Electronic Supplementary Material (ESI) for ChemComm.

Supporting information

An oxidatively damaged G-quadruplex/hemin DNAzyme

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Materials

Oligonucleotides purified by ultra-PAGE were purchased from Sangon (Shanghai, China) and dissolved in distilled and deionized water (18.2 MΩ) without further purification (**Table 1**). Strand concentration of each oligo was determined by measuring the UV absorption at 260 nm, using the corresponding molar extinction coefficient. Chemicals including hemin (>98.0%), triton X-100, H_2O_2 (30%), DMSO (>99%), tris-(hydroxymethyl)-amino-methane (Tris) (>99.9%), HCl (AR), KCl (>99%) and 2,2′-azino-bis (3-ethylben-zothiazoline-6-sulfonic acid) (ABTS) (>98.0%) were purchased from Sigma.

DNA stock samples in 10 mM Tris-HCl (pH 7.0) buffer supplemented with 100 mM KCl were heated at 95 ℃ for 5 min, then annealed slowly to room temperature during two hours and finally stored at 4 ℃ overnight or longer before use.

Spectroscopy Measurement

CD spectra were recorded by a Chirascan (Applied Photophysics, U.K.) equipped with a Peltier temperature control accessory, using a quartz cell of 1.0-cm light path length and 1.0 mL volume. All DNA samples were diluted to 5 μM in 10 mM pH 7.0 Tris-HCl buffer with 50 mM KCl. Each spectra were averaged from three scans within 220 to 350 nm wavelength range and 2 nm/s scan rate at 25 ℃.

TDS were performed on a Cary 100UV/Vis spectrophotometer (Agilent Technologies, USA) equipped with a Peltier temperature control accessory, using capped quartz cells of 1.0-cm light path length and 1.0 mL volume. All DNA samples were diluted to 5 μM in 10 mM pH 7.0 Tris-HCl buffer with 50 mM KCl, holding at 5 and 95 ℃ for 10 min respectively. Absorbance spectra at two temperatures both were recorded in the 220-350 nm range, with a scan speed of 600 nm/min and a data interval of 1 nm.

UV melting were measured via a Cary 100 UV/Vis spectrophotometer (Agilent Technologies, USA) equipped with a Peltier temperature control accessory, using capped quartz cells of 1.0-cm light path length and 1.0 mL volume. All DNA samples were diluted to 5 μM in 10 mM Tris-HCl buffer (pH 7.0) with 50 mM KCl. Absorbance at 295 nm was recorded, temperature was changed during the range between 5 and 95 ℃ within a temperature gradient of 0.5 ℃/min.

DNAzyme Activity Assay

Prior to measurement, 0.8 μM G-quadruplexes were incubated with 0.4 μM hemin in 10 mM pH 7.0 Tris-HCl buffer containing 0.05 % (v/v) Trion X-100 and 50 mM KCl for 2 h at 25 ℃. Then substrate ABTS (2.0 mM) and oxidation H_2O_2 (0.6 mM) were sequentially added. Absorbance of product $ABTS^{\cdot+}$ at 420 nm was monitored by a Cary 100 spectrophotometer (Agilent Technologies, USA) for 60 s. Initial reaction rates (V_0, nM/s) were calculated through the slope of the initial linear portion (the first 10 s) by using following equation (1):

$$V_0 = \frac{|dC|}{dt} = \frac{|dA|}{dt \times b \times \varepsilon} \qquad (1)$$

Where C is the real-time concentration of product; t is the reaction time; A is the absorbance of product; b is light path length, which is 1.0 cm; ε is the molar extinction coefficient of product $ABTS^{\cdot+}$, which is 36000 M^{-1} cm^{-1}. Each assay was repeated at least three times.

