Supplementary Information

Replication Protein A Unfolds G-Quadruplex Structures with a Varying Degree of Efficiency

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DNA Constructs: The following DNA constructs were purchased from Integrated DNA Technologies (www.idtdna.com) and used for FRET studies:

Stem: 5'-biotin-GCCTCGCTGCCGTCGCCA-Cy5-3'

TH-2: 5'-Cy3-**GGGGTGGGGGATGTAAGGAGGGG**TTTTTGGCGACGGCAGCGAGGC-3',

TR-3 : 5'-Cy3-TT**GGGTTAGGGTTAGGGTTAGGG**TTTGGCGACGGCAGCGAGGC - 3',

TH-4: 5'-Cy3-**GGGGTGGGGGATGTAAGGAGGGGAAGGTGGGGG**TTTTTGGCGACGGCAGCGAGGC-3'

DNA overhang sequence, in bold letters, corresponds to the sequence under investigation. The underlined thymines are used as spacers to minimize the interaction between the sequence of interest and the nearby fluorophore. The stem strand was annealed to TH-2, TR-3, and TH-4 and a partial duplex DNA with an 18 bp duplex was formed (see Figure 1). The two DNA oligonucleotides were annealed to form a partial duplex construct by mixing them at 2.0 μM concentration in 10 mM Tris buffer, pH 7.5 and 50 mM NaCl. The mixture was heated and maintained at 95°C for 5 minutes and cooled down gradually by placing a heated block at room temperature until the sample reached room temperature. All biochemical and biophysical studies were performed using oligonucleotides corresponding to the residues shown in bold only. The linker and DNA duplex were not necessary, as the oligos were not being attached to a surface.

Purification of DNA Samples: Oligonucleotides were resuspended in DEPC water and then purified by 17% denaturing PAGE. DNA was visualized using UV shadowing, and the band was subsequently excised and shaken in elution buffer (300 mM NaCl, 10 mM Tris-Cl pH 7.5, 1 mM EDTA) overnight at 4°C. Eluted DNA was then concentrated by *sec*-butanol, followed by ethanol precipitation. The concentration of the DNA was calculated by measuring the UV absorbance value at 260nm and extinction coefficients were calculated using the nearest neighbor approximation[1].

Preparation of 5'-end labeled Oligonucleotides: Oligonucleotides were 5'-end labeled by incubating the DNA at 37°C with γ-P^{32}-ATP (PerkinElmer) and T4 Polynucleotide Kinase (Promega) for one hour. After the completion of the labeling reaction, the unincorporated γ-P^{32}-ATP was removed by a G-25 mini-spin column (GE Healthcare). The labeled DNA eluted from the column was used without further purification.

FRET Sample preparation: Cleaned optical Quartz slides and glass cover slips were used for preparing the imaging chambers. Surfaces were coated with a mixture of polyethylene glycol and biotinylated polyethylene glycol (m-PEG-5000 and biotin-PEG-5000, Laysan Bio Inc.), to prevent non-specific binding of DNA and protein molecules to the surface. Biotinylated DNA, at 15 pM concentration, was immobilized onto biotin-PEG surface via neutravidin, at 0.5 mg/ml concentration. For RPA titration studies, the GQ structures were first formed by incubating the surface immobilized DNA constructs in 100 mM KCl for 15 minutes. RPA was then added to the chamber in an imaging solution (50 mM Tris pH 7.5, 0.8 mg/ml glucose, 0.1 mg/ml Bovine Serum Albumin, 140 mM β-mercaptoethanol, 0.1 mg/ml glucose oxidase, 0.02 mg/ml catalase, 2 mM Trolox, and 100 mM KCl). Images were acquired after 20 minutes of incubation.

Imaging and Data Analysis: Imaging was performed using prism-type total internal reflection configuration with an Olympus IX-71 microscope. 1000-2000 frame long movies were collected using Andor Ixon EMCCD camera (iXon DV 887-BI EMCCD, Andor Technology). An integration time of 100 ms was used for RPA titration experiments, and an integration time of 35 ms was used for flow

experiments. FRET time traces for individual molecules were analyzed to generate FRET histograms using a custom analysis program and Origin Pro 8.5 was used for statistical analysis and curve fitting of histograms.

