Electronic Supplementary Material (ESI) for RSC Advances.

SUPPORTING INFORMATION

Detection of Adenine-rich ssDNA based on Thymine-Substituted Tetraphenylethene with Aggregation-Induced Emission Characteristic

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1. Experimental Section

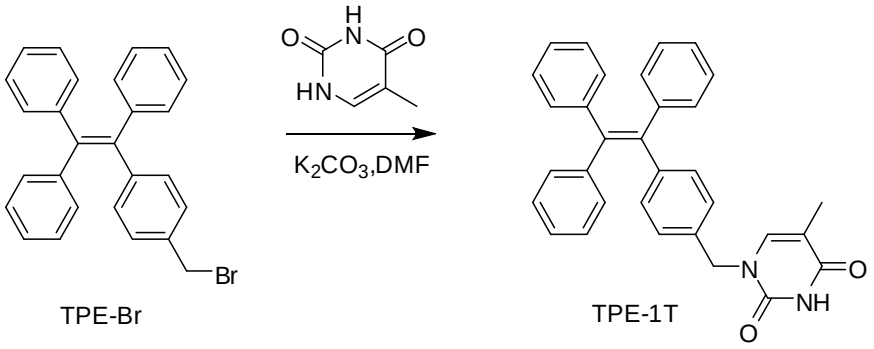
1.1 Materials and Method

Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under nitrogen immediately prior to use. Milli-Q water was used as deionized water. Other chemicals were purchased from Aldrich and used as received without further purification. ^{1}H and ^{13}C NMR spectra were measured on a Bruker ARX 400 NMR spectrometer using DMSO-d_6 as solvent and tetramethylsilane (TMS) as internal reference. UV absorption spectra were taken on a Milton Ray Spectronic 3000 array spectrophotometer. Photoluminescence (PL) spectra were recorded on a Perkin-Elmer LS 55 spectrofluorometer. Mass spectra were recorded on a GCT premier CAB048 mass spectrometer operated in MALDI-TOF mode. Elemental analysis was performed with an Elementar Vario Micro Cube. Thermogravimetric analysis (TGA) was carried out under nitrogen on a Perkin-Elmer TGA 7 analyzer at a heating rate of 10 ^{o}C/min. Minimum essential medium (MEM), fetal bovine serum (FBS), penicillin and streptomycin were purchased from Invitrogen.

1.2 Synthesis

TPE-T was prepared according to the synthetic route shown in Scheme S1.

5-methyl-1-(4-(1,2,2-triphenylvinyl)benzyl)pyrimidine-2,4(1H,3H)-dione (TPE-T)**.** A solution of TPE-Br (213 mg, 0.5 mmol), thymine (194 mg, 1.5 mmol) and K_2CO_3 (207 mg, 1.5 mmol) in dry DMF (15 mL) was refluxed under nitrogen for 24 h. After cooling to ambient temperature, the solvent was evaporated under reduced pressure. The residue was purified by a silica gel column chromatography using dichloromethane and ethyl acetate (3:1 v/v) as eluent to give a white powder in 41% yield. ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 11.27 (s, 1H), 7.55 (s, 1H), 7.07–7.15 (m, 9H), 7.02 (d, 2H), 6.93–6.97 (m, 8H), 4.73 (s, 2H), 1.72 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 164.05, 150.78, 142.92, 142.88, 142.25, 141.11, 140.55, 139.91, 130.67, 130.42, 130.40, 127.70, 127.67, 127.61, 126.56, 126.43, 126.36, 108.77, 49.54, 11.77. HRMS (MALDI-TOF): *m*/*z* 470.1949 [$(M+H)^+$, calcd 470.5610]. Elemental analysis calcd for $C_{30}H_{20}N_2$: C, 81.68; H, 5.57; N, 5.95. Found C, 80.06; H, 5.52; N, 5.81.



Scheme S1. Synthetic route to TPE-T.

Figure S1

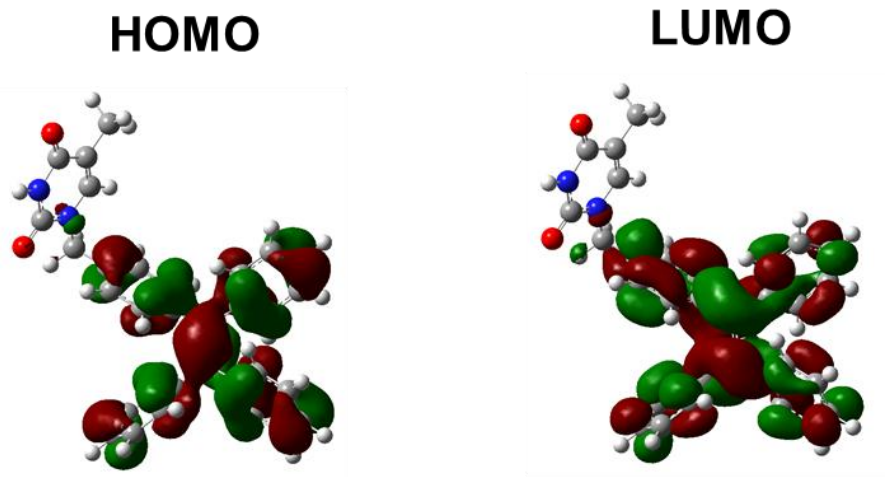


Figure S1. Optimized molecular structure and molecular orbital amplitude plots of HOMO and LUMO energy levels of the TPE-T calculated using the B3LYP/6-31G(d) basis set.

