Electronic Supplementary Material (ESI) for ChemComm.
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Supporting Information

Detection of AAG repeats through DNA triplex–induced G-cluster formation

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Contents

Experimental details

Synthesis of oligonucleotides

Natural DNA and FAM- and BHQ-modified oligonucleotides (ODNs) were purchased from Integrated DNA Technologies (IDT). ^{Py}A-modified ODNs were synthesized on a CPG support (scale: 1 μmol; pore size: 1000 Å) using standard phosphoramidite methods and an automated DNA synthesizer (POLYGEN DNA-Synthesizer). The synthesized ODNs were cleaved from the solid support through treatment with 28–30% aqueous NH_4OH (1.0 mL) for 13 h at 55 °C. After filtration of the CPG, the crude products from the automated ODN synthesis were lyophilized and diluted with distilled water (1 mL). The ODNs were purified through reversed-phase HPLC (Merck LichoCART C18 column; 10 × 250 mm; 10 μm; pore size: 100 Å). The HPLC mobile phase was held isocratically for 10 min with 5% MeCN/0.1 M triethylammonium acetate (TEAA) (pH 7.2) at a flow rate of 2.5 mL/min. The gradient was then increased linearly over 10 min from 5 to 50% MeCN/0.1 M TEAA at the same flow rate. The fractions containing the purified ODNs were cooled and lyophilized. 80% Aqueous AcOH was added to the ODNs. After 1 h at ambient temperature, the AcOH was evaporated under reduced pressure. The residue was diluted with water (1 mL); this solution was then purified through HPLC using the same conditions as those described above. The ODNs were analyzed through reversed-phase HPLC using almost the same eluent system (detection: 254 nm). The products were characterized using MALDI-TOF mass spectrometry.

ODN sample preparation

For fluorescence spectroscopy and circular dichroism (CD) measurements, 1.5 μM (fluorescence spectroscopy) or 3.0 μM (CD) of the ODN was added to a solution of 1 M Tris-HCl buffer (pH 7.2, 50 μL), 1 M NaCl (100 μL), 200 mM $MgCl_2$ (50 μL), and water (in a 1.5-mL microtube) to give a total volume of 1 mL, followed by vortex-mixing. To prepare annealed samples, the mixtures in a buffer solution were heated at 90 °C for 3 min, then cooled slowly under ambient conditions for 4 h.

Fluorescence spectra

Fluorescence spectra were recorded using an Eclipse spectrometer (Varian). Samples for fluorescence spectroscopy were prepared in a quartz cell (path length: 1 cm). Parameters for fluorescence spectra: excitation wavelength, 385 nm; scanning range, 400–750 nm; excitation and emission slits, 2.5 nm/10 nm; data interval, 1.0 nm.

CD spectroscopy

CD spectra of the ODNs were recorded using a J-810 apparatus (JASCO) equipped with a temperature controller. For each sample, five spectral scans were accumulated at 20 °C over the wavelength range from 200 to 450 nm.

Native polyacrylamide gel electrophoresis (PAGE)

30% Acrylamide (5 mL), 5X TBE buffer (2 mL), 200 mM $MgCl_2$ (0.5 mL), and distilled water (2.5 mL) were mixed for the 15% non-denaturing gel. 40% Acrylamide (5 mL), 5X TBE buffer (2 mL), and distilled water (3 mL) were mixed for the 20% non-denaturing gel. Ammonium persulfate (12 mg) was added to the solution. To initiate gel formation, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED, 10 μL) was added. Aliquots of the samples (200 pmol) were used for PAGE. Dried samples were dissolved in a buffer/formamide mixture (1:1, v/v; 10 μL) for sample loading. 15% PAGE was performed at 90 V, 26 mA, 3 W, and 4 °C for 4 h. 20% PAGE was performed at 90 V, 26 mA, 3 W, and room temperature for 3.5 h. After running, the gels were mixed with Stains-All (Sigma–Aldrich) in formamide for 30 min. The gels were dried and exposed to light for visualization of the DNA bands.

Table S1. Oligonucleotide sequences for the ^{Py}A-modified G-cluster system

Name	Sequence	Calculated *m/z*	Observed *m/z*
GC1	5′-GCT TCC AT ^{Py}A ACT ACC TT-3′	5301.9828	5301.1760
GC2	5′-AAG GTA GT ^{Py}A AT GGA AGC-3′	5529.0183	5529.2477
GC3	5′-GCT TCC AT G^{Py}AG A CTA CCT T-3′	5960.0590	5960.7996
GC4	5′-AAG GTA GT G^{Py}AG A TGG AAG C-3′	6187.1245	6186.3937
GC5	5′-GCT TCC AT GG^{Py}AGG AC TAC CTT-3′	6618.1652	6618.6078
GC6	5′-AAG GTA GT GG^{Py}AGG AT GGA AGC-3′	6845.2307	6845.1692

Figure S1. (A) Fluorescence emission spectra and (B) photograph of solutions of the GC1+GC2, GC3+GC4, and GC5+GC6 duplexes.