RPA preparation: RPA purification procedure was adapted from previous works[1,2]. Briefly, E.coli cells were transformed with a p11d-tRPA construct containing the coding sequences of RPA 70, RPA 14 and RPA 32. Upon reaching an OD_{600} of 0.6, protein expression was induced by adding IPTG to a final concentration of 0.4 mM. Cells were lysed by pelleting and sonication. Cellular debris was pelleted by centrifugation at 12,000rpm for 30 minutes. The supernatant was loaded onto an Affi-Gel Blue column (Bio-Rad). Protein was eluted using 1.5 M NaSCN in Hepes-Inositol buffer, pH 7.8. Eluted fractions containing protein were loaded onto a hydroxyapatite column to further concentrate the protein and eluted with HEPES-Inositol - 80 mM phosphate buffer, pH 7.5. RPA purity was assayed by SDS-PAGE (not shown). RPA functionality was confirmed in the context of its role in a DNA checkpoint complex using the assay described by Choi *et al.*[3].

Circular Dichroism: CD spectra were measured from 200 nm to 320 nm using Jasco-810 spectropolarimeter (Easton, MD). Samples were heated at 95 ^{0}C for 10 minutes, and then allowed to cool to room temperature over a period of two hours. CD samples contained 5 µM DNA, and either 100 mM potassium phosphate buffer, pH 7.4, or 100 mM LiCl and 10mM Tris-Cl, pH 7.4, as indicated in the figure legends. All measurements were performed at room temperature on a Jasco 810 Spectrapolarimeter (Easton, MD), using a quartz cuvette with a 1mm optical path length (Starna Cells,Inc.). The spectra obtained are representative of three scans taken over a range of 200nm to 320nm with a measurement taken every 0.5 nm at a scanning rate of 50nm/minute. Representative spectra have been baseline subtracted from a buffer-only sample, and smoothed using a Savitsky-Golay function. Interestingly, CD data on TH-2 and TH-4 is consistent with parallel GQ conformation even in the presence of Li^+, which is known not stabilize GQ as efficiently as K^+.

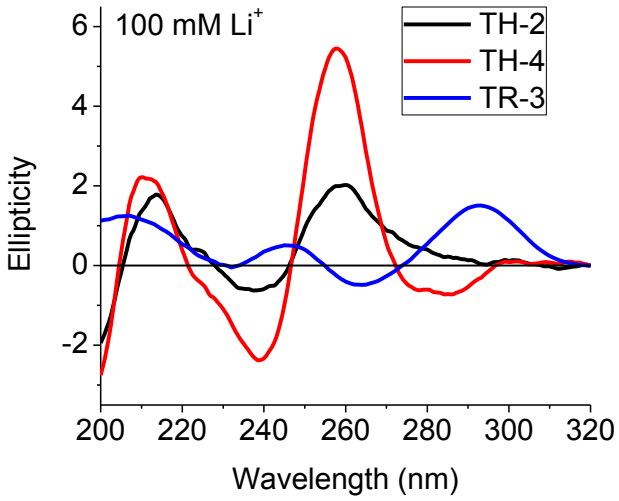

Figure S1: CD measurements for all three GQ constructs in 100 mM Li^+ concentration.

UV-Thermal Melting: Samples were prepared with a final concentration of 5µM purified DNA stock, and 100mM Potassium Phosphate Buffer, pH 7.4 in a final volume of 200µL. The sample was then heated at 95°C for 5 minutes to degas, and spun briefly to bring the condensation from the sides of the tube to the bottom. The sample was then transferred into a clean small volume quartz cuvette. The sample was overlaid with 100µL silicone oil and the cuvette was capped to prevent evaporation of the sample during heating. All UV experiments were performed on a Varian Cary 300 Spectrophotometer equipped with a Varian multi-cell Peltier temperature controller. All of the melting experiments were performed in a 50µL small volume quartz cuvette (Starna Cells Inc.). Samples were heated from 15°C-95°C at a rate of 0.5°C/min and cooled from 95°C-15°C at 0.5°C/min as well.

