Ensemble and Single Molecule FRET analysis of the Structure and Dynamics of the c-kit Promoter Quadruplexes.

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Experimental/Supporting Information

Materials

Oligonucleotides were purchased from IBA GmbH, Germany. All oligonulceotides were double HPLC purified and used as supplied. Stock solutions of 100 μM were prepared in molecular biology grade water.

Table 1. Oligonucleotides used in this study.

Name	Sequence (5′ to 3′)
kit 1-Cy5	Cy5-AGG GAG GGC GCT GGG AGG AGG GAG AGG TAA AAG GAT AAT GGC CAC GGT GCG GAC GGC
kit 1	AGG GAG GGC GCT GGG AGG AGG GAG AGG TAA AAG GAT AAT GGC CAC GGT GCG GAC GGC
kit 1-comp	CCC TCC TCC CAG CGC CCT CCC T
kit 2-Cy5	Cy5-GGG CGG GCG CGA GGG AGG GGA GAG GTA AAA GGA TAA TGG CCA CGG TGC GGA CGG C
kit 2	GGG CGG GCG CGA GGG AGG GGA GAG GTA AAA GGA TAA TGG CCA CGG TGC GGA CGG C
kit 2-comp	CCC CTC CCT CGC GCC CGC CC
overhang-comp-Cy3	GCC GTC CGC ACC GTG GCC ATT ATC CTT *T(Cy3)* TA CCT CT
overhang-comp	GCC GTC CGC ACC GTG GCC ATT ATC CTT TTA CCT CT
overhang	AGA GGT AAA AGG ATA ATG GCC ACG GTG CGG ACG GC

UV Melting

Samples were prepared with final concentrations of 4 μM DNA, 10 mM Tris.HCl, pH7.4, and 100 mM KCl. The samples were annealed by heating at 95 °C for 10 min then slowly cooled to 5 °C at 0.5 °C min^{-1} in a Varian Cary Bio100 UV-visible spectrophotometer equipped with a Peltier temperature controller. Absorption data was recorded at 295 nm, for quadruplex melting, and at 260 nm for duplex melting. Data was recorded as the samples were successively heated and cooled between 5 °C and 95 °C at 0.5 °C min^{-1}. Samples were covered with mineral oil to reduce evaporation and dry nitrogen was

passed through the sample chamber to prevent condensation at low temperatures. Tm values were determined by the first derivative method.

Table 2. Melting temperatures of quadruplex systems in 100 mM KCl and 10 mM Tris.HCl pH 7.0.

	kit 1	**kit 1: overhang-comp**	**kit 2**	**kit 2: overhang-comp**
Quadruplex T_m (°C)	50 ± 1	50 ± 3	70 ± 1	-[a]
Duplex $T_{1/2}$ (°C)	-	74 ± 2	-	77 ± 1

[a] Unable to determine due to overlapping transitions.

CD Spectroscopy

The spectra were recorded on a Chirascan (Applied Photophysics). Samples were prepared with a final concentration of 4 μM and were annealed by heating to 95 °C for 5 min and slowly cooled to room temperature at 0.1 °C $min^{-1}$and then stored at 4 °C. Scans were recorded at 20 °C with the wavelength varied from 220 to 320 nm at a rate of 50 nm min^{-1}. The spectra are an average of three scans with a background scan of the buffer subtracted and are presented as molar ellipticity.

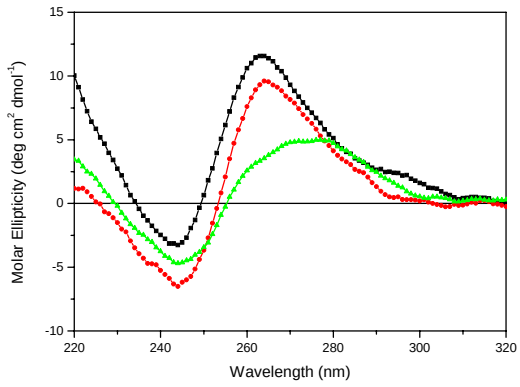

Figure S1. CD spectrum of c-kit1, **c-kit 1**, (black square), c-kit1 and duplex labelled, **c-kit 1:overhang-comp**, (red circle) and full duplex, **c-kit 1:overhang-comp:c-kit 1-comp**, (green triangle) in 100 mM KCl, 10 mM Tris HCl pH 7.0.