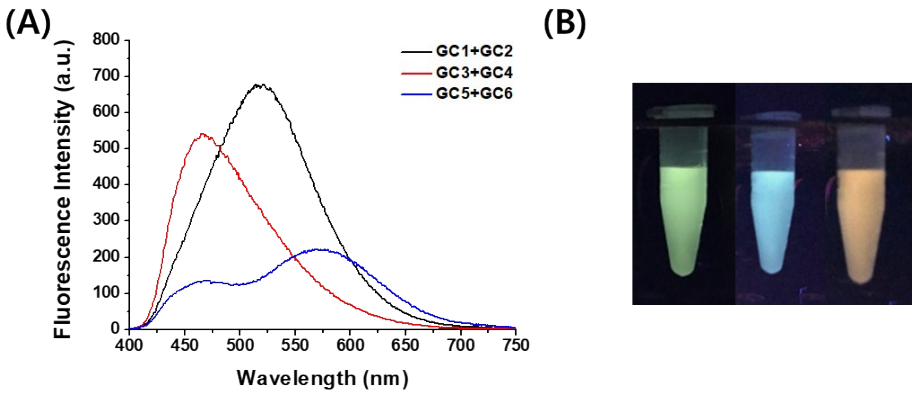


Figure S2. 20% Native polyacrylamide gel electrophoresis (PAGE)[a] images of GC1–GC6 (A) stained with Stains-All and (B) under UV irradiation. Lane 1: GC1; lane 2: GC2; lane 3: GC1+GC2; lane 4: GC3; lane 5: GC4; lane 6: GC3+GC4; lane 7: GC5; lane 8: GC6; lane 9: GC5+GC6.

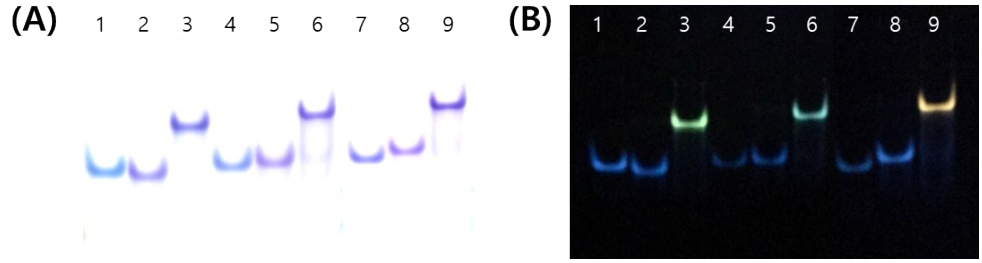


Figure S3. Circular dichroism (CD) spectra of GC1+GC2, GC3+GC4, and GC5+GC6.

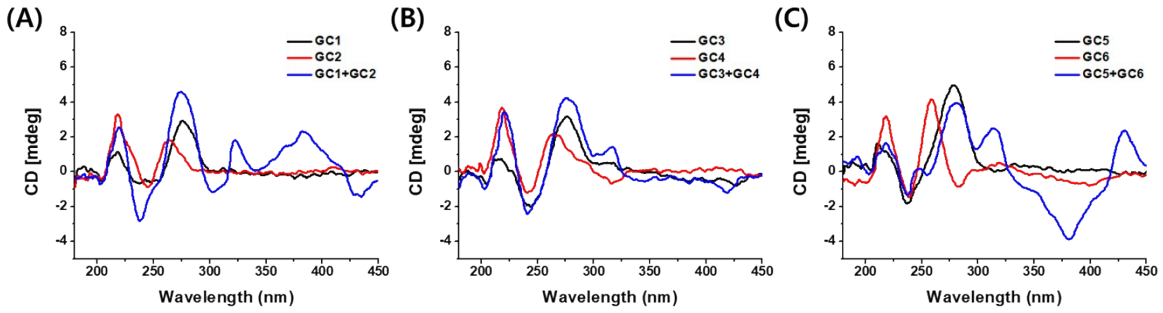


Table S2. Oligonucleotide sequences for the G-cluster triplex-forming probe.