Kinetic of DNAzyme degradation

Prior to measurement, 1.0 μM G-quadruplexes were incubated with 2.0 μM hemin in 10 mM pH 7.0 Tris-HCl buffer containing 0.05 % (v/v) Trion X-100 and 50 mM KCl for 2 h at 25 ℃. Absorbance of the Soret band (404 nm) of G-quadruplex/hemin complex was recorded after 0.4 μM H_2O_2 was added. Initial degradation velocity (V_d) was obtained from the slope of the initial linear portion (the first 5 s) by using the following equation (2).

$$V_d = \frac{|dC_{complex}|}{dt} = \frac{|dA|}{dt \times b \times \varepsilon_{complex}} \qquad (2)$$

Where $C_{complex}$ is the real-time concentration of G-quadruplex/hemin complex; t is the reaction time; A is the absorbance of complex at λ=404 nm; b is light path length, which is 1.0 cm; ε is the molar extinction coefficient of G-quadruplex/hemin complex at 404 nm. Each measurement was repeated in triplicate.

Dissociation Constant (K_d) Determination

To estimate the binding affinity between G-quadruplexes and hemin, a spectrophotometric titration of hemin by DNA was carried out. Hemin concertation is kept constant at 2.0 μM in 10 mM pH 7.0 Tris-HCl buffer at 25 °C, DNA concentration was varied from 0 to 4.0 μM. The saturation curve for the binding of hemin with G-quadruplexes was plotted by bound fraction (α, equation 3) of hemin versus DNA concentration. K_d was obtained by fitting of above curve with a one-site binding model (GraphPad Prism 8).

$$\alpha = \frac{Ax - A0}{A\infty - A0} \qquad (3)$$

Where A_x is the absorbance at 404 nm for hemin incubated with corresponding DNA, A_∞ and A_0 are the respective values in the presence of saturating DNA and in the absence of DNA, respectively.

Table S1 Characterization of G-quadruplexes and hemin.

Sequence	K_d (μM) [a]	K_a ($\times 10^6$ / M) [a]	Soret band		E band
			λ (nm)	ε ($M^{-1}cm^{-1}$)	λ (nm)
G3T	1.688	0.592	404	1.13×10^5	496
G3TC	2.059	0.486	404	1.07×10^5	495
TC-5	3.921	0.255	404	1.16×10^5	496
TC-9	3.405	0.294	404	1.03×10^5	498
TC-13	2.593	0.386	404	1.07×10^5	493
TC-17	4.035	0.248	404	1.05×10^5	500
G3A	2.205	0.454	404	1.07×10^5	494
A-5	5.627	0.178	404	1.18×10^5	494
A-9	4.753	0.210	404	1.20×10^5	496
A-13	3.540	0.282	404	1.23×10^5	495
A-17	4.005	0.250	404	1.20×10^5	496

[a] Binding constant between G-quadruplexes and hemin $K_a = 1 / K_d$, where K_d is dissociation constant between G-quadruplexes and hemin.

Figure S1 UV melting and annealing curves.

