Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Camera-based single-molecule FRET detection with improved time resolution

Shazia Farooq[1] and Johannes Hohlbein[1]

[1] Laboratory of Biophysics, Wageningen UR, Dreijenlaan 3, 6703 HA Wageningen, The Netherlands

Contact: johannes.hohlbein@wur.nl

SUPPLEMENTARY MATERIAL

Labelling, purification and immobilization of DNA

The DNA construct consisted of two DNA molecules: A primer strand (30 bases long, 5' CCT CAT TCT TCG TCC CAT TAC CAT ACA TCC) and a template DNA (75 bases long, 5' TGG ATT AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA TCC ATT GGA TGT ATG GTA ATG GGA CGA AGA ATG AGG) that forms a hairpin structure owing to sequence complementary of six consecutive bases in one region of the strand to another. The single stranded DNAs were prepared by automated synthesis (IBA, Germany). We labelled the 5' biotinylated primer strand with ATTO647N (ATTO-TEC, Germany) as the acceptor dye using the internally amino modified–dT base at position -12. The template DNA hairpin was labelled with Cy3B (GE Healthcare, UK) as the donor fluorophore on the 5' end. Both strands were first purified using denaturing polyacrylamide gel electrophoresis and then annealed by mixing equimolar amounts of top and bottom strand in annealing buffer (Tris-HCl pH 8, 500 mM NaCl, 1 mM EDTA) and heating to 95°C, followed by slow cooling to room temperature.

To ensure a controlled surface immobilisation of the DNA, the cover slips needed to be carefully modified. First, we placed the cover slips in a furnace for 1h at 500°C to remove any surface contaminations. We then silanized the glass surface with a mixture of 98% acetone and 2% Vectabond (Vectorlabs, USA). After rinsing the sample with deionised water and drying the cover slips under nitrogen, we mounted the cover slips to sticky, precast flow channels (sticky-slide VI, Ibidi, Germany). After forming the chambers, we dissolved 4 mg of NHS-PEG (mPEG-SPA MW 5000, Lyasan, USA) and 0.1 mg biotin-PEG-NHS (mPEG-SC MW 5000, Lyasan, USA) in 400 ml of 50 mM MOPS buffer (pH 7.5) and incubated the chambers for a few hours before rinsing with PBS buffer. To immobilise the biotinylated DNA, we incubated the chambers in 0.25 mg/ml Neutravitin that binds to the biotinylated PEG, and rinsed with PBS buffer after 10 minutes before adding the solution containing 10-50 pM of the DNA molecules containing a biotin for specific immobilisation. The surface density of the molecules was monitored with the camera. After reaching a desirable density, the remaining, non-bound molecules were washed off with PBS buffer. The imaging buffer consisted of 50mM Tris HCl pH 7.5, 1 mg/l BSA, 1 mM EDTA, 5% (v/v) glycerol, 1mM DTT, 1mM Trolox as a triplet-state quenching agent, 1% (v/v) of an oxygen scavenger system (0.1 mg/ml glucose oxidase and 7 mg/ml catalase) and 1% (w/v) D+ glucose.[1,2]