The following constructs were used for UV-melting measurements:

TH-2 with Spacer: **GGGGTGGGGGATGTAAGGAGGGG**TTTT
TH-2: **GGGGTGGGGGATGTAAGGAGGGG**
TR-3: TT**GGGTTAGGGTTAGGGTTAGGG**TT
TH-4 with Spacer: **GGGGTGGGGGATGTAAGGAGGGGAAGGTGGGGG**TTTT
TH-4: **GGGGTGGGGGATGTAAGGAGGGGAAGGTGGGGG**

The bold letters are the GQ forming regions and the underlined thymines are placed to replicate the smFRET constructs. We did not observe a significant difference in the thermal stabilities of the constructs with or without spacers. In smFRET experiments, these spacers are placed to minimize the interaction between the fluorophores and the GQ construct.

DMS Footprinting: DMS samples were prepared with 1µM unlabelled DNA, 10mM Tris-Cl buffer (pH 7.4), 100,000cpm γ-ATP labeled appropriate oligonucleotide per tube, and different salt conditions (indicated above each lane) in a final volume of 40µL. DMS samples were then heated at 95°C for ten minutes. TH-2 and TH-4 samples were then treated with 1% DMS for 2.5 minutes, while TR-3 was treated for 1.5 minutes. At the completion of the incubation, 30µL of the sample was placed into 300µL of DMS Stop Buffer (300 mM sodium acetate, 250 mg/mL sheared salmon sperm DNA, 2 M β-mercaptoethanol). Samples were immediately ethanol precipitated with ice cold 100% ethanol and frozen in a dry ice/acetone bath. Samples were removed from the ice bath after precipitation, and centrifuged at 13,200 rpm for ten minutes. The supernatant was removed, and the pellet was then subjected to two 70% ethanol washes. The pellet was dried to completion using a vacuum centrifuge and then treated with 70µL of freshly prepared 10% piperidine. Samples were allowed to incubate for 30 minutes at 95 °C with the piperidine before drying the sample to obtain a pellet. The pellet was washed with 150 µL H_2O, and again dried to completion. This pellet was resuspended in 2X urea loading buffer such that each sample contained the same number of counts per µL. Samples were loaded on a 10% denaturing gel and run for 55 minutes at 75 W. The gel was then dried and exposed to a phosphor-screen overnight. The gel images were obtained by scanning of the phosphor screens using a Typhoon™ 8600 Phosphorimager (GE Life Sciences).

Based on several measurements on each construct, one of each is shown in Figure S2, the following protection pattern is obtained (underlined guanines are protected):

TTGGGTTAGGGTTAGGGTTAGGGTT

GGGGTGGGGGATGTAAGGAGGGGA

GGGGTGGGGGATGTAAGGAGGGGAAGGTGGGGG

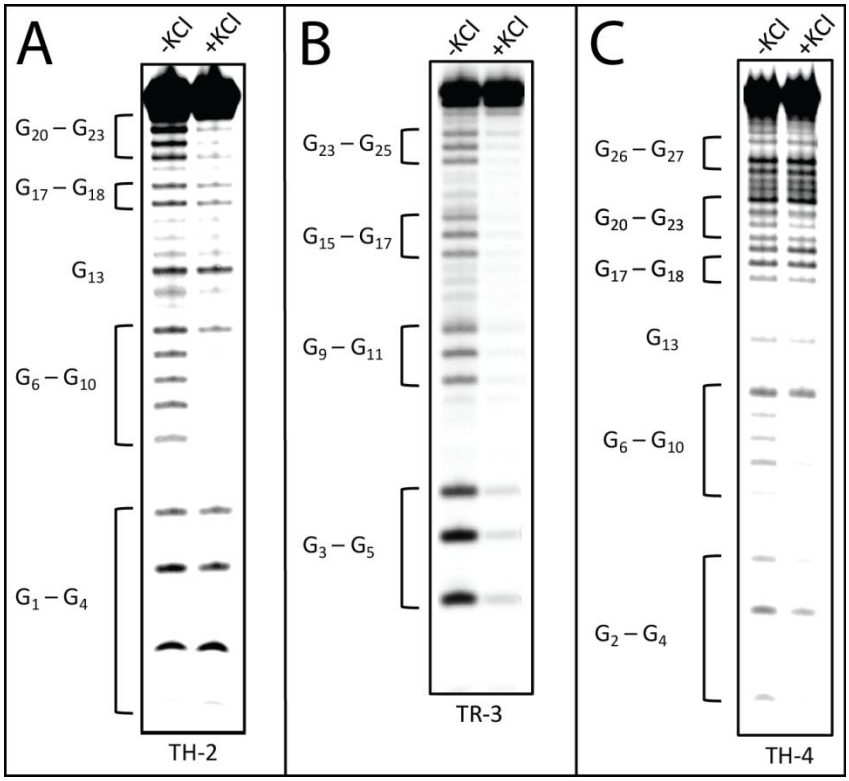

Figure S2: DMS footprinting data for A-TH-2, B-TR-3, and C-TH-4. Each gel is representative of several experiments which were performed in order to obtain the proposed protection pattern.

