Supporting Information

A Simple and Convenient G-quadruplex-Based Turn-On Fluorescence Assay for 3′ → 5′ Exonuclease Activity

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Materials and cell lines. Exonuclease III, exonuclease I and T7 exonuclease and their respective buffers were obtained from New England Biolabs (Beverely, MA, USA). DNase I, its respective buffer and crystal violet were obtained from Sigma-Aldrich (St. Louis, MO, USA). DNA oligomers were obtained from Tech Dragon Limited (Hong Kong). The sequences for the oligomers are:

G_{55}: [5′-AGGGTTAGGGTTAGGGTTAGGGCAGAAGGATAACCCTAACCCTAACCCTAACCCT-3′]
G_{55m}: [5′-AAGGTTAGCGTTAGGATTACGGCAGAAGGATAACCGTAATCCTAACGCTAACCTT-3′]

The oligonucleotides were hybridized by annealing at 95 °C for 10 min in 10 mM sodium phosphate, 1 mM EDTA and 46.25 mM sodium chloride, pH 7.4 and slowly cooling to 20 °C at 0.1 °C/s, followed by incubation at 20 °C for a further 30 min.

Expression and purification of recombinant human TREX1 protein. Human TREX1 encoding sequence was cloned for the expression of TREX1 protein with 314 amino acid residues. The recombinant protein bearing His-tag was expressed in *Escherichia coli* and purified using Ni-NTA column from Qiagen (Chatsworth, CA, USA). Further details can be found in Reference 14b.

Emission measurement. Emission spectra were recorded on a SPEX Fluorolog-2 Model fluorescence spectrophotometer. Oligonucleotide G_{55} (15 μM) was incubated at 37 °C for 30 min in a reaction volume of 16.7 μL with the indicated concentrations of enzyme (buffer): exonuclease III (10 mM Bis-Tris-Propane-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.0); exonuclease I (67 mM Glycine-KOH, 6.7 mM $MgCl_2$, 10 mM 2-mercaptoethanol, pH 9.5); T7 exonuclease (20 mM Tris-acetate, 50 mM potassium acetate, 10 mM magnesium acetate, 1 mM dithiothreitol, pH 7.9); DNase I (10 mM Tris-HCl, 2.5 mM $MgCl_2$, 0.5 mM $CaCl_2$, pH 7.6). The oligonucleotide digestion reactions were quenched by the addition of EDTA at a final concentration of 20 mM, and were diluted to 1 mL with Tris buffer (10 mM Tris-HCl, pH 7.5) containing CV (1 μM) and KCl (10 mM). Final

oligonucleotide concentration = 0.25 μM. The emission spectra were recorded in the 600–750 nm range, after equilibration at room temperature for 20 min. Excitation wavelength = 580 nm.

High-throughput emission measurements were recorded on a Tecan Infinite 200 multimode microplate reader. G_{55} (1 μM) was incubated at 37 °C for 30 min in a 100 μL reaction volume in a 96-well black microplate with exonuclease III (200 U/ml) in buffer solution (10 mM Bis-Tris-Propane-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.0). To each well was added 100 μL of Tris buffer solution (20 mM, pH 7.5) containing EDTA (40 mM), KCl (20 mM) and CV (4 μM). Final [CV] = 2 μM; final [G_{55}] = 0.5 μM. The emission intensity at λ = 635 nm was recorded after equilibration at room temperature for 20 min. For real-time measurement of ExoIII activity, G_{55} (0.5 μM) was incubated with the indicated concentrations of ExoIII in buffer solution (10 mM Bis-Tris-Propane-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.0) containing CV (2 μM) and KCl (10 mM) at 37 °C. For real-time measurement of TREX1 activity, G_{55} (0.5 μM) was incubated with the indicated concentrations of TREX1 in buffer solution (10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.0) containing CV (2 μM) and KCl (10 mM) at 25 °C. The emission intensity of each well at 635 nm was recorded every 1 min. Excitation wavelength = 590 nm.

