Electronic Supporting Information

A G-quadruplex-based, label-free, switch-on luminescent detection assay for Ag^+ ions based on the exonuclease III-mediated digestion of C-Ag^+-C DNA†

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Experimental section

Materials. Reagents, unless specified, were purchased from Sigma Aldrich (St. Louis, MO) and used as received. Iridium chloride hydrate ($IrCl_3.xH_2O$) was purchased from Precious Metals Online (Australia). exonuclease III (ExoIII) were purchased from New England Biolabs Inc. (Beverly, MA, USA). All oligonucleotides were synthesized by Techdragon Inc. (Hong Kong, China). DNA sequences used in this project:

ssDNA: 5′-$C_2AGT_2CGTAGTA_2C_3$-3′, ds17: 5′-$C_2AGT_2CGTAGTA_2C_3$-3′ and 5′-$G_3T_2ACTACGA_2CTG_2$-3′, Pu27: 5′-$TG_4AG_3TG_4AG_3TG_4A_2G_2$-3′, Pu22: 5′-$GAG_3TG_4AG_3TG_4A_2G$-3′, Hairpin DNA: 5′-$C_3TG_4AG_3TG_4AG_3TG_4A_2G_2CAGA_2G_2ATA_2C_2T_2C_4AC_3TC_4AC_3TC_4AC_3$-3′, DNA used in Figure 3b: 5'-A_5TCGATCGATACACAGCACAC$_3$-3' and 5'-C_3TGTGCTGTGTATCGATCGA-3'.

General experimental. Mass spectrometry was performed at the Mass Spectroscopy Unit at the Department of Chemistry, Hong Kong Baptist University, Hong Kong (China). Deuterated solvents for NMR purposes were obtained from Armar and used as received. ^1H and ^{13}C NMR were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz (^1H) and 100 MHz (^{13}C). ^1H and ^{13}C chemical shifts were referenced internally to solvent shift (CD_3CN: ^1H, δ 1.94, ^{13}C δ 118.7; d_6-DMSO: ^1H δ 2.50, ^{13}C δ 39.5). Chemical shifts (δ) are quoted in ppm, the downfield direction being defined as positive. Uncertainties in chemical shifts are typically ±0.01 ppm for ^1H and ±0.05 for ^{13}C. Coupling constants are typically ± 0.1 Hz for ^1H-^1H and ±0.5 Hz for ^1H-^{13}C couplings. The following abbreviations are used for convenience in reporting the multiplicity of NMR resonances: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. All NMR data was acquired and processed using standard Bruker software (Topspin).