Name	Sequence	Calculated *m/z*	Observed *m/z*
T4G0a	5′-CTT CTT CTT CTT GG^{Py}AGG-3′	5379.9372	5379.5981
T4G0b	5′- GG^{Py}AGG TTC TTC TTC TTC-3′	5379.9372	5379.2251
T4G1a	5′-CTT CTT CTT CTT GG^{Py}AGG T-3′	5683.9838	5686.9084
T4G1b	5′-A GG^{Py}AGG TTC TTC TTC TTC-3′	5692.9953	5694.7967
T4G2a	5′-CTT CTT CTT CTT GG^{Py}AGG AT-3′	5997.0419	5999.9090
T4G2b	5′-AT GG^{Py}AGG TTC TTC TTC TTC-3′	5997.0419	5999.6725
T4G3a	5′-CTT CTT CTT CTT GG^{Py}AGG AAT-3′	6310.1000	6313.6634
T4G3b	5′-ATT GG^{Py}AGG TTC TTC TTC TTC-3′	6301.0885	6306.7623
T4G4a	5′-CTT CTT CTT CTT GG^{Py}AGG C AAT-3′	6599.1469	6599.5795
T4G4b	5′-ATT G GG^{Py}AGG TTC TTC TTC TTC-3′	6630.1416	6630.0248
T4G5a	5′-CTT CTT CTT CTT GG^{Py}AGG A CAA T-3′	6912.2050	6916.1330
T4G5b	5′-ATT GT GG^{Py}AGG TTC TTC TTC TTC-3′	6934.1882	6937.7014
T4G6a	5′-CTT CTT CTT CTT GG^{Py}AGG T ACA AT-3′	7216.2516	7221.5794
T4G6b	5′-ATT GTA GG^{Py}AGG TTC TTC TTC TTC-3′	7247.2463	7253.0972
T4G4a-m	5′-CTT *TC*T CTT CTT GG^{Py}AGG C AAT-3′	6599.1469	6601.2239
T4G4b-m	5′-ATT G GG^{Py}AGG TTC TTC T*CT* TTC-3′	6630.1416	6630.9850
T4A4a	5′-CTT CTT CTT CTT AA^{Py}AAA C AAT-3′	6535.1669	6537.9799
T4A4b	5′-ATT G AA^{Py}AAA TTC TTC TTC TTC-3′	6566.1616	6567.7619
T4G4H	5′-AAG AAG AAG AAG TTT CTT CTT CTT CTT GG^{Py}AGG C AAT-3′	11331.9639	11330.8783

Target sequences

d$(AAG)_4$: 5′-AAG AAG AAG AAG-3′

AAG4-m: 5′-AAG AAG A*GA* AAG-3′

R12: 5′-TAC CAG TCA ATG-3′

Figure S4. 15% Native PAGE images of T4G4a and T4G4b (A) stained with Stains-All and (B) under UV irradiation. Lane 1: T4G4a; lane 2: T4G4b; lane 3: T4G4a+T4G4b; lane 4: T4G4a+T4G4b+d$(AAG)_4$; lane 5: T4G4a+d$(AAG)_4$; lane 6: T4G4b+d$(AAG)_4$; lane 7: d$(AAG)_4$.

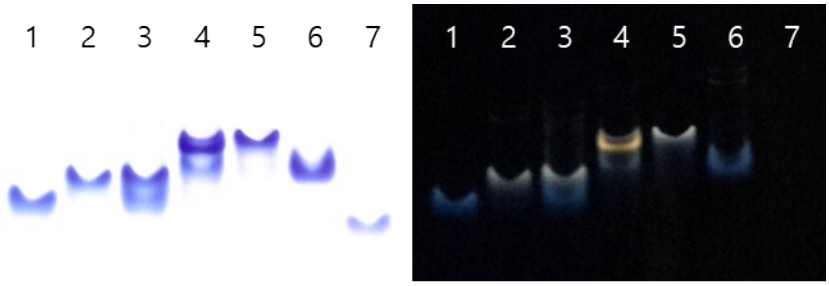


Figure S5. Fluorescence emission spectra of triplex probes featuring various numbers of base pairs, recorded in the absence and presence of $d(AAG)_4$. (A) T4G0a+T4G0b, (B) T4G1a+T4G1b, (C) T4G2a+T4G2b, (D) T4G3a+T4G3b, (E) T4G4a+T4G4b, (F) T4G5a+T4G5b, and (G) T4G6a+T4G6b.