Figure S2

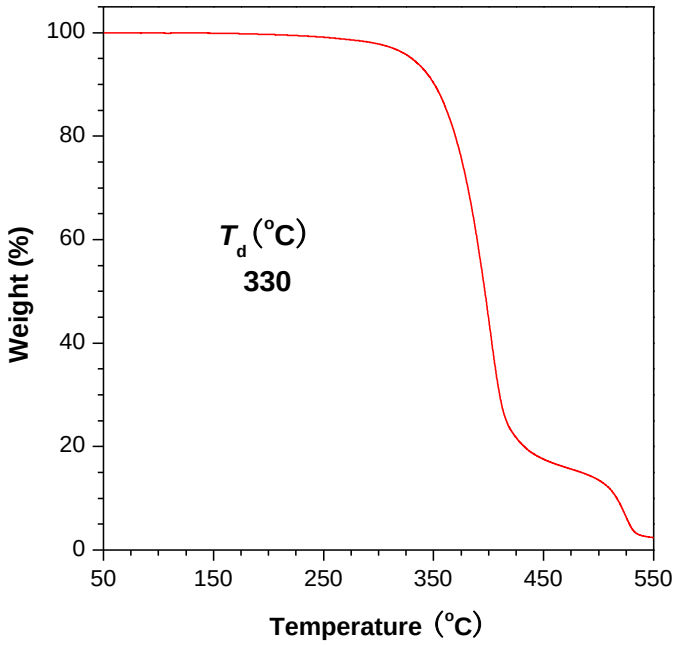


Figure S2. TGA thermograms of TPE-T recorded under nitrogen at a heating rate of 10 °C/min.

Figure S3

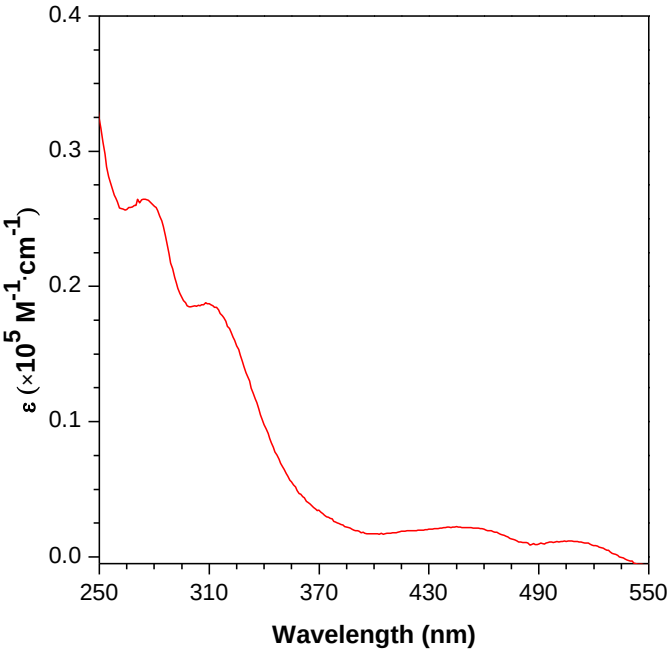


Figure S3. Absorption spectra of TPE-T in ethanol-water mixture (36:64, v/v; 10 μM).

Figure S4

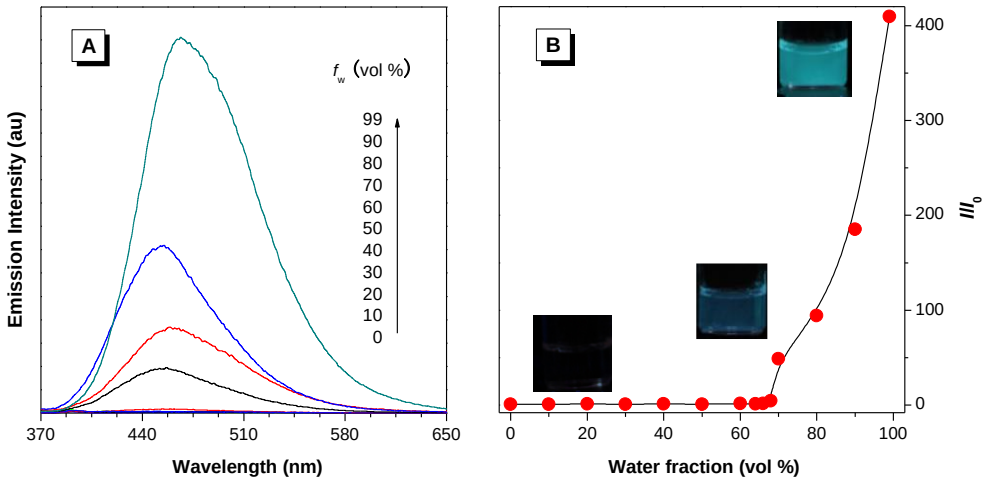


Figure S4. (A) PL spectra of TPE-T (10 μM) in ethanol-water mixtures with different water fractions (f_w). (B) Plot of I/I_0 value versus the composition of the ethanol–water mixture of TPE-T. I_0 = intensity in pure ethanol solution. Inset: Photographs of TPE-T in ethanol–water mixtures with different f_w values taken under UV illumination. Excitation wavelength: 350 nm.

Figure S5

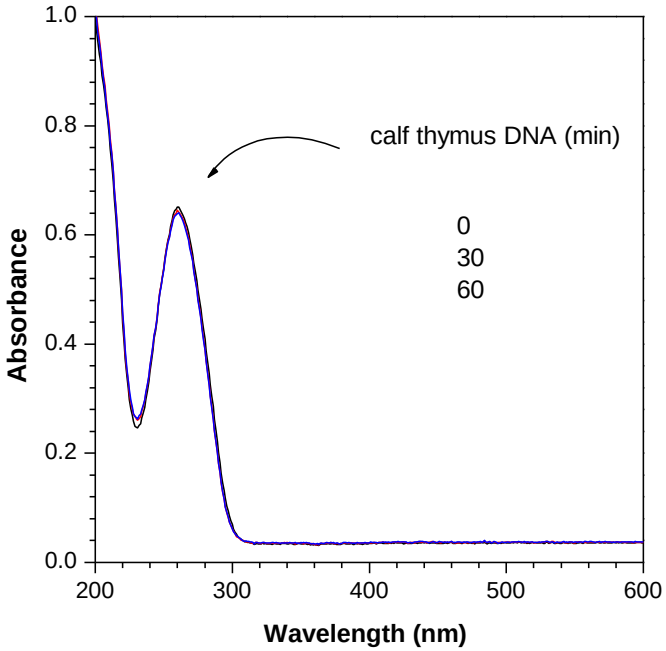


Figure S5. Absorption spectra of calf thymus DNA (dsDNA, 30 μg/mL) in ethanol/water mixture (36:64, v/v) at different incubation time.