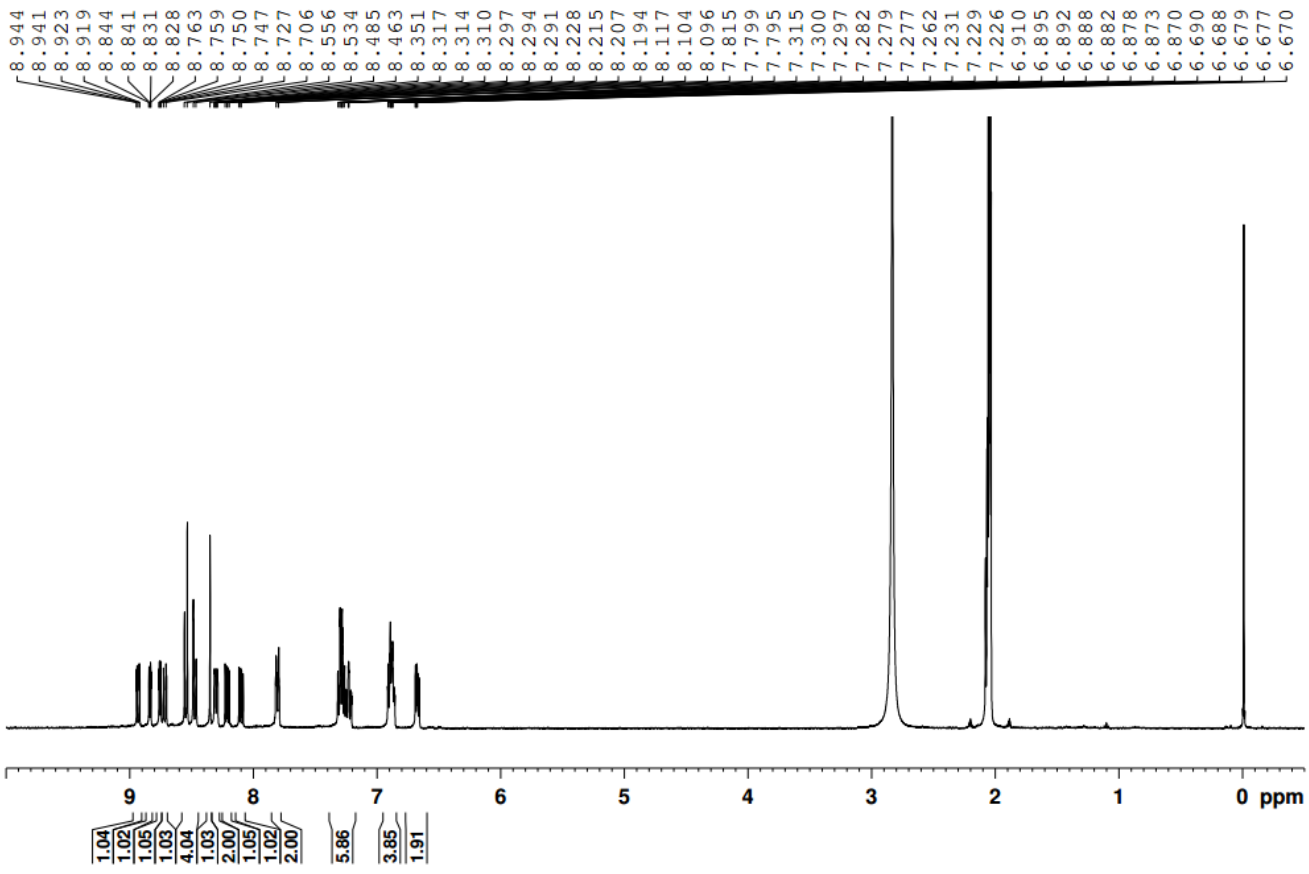
Photophysical measurement. Emission spectra and lifetime measurements for complexes were performed on a PTI TimeMaster C720 Spectrometer (Nitrogen laser: pulse output 337 nm) fitted with a 380 nm filter. Error limits were estimated: λ (±1 nm); τ (±10%); φ (±10%). All solvents used for the lifetime measurements were degassed using three cycles of freeze-vac-thaw. Luminescence quantum yields were determined using the method of Demas and Crosby $[Ru(bpy)_3][PF_6]_2$ in degassed acetonitrile as a standard reference solution ($\Phi_r = 0.062$) and calculated according to the following equation: $\Phi_s = \Phi_r(B_r/B_s)(n_s/n_r)^2(D_s/D_r)$ where the subscripts s and r refer to sample and reference standard solution

