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Detecting Secondary Structure Formation with FRET-PAINT

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MATERIALS AND METHODS

Nucleic Acid Constructs: DNA oligonucleotides were purchased as polyacrylamide gel electrophoresis (PAGE) or high-performance liquid chromatography (HPLC) purified from Integrated DNA Technologies (IDT). Cy5-PNA (TAACCCTT-Cy5 where underlined nucleotides are complementary to 7-nt long telomeric sequence AGGGTTA) was purchased as HPLC-purified from PNA-Bio Inc. The sequences of all nucleic acid strands are given in Table S1. Partial duplex DNA constructs (pdDNA) for FRET-PAINT experiments were created by annealing a biotinylated and Cy3-labeled short strand (18 nt) and a longer complementary strand (28-75 nt) that has a single stranded overhang. The overhangs contain 1-3 GGGTTA telomeric repeats and may contain a G4 confirming sequence GGGTGGGTGGGTGGG on one or both sides of the telomeric repeats. For smFRET experiments, the Cy5-labeled short strand (30 nt) and biotin on 3′-end was annealed with Cy3-labeled long strands with overhang containing 1-3 telomeric repeats on 3′-end. The two strands were annealed in a thermal cycler in 150 mM KCl and 10 mM $MgCl_2$ at 95 °C for 3 min, followed by a slow cooling to 30 °C (1 °C decrease every 3 min). The $MgCl_2$ concentration was reduced to 2 mM at all following steps of the process, including during the FRET-PAINT or smFRET studies.

FRET-PAINT Assay: FRET-PAINT measurements were performed on a home-built prism-type total internal reflection microscope. The setup includes an Olympus IX-71 microscope, an Olympus water objective (60x, 1.20 NA), and an Andor Ixon EMCCD camera. Quartz slides and glass microscope coverslips were cleaned and coated with two layers of polyethylene glycol (5000 Da and 333 Da) and 2% (v/v) Tween-20 as described before [1]. Annealed partial duplex DNA constructs are immobilized on the surface using biotin-streptavidin attachment. FRET-PAINT measurements were performed in an imaging buffer that contains 50 mM Tris–HCl pH 7.5, 2 mM Trolox, 0.8 mg/ml glucose, 0.1 mg/ml glucose oxidase, 0.1 mg/ml bovine serum albumin (BSA), 2 mM $MgCl_2$, 150 mM KCl (or 150 mM LiCl) and 40 nM Cy5-PNA. The Cy5-PNA strand was preheated to 85°C for 10 minutes before it was added to the imaging buffer.

The FRET-PAINT measurements were performed by exciting the donor fluorophore with a 532 nm laser beam (Spectra-Physics Excelsior). Movies with 1000-2000 frames at a frame acquisition rate of 100 ms/frame were recorded. An automated, bias-free algorithm, AutoStepfinder[2] was used to determine the FRET bursts representing Cy5-PNA binding. The binding frequencies were determined based on these detected binding events by dividing the total number of binding events by the total observation time (the total time of each trajectory that shows at least one binding event or donor photobleaching). The error bars for frequencies were determined by Bootstrapping analysis [3] with a sample size of 2000 and 95% confidence level [4].

Table S1. Sequences of nucleic acid strands used in the study. All data except that in Figure 1E (the traditional smFRET data) is attained by hybridizing an 18-nt short strand (Stem-18) with a longer, unlabeled strand. Data using traditional smFRET assay (Figure 1E) was obtained by hybridizing a 30-nt long short strand (Stem-30) with a Cy5-labeled long strand.