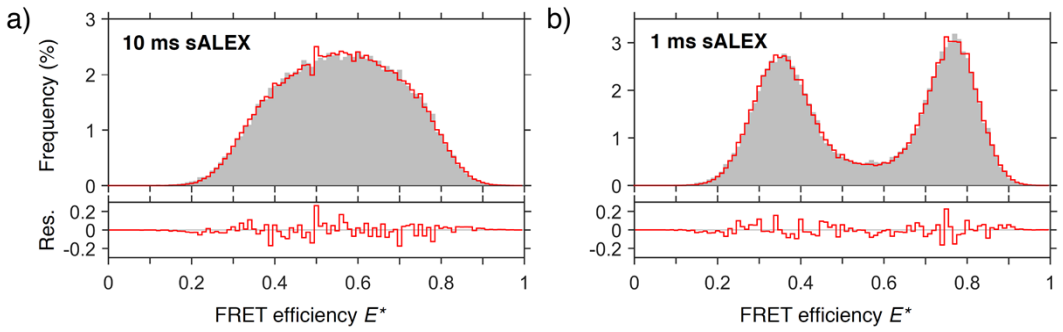

Figure S1. Simulations. The cumulated *E** histogram (grey bars; Figure 2b**:** 10 ms and 1 ms excitation time) and fitted using a dynamic two-species model. After optimization based on a Levenberg-Marquardt algorithm, the final fit (red line) shows small residuals and yielded **a)** $E^*_o = 0.345 \pm 0.008$, $E^*_c = 0.769 \pm 0.007$, $k_{oc} = (201 \pm 10)\ s^{-1}$, $k_{co} = (190 \pm 10)\ s^{-1}$ and $\sigma_o = 0.053 \pm 0.007$ and $\sigma_c = 0.053 \pm 0.012$ with $\chi^2 = 2.0$ and **b)** $E^*_o = 0.352 \pm 0.001$, $E^*_c = 0.773 \pm 0.001$, $k_{oc} = (189 \pm 7)\ s^{-1}$, $k_{co} = (188 \pm 7)\ s^{-1}$ and $\sigma_o = 0.057 \pm 0.002$ and $\sigma_c = 0.051 \pm 0.002$ with $\chi^2 = 2.0$

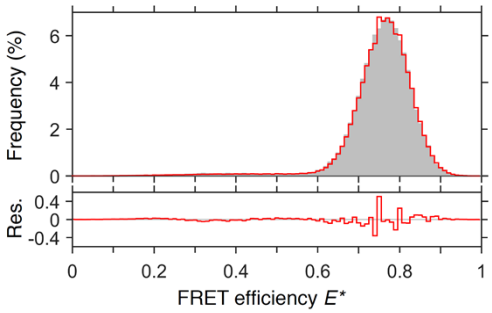

Figure S2. Simulations. Simulation using a forward rate of k_{oc} = 5 s^{-1} and a backward rate of k_{co} = 200 s^{-1}. All other parameters were kept as described previously. The final fit (red line) shows small residuals and yielded E^*_o = 0.350 ± 0.058, E^*_c = 0.771 ± 0.001, k_{oc} = (216 ± 15) s^{-1}, k_{co} = (6 ± 2) s^{-1} and σ_o = 0.115 ± 0.038 and σ_c = 0.050 ± 0.002 with χ^2 = 9.1