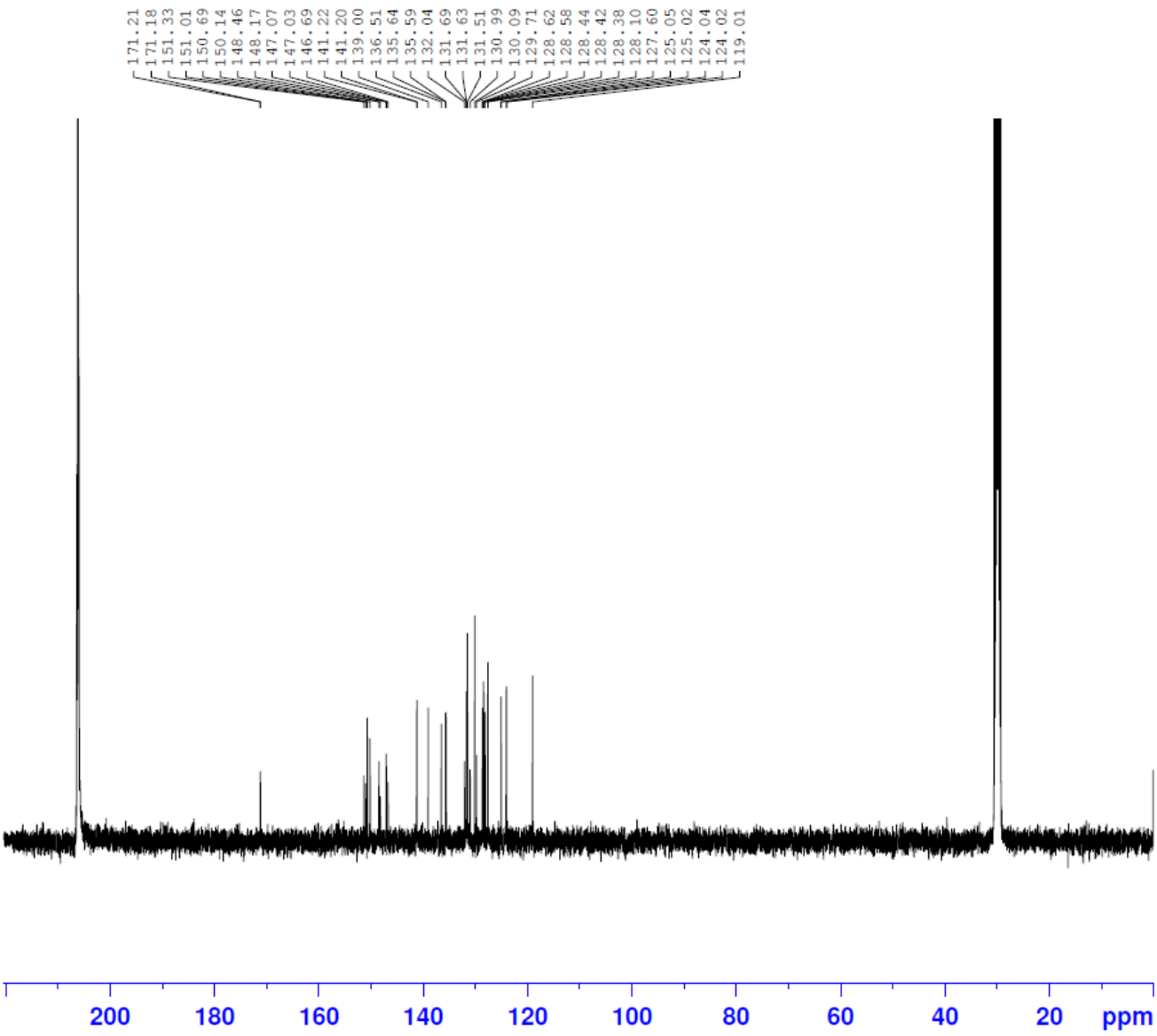
respectively, n is the refractive index of the solvents, D is the integrated intensity, and Φ is the luminescence quantum yield. The quantity B was calculated by $B = 1 - 10^{-AL}$, where A is the absorbance at the excitation wavelength and L is the optical path length.[1]

Synthesis

The complexes **2**–**4**,[2-4] **5**,[5] **6**,[6] **7**,[2] **8**,[2] and **9**–**10**[2, 7] were prepared according to (modified) literature methods. All complexes are characterized by ^{1}H-NMR, ^{13}C-NMR and high resolution mass spectrometry (HRMS).

Complex **1**. Yield: 62%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.93 (d, J = 8.4 Hz, 1H), 8.83 (d, J = 5.2 Hz, 1H), 8.75 (d, J = 5.2 Hz, 1H), 8.71 (d, J = 8.4 Hz, 1H), 8.56-8.46 (m, 4H), 8.35 (s, 1H), 8.30 (d, J = 8.0 Hz, 2H), 8.21 (q, J = 3.2 Hz, 1H), 8.10 (q, J = 3.2 Hz, 1H), 7.80 (d, J = 8.0 Hz, 2H), 7.31-7.21 (m, 6H), 6.91-6.85 (m, 4H), 6.69-6.66 (m, 2H); ^{13}C NMR (400 MHz, Acetone-d_6) δ 171.21, 171.18, 151.33, 151.01, 150.69, 150.14, 148.46, 148.17, 147.07, 147.03, 146.69, 141.22, 141.20, 139.00, 136.51, 135.64, 135.59, 132.04, 131.69, 131.63, 131.51, 130.99, 130.09, 129.71, 128.62, 128.58, 128.44, 128.42, 128.38, 128.10, 127.60, 125.05, 125.02, 124.04, 124.02, 119.01; HRMS: Calcd. for $C_{42}H_{27}ClIrN_4$ $[M–PF_6]^+$: 815.1553 Found: 815.1550; Anal. ($C_{42}H_{27}ClF_6IrN_4P·3H_2O$) C, H, N: calcd 49.73, 3.28, 5.52; found 49.51, 3.1, 5.72.