Figure S6

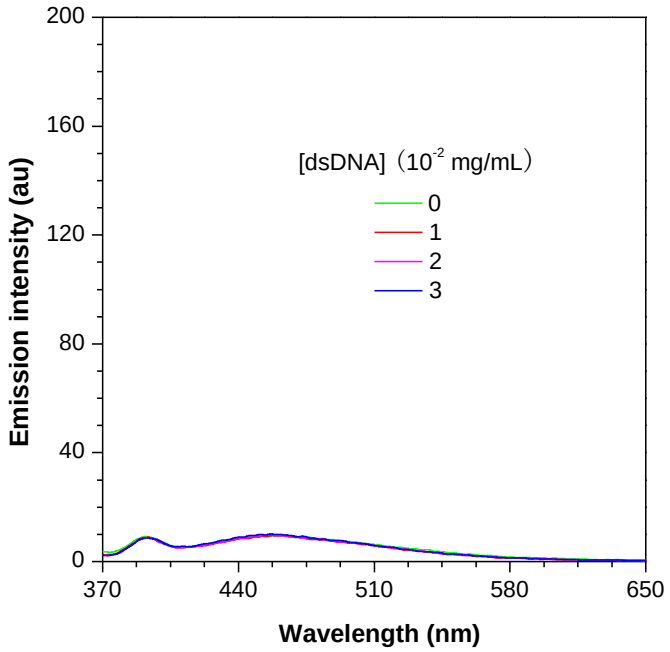


Figure S6. PL spectra of TPE-T (10 μM) in ethanol-water mixture (36:64, v/v) with different concentrations of calf thymus DNA (dsDNA). Excitation wavelength: 350 nm.

Figure S7

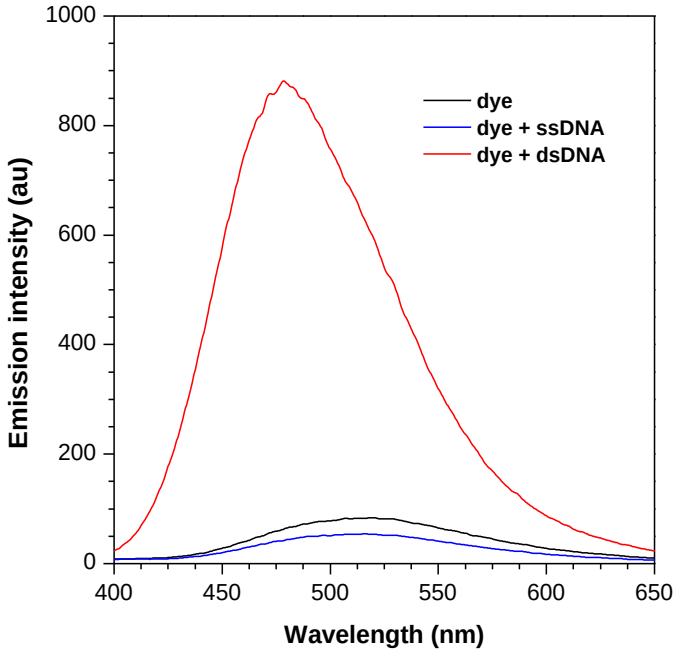


Figure S7. PL spectra of Hoechst 33342 in PBS buffer with calf thymus DNA (dsDNA, 30 μg/mL) or ssDNA (AA, 2 μM). Excitation wavelength: 350 nm.

Figure S8

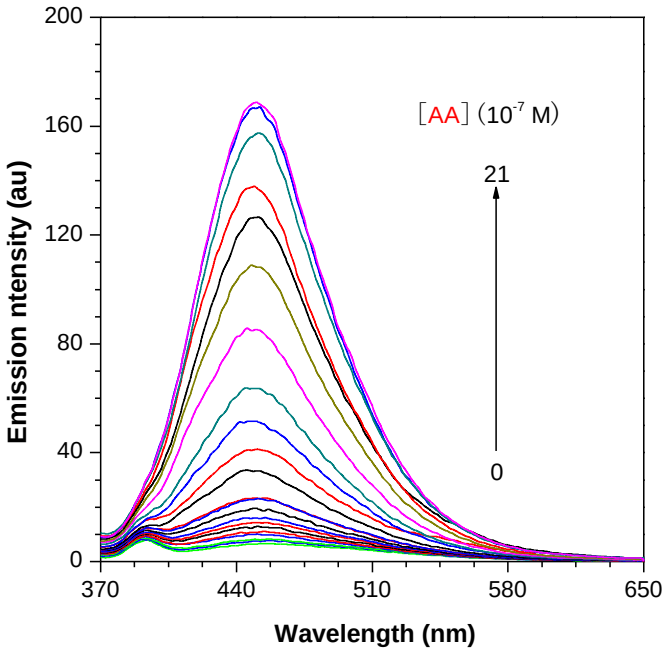


Figure S8. PL spectra of TPE-T (10 μM) in ethanol-water mixture (36:64, v/v) with different concentrations of AA. From bottom to top (μM): 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0 and 2.1. Excitation wavelength: 350 nm.

