Supporting Information

Thioflavin T Specifically Brightening "Guanine Island" in Duplex-DNA: A Novel Fluorescent Probe for Single-Nucleotide Mutation

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Table S1. Oligonucleotide sequences Used in This Work [a]

DNA Name	**DNA Sequences**
DNA (S1)	5’ -T*GGG*ATTATTATT- 3’
DNA (S2)	5’ -AATAATAAT*G*A- 3’
DNA (S3)	5’ -TTT*GGG*ATTATTATT- 3’
DNA (S4)	5’ -AATAATAAT*G*AAA- 3’
DNA (S5)	5’ -TTTTTT*GGG*ATTATTATT- 3’
DNA (S6)	5’ -AATAATAAT*G*AAAAAA- 3’
DNA (S7)	5’ -TTTTTTTTT*GGG*A- 3’
DNA (S8)	5’ -T*G*AAAAAAAAA- 3’
DNA (S9)	5’ -TTTTTTTTT*GGG*ATT- 3’
DNA (S10)	5’ -AAT*G*AAAAAAAAA- 3’
DNA (S11)	5’ -TTTTTTTTT*GGG*ATTATT- 3’
DNA (S12)	5’ -AATAAT*G*AAAAAAAAA- 3’
DNA (S13)-(S15)	5’ -AATAATAAT*G*YAAAAAAAA- 3’ Y= T (S13), C (S14) or G (S15)
DNA (S16)-(S18)	5’ –TTTTTTTTY’*GGG*ATTATTATT- 3’ Y’=A (S16), G (S17) or C (S18)
DNA (S19)-(S22)	5’ -AATAATAAA*G*YAAAAAAAA- 3’ Y= A (S19), T (S20), C (S21) or G (S22)
DNA (S23)-(S26)	5’ –TTTTTTTTY’*GGG*TTTATTATT- 3’ Y’=T (S23), A (S24), G (S25) or C (S26)
DNA (S27)-(S30)	5’ -AATAATAAC*G*YAAAAAAAA- 3’ Y= A (S27), T (S28), C (S29) or G (S30)
DNA (S31)-(S34)	5’ –TTTTTTTTY’*GGG*GTTATTATT- 3’ Y’=T (S31), A (S32), G (S33) or C (S34)
DNA (S35)-(S38)	5’ -AATAATAAG*G*YAAAAAAAA- 3’ Y= A (S35), T (S36), C (S37) or G (S38)
DNA (S39)-(S42)	5’ –TTTTTTTTY’*GGG*CTTATTATT- 3’ Y’=T (S39), A (S40), G (S41) or C (S42)
DNA (S43)	5'-TTTTTT *G* ATTTTT *G* ATTTTT CGAC TTTTTT *GGG* ATTTTT *GGG* ATTTTT -3'

DNA (S44)-(S46)	5’ -TTTTTTTTT*GXG*ATTATTATT- 3’ X=A (S44), T (S45) or C (S46)
DNA (S47)-(S49)	5’ -TTTTTTTTT*XGG*ATTATTATT- 3’ X=A, T or C
DNA (S50)-(S52)	5’ -TTTTTTTTT*GGX*ATTATTATT- 3’ X=A, T or C
22AG	5'-AGGGTTAGGGTTAGGGTTAGGG-3'
PW17	5'-GGGTAGGGCGGGTTGGG-3'
PS2.M	5'-GTGGGTAGGGCGGGTTGG-3'
T30695	5'-GGGTGGGTGGGTGGGT-3'
T3TT	5'-GGGTTTGGGTGGGTGGG-3'
TBA	5’ -GGTTGGTGTGGTTGG- 3’

[a] The underlined letters represent the position of forming “Guanine Island”.