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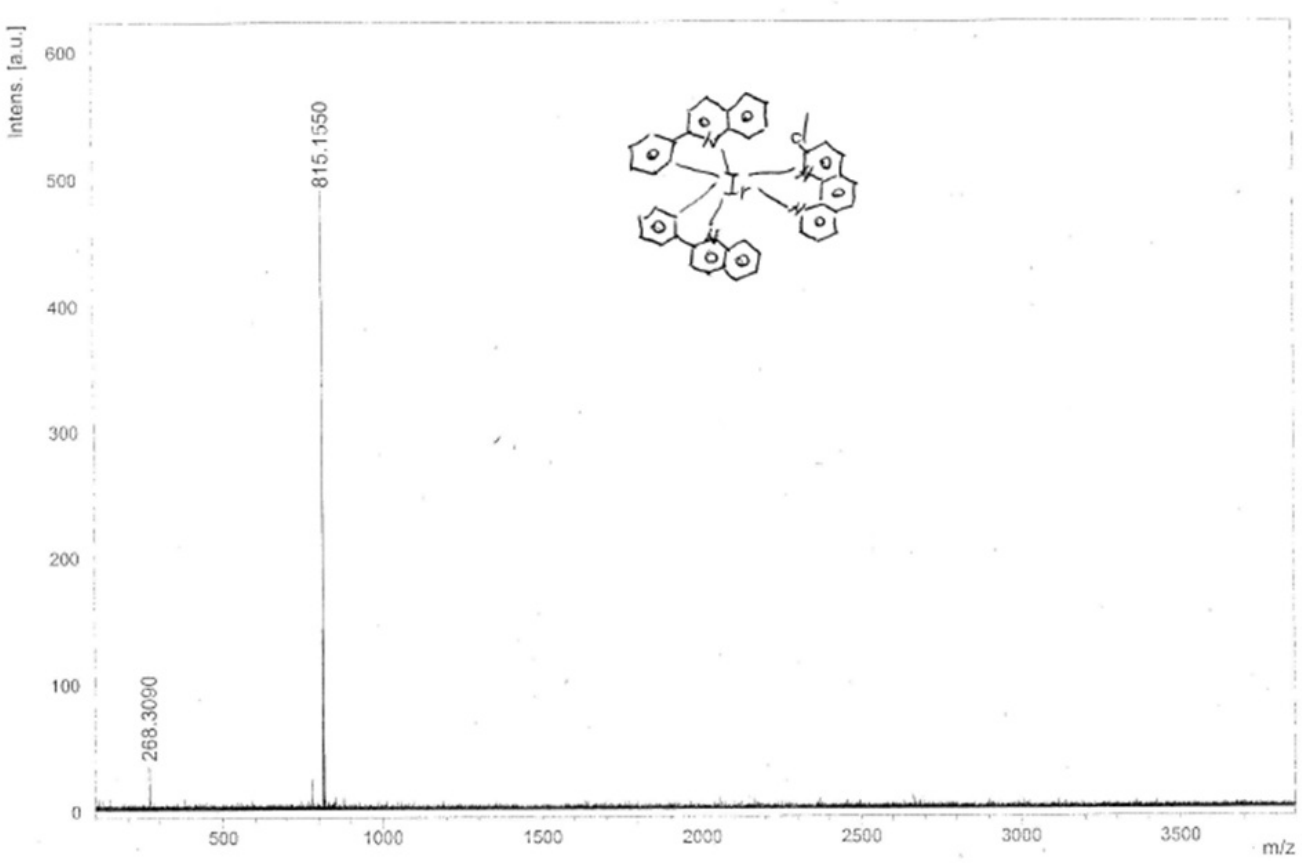


Luminescence response of complexes towards different forms of DNA.
The G-quadruplex DNA-forming sequences were annealed in Tris-HCl buffer (20 mM Tris, 100 mM KCl, pH 7.0) and were stored at –20 °C before use. Complexes (1 μM) were added to 5 μM of ssDNA, ctDNA or G-quadruplex DNA in Tris-HCl buffer (20 mM Tris, pH 7.0).

