Electronic Supporting Information

Detection of base excision repair enzyme activity using a luminescent G-quadruplex selective switch-on probe†

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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). Uracil-DNA Glycosylase (UDG) and Uracil Glycosylase inhibitor (UGI) 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:

	Sequence
ss DNA	5′-CTCAT$_4$C$_2$ATACAT$_2$A$_3$GATAGTCAT-3′
ds26	5′-CA$_2$TCG$_2$ATCGA$_2$T$_2$CGATC$_2$GAT$_2$G-3′
ds17	5′-C$_2$AGT$_2$CGTAGTA$_2$C$_3$-3′ 5′-G$_3$T$_2$ACTACGA$_2$CTG$_2$-3′
PS2.M	5′-GTG$_3$TAG$_3$CG$_3$T$_2$G$_2$-3′
HTS	5′-T$_2$AG$_3$T$_2$AG$_3$T$_2$AG$_3$T$_2$AG$_3$-3′
Pu27	5′-TG$_4$AG$_3$TG$_4$AG$_3$TG$_4$A$_2$G$_2$-3′
Pu22	5′-GAG$_3$TG$_4$AG$_3$TG$_4$A$_2$G-3′
ON1	5′-G$_3$TAG$_3$A$_3$T$_2$CT$_2$A$_2$GTGCG$_3$T$_2$G$_3$-3′
ON2	5′-CGCACU$_2$A$_2$GA$_2$U$_2$TC-3′
ON1m	5′-<u>TA</u>GTAG<u>A</u>GA$_3$T$_2$CT$_2$A$_2$GTGCG<u>A</u>GT$_2$G<u>TA</u>-3′
F21T	5′-FAM-d(G$_3$[T$_2$AG$_3$]$_3$)-TAMRA-3′
F10T	5′-FAM-dTATAGCTA-HEG-TATAGCTATAT -TAMRA-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. Circular dichroism (CD) spectra were collected on an OLIS 1000 CD (OLIS, Jefferson, GA).

^{1}H and ^{13}C NMR were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz (^{1}H) and 100 MHz (^{13}C). ^{1}H and ^{13}C chemical shifts were referenced internally to solvent shift (CD_3CN: ^{1}H, δ 1.94, ^{13}C δ 118.7; d_6-DMSO: ^{1}H 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 ^{1}H and ±0.05 for ^{13}C. Coupling constants are typically ± 0.1 Hz for ^{1}H-^{1}H and ±0.5 Hz for ^{1}H-^{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 complex **1** 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[1] $[Ru(bpy)_3][PF_6]_2$ 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.

Synthesis
The following complexes were prepared according to (modified) literature methods: the precursor complex $[Ir_2(bzq)_4Cl_2]$, $[Ir(bzq)_2(5\text{-Clphen})]PF_6$ (**1**),[2] $[Ir(bzq)_2(phen)]PF_6$ (**2**),[3] $[Ir(ppy)_2(dpp)]PF_6$ (**3**),[4] $[Ir(phq)_2(dpp)]PF_6$ (**4**)[5] and $[Ir(ppy)_2(biq)]PF_6$ (**5**).[6] All complexes are characterized by ^{1}H-NMR,^{13}C-NMR and high resolution mass spectrometry (HRMS).

$[Ir(bzq)_2(5\text{-Clphen})]PF_6$ (1). A suspension of $[Ir_2(bzq)_4Cl_2]$ (0.2 mmol) and 5-chloro-1,10-phenanthroline (0.44 mmol) in a mixture of DCM:methanol (1:1, 20 mL) was refluxed overnight under a ntirogen atmosphere. The resulting solution was then allowed to cool to room temperature, and filtered to remove unreacted cyclometallated dimer. To the filtrate, an aqueous solution of ammonium hexafluorophosphate (excess) was added and the filtrate was reduced in volume by rotary evaoration until precipitation of the crude product occurred. The precipiate was then filtered and washed with several portions of water (2 × 50 mL) followed by diethyl ether (2 × 50 mL). The product was recrystallized by acetonitrile:diethyl ether vapor diffusion to yield the titled compound as a brownish orange solid.

Yield: 57%. ^{1}H NMR (400 MHz, CD_3CN) δ 9.07-9.04 (d, J = 8.0 Hz, 1H), 8.87-8.85 (d, J = 8.0 Hz, 1H), 8.67 (s, 1H), 8.52-8.50 (d, J = 8.0 Hz 3H), 8.44-8.42 (d, J = 8.0 Hz, 1H), 8.16-8.15 (t, J = 8.0 Hz, 2H), 8.11-8.08 (q, J = 8.0 Hz, 1H), 8.01-7.97 (d, J = 8.0 Hz, 3H), 7.88-7.86 (d, J = 8.0 Hz, 2H), 7.60-7.58 (d, J = 8.0 Hz, 2H), 7.45-7.40 (m, 2H), 7.24-7.20 (t, J = 8.0Hz, 2H), 6.47-6.44 (q, J = 8.0Hz, 2H); ^{13}C NMR (400 MHz, CD_3CN) δ 157.2, 152.9, 152.4, 150.2, 148.5, 147.4, 147.0, 141.3, 139.1, 138.5, 136.5, 134.7, 131.9, 131.4, 130.6, 130.4, 129.0, 128.7, 128.4, 127.6, 125.2, 123.7, 121.4; HRMS: Calcd. for $C_{38}H_{23}ClIrN_4$ $[M–PF_6]^+$: 763.2860 Found: 763.9931