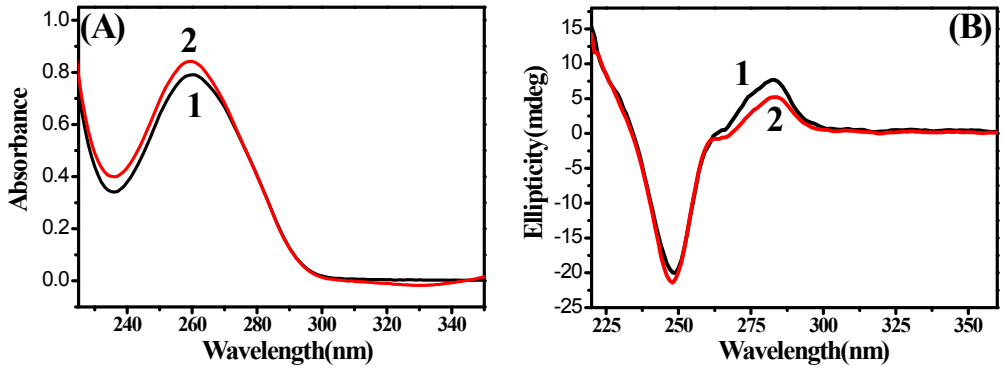

Fig. S1 (A) UV/Vis and (B) CD spectra of 2 μM ds-DNA (1+3) in the absence (curve 1) and presence (curve 2) 16 μM ThT in buffer solution.

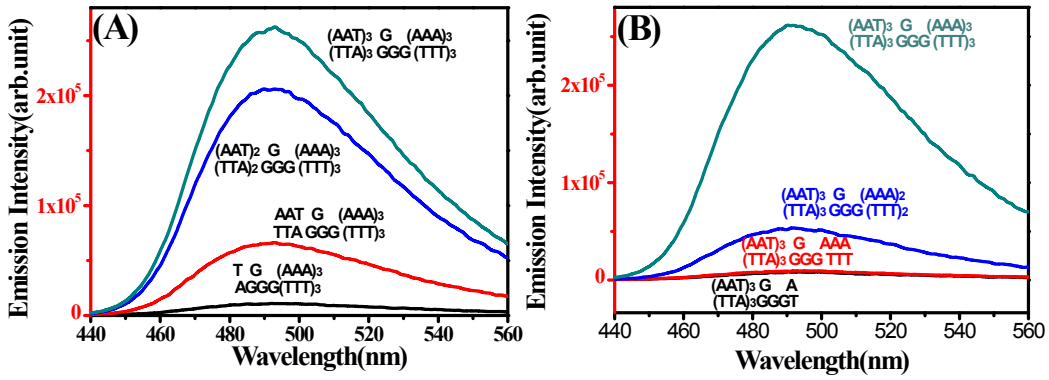

Fig. S2. (A) Fluorescent spectra of 8 μM ThT in the presence of 1 μM ds-DNA with different numbers of hybridized base pairs in the left side of "Guanine Island'': ds-DNA (S1+S2) (black curve); ds-DNA (S3+S4) (red curve); ds-DNA (S5+S6) (blue curve); ds-DNA (1+2) (green curve). (C) Fluorescent spectra of 8 μM ThT in the presence of 1 μM ds-DNA with different numbers of hybridized base pairs in the right side of "Guanine Island'': ds-DNA (S7+S8) (black curve); ds-DNA (S9+S10) (red curve); ds-DNA (S11+S12) (blue curve); ds-DNA (1+2) (green curve).

As shown in Figure S3, the excitation wavelengths were set to 425, 580, 410, 410 and 410 nm to measure the emission intensity of ThT, CV, NMM, PPIX and ZnPPIX at 495, 640, 610, 630 and 590 nm, respectively.

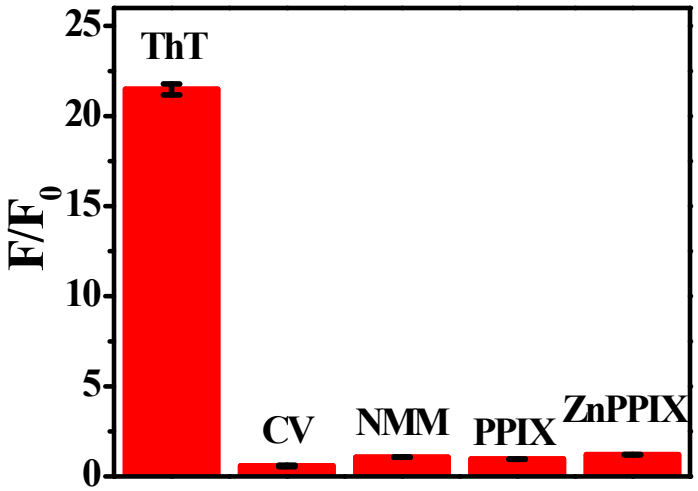

Fig. S3 Fluorescence intensity ratios of 8 μM fluorescent dyes in buffer solution. F_0 and F denote the maximized emission intensity of each fluorescent dye in the presence of 1 μM fully hybridized ds-DNA (1+3) and 1 μM ds-DNA (1+2) containing a "Guanine Island". Error bars are standard deviation of the mean (n = 3).