Circular dichroism measurement. CD spectra were recorded on a spectropolarimeter at room temperature under an atmosphere of nitrogen. G_{55} (15 μM) was incubated at 37 °C for 30 min with the exonuclease III (200 U/ml) in buffer (10 mM Bis-Tris-Propane-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.0). The oligonucleotide digestion reactions were diluted 3-fold into Tris buffer (10 mM Tris-HCl, 10 mM KCl, pH 7.5). Final oligonucleotide concentration = 5 μM. Spectra were recorded in the 200–320 nm range in 0.5 mm pathlength cuvettes after equilibration at room temperature for 20 min, using a scanning speed of 100 nm/min, a response time of 1 s and a bandwidth of 0.2 nm. Spectra were averaged from 3 scans.

For the real-time measurement, the oligonucleotides (1.5 μM) were incubated with exonuclease III (75 U/ml) at 37 °C in buffer (10 mM Bis-Tris-Propane-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol,10 mM KCl, pH 7.0). Scans were recorded every 1 min using the parameters above.

Gel electrophoresis. 5 mL of 30% acrylamide solution, 0.2 mL of KCl (1 M), 1 mL of 10 × TBE buffer and 3.8 mL of H_2O was mixed carefully to avoid formation of bubbles. 5 uL of TEMED and 80 uL of 10% APS was added to the mixture, which was poured into the glass panels and allowed to set at room temperature for 30 min. For the hydrolysis assay, the oligonucleotide (1.5 μM) was incubated with ExoIII (75 U/ml) in buffer (10 mM Bis-Tris-Propane-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol,10 mM KCl, pH 7.0) at 37 °C for the indicated time. The reactions were quenched by the addition of 20 mM EDTA and 10 mM KCl. The digestion mixture was loaded onto the gel and electrophoresed using 1× TBE containing 10 mM KCl, and visualized using SYBR Green I.

Figure S1. Design of G_{55} oligonucleotide. a) The G_{55} oligonucleotide sequence comprises a telomeric G-quadruplex sequence and the complimentary cytosine-rich sequence connected by a flexible linker. b) The oligonucleotide is reannealed by heating at 100 °C for 10 min followed by slow cooling to form the stem-loop DNA secondary structure.

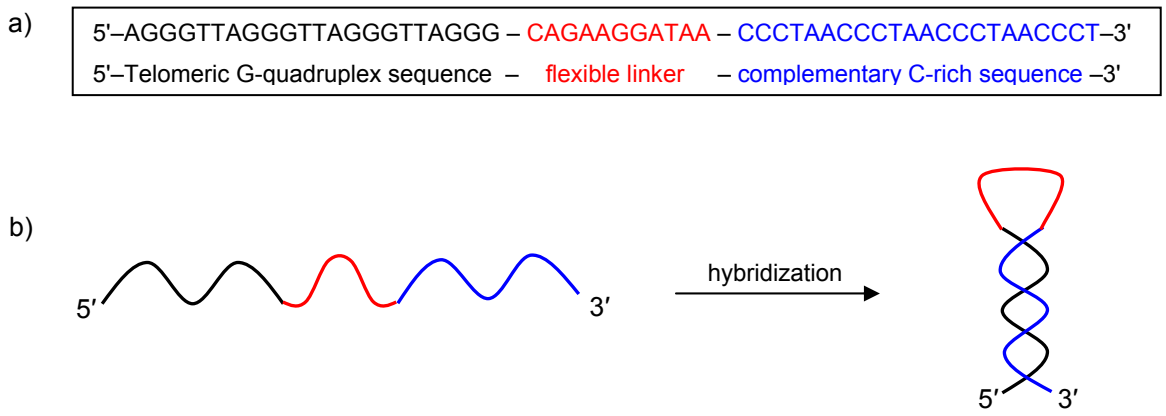

Figure S2. Fluorescence response of CV (1 µM) in the presence of oligonucleotide G_{55} or G_{55m} (0.25 µM). G_{55} or G_{55m} (15 µM) was incubated with ExoIII (2000 U/mL) 37 °C for 30 min