FRET melting assay.
The ability of **1** to stabilize G-quadruplex DNA was investigated using a fluorescence resonance energy transfer (FRET) melting assay. The labelled G-quadruplex-forming oligonucleotide F21T (5′-*FAM*-d($G_3[T_2AG_3]_3$)-*TAMRA*-3′; donor fluorophore *FAM*: 6-carboxyfluorescein; acceptor fluorophore *TAMRA*: 6- carboxytetramethylrhodamine) was diluted to 200 nM in a potassium cacodylate buffer (100 mM KCl, pH 7.0), and then heated to 95 °C in the presence of the indicated concentrations of 11. The labeled duplex-forming oligonucleotide F10T (5′-FAM-dTATAGCTA-HEG-TATAGCTATAT-TAMRA-3′) (HEG linker: [(-CH_2-CH_2-O-)$_6$]) was treated in the same manner, except that the buffer was changed to 10 mM lithium cacodylate (pH 7.4). Fluorescence readings were taken at intervals of 0.5 °C over the range of 25 to 95 °C.

Absorption titration. A solution of complex **1** (20 μM) was prepared in Tris-HCl buffer (20 mM, pH 7.0). Aliquots of a millimolar stock solution of pre-annealed Pu27 (0–9 μM), ds26 (0–9 μM), or ssDNA CCR5-DEL (0–9 μM) were added. Absorption spectra were recorded in the spectral range λ = 200–600 nm after equilibration at 20.0 °C for 10 min. The intrinsic binding constant, *K*, was determined from a plot of $D/\Delta\varepsilon$ap vs *D* according to equation (**1**):[8]

$$D/\Delta\varepsilon\text{ap} = D/\Delta\varepsilon + 1/(\Delta\varepsilon \times K) \quad (\mathbf{1})$$

where D is the concentration of DNA, $\Delta\varepsilon ap = |\varepsilon A - \varepsilon F|$, $\varepsilon A = A_{obs}/[ligand]$, and $\Delta\varepsilon = |\varepsilon B - \varepsilon F|$; εB and εF correspond to the extinction coefficients of DNA−ligand adduct and unbound ligand, respectively.

G4-FID assay.

The FID assay was performed as previously described. 0.25 μM pre-folded DNA target is mixed with thiazole orange (0.50 μM for Pu27 and ds17) in Tris-HCl buffer (10 mM, pH 7.3) containing 100 mM KCl, in a total volume of 3 mL. Each ligand addition is followed by a 3-min equilibration period after which the fluorescence spectrum is recorded. The percentage of displacement is calculated from the fluorescence area (FA, 510–750 nm, excitation, 501 nm).[9]

Ag^+ ion detection.

The DNA substrate (100 μM) was dissolved in Tris-acetate buffer (10 mM, pH 7). The solution was heated to 95 °C for 10 min and then cooled at 0.1 °C/s to room temperature to allow the formation of the hairpin structure. The annealed product was stored at –20 °C before use. For Ag^+ detection, 50 μL of the DNA substrate in Tris-acetate buffer (10 mM, pH 7) and the indicated concentrations of Ag^+ ions were added to a solution containing 20 U/mL of ExoIII. After incubation at 37 °C for 30 min, the reaction was stopped by heat inactivation (70 °C for 20 min), and complex **1** (1 μM) was added to the solution. The mixture was cooled down and was subsequently diluted using Tris buffer (10 mM Tris, 50 mM KNO_3, pH 7.0) to a final volume of 500 μL. Finally, 1 μM of complex **1** was added to the mixture. Emission spectra were recorded in the 450−750 nm range using an excitation wavelength of 310 nm.

Table S1. Photophysical properties of complex **1**−**10** in CH_3CN at 298 K.