Primer: 5'AAAAAAAAAAAAAAAAAAAA3'

Figure S9

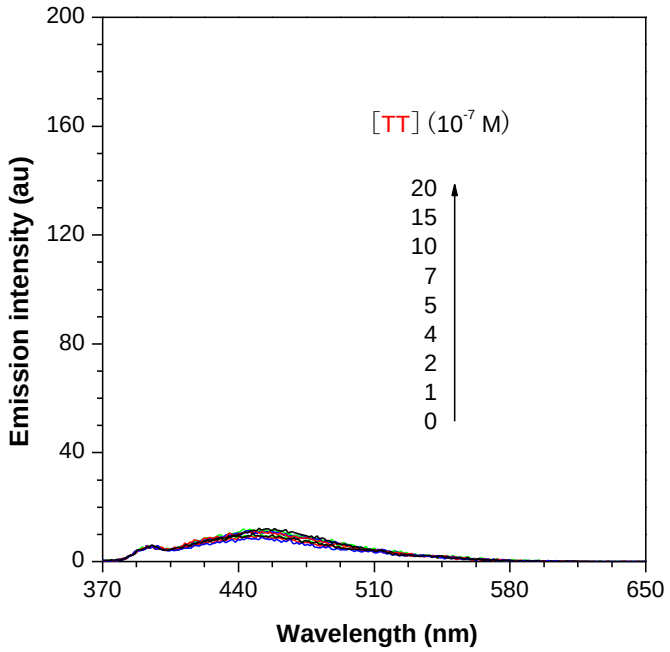


Figure S9. PL spectra of TPE-T (10 μM) in ethanol-water mixture (36:64, v/v) with different concentrations of TT. From bottom to top (μM): 0, 0.1, 0.2, 0.4, 0.5, 0.7, 1.0, 1.5 and 2.0. Excitation wavelength: 350 nm.

Primer: 5'TTTTTTTTTTTTTTTTTTTT3'

Figure S10

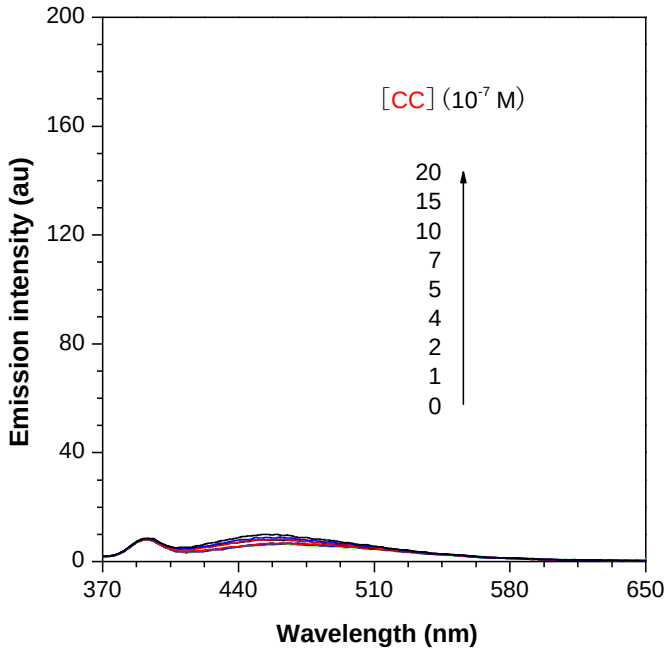


Figure S10. PL spectra of TPE-T (10 μM) in ethanol-water mixture (36:64, v/v) with different concentrations of CC. From bottom to top (μM): 0, 0.1, 0.2, 0.4, 0.5, 0.7, 1.0, 1.5 and 2.0. Excitation wavelength: 350 nm.

Primer: 5'CCCCCCCCCCCCCCCCCCCCC3'

Figure S11

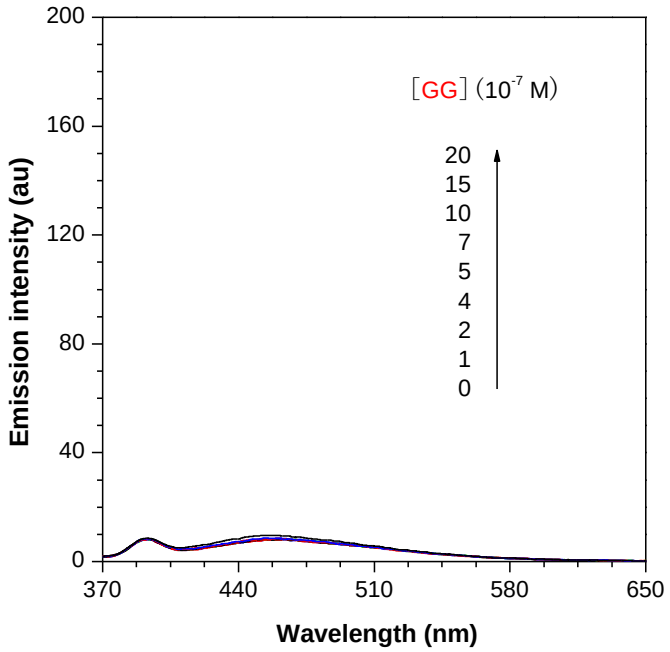


Figure S11. PL spectra of TPE-T (10 μM) in ethanol-water mixture (36:64, v/v) with different concentrations of GG. From bottom to top (μM): 0, 0.1, 0.2, 0.4, 0.5, 0.7, 1.0, 1.5 and 2.0. Excitation wavelength: 350 nm.

Primer: 5'GGGGGGGGGGGGGGGGGGGGG3'

Figure S12

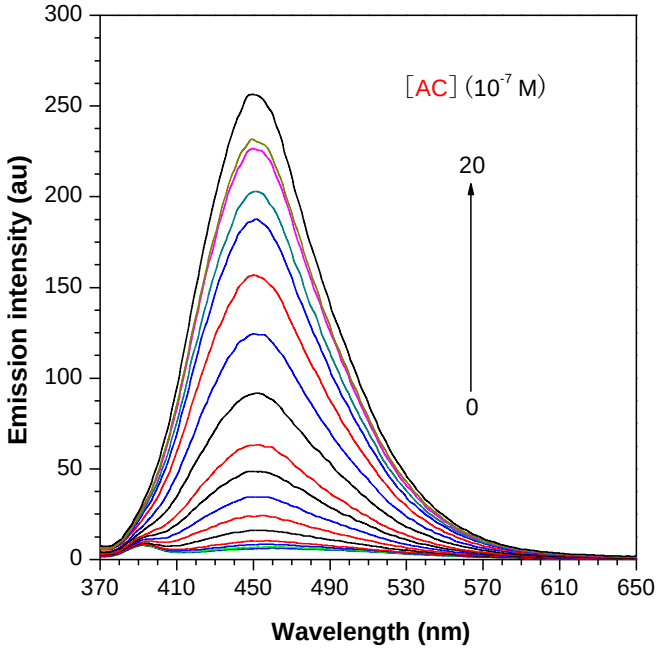


Figure S12. PL spectra of TPE-T (10 μM) in ethanol-water mixture (36:64, v/v) with different concentrations of AC. From bottom to top (μM): 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6 and 2.0. Excitation wavelength: 350 nm. Primer: 5'ACACACACACACACACACACA3'