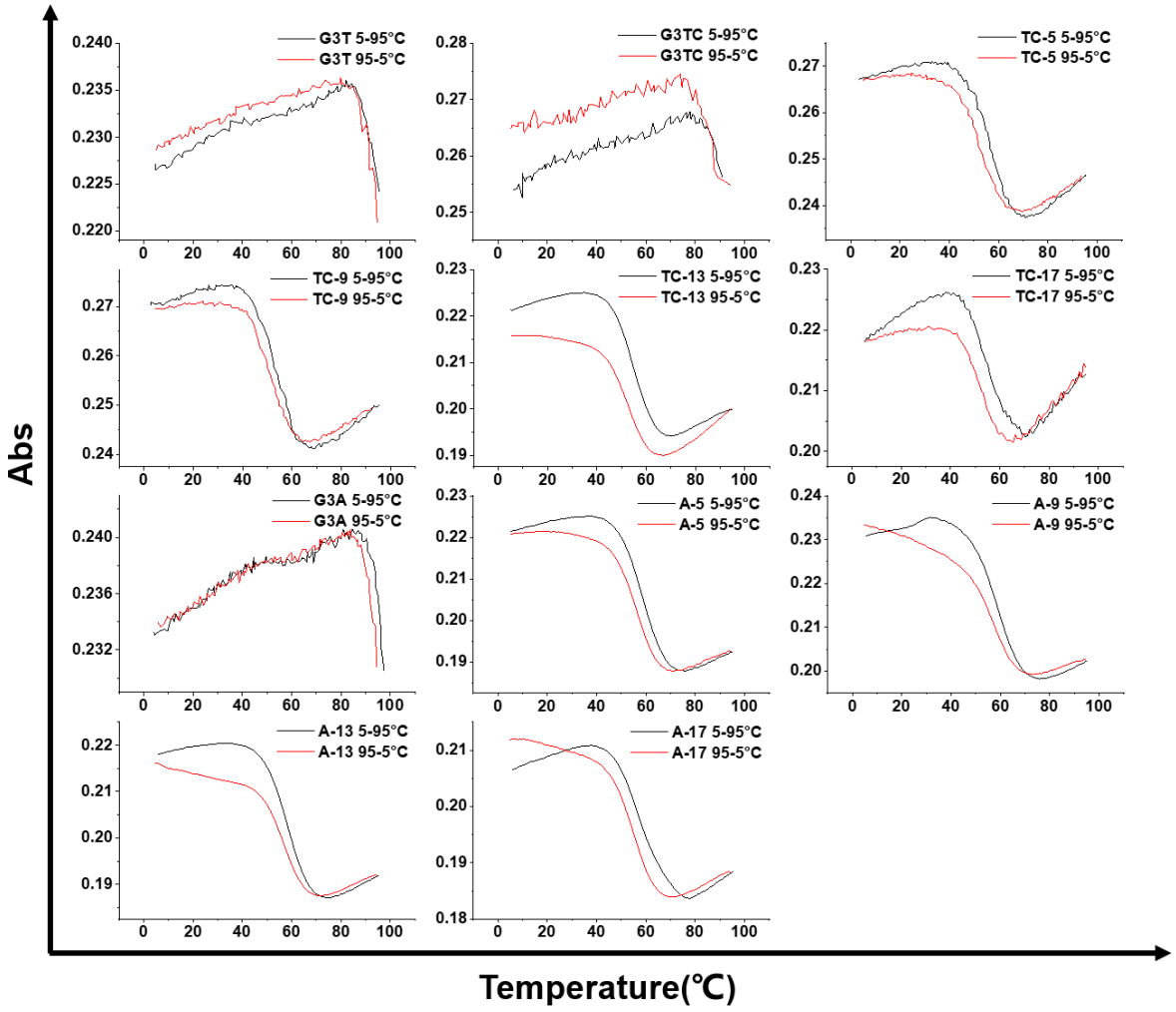

Figure S1 UV melting (black line) and annealing (red line) curves of G-quadruplexes. DNA samples were prepared as 5 μM in 10 mM Tris-HCl (pH 7.0) buffer with 50 mM KCl. Absorbance at 295 nm was recorded, temperature was changed during the range between 5 and 95 ℃ within a temperature gradient of 0.5 ℃/min.

Figure S2 DNAzyme activity assays.