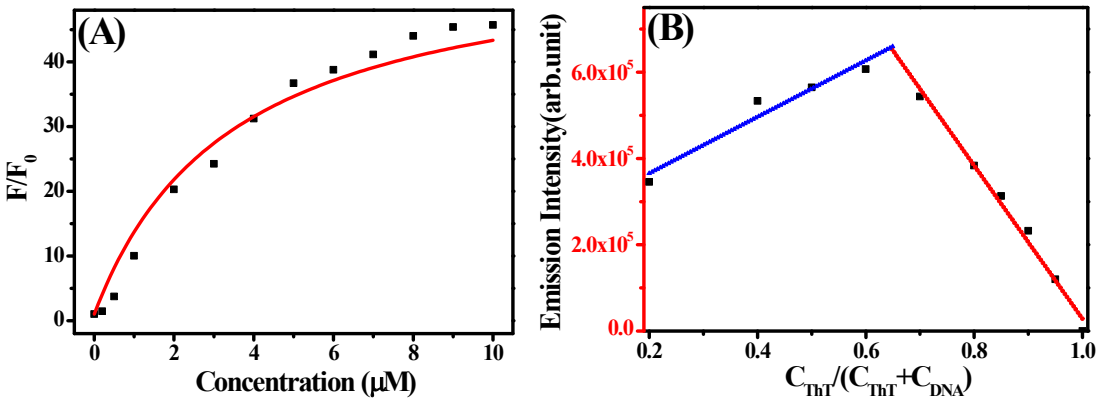

Fig. S4 (A) Fluorescence intensity of 100 nM ThT at 495 nm in the presence of ds-DNA (1+2) ranging from 0 to 10 μM. The red line is the fitted curve assuming a 2:1 binding of ThT to ds-DNA (1+2). F_0 and F denote the fluorescence intensity of ThT in the absence and presence of ds-DNA (1+2). (B) Jobs' plot obtained from fluorimetric analysis of mixtures of ThT with ds-DNA (1+2) (ThT+ DNA = 8 μM). X axis = mole fraction of ThT.

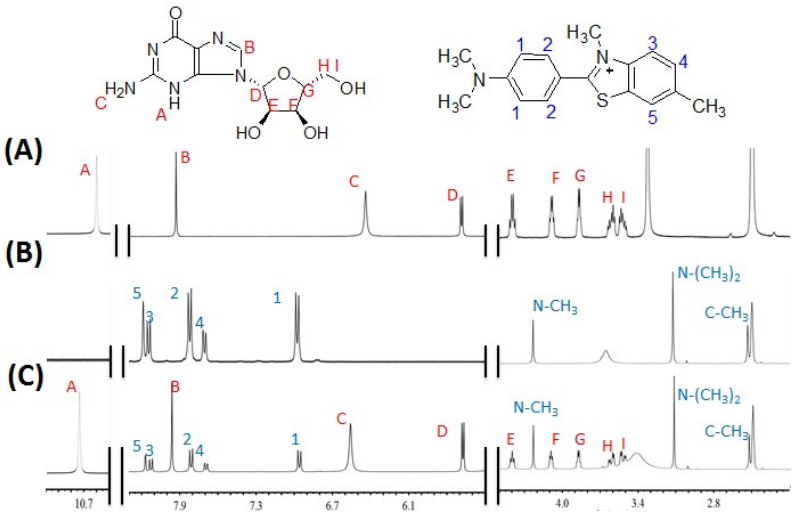

Fig. S5 ^{1}H NMR spectra of 15 μM ThT (A), 45 μM guanosine (B), and a mixture containing 15 μM ThT and 45 μM guanosine (C), respectively.