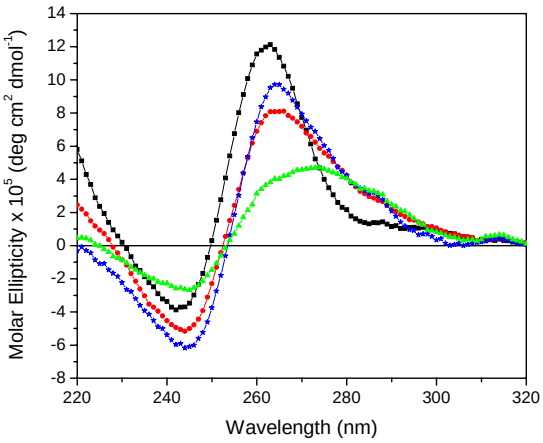

Figure S2. CD spectrum of c-kit2, **c-kit 2**, (black squares), c-kit2 and duplex, **c-kit 2:overhang-comp**, (red circles), c-kit2 and duplex labelled, **c-kit 2-Cy5:overhang-comp-Cy3**, (blue stars) and full duplex, **c-kit 2:overhang-comp:c-kit 2-comp**, (green triangles) in 100 mM KCl, 10 mM Tris HCl pH 7.0.

The CD spectrum of c-kit2 (S2, black squares) corresponds with that previously published,[1] showing a large positive peak at 260 nm and a negative signal at 240 nm, suggesting a mostly parallel structure. When the systems with the duplex are scanned, **c-kit 2:overhang-comp**, (S2, red circles), c-kit2 and duplex labelled, **c-kit 2-Cy5:overhang-comp-Cy3**, (S2, blue stars), a positive peak at 280 nm is observed as a shoulder on the peak at 260 nm. There is no difference between the labelled and unlabelled systems. The system annealed with the complement to the G-rich region shows a spectrum expected for a duplex, a small amount of unfolded quadruplex may be present but the broad peak at 280 nm would hide this smaller signal.

For c-kit1 the CD spectra shows a mostly parallel structure too (S1, black squares), however there is a small peak at 295 nm. This peak, in the absence of those at 240 and 260nm, is usually indicative of an anti parallel structure. However, when both sets of peaks are observed this may be due to a mixed population or a mixed type structure. In the presence of the duplex (**c-kit 1:overhang-comp**, (S1, red circles)) there is no obvious peak at 295 nm, suggesting that the structure is parallel, which agrees with the published NMR structure.[2]

Fluorescence Spectroscopy

Stock solutions of 500 nM oligonucleotide strand concentration were prepared with the buffer containing either no added salt or 100 mM KCl. Samples were annealed by heating to 95 °C for 5 min and slowly cooled to room temperature at 0.1 °C $min^{-1}$and then stored in the dark at 4 °C. For bulk measurements a sample of the stock solution was diluted to 100 nM with the appropriate buffer solution. Fluorescence data was recorded on a Varian Cary Eclipse Fluorescence Spectrometer fitted with a Peltier temperature controller. Kinetics data was recorded using the kinetics programme supplied with the software. The samples were excited at 520 nm and observed at 660 nm with data recorded every two minutes until no further change in signal was observed. Samples were prepared as for the bulk scans and were allowed to equilibrate at the given temperature during which time the fluorescence emission was recorded. Samples were allowed to equilibrate for ten minutes or until the signal was constant. Complementary strand was added as a small volume of solution at t = 0. After addition of the complementary strand the solution was mixed and the decay of the emission of Cy5 observed. Data was fitted in Origin for the portion for which a change in the fluorescence was observed.