Luminescence response of 1 towards different forms of DNA

The G-quadruplex DNA-forming sequences (PS2.M, HTS, Pu27) were annealed in Tris-HCl buffer (20 mM Tris, 100 mM KCl, pH 7.0) and were stored at –20 °C before use. Complex **1** (1 μM) was added to 5 μM of ssDNA, ctDNA or G-quadruplex DNA in Tris-HCl buffer (20 mM Tris, pH 7.0). Emission spectra were recorded in 500−700 nm range using an excitation wavelength of 360 nm.

FRET melting assay

The ability of the **1** to stabilize G-quadruplex DNA was investigated using a fluorescence resonance energy transfer (FRET) melting assay. The labelled oligonucleotides F21T (5′-*FAM* d($G_3[T_2AG_3]_3$)-*TAMRA*-3′; donor fluorophore *FAM*: 6-carboxyfluorescein; acceptor fluorophore *TAMRA*: 6-carboxytetramethylrhodamine) used as the FRET probes was diluted to 200 nM in a potassium cacodylate buffer (100 mM KCl, pH 7.0) and then annealed by heating to 95 °C for 10 min in the presence of indicated concentrations of **1**. The F10T (5′-FAM- dTATAGCTA-HEG-TATAGCTATAT -TAMRA-3′) was used as double stranded FRET probe (HEG linker: [(-CH_2-CH_2-O-)$_6$]) and annealed under the same conditions as the other F21T unless 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.

G4-FID assay

The FID assay was performed as previously described.[7] The quadruplex forming DNA (0.25 μM) in Tris-HCl buffer (50 mM Tris, 100 mM KCl, pH 7.0) were annealed by heating at 95 °C for 10 min. Indicated concentration of thiazole orange (0.5 μM for quadruplex DNA; 0.75 μM for duplex DNA) was added and the mixture was incubated for 1 h. Emission measurement was taken after addition of each indicated concentration of **1** followed by an equilibration time for 5 min. The fluorescence area was converted into percentage of displacement (PD) by using the following equation. PD = 100 − [(FA/FA_0) × 100] (FA_0 = fluorescence area of DNA-TO complex in the absence of **1**; FA = fluorescence area in the presence of **1**)

Absorption titration. A solution of complex **1** (20 μM) was prepared in Tris-HCl buffer (50 mM, 100 mM KCl, pH 7.4). Aliquots of a millimolar stock solution of Pu27 (0–10 μM) or ds17 (0–20 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, $\Delta\varepsilon\text{ap} = |\varepsilon\text{A} - \varepsilon\text{F}|$, $\varepsilon\text{A} = A_{obs}/[\text{ligand}]$, and $\Delta\varepsilon = |\varepsilon\text{B} - \varepsilon\text{F}|$; εB and εF correspond to the extinction coefficients of DNA−ligand adduct and unbound ligand, respectively.

Detection of UDG activity

The probe DNA (100 μM) and its partially complementary damaged DNA strand with several U bases (100 μM) were first added in a hybridization buffer (50 mM Tris, 150 mM NaCl, pH 7.0) with a final volume of 30 μL. 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 duplex substrate (ON1-ON2). The annealed product was stored at –20 °C before use. For assaying UDG activity, 50 μL of 1×NEB UDG reaction buffer (20 mM Tris–HCl, 1 mM EDTA, 1 mM DTT, pH 8.0) and indicated concentrations of UDG were added to the solution containing the duplex substrate. The mixture was heated to 37 °C for 30 min to allow the base cleavage reaction to take place. The mixture was cooled down and was subsequently diluted using Tris buffer (50 mM Tris, 20 mM KCl, 150 mM NH_4OAc, pH 7.0) to a final volume of 500 μL. Finally, 0.5 μM of

complex **1** was added to the mixture. Emission spectra were recorded in the 500−700 nm range using an excitation wavelength of 360 nm.

For UDG inhibitor screening, the duplex substrate was incubated with the indicated concentrations of UDG and an equal amount of UDG inhibitor. The mixture was heated to 37 °C for 30 min and the mixture was cooled to room temperature. The mixture was then diluted with Tris buffer (50 mM Tris, 20 mM KCl, 150 mM NH_4OAc, pH 7.0) with a final volume of 500 µL. 0.5 µM of complex **1** was subsequently added to the mixture. Emission spectra were recorded in the 500−700 nm range using an excitation wavelength of 360 nm.

Gel electrophoresis to investigate the dissociation of the duplex substrate

The UDG-treated product was obtained according to the procedure described above. The products were resolved on 20% polyacrylamide gel to separate the cleaved products from the substrate using 1×TBE as running buffer at a constant voltage of 100 V for 60 min. The separated products were visualized by SYBR green I staining.