Figure S13

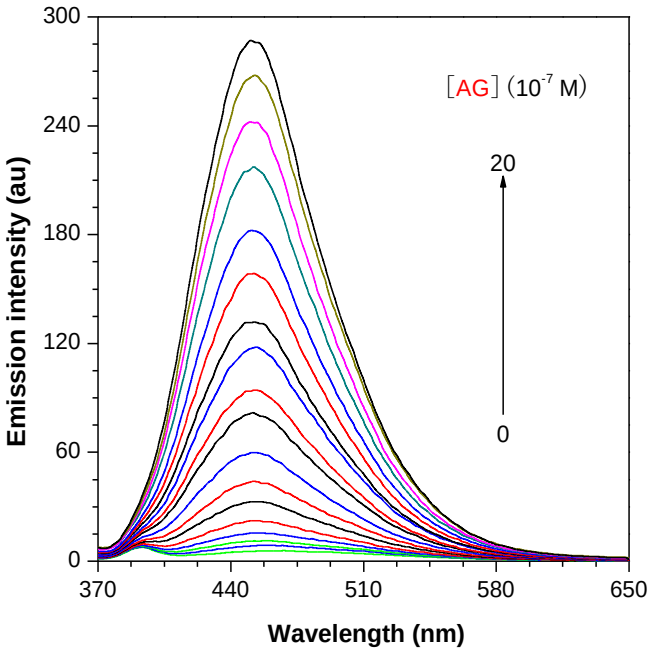


Figure S13. PL spectra of TPE-T (10 μM) in ethanol-water mixture (36:64, v/v) with different concentrations of AG. From bottom to top (μM): 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6 and 2.0. Excitation wavelength: 350 nm. Primer: 5'AGAGAGAGAGAGAGAGAGAGA3'

Figure S14

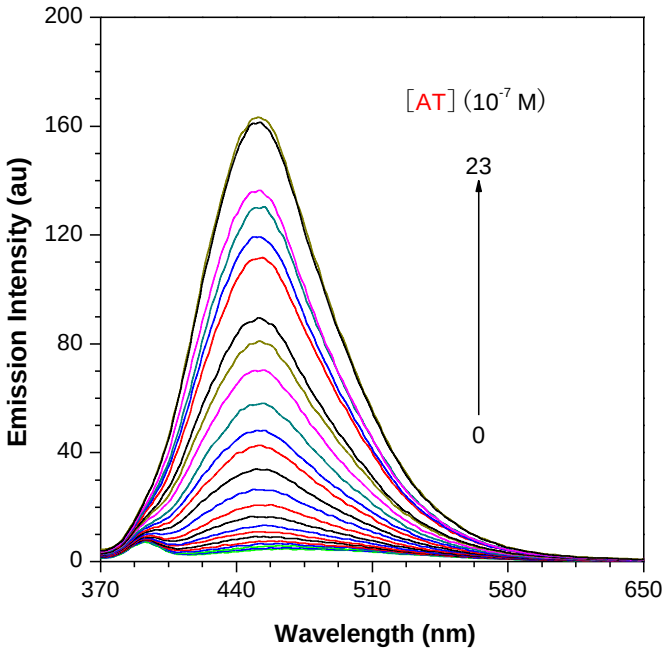


Figure S14. PL spectra of TPE-T (10 μM) in ethanol-water mixture (36:64, v/v) with different concentrations of AT. From bottom to top (μM): 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2 and 2.3. Excitation wavelength: 350 nm.

Primer: 5'ATATATATATATATATATATA3'

Figure S15

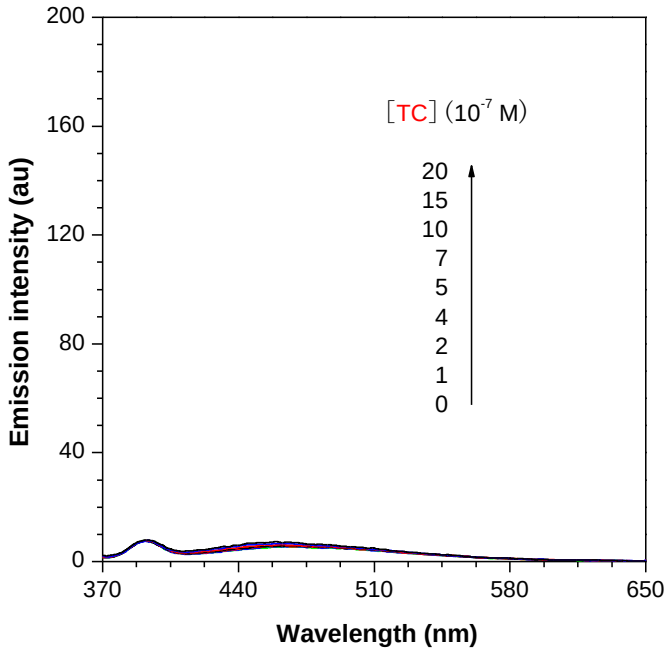


Figure S15. PL spectra of TPE-T (10 μM) in ethanol-water mixture (36:64, v/v) with different concentrations of TC. From bottom to top (μM): 0, 0.1, 0.2, 0.4, 0.5, 0.7, 1.0, 1.5 and 2.0. Excitation wavelength: 350 nm.