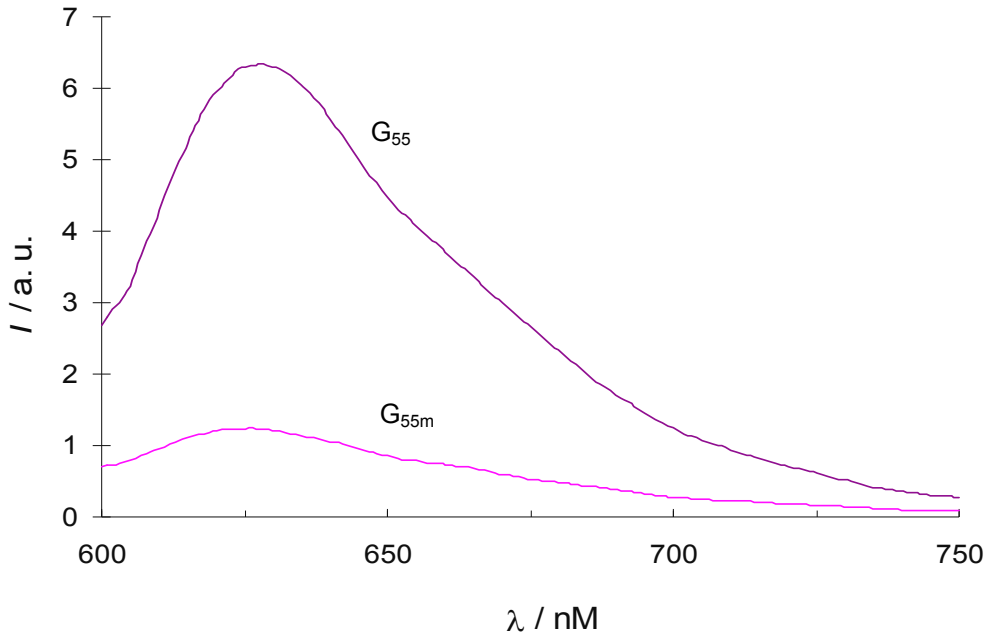

Figure S3. Fluorescence response of CV (1 μM) in the presence of oligonucleotide G_{55} (0.25 μM). G_{55} (15 μM) was previously incubated with ExoIII (2×10^3 U/mL) or heat-inactivated ExoIII (2×10^3 U/mL) 37 °C for 10 min. ExoIII was inactivated by heating at 100 °C for 20 min.

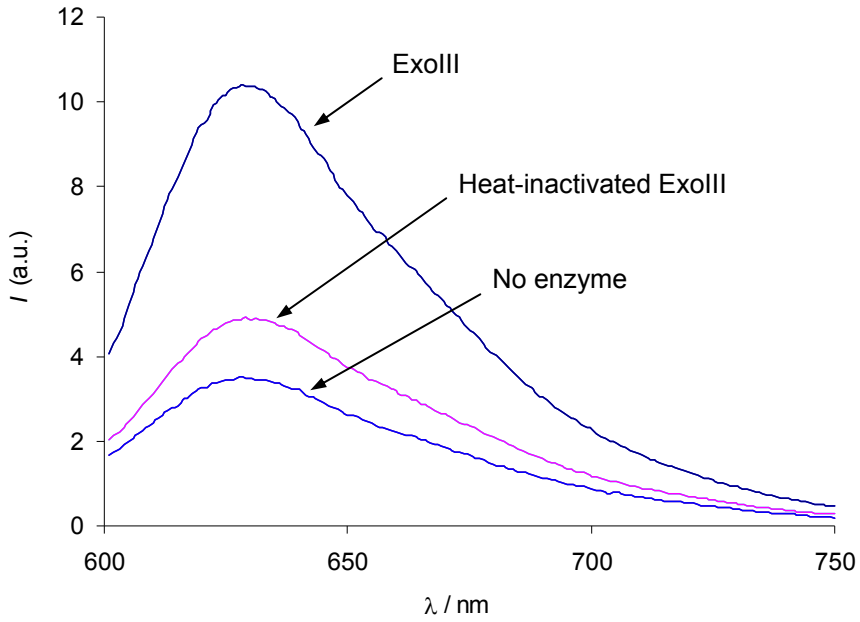

Figure S4. Fluorescence response of CV (1 μM) in the presence or absence of ExoIII (33 U/mL).