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

Complex	UV/vis absorption	Emission	
	λ_{abs} [nm] (ε [$dm^3mol^{-1}cm^{-1}$])	λ_{em} [nm] (τ [µs])	Quantum yield Φ
1	267 (3.8×10^4), 273 (sh),[a] 320 (1.6×10^4), 416 (sh)	580 (4.492)	0.054

[a] sh = shoulder peak

Fig. S1 Thermal difference spectrum of ON1 G-quadruplex resulting from the difference between the absorbance recorded at 95 °C and 20 °C.

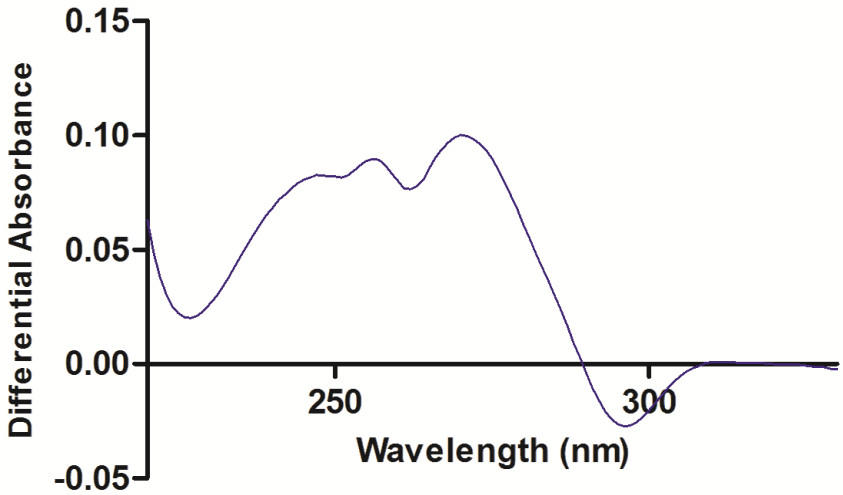


Fig. S2 Chemical structures of other iridium(III) complexes investigated in this study.

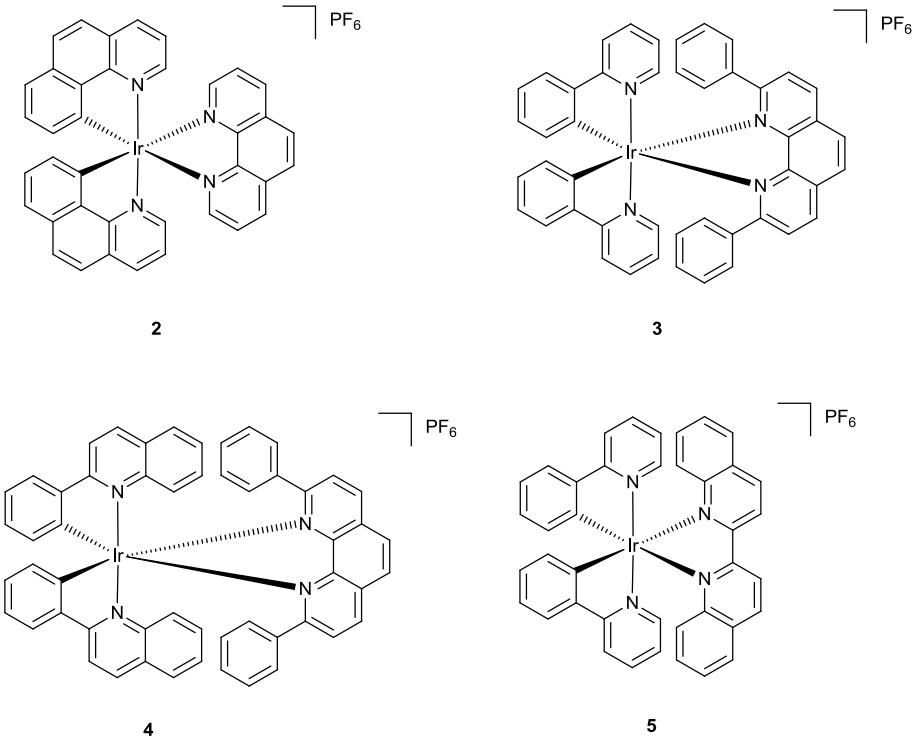


Fig. S3 UV/vis absorption and normalized emission spectra of complex **1** (20 μM) in acetonitrile solution at 298 K.

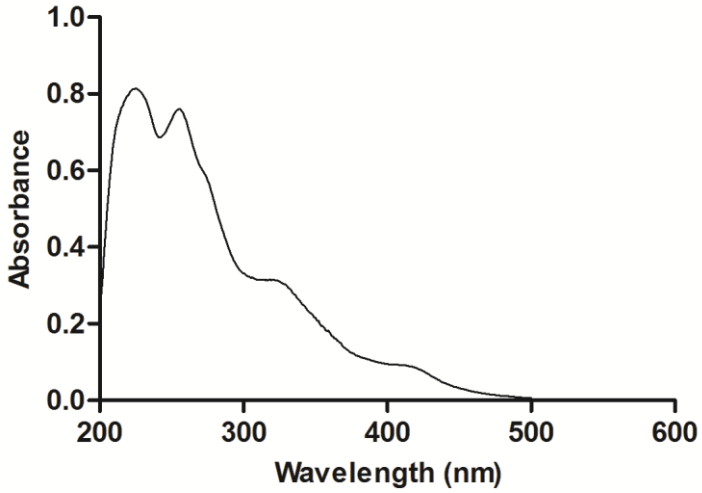


Fig. S4 Normalized excitation and emission spectra of complex **1** (20 μM) in acetonitrile solution at 298 K.

