Electronic Supporting Information

Label-free luminescence switch-on detection of T4 polynucleotide kinase activity using a G-quadruplex-selective probe†

Hong-Zhang He,[a] Ka-Ho Leung,[a] Wei Wang,[a] Daniel Shiu-Hin Chan,[a] Chung-Hang Leung,*[b] and Dik-Lung Ma*[a]

[a] *Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China.*
E-mail: edmondma@hkbu.edu.hk
[b] *State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macao, China.*
E-mail: duncanleung@umac.mo

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). Lambda Exonuclease (λ exo) and T4 Polynucleotide Kinase (T4 PNK) were purchased from New England Biolabs Inc. (Beverly, MA, USA). All oligonucleotides were synthesized by Techdragon Inc. (Hong Kong, China)

Table1. DNA sequences used in this project:

	Sequence
CCR5-DEL	5′-CTCAT$_4$C$_2$ATACAT$_2$A$_3$GATAGTCAT-3′
HTS	5′- AG$_3$(T$_2$AG$_3$)$_3$-3′
HG$_{55}$	5′- (C$_3$TA$_2$)$_3$C$_3$TA$_2$TAG$_2$A$_2$GACAG$_3$(T$_2$AG$_3$)$_3$-3′
HG$_{55m}$	5′- A$_2$G$_2$T$_2$AGCGT$_2$AG$_2$AT$_2$ACGGCAGA$_2$G$_2$ATA$_2$C$_2$GTA$_2$TC$_2$TA$_2$CGCTA$_2$C$_2$T$_2$-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′

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 a JASCO-815 spectrometer.

^{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.

G4-FID assay
The FID assay was performed as previously described.[2] The HTS G-quadruplex DNA (0.25 μM) in Tris-HCl buffer (20 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 HTS G-quadruplex DNA; 0.5 μM for ds17; 0.75 μM for ds26) 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. $PD = 100 - [(FA/FA_0) \times 100]$ (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 (20 mM, pH 7.0). Aliquots of a millimolar stock solution of pre-annealed HTS (0–20 μM), ds17 (0–20 μM), or ssDNA CCR5-DEL (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**):[3]

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

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.

Synthesis
The following complexes were prepared according to (modified) literature methods: the precursor complex $[Ir_2(ptpy)_4Cl_2]$[4], $[Ir_2(ppy)_4Cl_2]$[5], $[Ir(ptpy)_2(dpp)]PF_6$ (**1**), $[Ir(ptpy)_2(bpy)]PF_6$ (**2**)[6], $[Ir(ptpy)_2(Phen)]PF_6$ (**3**), $[Ir(ptpy)_2(dip)]PF_6$ (**4**) and $[Ir(ptpy)_2(BCP)]PF_6$ (**5**), $[Ir(ppy)_2(dpp)]PF_6$ (**6**)[7]. All complexes are characterized by ^{1}H-NMR, ^{13}C-NMR, high resolution mass spectrometry (HRMS) and elemental analysis.

$[Ir(ptpy)_2(dpp)]PF_6$ (1), **$[Ir(ptpy)_2(Phen)]PF_6$ (3)**, **$[Ir(ptpy)_2(dip)]PF_6$ (4) and $[Ir(ptpy)_2(BCP)]PF_6$(5)**. A suspension of $[Ir_2(ptpy)_4Cl_2]$ (0.2 mmol) and corresponding N^N ligands 2,9-diphenyl-1,10-phenanthroline (dpp), 1,10-phenanthroline (Phen), 4,7-diphenyl-1,10-phenanthroline

(dip), or 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline (BCP) (0.44 mmol) in a mixture of DCM:methanol (1:1, 20 mL) was refluxed overnight under a nitrogen 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.

[Ir(ptpy)$_2$(dpp)]PF$_6$ (1). Yield: 56%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.74 (d, J = 8.3 Hz, 2H), 8.29 (s, 4H), 7.90-7.83 (m, 6H), 7.76-7.58 (m, 4H), 7.00-6.85 (m, 4H), 6.65 (dd, J = 21.2, 13.7 Hz, 4H), 6.21-6.13 (m, 4H), 1.92 (dt, J = 4.4, 2.2 Hz, 6H). ^{13}C NMR (400 MHz, Acetone-d_6) δ 21.9, 120.1, 122.2, 123.0, 124.9, 128.1, 128.8, 129.9, 131.5, 132.5, 138.8, 139.2, 139.5, 148.2, 149.3, 151.5, 166.9, 169.3. HRMS: Calcd. for $C_{48}H_{36}IrN_4$ [M–PF$_6$]$^+$: 861.2562 Found: 861.2584. Elemental analysis: ($C_{48}H_{36}IrN_4$ + 0.5 H_2O) C, H, N: calcd 56.8, 3.64, 5.52; found 56.78, 3.52, 5.44.