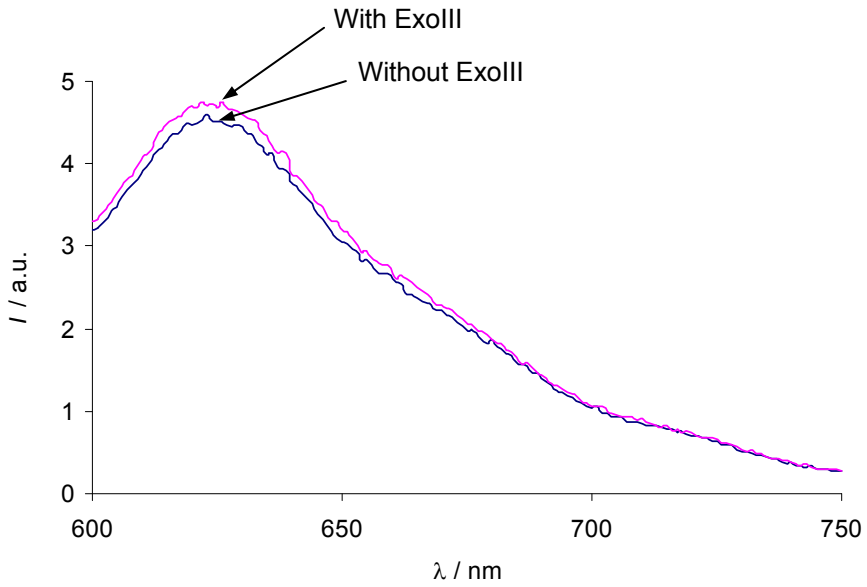

Figure S5. Real-time CD measurement of indicated oligonucleotide (1.5 μM) with exonuclease III (75 U/ml) at 37 °C in buffer (10 mM Bis-Tris-Propane-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 10 mM KCl, pH 7.0). a) G_{55}. Inset: CD intensity at 268 / 295 nm *vs.* time. b) G_{55m}.