Primer: 5'TCTCTCTCTCTCTCTCTCT3'

Figure S16

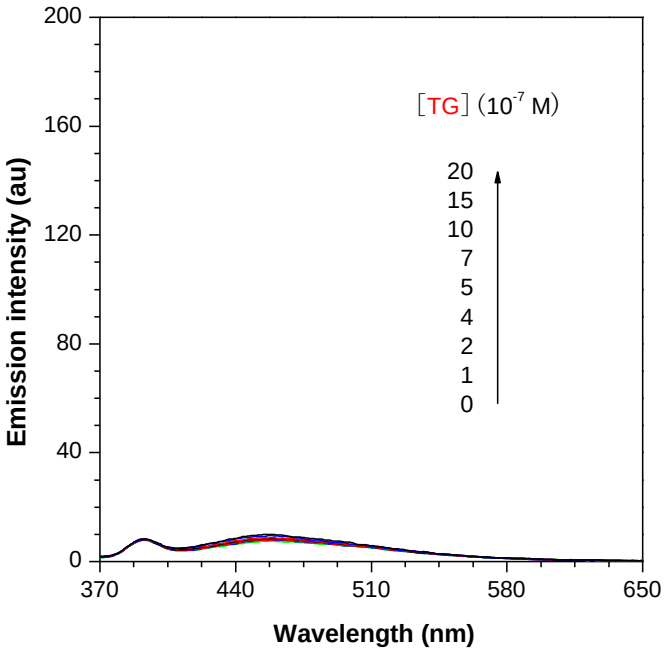


Figure S16. PL spectra of TPE-T (10 μM) in ethanol-water mixture (36:64, v/v) with different concentrations of TG. From bottom to top (μM): 0, 0.1, 0.2, 0.4, 0.5, 0.7, 1.0, 1.5 and 2.0. Excitation wavelength: 350 nm.

Primer: 5'TGTGTGTGTGTGTGTGTGTGT3'

Figure S17

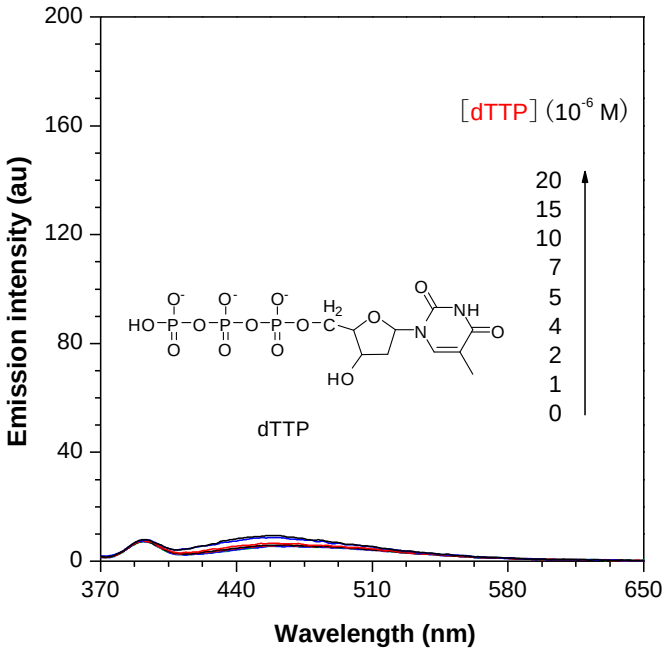


Figure S17. PL spectra of TPE-T (10 μM) in ethanol-water mixture (36:64, v/v) with different concentrations of dTTP. From bottom to top (μM): 0, 0.1, 0.2, 0.4, 0.5, 0.7, 1.0, 1.5 and 2.0. Inset: structure of dTTP. Excitation wavelength: 350 nm.

Figure S18

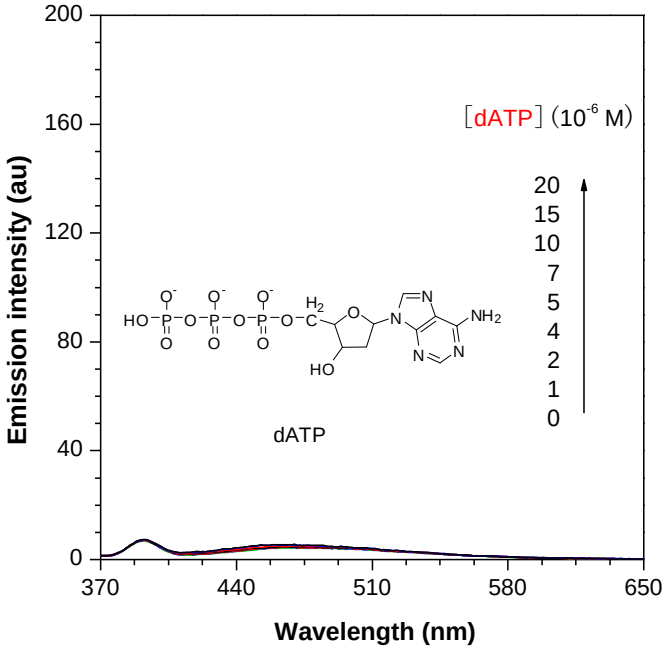


Figure S18. PL spectra of TPE-T (10 μM) in ethanol-water mixture (36:64, v/v) with different concentrations of dATP. From bottom to top (μM): 0, 0.1, 0.2, 0.4, 0.5, 0.7, 1.0, 1.5 and 2.0. Inset: structure of dATP. Excitation wavelength: 350 nm.

Figure S19

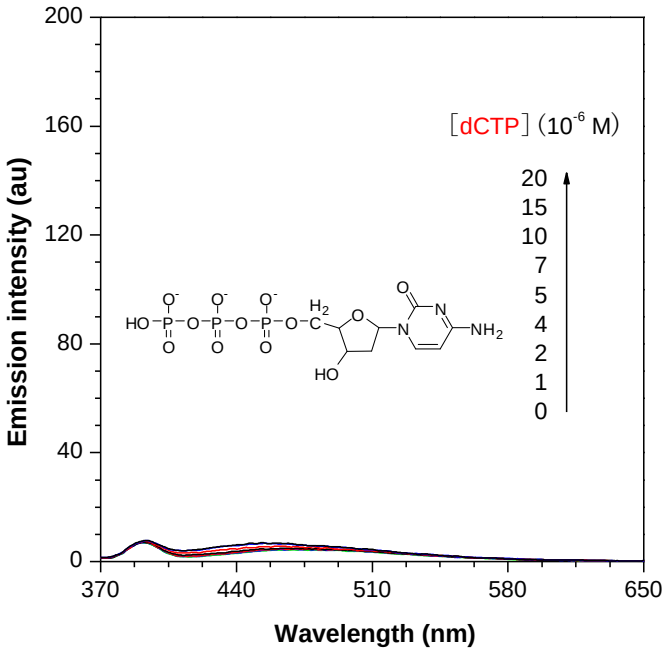


Figure S19. PL spectra of TPE-T (10 μM) in ethanol-water mixture (36:64, v/v) with different concentrations of dCTP. From bottom to top (μM): 0, 0.1, 0.2, 0.4, 0.5, 0.7, 1.0, 1.5 and 2.0. Inset: structure of dCTP. Excitation wavelength: 350 nm.