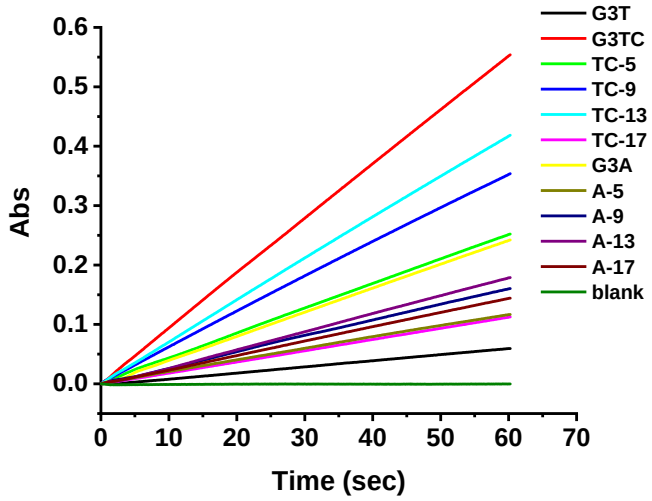

Figure S2 DNAzyme activity assays. Time-dependent absorbance at 420 nm of product $ABTS^{\cdot+}$ was recorded. 'Blank' represents the V_o of hemin alone. Reaction conditions: 0.8 μM DNA, 0.4 μM hemin, 0.05 % Triton X-100, 2.0 mM ABTS and 0.6 mM H_2O_2, 10 mM Tris-HCl (pH 7.0) containing 50 mM KCl, 25 ℃. Each assay was repeated in triplicate.

Figure S3 Oxidative lesions on other sites.