Single Molecule Measurements

A home-built dual-channel confocal fluorescence microscope was used to detect freely diffusing single molecules. The donor, Cy3, was excited by an Argon ion laser (Model 35LAP321-230, Melles Griot) with 150 micro Watts at 514.5 nm. Donor and acceptor fluorescence were collected through an oil-immersion objective (Nikon Plan Apo X60 NA 1.45 objective, MCS-PCI) and detected separately by two photon-counting modules (SPCM-AQR14, Perkin Elmer). The outputs of the two detectors were recorded by two computer-implemented multichannel scalar cards (MCS-plus, EG&G). Sample solutions of 50pM were used to achieve single molecule detection. All the samples contained 200 μM ascorbic acid and 0.01% Tween 20 to reduce photobleaching and adsorption of DNA molecules onto the glass surface respectively. The temperature of the sample was controlled by a thermostage (PE60 Linkam Scientific Instruments Ltd, UK). A threshold of 30 counts per ms bin for the sum of the donor and acceptor fluorescence signals was

used to differentiate single molecule bursts from the background. A background obtained from independent measurements of buffer solutions without labeled samples, was subtracted from each burst. The FRET ratio, E_{app}, of each burst was calculated according to $E_{app} = \frac{n_A}{n_A + n_D}$ where n_A and n_D are the acceptor and donor counts respectively. The percentage of crosstalk from the donor signal to the acceptor signal was measured and corrected for in nA.

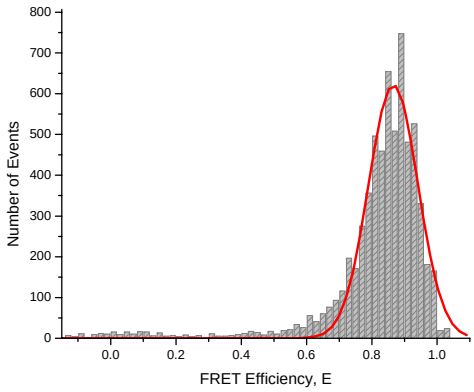

Figure S3. c-kit 1 in 100 mM KCl at 37 °C.

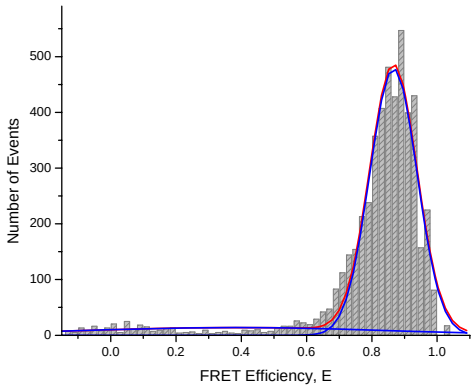

Figure S4. c-kit 2 in 100 mM KCl at 37 °C.

Single molecule histograms for both c-kit 1 and c-kit 2 in 100 mM KCl at 37 °C (S3 and S4 respectively) show one high FRET species (E ~ 0.90) and are similar to those shown in the main paper recorded at 20 °C (Figure 2 b & d).