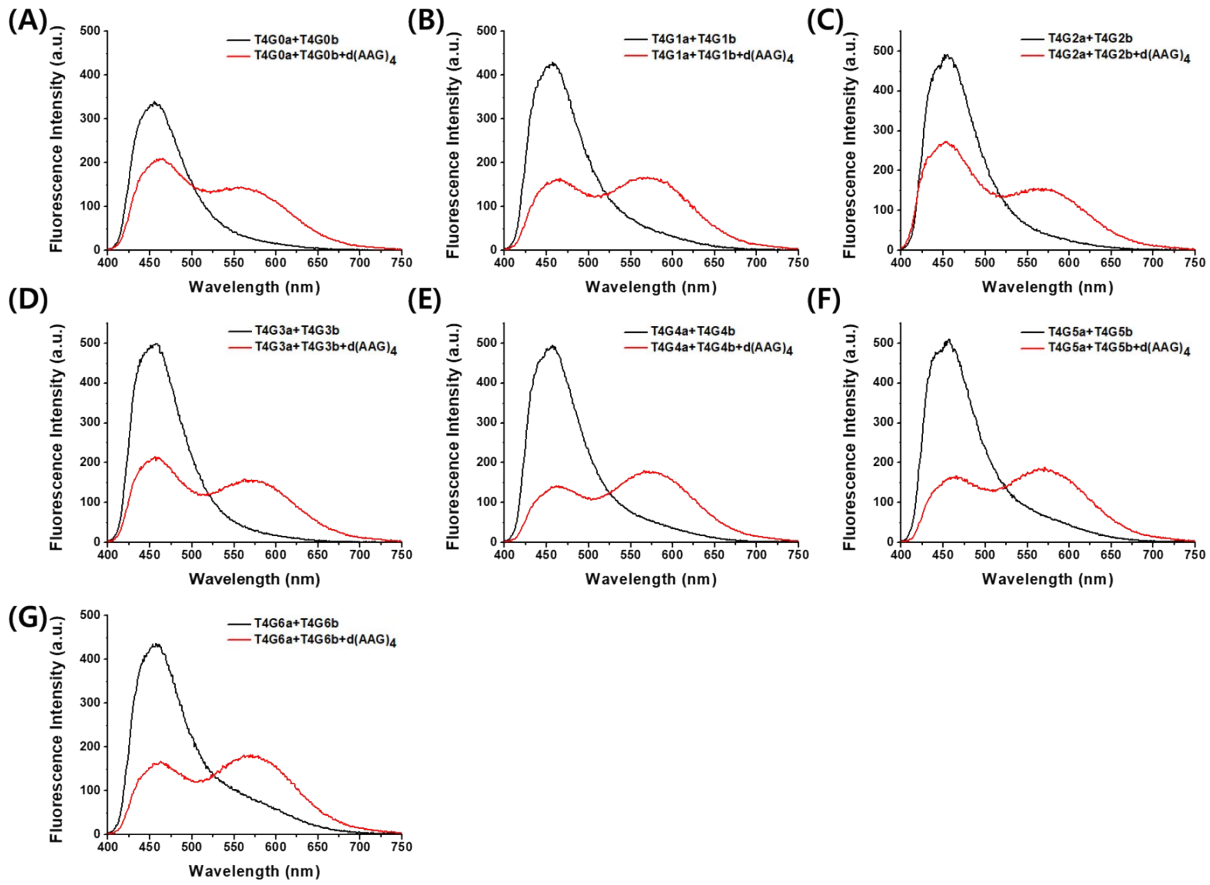


Table S3. Fluorescence emission spectral analysis of the probes.

Sample	1st Area (A_1)[a]	2nd Area (A_2)[b]	1st Area (%)	2nd Area (%)	Ratio (A_2/A_1)
T4G0a+T4G0b+d(AAG)$_4$	49.3	111.4	30.7	69.3	2.26
T4G1a+T4G1b+d(AAG)$_4$	53.3	148.3	26.4	73.6	2.78
T4G2a+T4G2b+d(AAG)$_4$	58.0	95.5	37.7	62.3	1.65
T4G3a+T4G3b+d(AAG)$_4$	55.2	112.8	32.9	67.1	2.04
T4G4a+T4G4b+d(AAG)$_4$	53.2	169.0	24.0	76.0	3.17
T4G5a+T4G5b+d(AAG)$_4$	51.6	158.1	24.6	75.4	3.06
T4G6a+T4G6b+d(AAG)$_4$	51.6	152.2	25.3	74.7	2.95
T4A4a+T4A4b+d(AAG)$_4$	–	–	–	–	–
T4G4a-m+T4G4b-m+AAG4-m	52.1	189.8	21.5	78.5	3.64
T4G4H+T4G4b	35.2	85.4	29.2	70.8	2.43

[a] Area of the fluorescence emission peak at 455 nm.

[b] Area of the fluorescence emission peak at 580 nm.

Figure S6. Fluorescence emission spectra of T4G4a+T4G4b recorded at acidic and neutral pH. Buffer: 10 mM sodium phosphate, 10 mM $MgCl_2$.

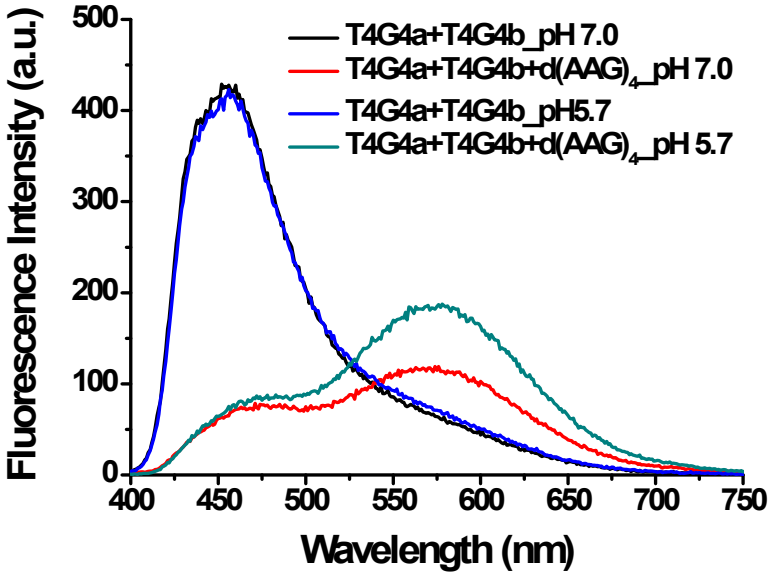


Figure S7. 15% Native PAGE images of T4G4a and T4G4b (A) stained with Stains-All and (B) under UV irradiation. Lane 1: T4G4a; lane 2: T4G4b; lane 3: T4G4a+T4G4b; lane 4: T4G4a+T4G4b+d$(AAG)_4$ (0.5 eq.); lane 5: T4G4a+T4G4b+d$(AAG)_4$ (1.0 eq.); lane 6: T4G4a+T4G4b+d$(AAG)_4$ (1.5 eq.); lane 7: T4G4a+T4G4b+d$(AAG)_4$ (2.0 eq.); lane 8: d$(AAG)_4$.