Complex	Quantum yield	λ_{em} / nm	Lifetime / μs	UV/vis absorption λ_{abs} / nm (ε / $dm^3mol^{-1}cm^{-1}$)
1	0.1363	560	4.80	209 (6.5×10^4), 232(4.7×10^4), 275 (5.8×10^4), 336 (1.6×10^4)
2	0.382	566	4.84	228 (6.7×10^3), 282 (1.2×10^4)
3	0.2573	569	186	326 (2.06×10^4), 417 (5.7×10^3)
4	0.04179	566	4.7	330 (6.9×10^3), 419 (1.89×10^3)
5	0.0783	588	4.45	212 (1.6×10^5), 250 (1.1×10^5), 310 (4.7×10^4), 413 (1.1×10^4)
6	0.35	651	3.61	260 (4.57×10^4), 324 (3.74×10^4), 429 (5.24×10^3)
7	0.05493	561	0.98	277 (1.46×10^4), 322 (6.8×10^3), 416 (1.77×10^3)
8	0.10674	535	4.27	267 (1.96×10^4), 299 (1.33×10^4), 322 (7.67×10^3), 429 (1.4×10^3)
9	0.05722	577	0.74	261 (3.3×10^4), 268 (3.2×10^3), 296 (1.9×10^4), 371 (9.05×10^3)
10	0.090	586	4.37	227 (5.41×10^4), 267 (9.97×10^4)

Table S2. Quantum yield of complex **1** in Tris buffer in the presence of various DNA structures.

	Tris buffer	5 μM of ssDNA	5 μM of ds17	5 μM of Pu27
Quantum yield Φ	0.041	0.078	0.030	0.192

Table S3. Comparison of detection limits of Ag^+ of recently reported analytical techniques

Method	Detection limit	Ref.	Modified DNA?
C-Ag^+-C DNA probe - SYBR Green I	32 nM	[10]	No
C-Ag^+-C DNA probe - platinum(II) complex	20 nM	[11]	No
C-Ag^+-C DNA probe - platinum(II) complex	20 nM	[12]	No
C-Ag^+-C DNA probe - Thioflavine T	16 nM	[13]	No
C-Ag^+-C DNA probe - SYBR Green I	1 nM	[14]	No
G-quadruplex–hemin DNAzyme colorimetric detection	6.3 nM	[15]	No
G-quadruplex–hemin DNAzyme colorimetric detection	2.5 nM	[16]	No
Ru(II) complex and unmodified quantum dots	100 nM	[17]	No
G-quadruplex probe - triphenylmethane dye switch off detection	80 nM	[18]	No
Nano-graphite-DNA hybrid and DNase I fluorescence detection	0.3 nM	[19]	Yes
Labeled DNA fluorescence detection	10 nM	[20]	Yes
Labeled DNA fluorescence detection	5 nM	[21]	Yes
Labeled DNA fluorescence detection	2.5 nM	[22]	Yes
Labeled DNA fluorescence detection	693 pM	[23]	Yes
Labeled DNA fluorescence detection	50 pM	[24]	Yes
Labeled DNA fluorescence detection with enzyme amplification	16 pM	[25]	Yes
Single-walled carbon-nanotube-based fluorescent detection	1 nM	[26]	Yes
Graphene-based fluorescent nanoprobe	5 nM	[27]	Yes
Ag^+-assisted isothermal exponential degradation reaction	3 pM	[28]	Yes
electrically contacted enzymes on duplex-like DNA scaffolds	3 pM	[29]	Yes
Gold nanoparticles cleavage-based colorimetric detection	470 fM	[30]	Yes

Fig. S1 Diagrammatic bar array representation of the luminescence enhancement selectivity ratio of complexes **1**–**10** for G-quadruplex DNA over dsDNA.

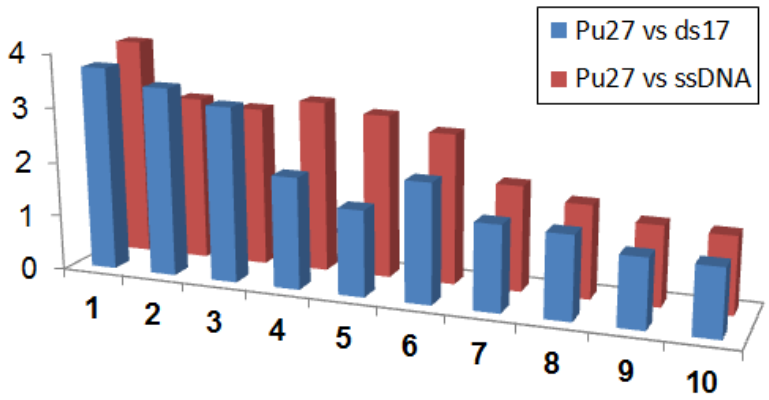


Fig. S2 UV/Vis spectrophotometric titration of complex **1** with increasing concentrations of Pu27.

