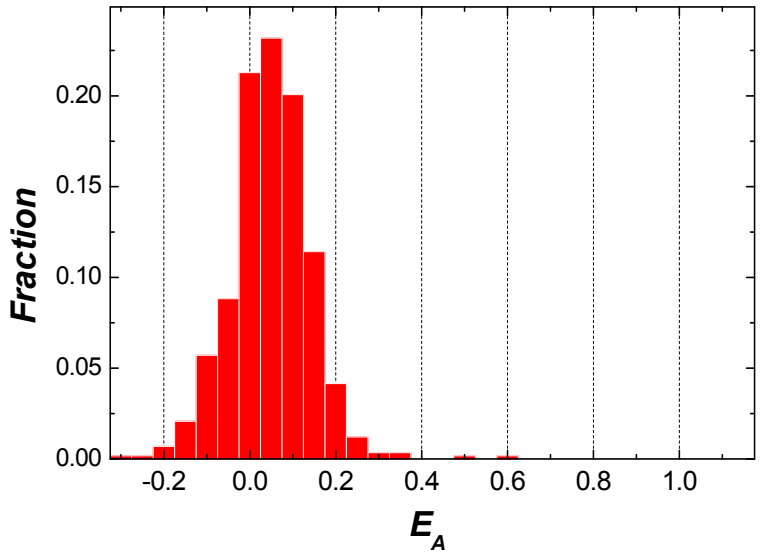

Figure S5. FRET histograms (obtained in the image scanning mode) of TAR-cTAR duplexes in 2 μM NC and 40 mM $MgCl_2$.

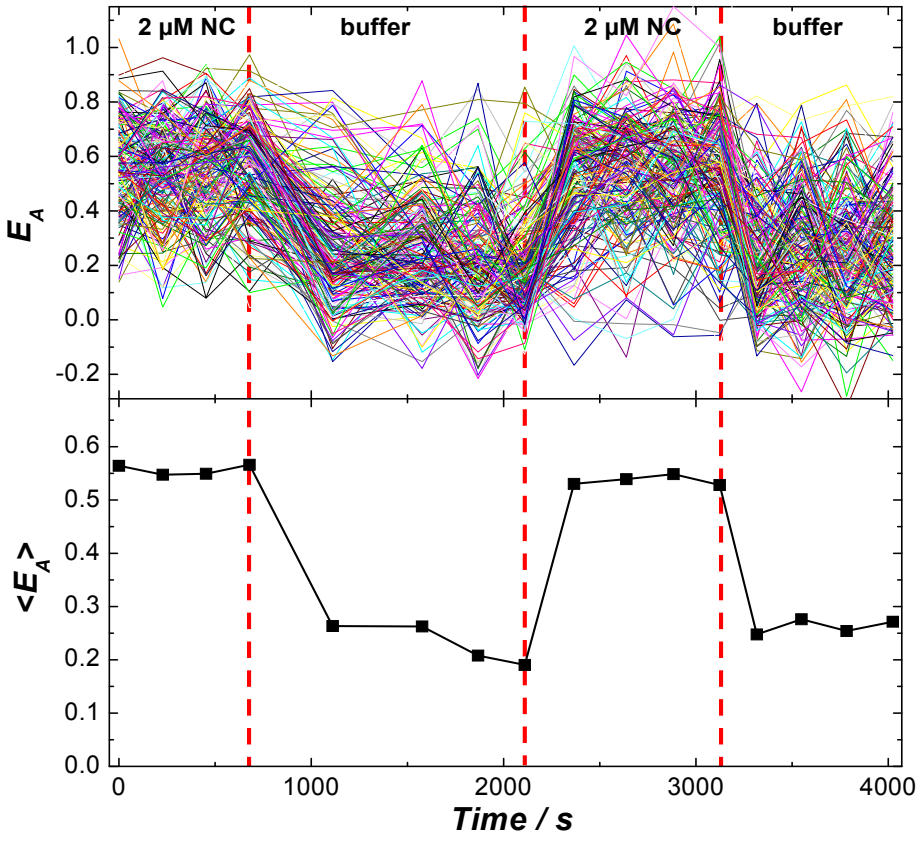

Figure S6. Conformational dynamics of TAR-cTAR DNA mismatch1 duplexes during multiple cycles of switching between 2 μM NC and buffer. Single-molecule FRET trajectories (obtained in the image scanning mode) of 209 molecules found in a 30 μm X 30 μm region (*upper panel*) and the corresponding molecularly-averaged FRET trajectory (*lower panel*).