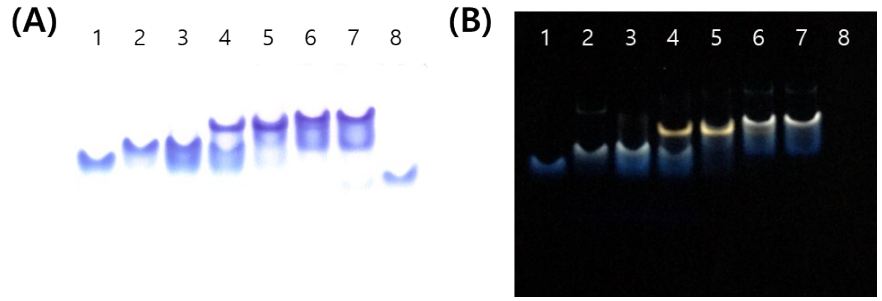


Figure S8. Fluorescence emission spectra of T4G4a-m+T4G4b-m in the absence and presence of AAG4-m.

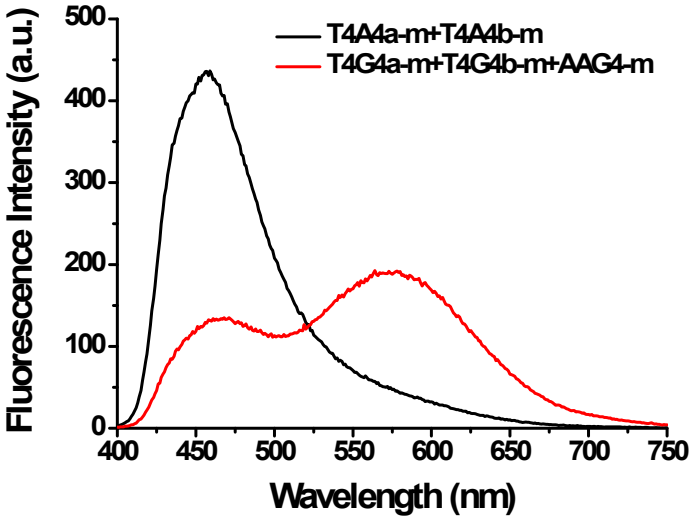


Figure S9. 15% Native PAGE images of T4G4a-m and T4G4b-m (A) stained with Stains-All and (B) under UV irradiation. Lane 1: T4G4a-m; lane 2: T4G4b-m; lane 3: T4G4a-m+T4G4b-m; lane 4: T4G4a-m+T4G4b-m+AAG4-m; lane 5: T4G4a-m+AAG4-m; lane 6: T4G4b-m+AAG4-m; lane 7: AAG4-m.

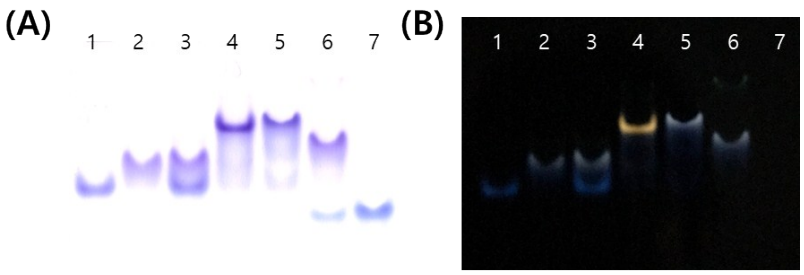


Figure S10. 15% Native PAGE images of T4G4a-m and T4G4b-m (A) stained with Stains-All and (B) under UV irradiation. Lane 1: T4G4a-m; lane 2: T4G4b-m; lane 3: T4G4a-m+T4G4b-m; lane 4: T4G4a-m+T4G4b-m+AAG4-m (0.5 eq.); lane 5: T4G4a-m+T4G4b-m+AAG4-m (1.0 eq.); lane 6: T4G4a-m+T4G4b-m+AAG4-m (1.5 eq.); lane 7: T4G4a-m+T4G4b-m+AAG4-m (2.0 eq.); lane 8: AAG4-m.

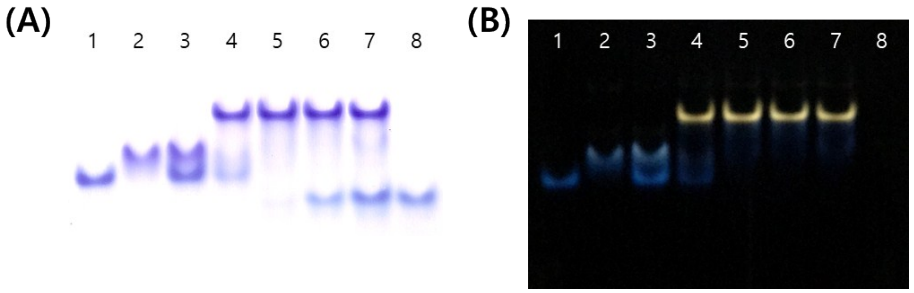


Figure S11. CD spectra of (A) T4G4a+T4G4b and (B) T4G4H recorded in the presence and absence of $d(AAG)_4$ and T4G4b, respectively.