Pd-DNA	**Long Strands (Sequence in 5'- 3')**	**Stem**
1GTr	TGGCGACGGCAGCGAGGCTTAGGGTTA	Stem-18
2GTr	TGGCGACGGCAGCGAGGCTTAGGGTTAGGGTTA	Stem-18
3GTr	TGGCGACGGCAGCGAGGCTTAGGGTTAGGGTTAGGGTTA	Stem-18
1GTr-G4	TGGCGACGGCAGCGAGGCTTAGGGTTAGGGTGGGTGGGTGGGTTA	Stem-18
2GTr-G4	TGGCGACGGCAGCGAGGCTTAGGGTTAGGGTTAGGGTGGGTGGGTGGGTTA	Stem-18
3GTr-G4	TGGCGACGGCAGCGAGGCTTAGGGTTAGGGTTAGGGTTAGGGTGGGTGGGTGGGTTA	Stem-18
G4-1GTr	TGGCGACGGCAGCGAGGCTTAGGGTGGGTGGGTGGGTTAGGGTTA	Stem-18
G4-2GTr	TGGCGACGGCAGCGAGGCTTAGGGTGGGTGGGTGGGTTAGGGTTAGGGTTA	Stem-18
G4-3GTr	TGGCGACGGCAGCGAGGCTTAGGGTGGGTGGGTGGGTTAGGGTTAGGGTTAGGGTTA	Stem-18
G4-1GTr-G4	TGGCGACGGCAGCGAGGCTTAGGGTGGGTGGGTGGGTTAGGGTTAGGGTGGGTGGGTGGGTTA	Stem-18
G4-2GTr-G4	TGGCGACGGCAGCGAGGCTTAGGGTGGGTGGGTGGGTTAGGGTTAGGGTTAGGGTGGGTGGGT GGGTTA	Stem-18
G4-3GTr-G4	TGGCGACGGCAGCGAGGCTTAGGGTGGGTGGGTGGGTTAGGGTTAGGGTTAGGGTTAGGGTGG GTGGGTGGGTTA	Stem-18
1GTr-FRET	TGGCGACGGCAGCGAGGCTTAGGGTTAGGGTTAGGGTTAG-Cy3	Stem-30
2GTr-FRET	TGGCGACGGCAGCGAGGCTTAGGGTTAGGGTTAGGGTTAGGGTTAG-Cy3	Stem-30
3GTr-FRET	TGGCGACGGCAGCGAGGCTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAG-Cy3	Stem-30
$(SpGTR)_1$	TGGCGACGGCAGCGAGGCTTTTTTTTTTTTAGGGTTA	Stem-18
$(SpGTR)_2$	TGGCGACGGCAGCGAGGCTTTTTTTTTTTTAGGGTTATTTTTTTTTTTTAGGGTTA	Stem-18
$(SpGTR)_3$	TGGCGACGGCAGCGAGGCTTTTTTTTTTTTAGGGTTATTTTTTTTTTTTAGGGTTATTTTTTTTTT TTAGGGTTA	Stem-18
	Short Strands (Sequence in 5'- 3')	**Assay**
Stem-18	Cy3-GCCTCGCTGCCGTCGCCA-biotin	FRET-PAINT
Stem-30	Cy5-CCCTAACCCTAAGCCTCGCTGCCGTCGCCA-biotin	smFRET

Table S2. One-way ANOVA analysis was performed to compare the relative frequencies for different groups of constructs (nGTr, nGTr-G4, G4-nGTr, G4-nGTr-G4). The analysis was based on the data in Fig. 2B.

DNA construct Group	ANOVA test
nGTr	$F(2, 594)= 3346.30991$, $p=0.001$
nGTr-G4	$F(2, 594)= 5573.87954$, $p=0.001$
G4-nGTr	$F(2, 594)= 5731.32209$, $p=0.001$
G4-nGTr-G4	$F(2, 594)= 2270.43863$, $p=0.001$

Table S3. Non-normalized binding frequencies of the imager probe in the FRET-PAINT studies presented in Fig. 2.

Construct	**Frequency ($\times 10^{-3}$ s^{-1})**	**Error ($\times 10^{-3}$ s^{-1})**
1GTr	10.1	0.9
2GTr	20.3	1.9
3GTr	14.9	1.6
1GTr-G4	19.7	2.2
2GTr-G4	36.6	4.3
3GTr-G4	13.9	1.5
G4-1GTr	25.1	4.1
G4-2GTr	47.8	5.7
G4-3GTr	9.6	1.6
G4-1GTr-G4	7.5	1.0
G4-2GTr-G4	14.8	1.6
G4-3GTr-G4	10.7	0.6