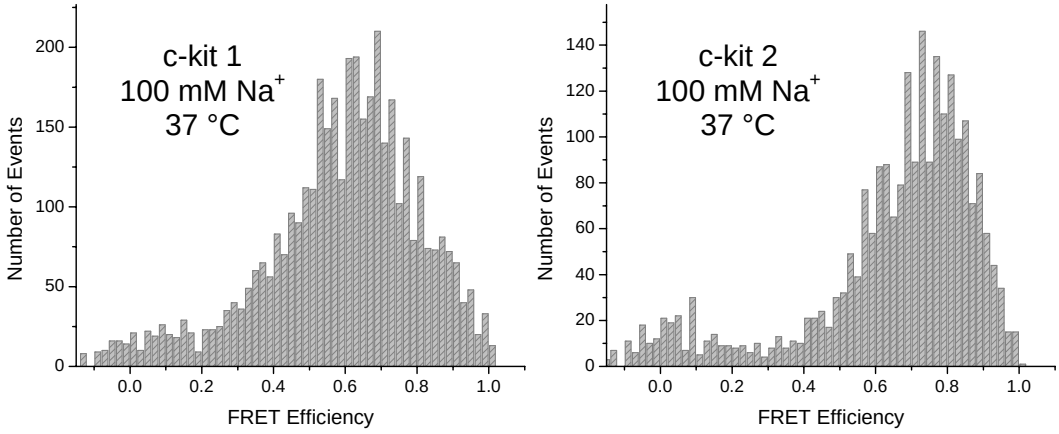

Figure S5. c-kit 1 and c-kit 2 in 100 mM Na^+ at 37 °C

Single molecule histograms for both c-kit 1 and c-kit 2 in 100 mM NaCl at 37 °C (S5) show medium and high FRET species.

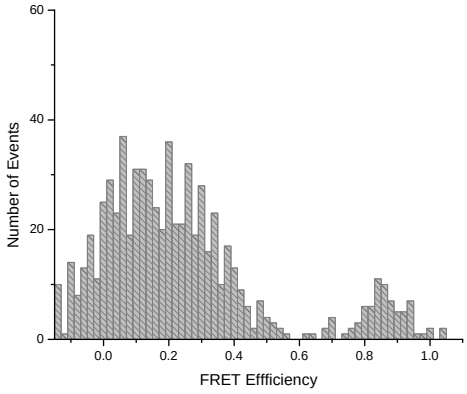

Figure S6. c-kit 1 annealed in the presence of 100 equivalents of complement.

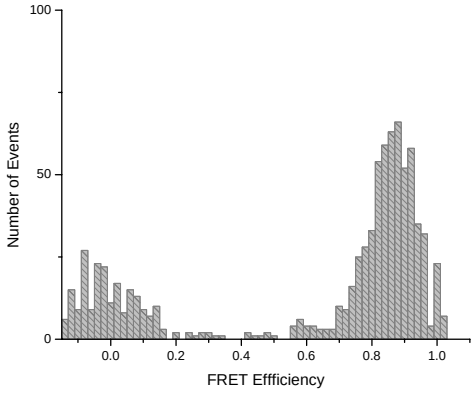

Figure S7. c-kit 2 annealed in the presence of 100 equivalents of complement.

Figures S6 and S7 show the spectra recorded for the full duplex obtained by annealing the samples in the presence of excess complement to the G-rich strand (100 eq). In both cases some of the high FRET species was observed. This may be due to intramolecular folding to give the quadruplex being more favourable to intermolecular duplex formation. This is supported by the formation of more of the high FRET species with c-kit 2 which is the more stable quadruplex, compared to c-kit 1.

Temp	**Conc. of Comp**	**τ (s)**	**k ×10^{-5} (s^{-1})**
37 °C	1 μM	8200 ± 400	12 ± 1
	4 μM	8400 ± 400	12 ± 1

Table S1. Varying the concentration of complementary strand for c-kit 1 quadruplex.

Temp	**Conc. of Comp**	**τ (s)**	**k ×10^{-5} (s^{-1})**
45 °C	1 μM	13500 ± 980	7.7 ± 0.6
	4 μM	13000 ± 160	7.4 ± 0.1

Table S2. Varying the concentration of complementary strand for c-kit 2 quadruplex.

The concentration of added C-rich complement was varied to ensure that the rate measured was independent of this concentration, data shown in table S1 and table S2.

1. Fernando, H.; Reszka, A. P.; Huppert, J.; Ladame, S.; Rankin, S.; Venkitaraman, A. R.; Neidle, S.; Balasubramanian, S. Biochemistry 2006, 45, 7854.
2. Phan, A. T.; Kuryavyi, V.; Burge, S.; Neidle, S.; Patel, D. J., J. Am. Chem. Soc. 2007, 129, 4386-4392.