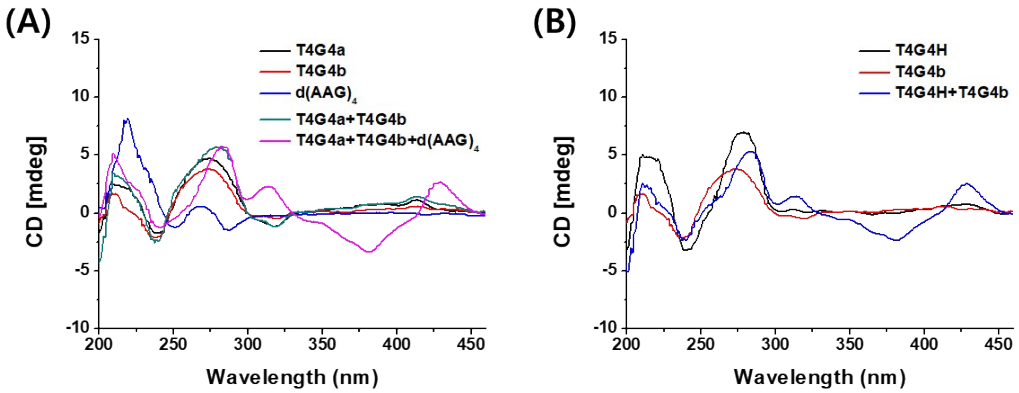


Figure S12. (A) Schematic representation and (B) fluorescence emission spectra of the hairpin-type triplex probe T4G4H.

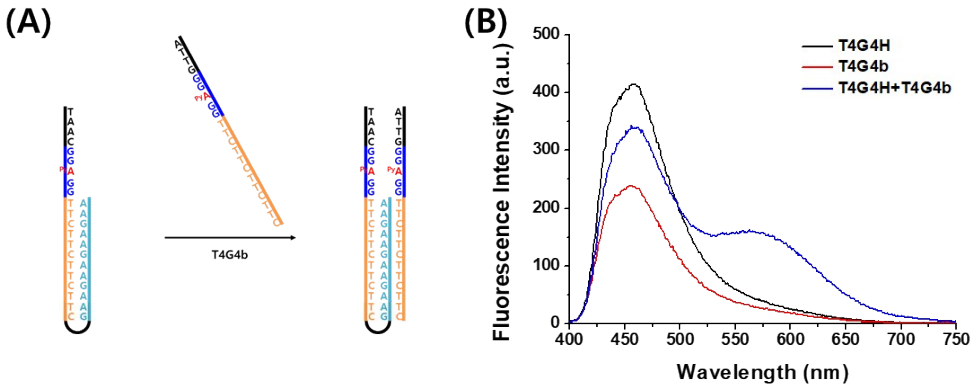


Figure S13. 15% Native PAGE images of T4G4H and T4G4b (A) stained with Stains-All and (B) under UV irradiation. Lane 1: T4G4H; lane 2: T4G4b; lane 3: T4G4H+T4G4b.

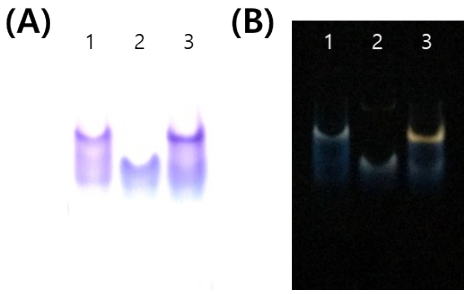


Figure S14. (A) Schematic representation of the FAM-/BHQ-modified triplex-forming probe; (B) fluorescence emission spectra of T4N4a-F+T4N4b-Q in the absence and presence of $d(AAG)_4$. Excitation wavelength: 495 nm; Ex/Em slit: 2.5/5 nm.