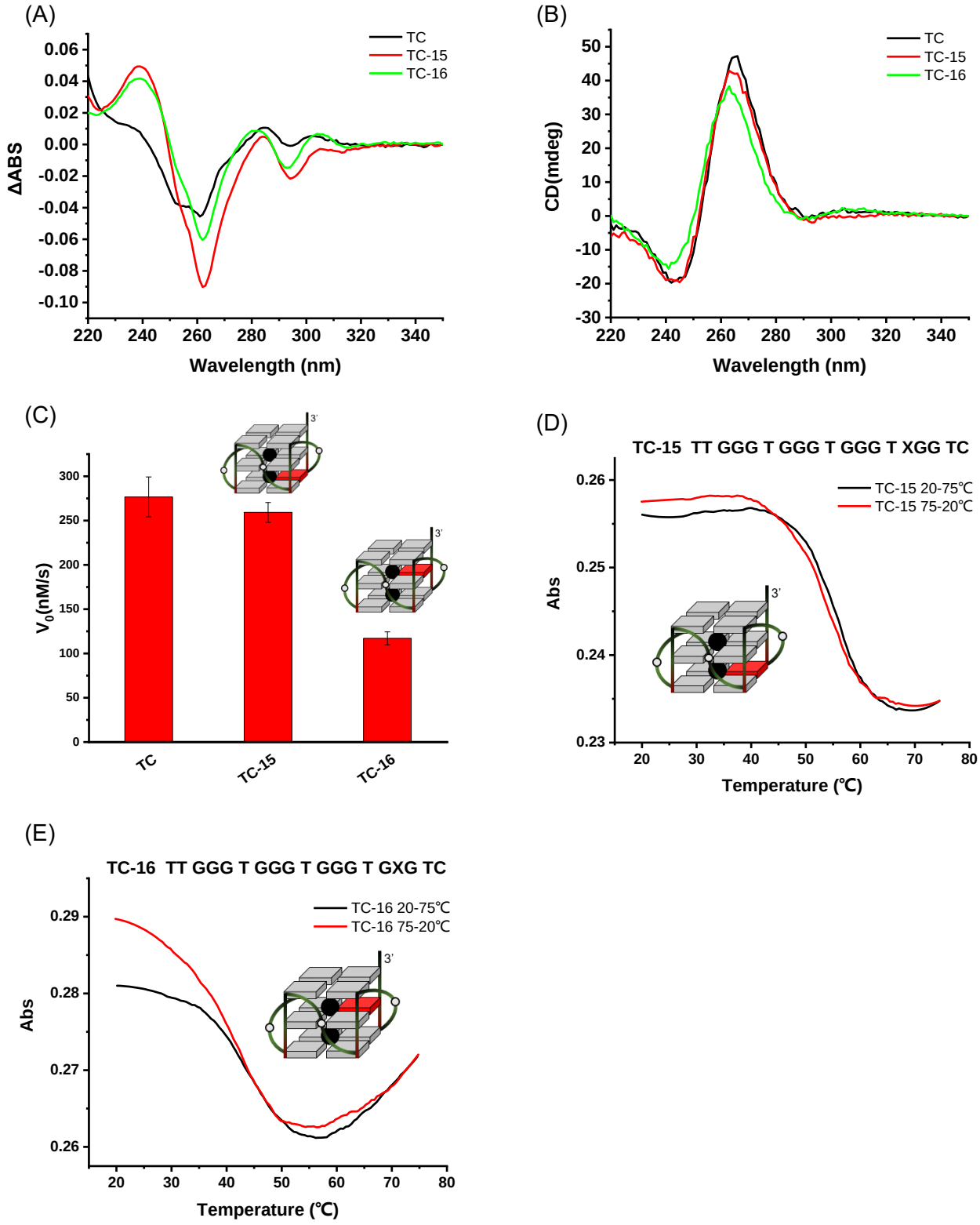

Figure S3 8-oxoG (indicated by red brick and represented by X in the sequences) were also introduced in positions 15 (TC-15) or 16 (TC-16) of G3TC (TT GGG T GGG T GGG T GGG TC). (**A**) Thermal difference spectra (TDS) and (**B**) CD spectra of 5.0 μM TC-15 and TC-16 collected in 10 mM pH 7.0 Tris-HCl containing 50 mM KCl. (**C**) Initial reaction velocities (V_o, nM/s) of TC-15 and TC-16. Reaction conditions: 0.8 μM DNA, 0.4 μM hemin, 0.05 % Triton X-100, 2.0 mM ABTS and 0.6 mM H_2O_2, 10 mM pH 7.0 Tris-HCl containing 50 mM KCl, 25 ℃. (**D** and **E**) UV melting (black line) and annealing (red line) curves of 5.0 μM TC-15 and TC-16 in 10 mM pH 7.0 Tris-HCl containing 50 mM KCl.

Figure S4 G-quadruplex titration to hemin.