Table S2. ^{1}H Chemical Shifts of ThT and Guanosine

Proton	δ			Δδ
	ThT	Guanosine	ThT+Guanosine	
1 (ThT)	6.976	/	6.969	-0.007
2 (ThT)	7.822	/	7.814	-0.008
3 (ThT)	8.144	/	8.128	-0.016
4 (ThT)	7.706	/	7.697	-0.009
5 (ThT)	8.188	/	8.171	-0.017
A (Guanosine)	/	10.608	10.743	0.135
B (Guanosine)	/	7.930	7.964	0.034
C (Guanosine)	/	6.442	6.570	0.128

For Hg^{2+} Analysis

As shown in Figure S6A, DNA (S43) could form a hairpin structure in the presence of Hg^{2+} though T–Hg^{2+}–T interaction, which contained two "Guanine Island" units. Figure S6B showed fluorescence emission spectra of ThT was gradually enhanced upon increasing Hg^{2+} concentration ranging from 0 to 3 μM. A linear relationship between emission intensity at 495 nm and Hg^{2+} concentration (from 50 nM to 3 μM) was obtained (Figure S6C). The detection limit is calculated to be 10 nM. The variation in the fluorescence intensity was much smaller for other metal ions compared to that of Hg^{2+} (Figure S6D), exhibiting a highly selectivity to Hg^{2+}.

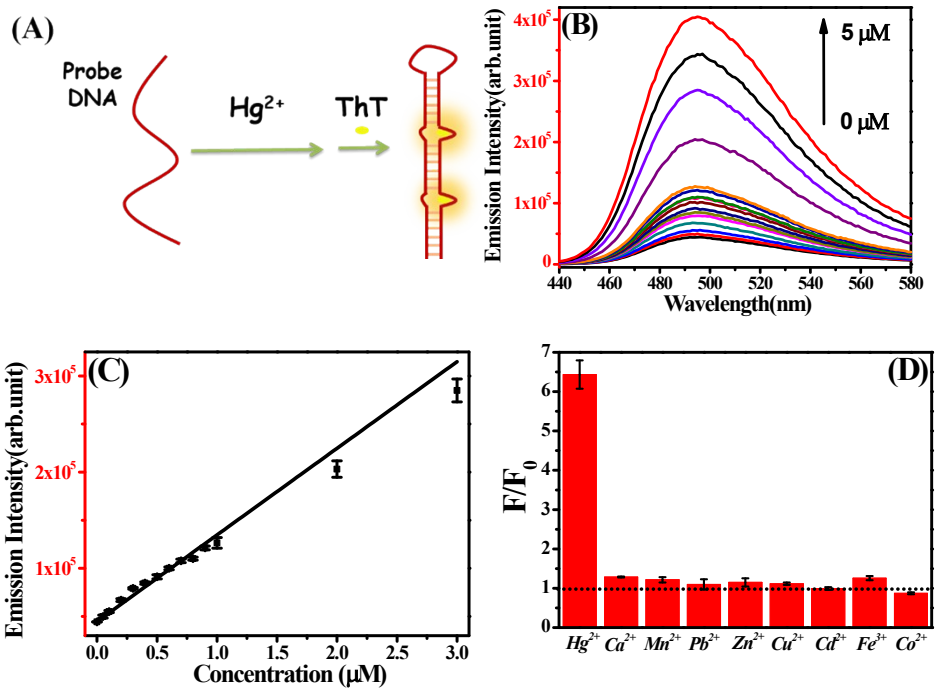

Fig. S6 (A) Schematic illustration of probe DNA (S43) for analyzing Hg^{2+} with "Guanine Island" as a ThT lighting up probe. (B) Fluorescence emission spectra of ThT (4 μM) in the presence of 0.5 μM DNA (S43) and Hg^{2+} (ranging from 0 to 5 μM) in 10 mM Tris-Ac buffer solution. (C) Relationship of ThT emission intensity with the concentration of Hg^{2+}. (D) Selectivity of Hg^{2+} (3 μM) over other metal ions (30 μM). F_0 and F denote the fluorescence intensity of ThT in the absence and presence of metal ions, respectively. Error bars are standard deviation of the mean (n = 3).