Name	Sequence
T4N4a-F	5′-**FAM**-CTT CTT CTT CTT GCG CGC AAT-3′
T4N4b-Q	5′-ATT GCG CGC TTC TTC TTC TTC-**Q**-3′

FAM: 6-fluorescein

Q: blackhole quencher

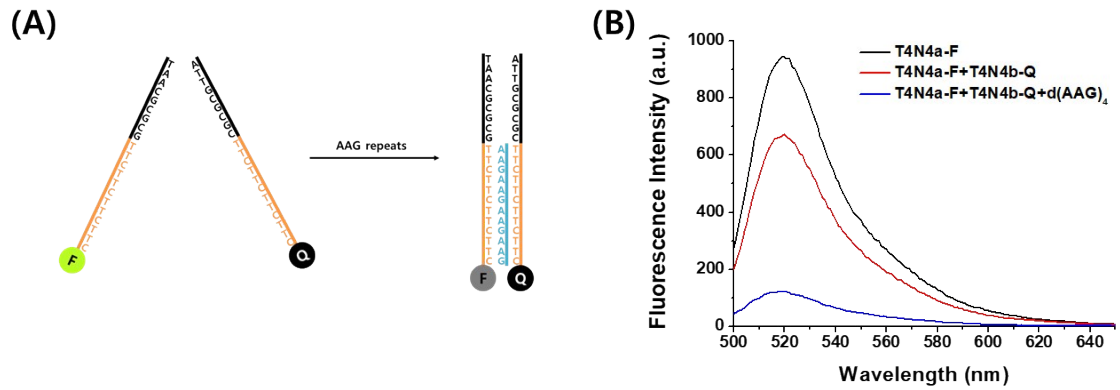


Figure S15. 15% Native PAGE images of T4N4a-F and T4N4b-Q (A) stained with Stains-All and (B) under UV irradiation. Lane 1: T4N4a-F; lane 2: T4N4b-Q; lane 3: T4N4a-F+T4N4b-Q; lane 4: T4N4a-F+T4N4b-Q+$d(AAG)_4$; lane 5: $d(AAG)_4$.

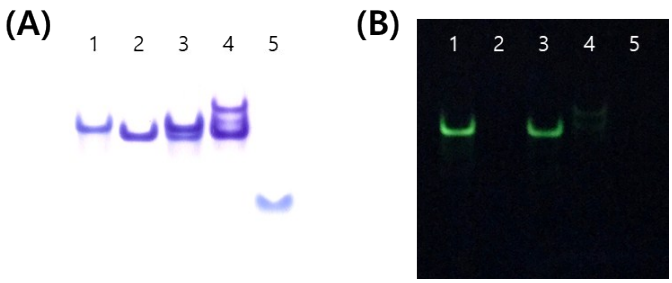


Figure S16. Fluorescence emission spectra of T4A4a+T4A4b recorded in the absence and presence of $d(AAG)_4$. Excitation wavelength: 385 nm; Ex/Em slit: 2.5/5 nm.

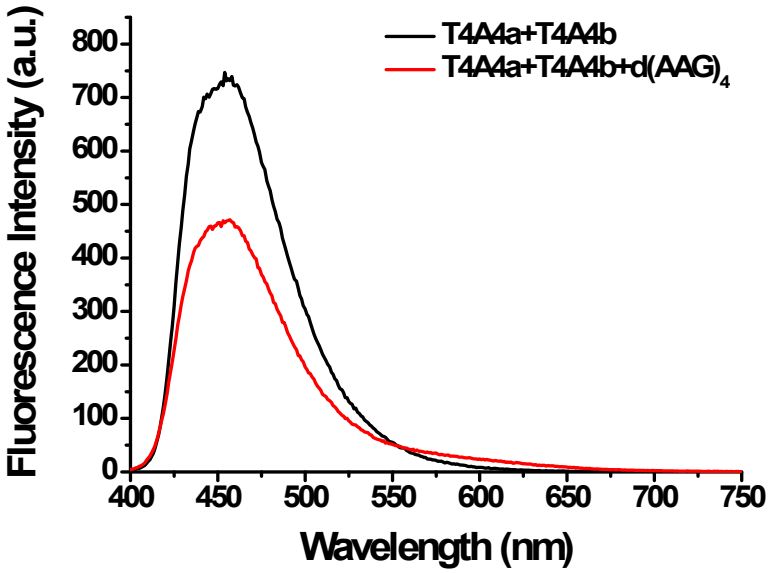


Figure S17. 15% Native PAGE images of T4A4a and T4A4b (A) stained with Stains-All and (B) under UV irradiation. Lane 1: T4A4a; lane 2: T4A4b; lane 3: T4A4a+T4A4b; lane 4: T4A4a+T4A4b+$d(AAG)_4$; lane 5: T4A4a+$d(AAG)_4$; lane 6: T4A4b+$d(AAG)_4$; lane 7: $d(AAG)_4$.

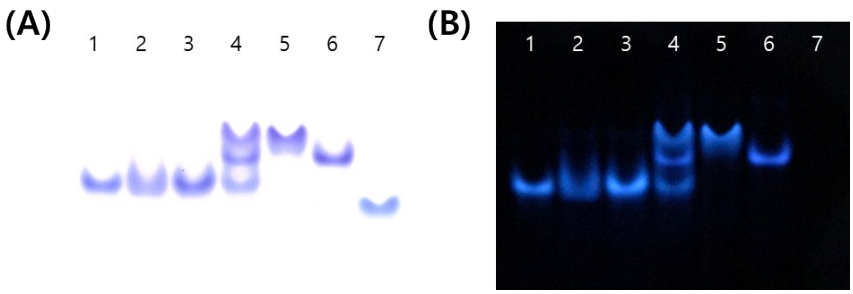


Figure S18. Selectivity of the probe T4G4a+T4G4b toward the excess amount of various repeat and random sequences. (A) Fluorescence emission spectra; (B) fluorescence intensity at 580 nm. Sample concentration: 1.5 μM of T4G4a and T4G4b and 4.5 μM of repeat or random sequences.