Folding of the GQ structure as a function of Potassium

smFRET is capable of detecting the various folding conformations during attained by the DNA at different K^+ conformations as shown in Figure S3.

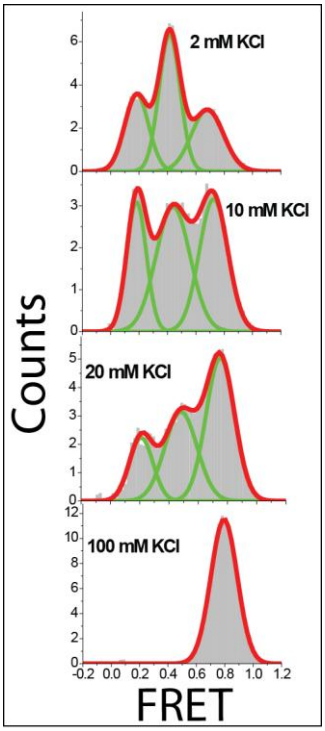

Figure S3: Different folding conformations adopted by TH-2 as K^+ concentration is titrated to 100 mM.

RPA Unfolding of GQ Imaged at 18 millisecond Image Acquisition Time

The data presented in Figure 4 on the time it takes for RPA to unfold GQ structures was acquired at 35 millisecond image acquisition time, limited the accuracy of the method to ±0.10 sec. In order to check our results with a higher accuracy method, we used half of the CCD sensor on the camera and reduced the image acquisition time to 18 msec. This improved the accuracy of the method by a factor of two, but made it twice as difficult to collect statistically significant data. We performed this control measurement on the TR-3 construct and obtained the data presented in Figure S4. The average unfolding times were consistent for the two cases within the experimental uncertainties of the methods: 0.38±0.10 sec for 35 msec image acquisition and 0.27±0.05 sec for 18 msec image acquisition.

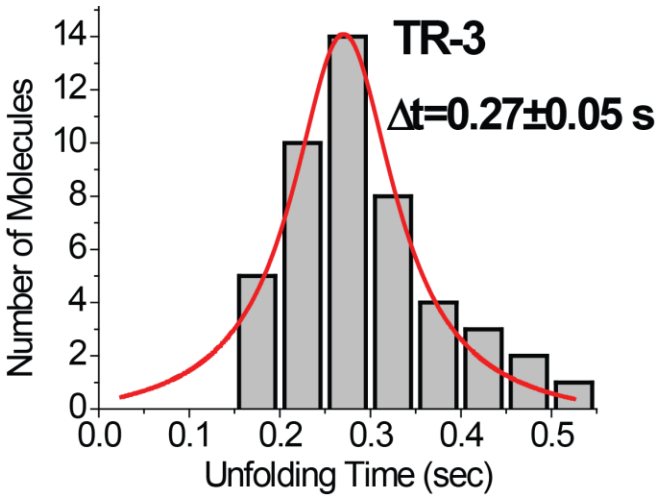

Figure S4: RPA unfolding of TR-3 imaged at 18 millisecond image acquisition time. The results of this measurement are consistent with those measured with 35 millisecond image acquisition time within the experimental uncertainties of the methods.

References

(1) Binz, S. K.; Dickson, A. M.; Haring, S. J.; Wold, M. S. *Methods Enzymol* **2006**, *409*, 11-38.
(2) Henricksen, L. A.; Umbricht, C. B.; Wold, M. S. *J Biol Chem* **1994**, *269*, 11121-11132.
(3) Choi, J. H.; Lindsey-Boltz, L. A.; Kemp, M.; Mason, A. C.; Wold, M. S.; Sancar, A. *Proc Natl Acad Sci U S A* **2010**, *107*, 13660-13665.