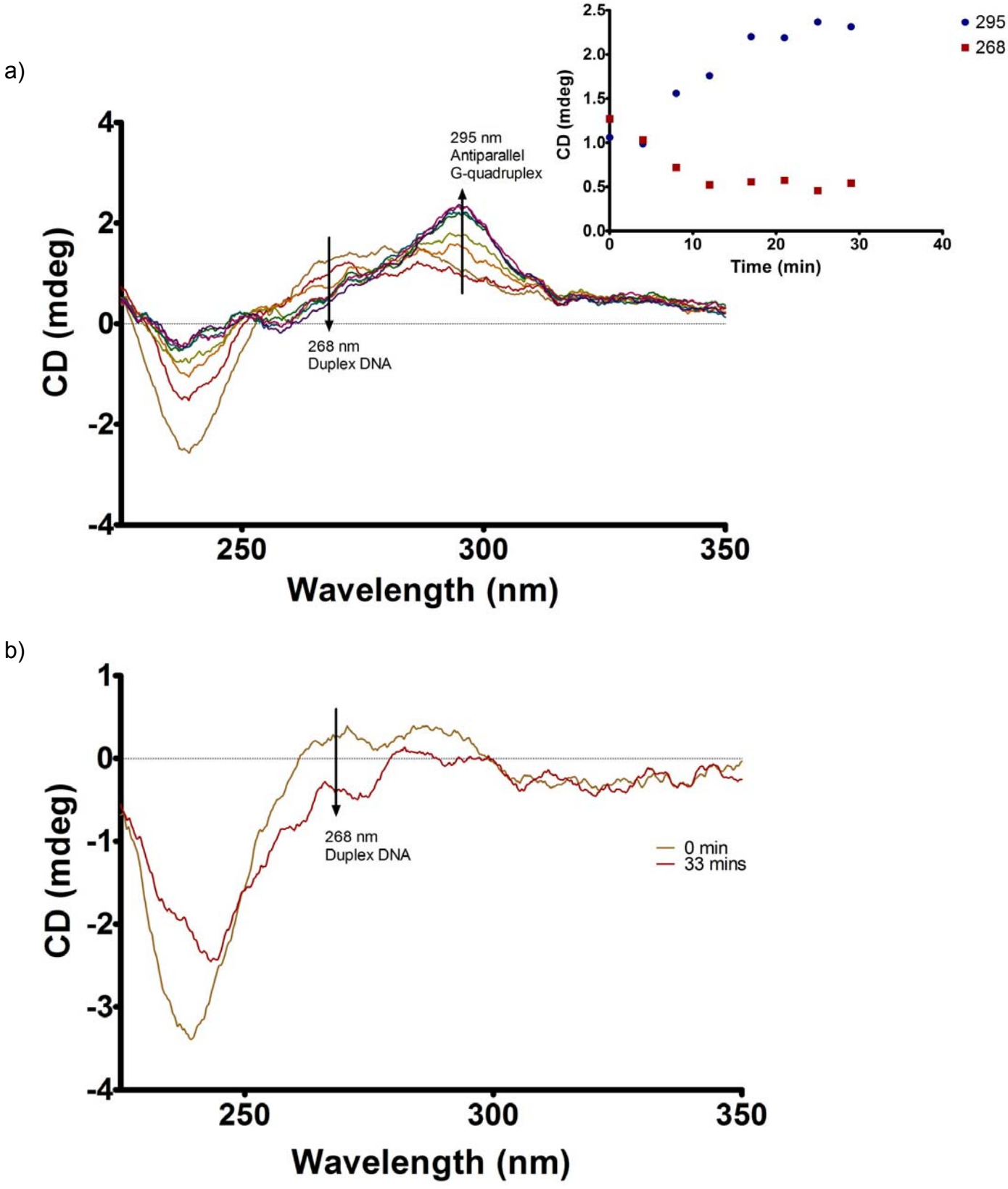

Figure S6. Gel electrophoresis of DNA hydrolysis samples. G_{55} (1.5 μM) was incubated with ExoIII (75 U/ml) in buffer (10 mM Bis-Tris-Propane-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol,10 mM KCl, pH 7.0) at 37 °C for the indicated time, then were quenched with 20 mM EDTA and 10 mM KCl. The gel was visualized with SYBR Green. The positive control contains the expected hydrolysis product: Quad = [5′-AGGGTTAGGGTTAGGGTTAGGGCAGAAGGATAA-3′].

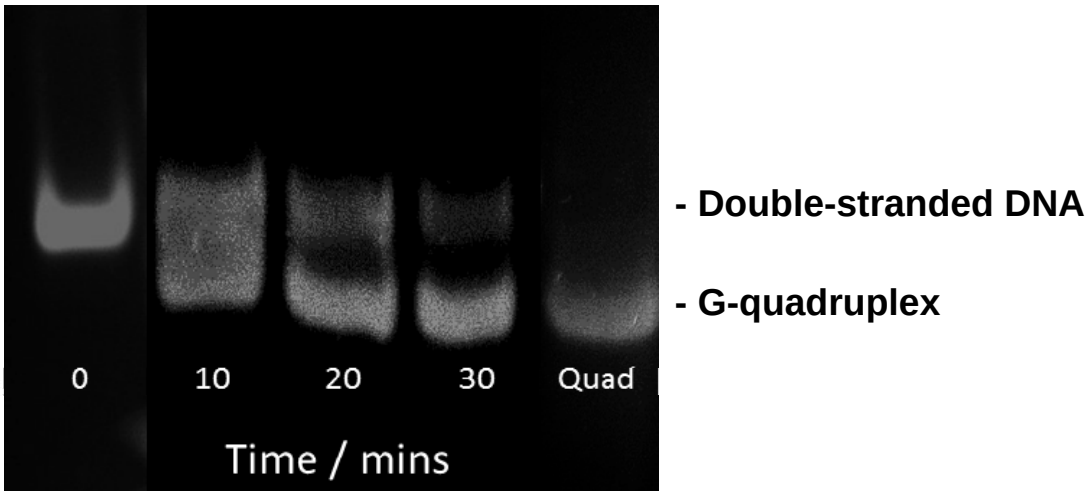

Figure S7. Time-course of ExoIII activity. Emission intensity of CV (2 μM) with G55 (0.5 μM) vs. time in the presence of ExoIII (0, 25, 50, 100 U / mL) at 37 °C. Buffer conditions: 10 mM Bis-Tris-Propane-HCl, 10 mM MgCl2, 1 mM DTT, 10 mM KCl.