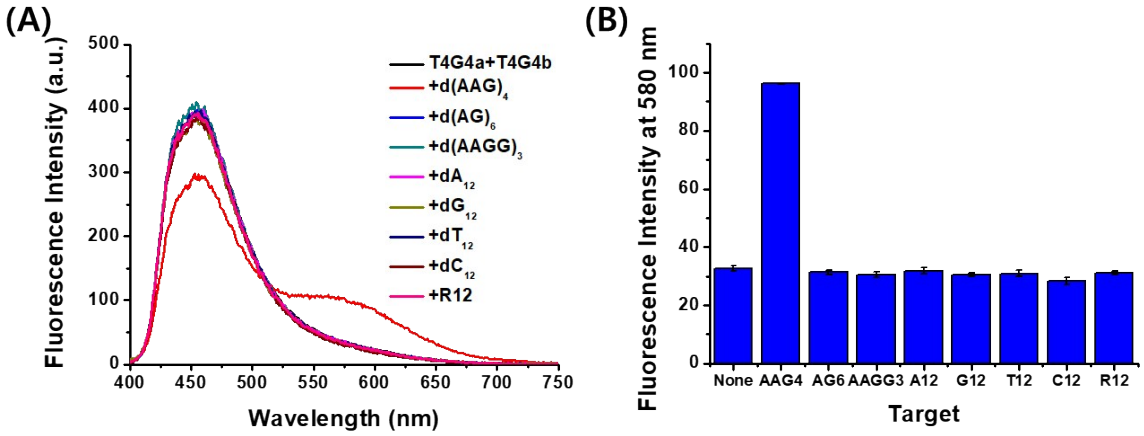


Figure S19. Fluorescence emission spectra of T4G4a+T4G4b recorded in the absence and presence of repeat and random sequence mixture. Sample concentration: 1.5 μM of T4G4a, T4G4b, $d(AAG)_4$, $d(AG)_6$, $d(AAGG)_3$, dA_{12}, dG_{12}, dT_{12}, dC_{12} and R12.

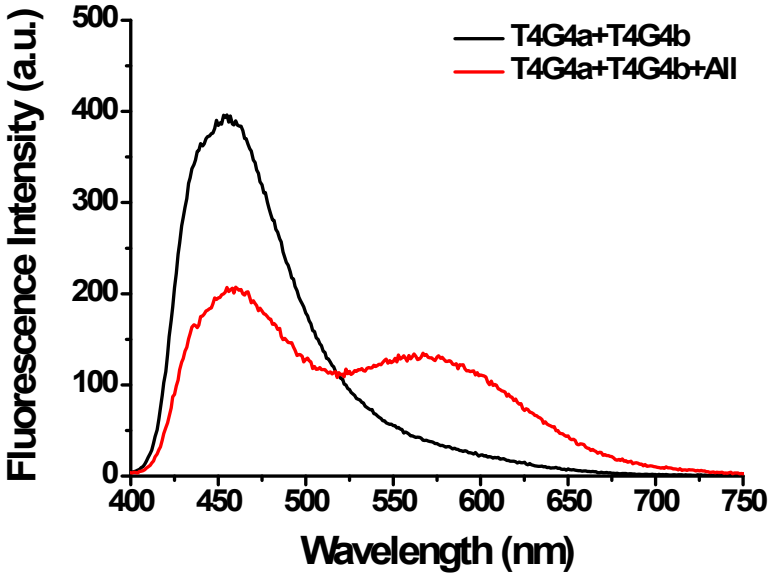
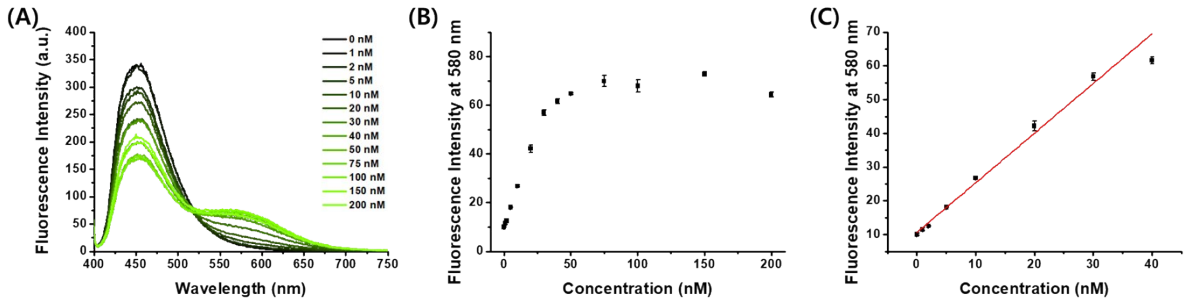


Figure S20. Concentration-dependent fluorescence emission spectra and analyses of T4G4a+T4G4b in response to $d(AAG)_4$. Sample concentration: 100 nM of T4G4a and T4G4b and 200 nM of $d(AG)_6$, $d(AAGG)_3$, dA_{12}, dG_{12}, dT_{12}, dC_{12} and R12 mixture; Ex./Em. Slit: 10/10 nm.



Limit of detection (LOD) : 7.1 nM