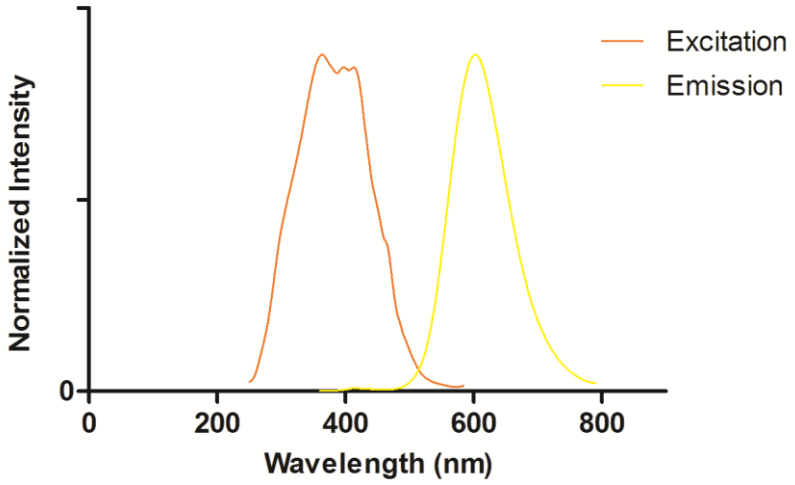


Fig. S5 Plot of $D/\Delta\varepsilon_{ap}$ *vs.* concentration of DNA of complex **1** (20 μM) for calculating the intrinsic binding constant (*K*). Absorbance of **1** at 325 nm was used for calculation. Intrinsic binding constant of **1** to Pu27 G-quadruplex = 4.50×10^5 M^{-1}; ds17 duplex DNA = 7.26×10^4 M^{-1}; ssDNA = 4.76×10^4 M^{-1}.

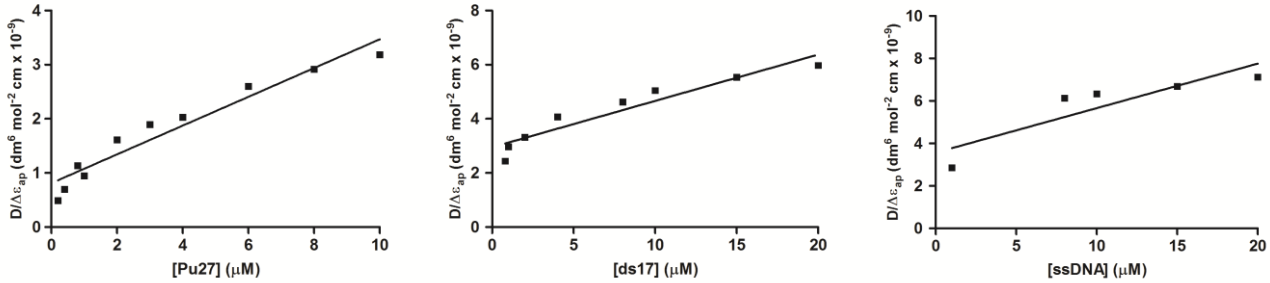


Fig. S6 Scatter plot showing the melting profile of 200 nM F21T G-quadruplex DNA in the absence of **1** (black rectangle) and with increasing concentration of **1**: 1:1 (blue triangle) and 1:2 (red circle). The effect of stabilization of F21T by acetonitrile was also examined but the effect is minimal (data not shown).

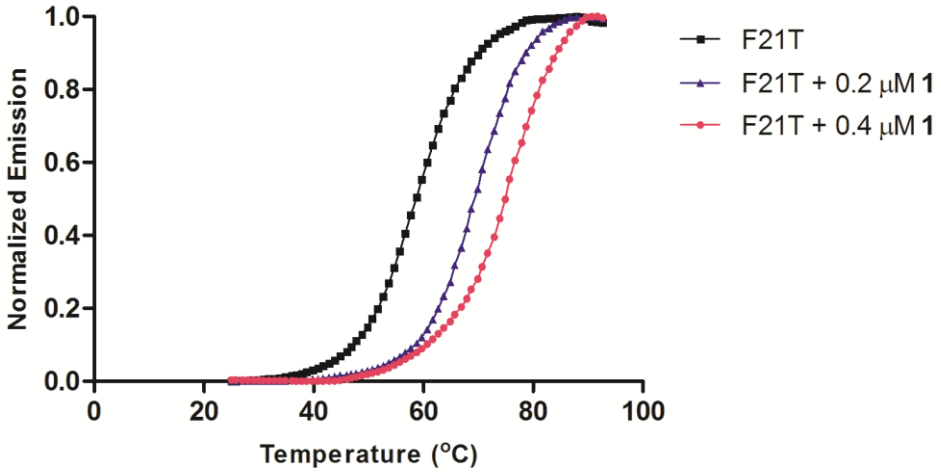


Fig. S7 Scatter plot showing the melting profile of 200 nM F10T double-stranded DNA in the absence of **1** (black rectangle) and with increasing concentration of **1**: 1:1 (blue triangle) and 1:2 (red circle). The effect of stabilization of F10T by acetonitrile was also examined but the effect is minimal (data not shown).