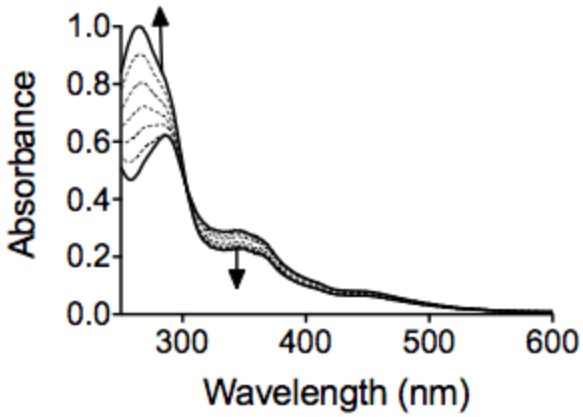


Fig. S3 Plot of $D/\Delta\varepsilon_{ap}$ *vs.* concentration of DNA of complex **1** (20 μM) for calculating the intrinsic binding constant (*K*). Absorbance of **1** at 325 nm was used for calculation. Intrinsic binding constant of **1** to Pu27 G-quadruplex = 1.29×10^5 M^{-1}; ds17 duplex DNA = 4.49×10^4 M^{-1}; ssDNA = 4.02×10^4 M^{-1}.

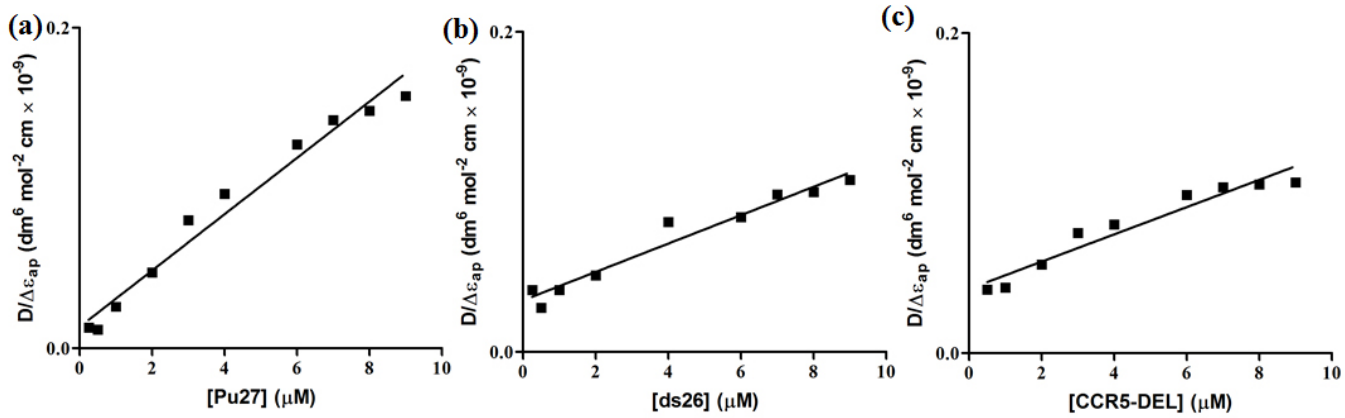
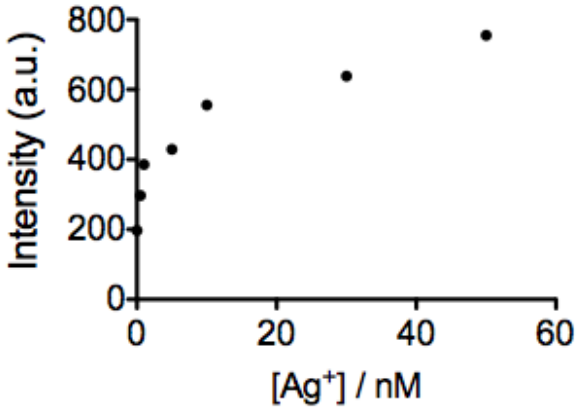


Fig. S4 Luminescence response of the system at λ = 562 nm *vs.* Ag^+ ion concentration in 50-fold diluted river water sample.



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