Figure S20

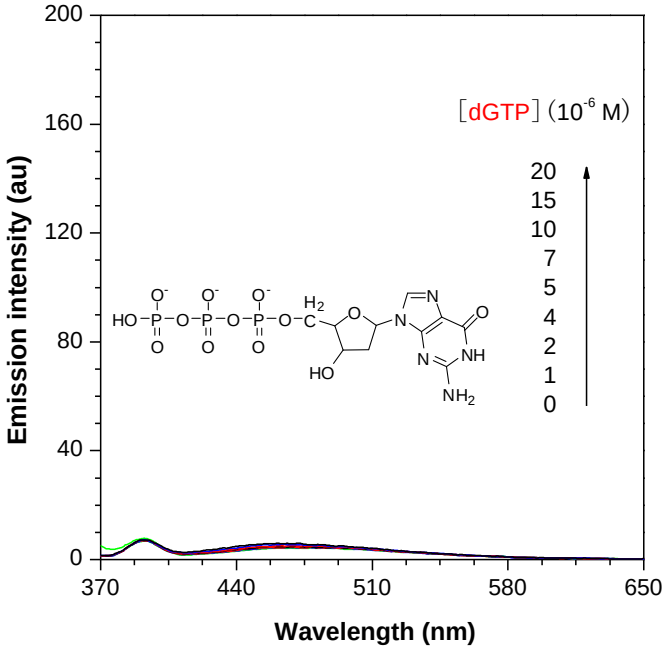


Figure S20. PL spectra of TPE-T (10 μM) in ethanol-water mixture (36:64, v/v) with different concentrations of dGTP. From bottom to top (μM): 0, 0.1, 0.2, 0.4, 0.5, 0.7, 1.0, 1.5 and 2.0. Inset: structure of dGTP. Excitation wavelength: 350 nm.

Figure S21

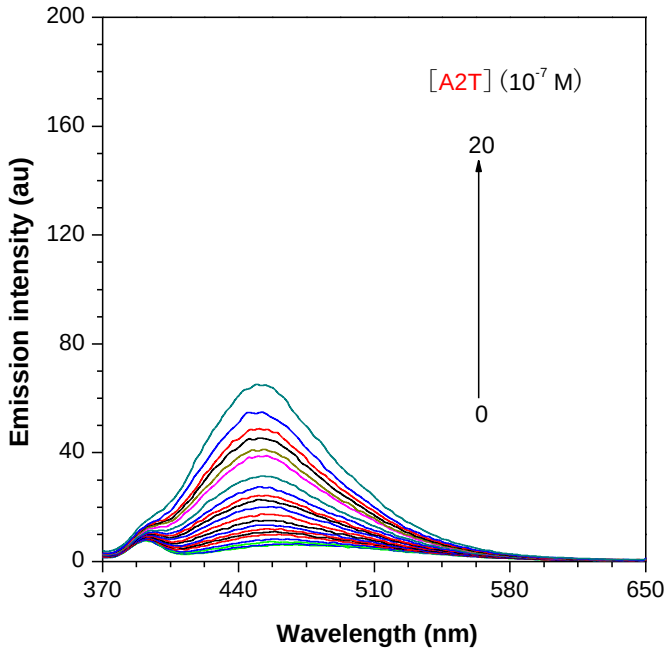


Figure S21. PL spectra of TPE-T (10 μM) in ethanol-water mixture (36:64, v/v) with different concentrations of A2T. From bottom to top (μM): 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 and 2.0. Excitation wavelength: 350 nm.

Primer: 5'ATTATATTATATTATATTATA3'

Figure S22

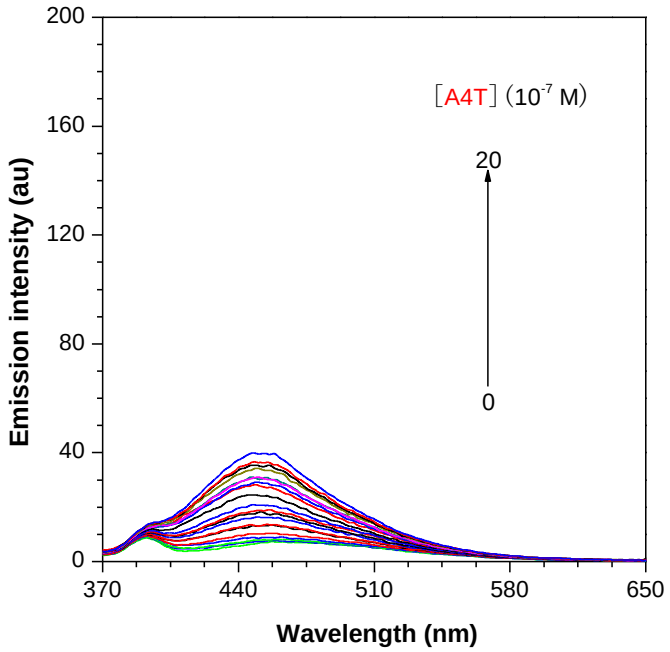


Figure S22. PL spectra of TPE-T (10 μM) in ethanol-water mixture (36:64, v/v) with different concentrations of A4T. From bottom to top (μM): 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 and 2.0. Excitation wavelength: 350 nm.