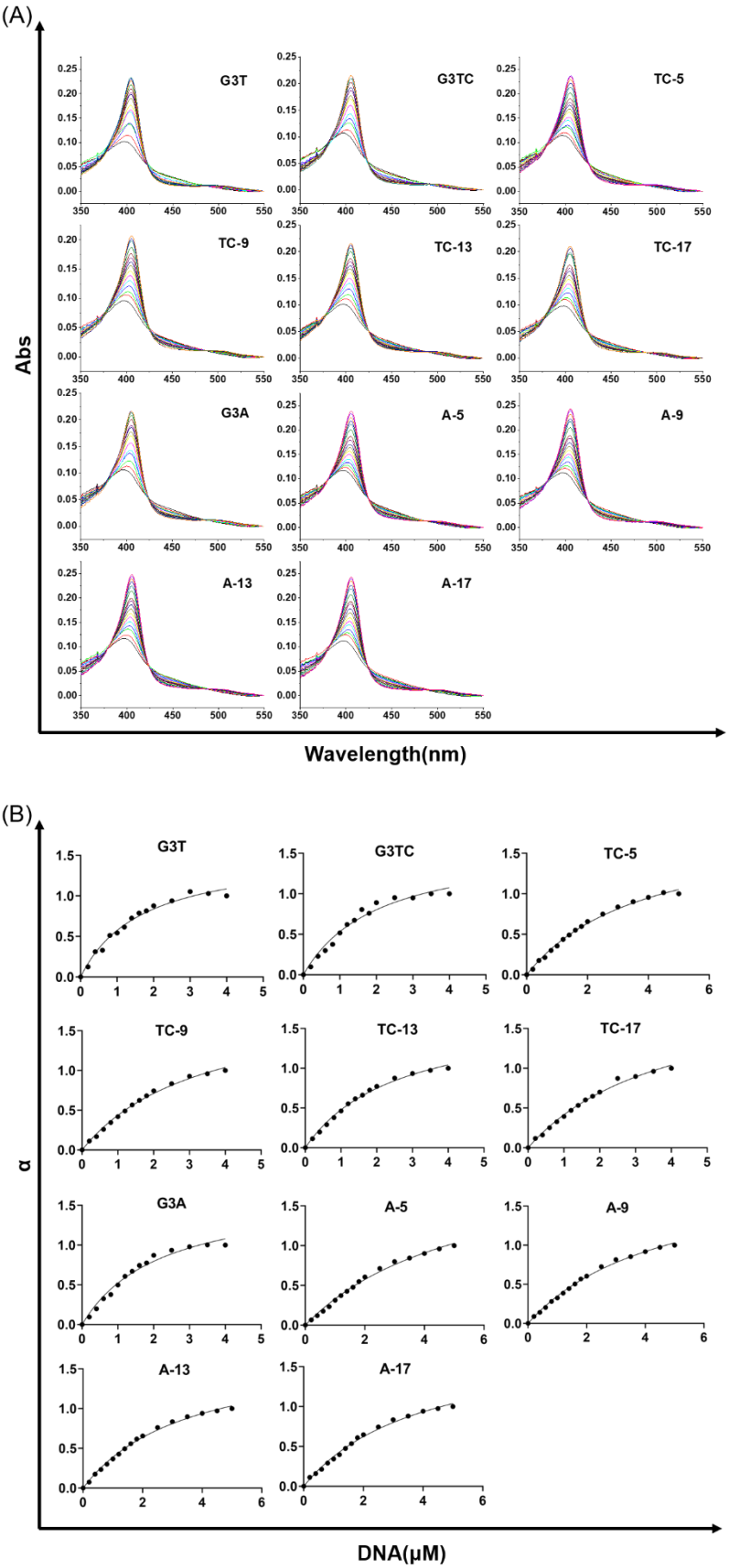

Figure S4 (**A**) UV-vis spectra of 2 μM hemin with increasing concentrations of G-quadruplexes from 0 to 4 μM; (**B**) Plots of the fraction of bound hemin as a function DNA concentration. α is the bound fraction of hemin by G-quadruplex.

Figure S5 Degradation of G-quadruplex/hemin complexes.

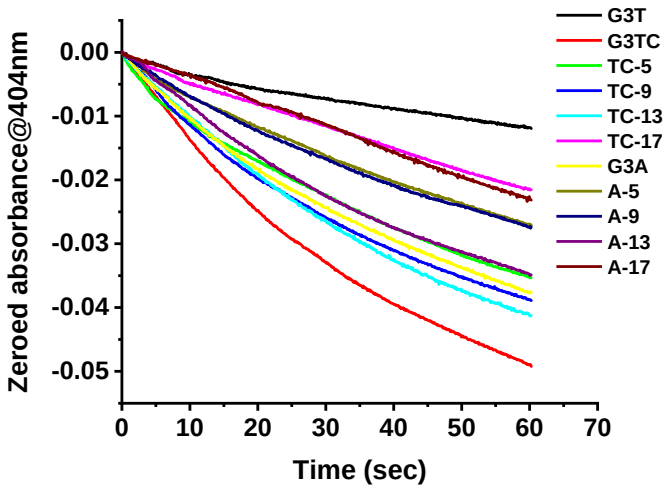

Figure S5 Degradation of G-quadruplex/hemin complexes. Plot of absorbance at 404 nm of G-quadruplex/hemin complex versus reaction time. Reaction conditions: 1.0 μM DNA, 2.0 μM hemin, 0.05 % (v/v) Triton X-100 and 0.4 mM H_2O_2, 10 mM Tris-HCl (pH 7.0) containing 50 mM KCl, 25 ℃. Each assay was repeated in triplicate.