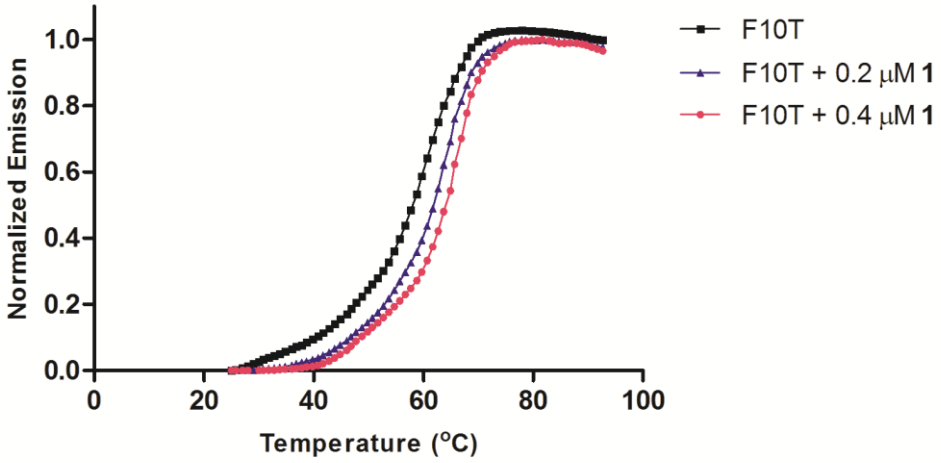


Fig. S8 G4-FID titration curves of DNA duplex ds17, ds26 and G-quadruplexes (Pu22, Pu27 and HTS) in the presence of increasing concentration of complex **1** in Tris-HCl buffer (50 mM Tris, pH 7.0, 100 mM KCl). DC_{50} value is determined by half-maximal concentration of compound required to displace 50% TO from DNA.

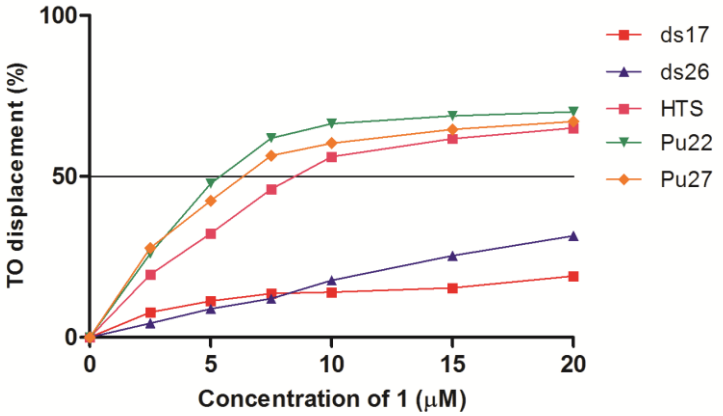


Fig. S9 Emission spectrum of complex **1** (1 μM) in the presence of 5 μM of ss DNA, ct DNA or various G-quadruplexes.

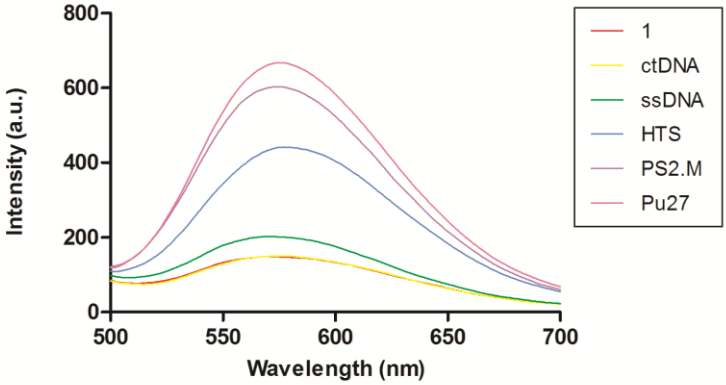


Fig. S10 Luminescence response of the system ([complex **1**] = 1 μM, [duplex substrate] = 0.5 μM) with or without 5 U/mL UDG.

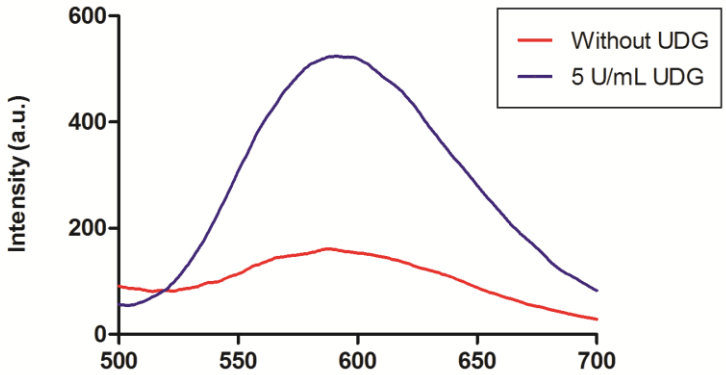


Fig. S11 Luminescence intensity of the system ([complex **1**] = 1 μM, [UDG] = 5 U/mL) in the presence of duplex substrate or ON1m-ON2 duplex. Error bars represent the standard deviations of the results from three independent experiments.