FRET Distributions of 3GTr Construct at High Salt Concentrations

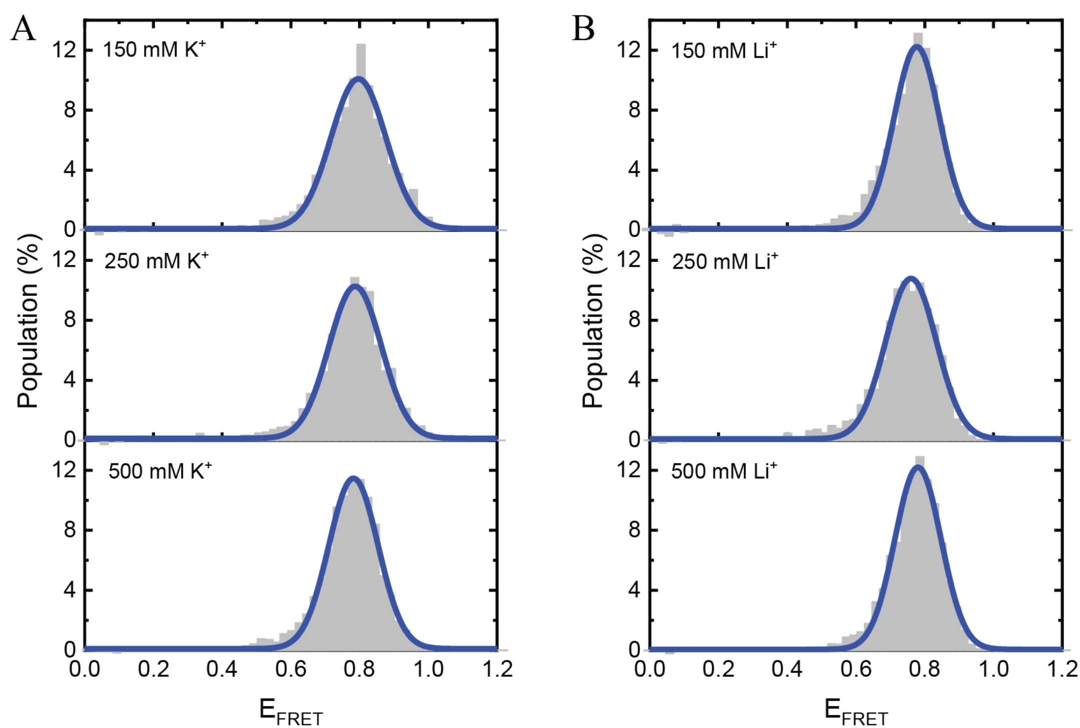

Figure S1. FRET histograms for 3GTr construct at 150 mM, 250 mM, and 500 mM of (A) K^+ and (B) Li^+. The peak positions in respective K^+ and Li^+ concentrations are effectively indistinguishable from each other, demonstrating the challenges associated with detecting G3 formation using smFRET. For the K^+ case, the peak positions are at x_c=0.80±0.09 for 150 mM K^+, x_c=0.79±0.09 for 250 mM K^+, and x_c=0.78±0.08 for 500 mM K^+. For the Li^+ case, the peak positions are at x_c=0.78±0.08 for 150 mM Li^+, x_c=0.76±0.09 for 250 mM Li^+, and x_c=0.78±0.08 for 500 mM Li^+. The fitting errors in determining the peak position were consistently ±0.01 or less for all constructs.

FRET Distributions of (SpGTr)n Constructs

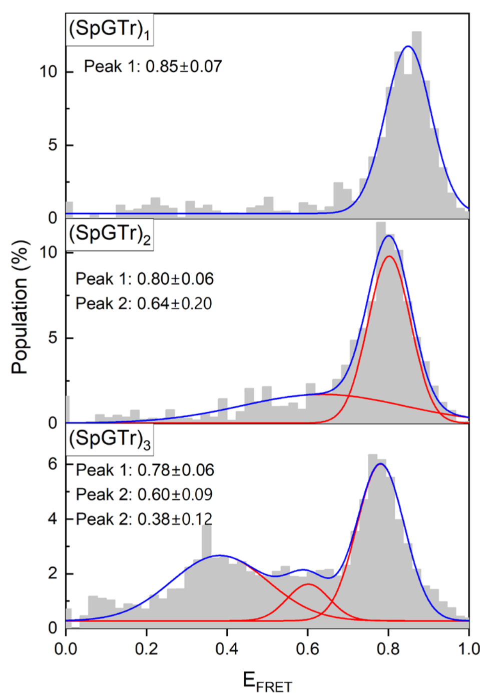

Figure S2. FRET histograms for constructs shown in Fig. 2C. The FRET histograms were created based on Cy5-PNA binding events to the G-Tracts on the overhang. The relative frequencies of these binding events are presented in Fig. 2D, while the number of binding events are given in the caption of Fig. 2.

References

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