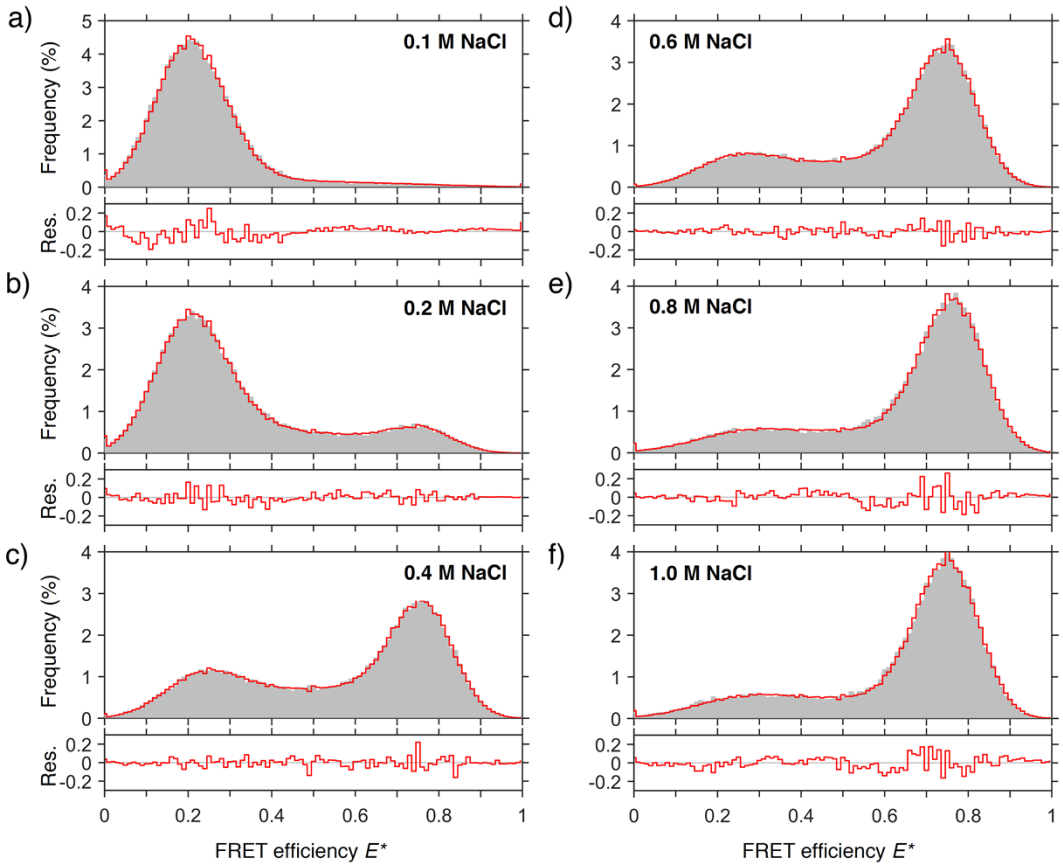

Figure S3. Experimental data. Additional FRET histograms for analysing conformational dynamics of DNA hairpins with dynamic probability distribution analysis. **(a) 100 mM NaCl**, $E^*_o = 0.210 \pm 0.001$, $E^*_c = 0.700 \pm 0.044$, $k_{oc} = (23 \pm 3)$ s^{-1} and $k_{co} = (324 \pm 10)$ s^{-1}, ($\chi^2 = 12$). **(b) 200 mM NaCl**, $E^*_o = 0.210 \pm 0.001$, $E^*_c = 0.768 \pm 0.006$, $k_{oc} = (76 \pm 2)$ s^{-1} and $k_{co} = (291 \pm 8)$ s^{-1} ($\chi^2 = 2.0$). **(c) 400 mM NaCl** $E^*_o = 0.235 \pm 0.004$, $E^*_c = 0.767 \pm 0.001$, $k_{oc} = (207 \pm 7)$ s^{-1} and $k_{co} = (118 \pm 4)$ s^{-1} ($\chi^2 = 1.9$). **(d) 600 mM NaCl** $E^*_o = 0.240 \pm 0.005$, $E^*_c = 0.751 \pm 0.001$, $k_{oc} = (241 \pm 8)$ s^{-1} and $k_{co} = (89 \pm 3)$ s^{-1} ($\chi^2 = 1.6$). **(e) 800 mM NaCl** $E^*_o = 0.258 \pm 0.013$, $E^*_c = 0.768 \pm 0.001$, $k_{oc} = (233 \pm 9)$ s^{-1} and $k_{co} = (67 \pm 3)$ s^{-1} ($\chi^2 = 2.6$). **(f) 1 M NaCl** $E^*_o = 0.261 \pm 0.011$, $E^*_c = 0.755 \pm 0.001$, $k_{oc} = (205 \pm 8)$ s^{-1} and $k_{co} = (56 \pm 3)$ s^{-1} ($\chi^2 = 4.4$).

REFERENCES

1 T. Cordes, J. Vogelsang and P. Tinnefeld, *J. Am. Chem. Soc.*, 2009, **131**, 5018–5019.

2 I. Rasnik, S. McKinney and T. Ha, *Nat. Meth.*, 2006, **3**, 891–893.