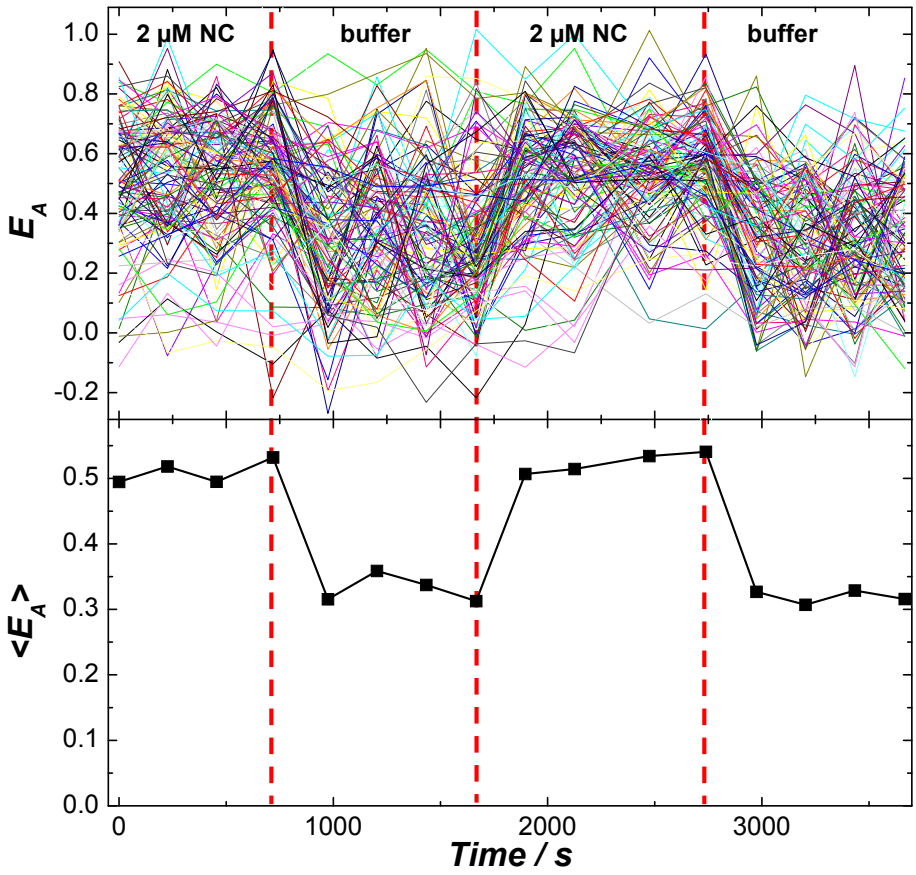

Figure S7. Conformational dynamics of TAR-cTAR DNA mismatch2 duplexes during multiple cycles of switching between 2 μM NC and buffer. Single-molecule FRET trajectories (obtained in the image scanning mode) of 125 molecules found in a 30 μm X 30 μm region (*upper panel*) and the corresponding molecularly-averaged FRET trajectory (*lower panel*).

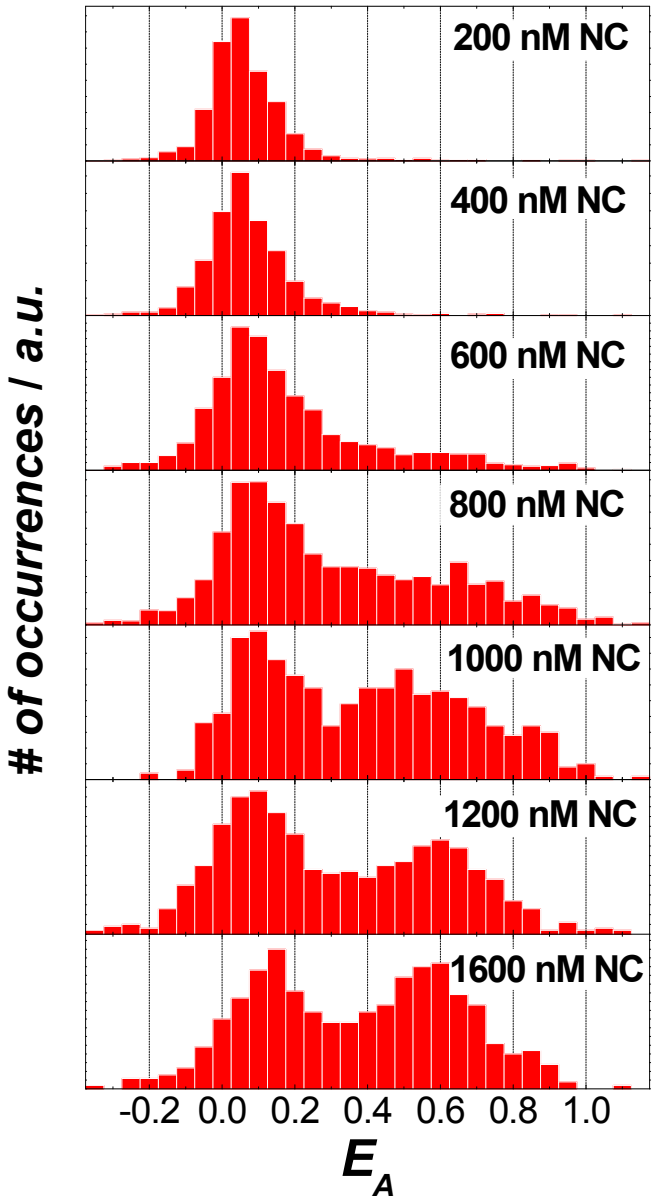

Figure S8. FRET histograms (obtained in the image scanning mode) of the TAR-cTAR DNA duplexes in the presence of NC at different concentrations.