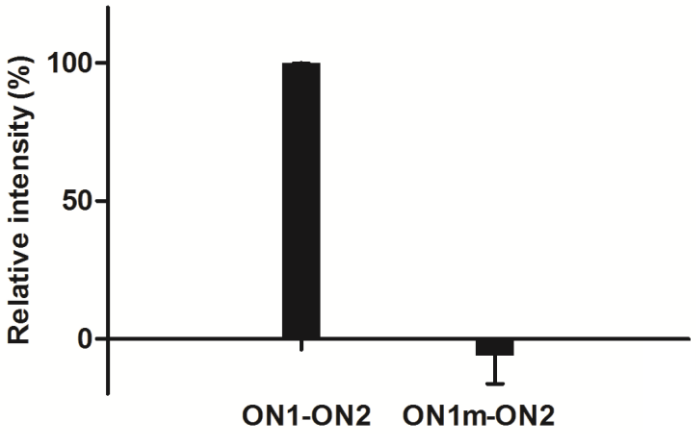


Fig. S12 Luminescence response of the system with the complex alone ([complex **1**] = 1 μM) in the presence of increasing concentrations of UDG (0, 10, 20 and 30 U/mL).

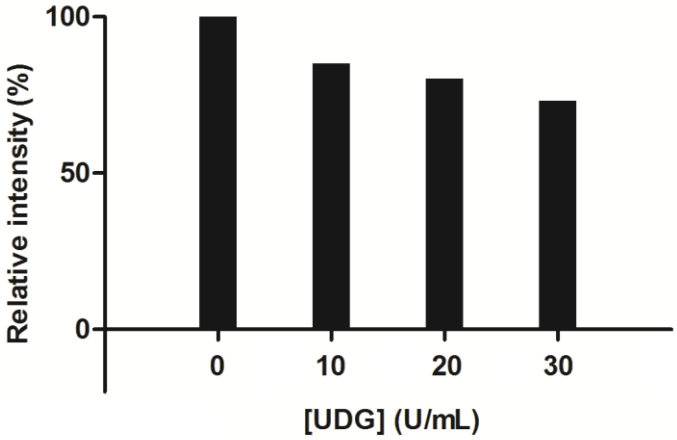


Fig. S13 Non-denaturing gel electrophoresis of samples in 20% polyacrylamide gel. Lane 1 contains 15 μM ON1. Lane 2 contains 10 μM ON2. Lanes 3 contains 15 μM ON1-ON2 duplex substrate without UDG. Lane 4 contains 15 μM ON1-ON2 duplex substrate with 5 U/mL UDG.

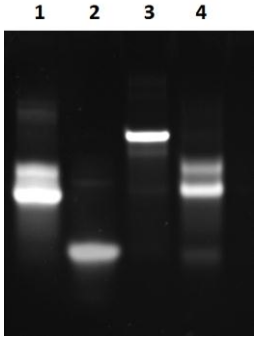


Fig. S14 Circular dichroism (CD) spectrum of blank (red) or 5 U/mL UDG (blue) recorded in Tris buffer (50 mM Tris, pH 7.0).

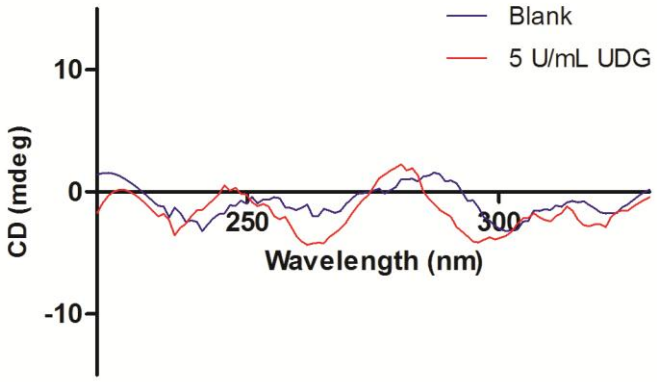


Fig. S15 Circular dichroism (CD) spectrum of 2.5 μM ON1-ON2 duplex substrate in the absence (red) or presence (blue) of 5 U/mL UDG recorded in Tris buffer.