Detection of GGG triad

Consecutive G bases, especially GGG triad, exist in many biologically important DNA strands. They could potentially form G4 structures in the telomeric DNA strands, oncogenes or proto-oncogenes, and bind with transcription factor in the promotor region.[1] Any G mutation appeared in GGG triad could disrupt the G4 formation,[2, 3] resulting in expression of cancer-related genes[4] and altering the conformation of transcription factor on the binding site.[5] Thus, the detection of GGG triad was pivotal, but the related recognizing assay was rarely reported.[6] Inspired by the results discussed above, "Guanine Island" in ds-DNA as a ThT lighting up probe was also applied to detect GGG triad. DNA (2) as a probe DNA was selected to hybridize with GGG triad-contained target DNA (1) forming ds-DNA (1+2), which contained a "Guanine Island". Upon binding with ThT, ds-DNA (1+2) exhibited a greatly enhanced fluorescence as shown in Figure S7A. With 1 μM DNA (2) as a probe, the fluorescent detection of GGG triad was achieved. Figure S7B implied that ThT fluorescence gradually enhanced with increasing target DNA (1) ranged from 0 to 1 μM. Figure S7C outlined the relationship between fluorescence intensity at 495 nm and concentration of target DNA (1). A linear range was observed from 0 to 1 μM. The inset of Figure S7C showed the solution photos with the varying target DNA (1) concentration. The detection limit was 10 nM. If a G base of GGG triad in target (1) freely mutated to A [DNA (S44), DNA (S47) and DNA (S50)], T [DNA (S45), DNA (S48) and DNA (S51)] or C [DNA (S46), DNA (S49) and DNA (S52)], upon further hybridizing with probe DNA (2), ds-DNA was formed, respectively. After binding with ThT, the corresponding fluorescence intensity was shown in Figure S7D. Interestingly, only GGG triad produced the strongest fluorescence. The results demonstrated that GGG triad could be sensitively and selectively detected with "Guanine Island" as a ThT lighting up probe.

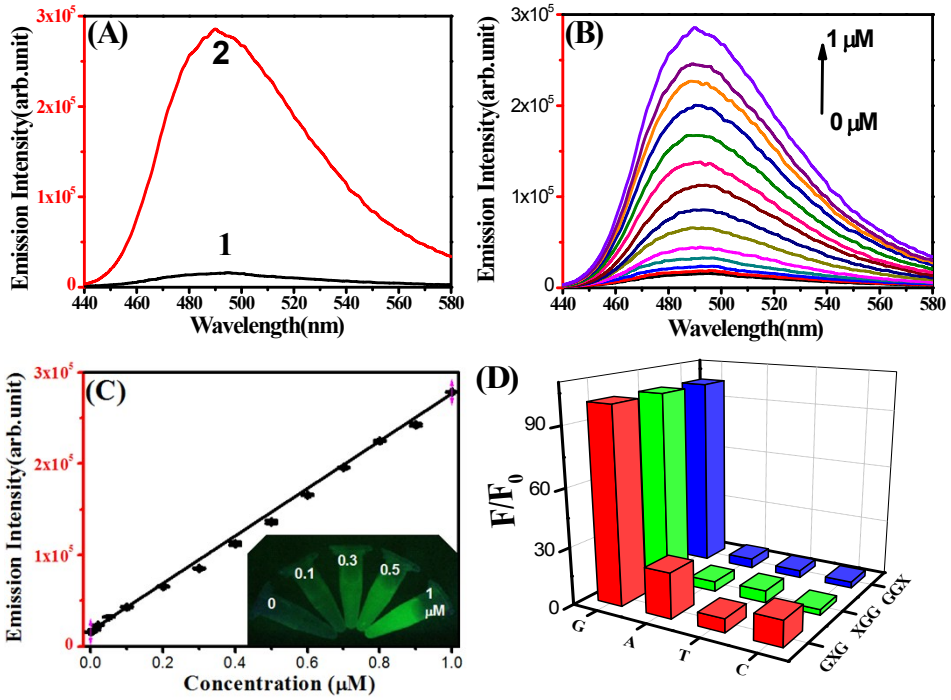

Fig. S7 (A) Fluorescent emission spectra of 8 μM ThT with DNA (2) as a probe in the absence (curve 1) and presence (curve 2) of target DNA (1). (B) Fluorescence responses of ThT upon adding target DNA (1) ranging from 0 to 1 μM. (C) Relationship between ThT emission intensity and concentration of target DNA (1). Inset: the photos of ThT with probe DNA (2) before and after hybridizing with different concentrations of target DNA (1). (D) Fluorescence intensities of ThT with probe DNA (2) hybridizing with complementary triad sequences (DNA (S44)-DNA (S52)), respectively, X = G, A, T or C. F_0 and F denote the fluorescence intensity of ThT in the absence and presence of ds-DNA. Error bars are standard deviation of the mean (n = 3).

References:

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