Primer: 5'ATTTTATTTTATTTTATTTTA3'

Figure S23

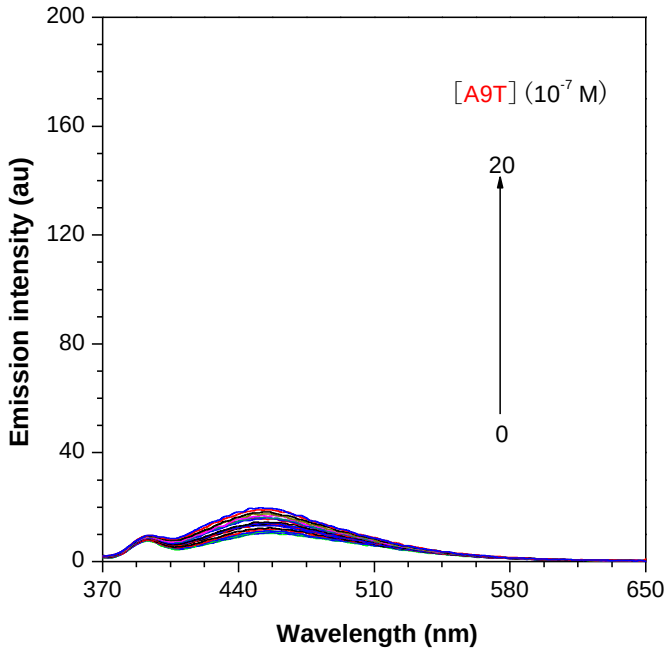


Figure S23. PL spectra of TPE-T (10 μM) in ethanol-water mixture (36:64, v/v) with different concentrations of A9T. From bottom to top (μM): 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 and 2.0. Excitation wavelength: 350 nm.

Primer: 5'ATTTTTTTTTATTTTTTTTTA3'

Figure S24

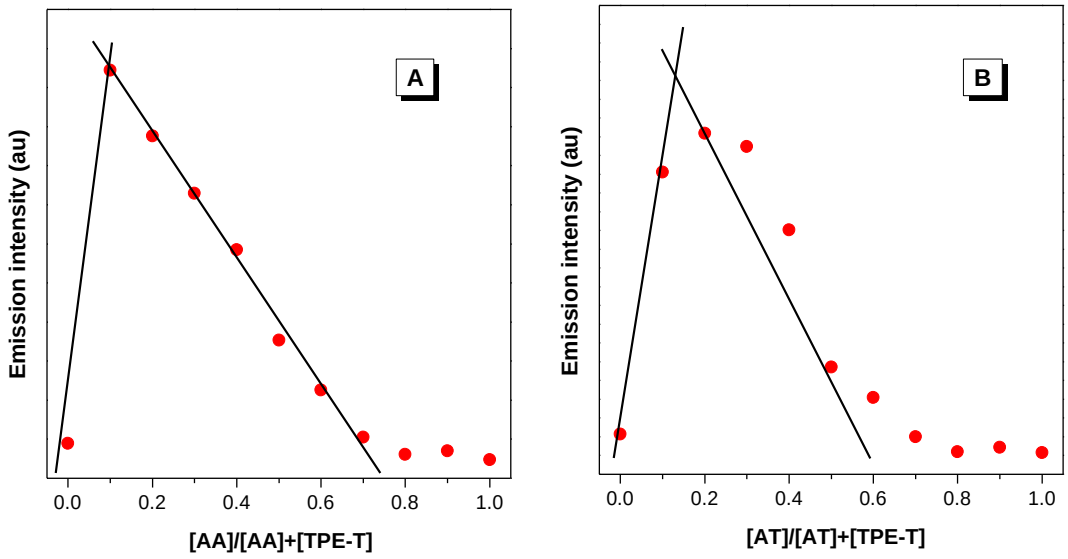


Figure S24. Job plot for determination of binding ratio of TPE-T to ssDNA, (A) AA and (B) AT. Total concentration of ssDNA and TPE-T was kept at 10 μM in ethanol-water mixture (36:64, v/v). Excitation wavelength: 350 nm.

Figure S25

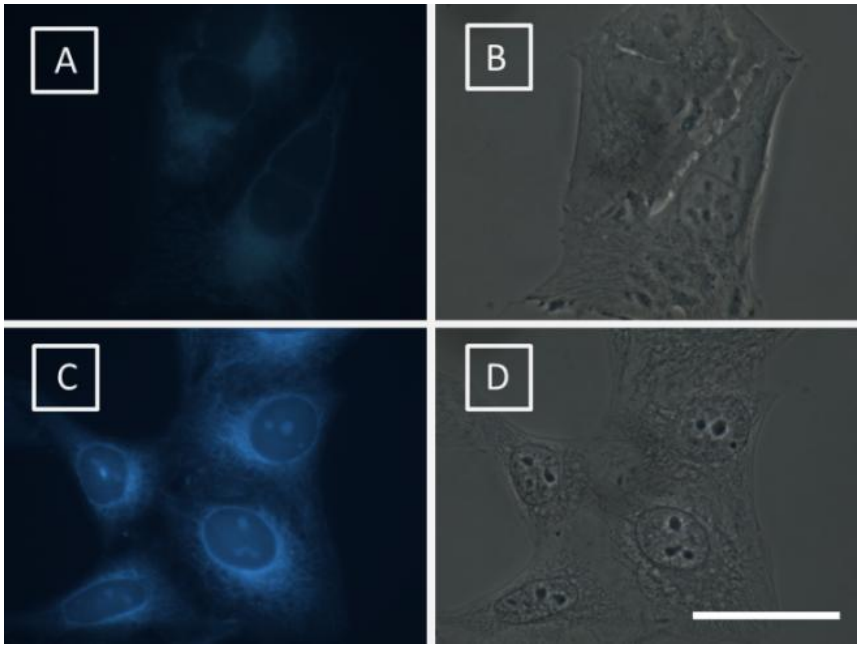


Figure S25. (A and C) Fluorescence and (B and D) bright-field images of (A and B) living and (C and D) fixed HeLa cells incubated with probe TPE-T (5 μg/mL) for 30 min. The HeLa cells were fixed with ethanol.