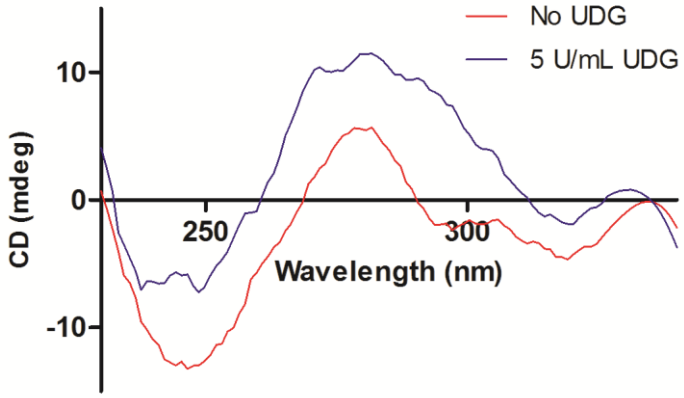


Fig. S16 Relative luminescence intensity at 585 nm of the system ([duplex substrate] = 1.5 μM, [UDG] = 2.5 U/mL) in the presence of different concentrations of complex **1** (0.25, 0.5, 1, 2 and 3 μM) in aqueous buffered solution (50 mM Tris, 20 mM KCl, 150 mM NH_4OAc, pH 7.0). Error bars represent the standard deviations of the results from three independent experiments.

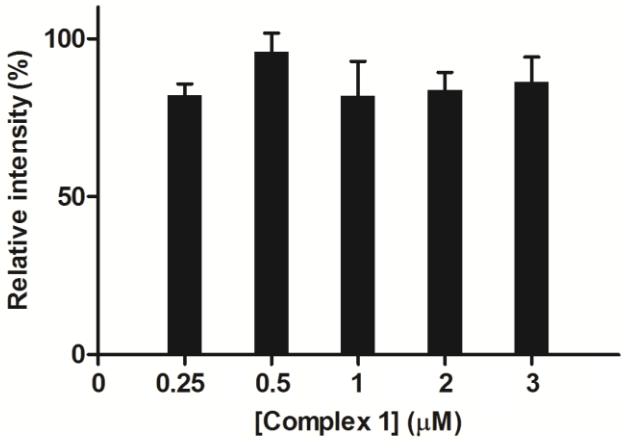


Fig. S17 Relative luminescence intensity at 585 nm of the system ([complex **1**] = 0.5 μM, [UDG] = 2.5 U/mL) at various concentrations of the duplex substrate (0.25, 0.5, 1.5 and 3 μM) in aqueous buffered solution (50 mM Tris, pH 7.0). Error bars represent the standard deviations of the results from three independent experiments.

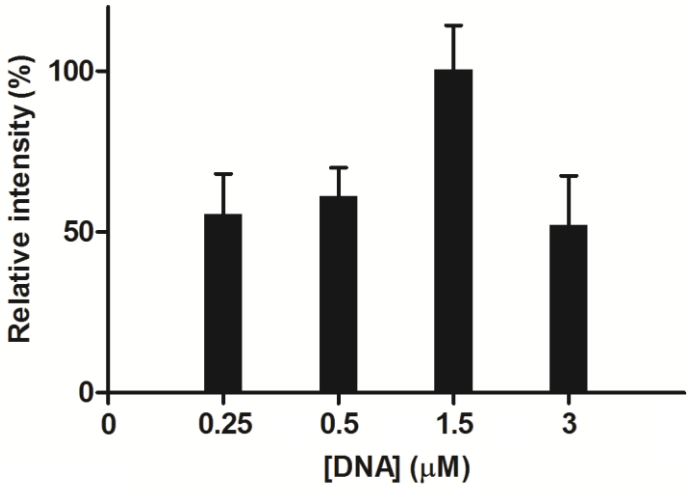


Fig. S18 Relative luminescence intensity at 585 nm of the system ([duplex substrate] = 1.5 μM, [UDG] = 2.5 U/mL, [complex **1**] = 0.5 μM) at various concentrations of KCl ([KCl] = 0, 20, 50, 100 mM). Error bars represent the standard deviations of the results from three independent experiments.

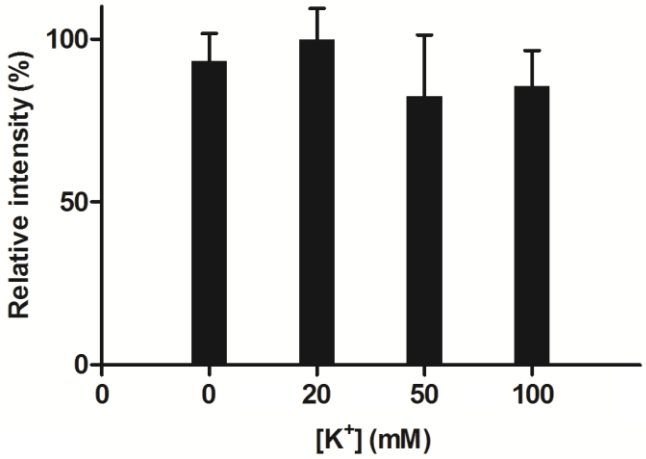


Fig. S19 Relative luminescence intensity at 585 nm of the system ([duplex substrate] = 1.5 μM, [UDG] = 2.5 U/mL, [complex **1**] = 0.5 μM) at various concentrations of NH_4OAc ([NH_4OAc] = 0, 50, 150, 300 mM). Error bars represent the standard deviations of the results from three independent experiments.

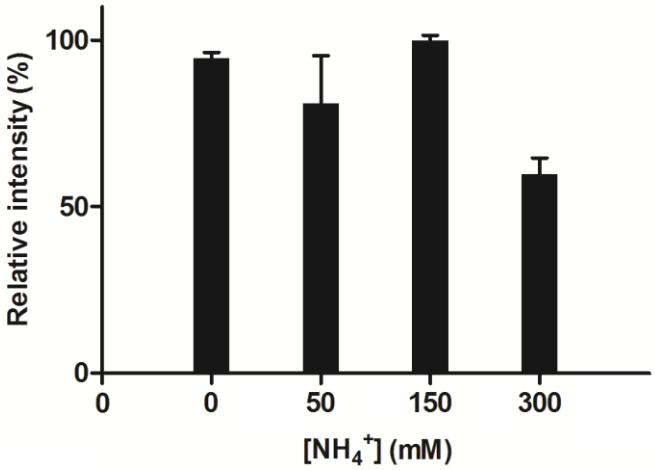


Fig. S20 Relative luminescence intensity at 585 nm of the system ([duplex substrate] = 1.5 μM, [UDG] = 2.5 U/mL, [complex **1**] = 0.5 μM) by varying the pH of the buffered system (50 mM Tris, 20 mM KCl, 150 mM NH_4OAc). Error bars represent the standard deviations of the results from three independent experiments.

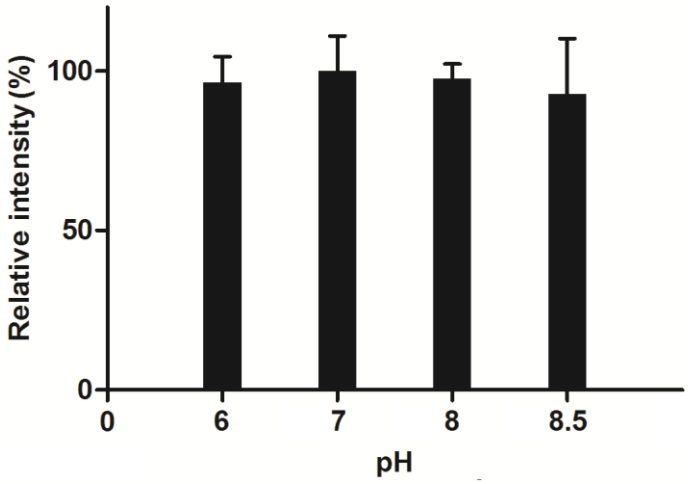


Fig. S21 Photograph image of the system in the presence (left) or absence (right) of UDG recorded in aqueous buffer (50 mM Tris, 20 mM KCl, 150 mM NH_4OAc, pH 7.0).

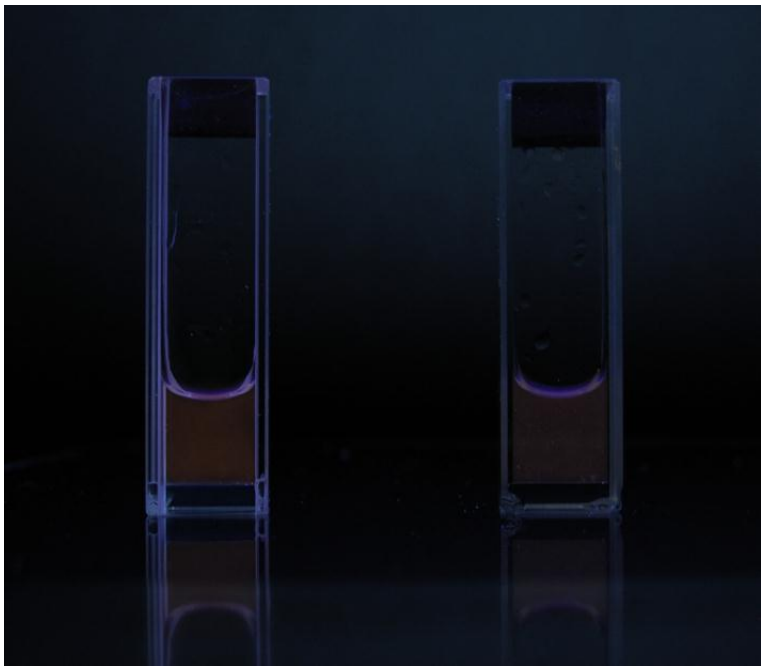
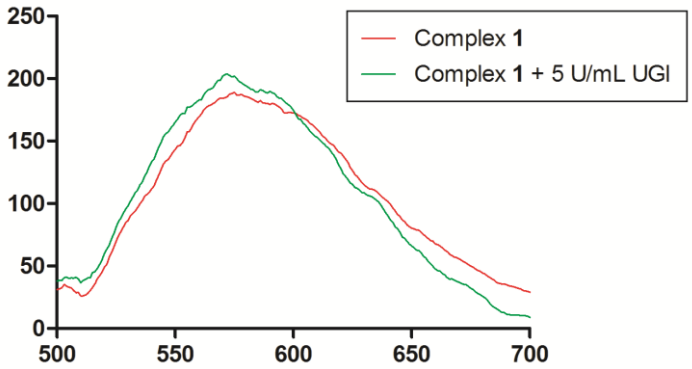


Fig. S22 Luminescence response of 0.5 μM of **1** in the presence (green line) or absence (red line) of 5 U/mL UGI.



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