[Ir(ptpy)$_2$(Phen)]PF$_6$ (3). Yield: 64%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.92 (dd, J = 8.3, 1.4 Hz, 2H), 8.53-8.41 (m, 4H), 8.19 (d, J = 8.0 Hz, 2H), 8.09 (dd, J = 8.3, 5.0 Hz, 2H), 8.01-7.80 (m, 4H), 7.64 (d, J = 5.2 Hz, 2H), 7.03-6.89 (m, 4H), 2.15 (s, 2H), 2.07 (dt, J = 4.4, 2.2 Hz, 6H). ^{13}C NMR (400 MHz, Acetone-*d6*) δ 168.7, 152.1, 151.1, 150.1, 147.8, 142.5, 141.0, 139.5, 139.2, 133.4, 132.5, 129.3, 127.8, 125.7, 124.4, 123.7, 120.3, 21.9. HRMS: Calcd. for $C_{36}H_{28}IrN_4$ [M–PF$_6$]$^+$: 709.1939 Found: 709.1945. Elemental analysis: ($C_{36}H_{28}IrN_4$ + 0.5 H_2O) C, H, N: calcd 50.11, 3.39, 6.49; found 50.17, 3.32, 6.47.

[Ir(ptpy)$_2$(dip)]PF$_6$ (4). Yield: 54%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.52 (d, J = 5.2 Hz, 2H), 8.28 (s, 2H), 8.19 (d, J = 8.1 Hz, 2H), 8.10-7.66 (m, 12H), 7.66 (s, 2H), 7.73-7.37 (m, 10H), 7.07-6.80 (m, 2H), 2.13 (s, 4H). ^{13}C NMR (400 MHz, Acetone-d_6) δ 21.0, 119.5, 122.9, 123.6, 124.9, 126.2, 127.1, 129.1, 129.4, 129.7, 129.9, 132.5, 135.9, 138.4, 140.2, 141.7, 147.6, 149.2, 150.7, 150.8, 150.9, 167.9. HRMS: Calcd. for $C_{48}H_{36}IrN_4$ [M–PF$_6$]$^+$: 861.2569 Found: 860.9312. Elemental analysis: ($C_{48}H_{36}IrN_4$ + H_2O) C, H, N: calcd 56.3, 3.74, 5.47; found 55.86, 4.06, 6.88.

[Ir(ptpy)$_2$(BCP)]PF$_6$(5). Yield: 52%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.17 (dd, J = 8.6, 1.2 Hz, 2H), 8.10 (s, 2H), 7.92 (ddd, J = 8.5, 5.0, 2.1 Hz, 4H), 7.76 (d, J = 16.2 Hz, 4H), 7.73-7.58 (m, 8H), 7.02 (ddd, J = 7.3, 6.0, 1.4 Hz, 4H), 6.79 (dd, J = 7.9, 1.1 Hz, 4H), 2.29 (s, 6H), 2.08-1.99 (m, 6H). ^{13}C NMR (400 MHz, Acetone-d6) δ 21.0, 26.9, 119.4, 122.5, 123.1, 124.7, 124.8, 127.8, 128.2, 129.0, 129.7, 132.3, 136.0, 1138.4, 139.5, 141.0, 148.9, 149.7, 150.1, 150.7, 164.6, 167.9. HRMS: Calcd. for $C_{50}H_{40}IrN_4$ [M–PF$_6$]$^+$: 889.2882 Found: 889.2908. Elemental analysis: ($C_{50}H_{40}IrN_4$ + H_2O) C, H, N: calcd 57.08, 4.02, 5.33; found 56.7, 3.84, 5.29.

Total cell extract preparation

The TRAMPC1 (ATCC® CRL2730™) cell line were purchased from American Type Culture Collection (Manassas, VA 20108 USA). Prostate cancer cells were trypsinized and resuspended in TE buffer (10 mM Tris-HCl 7.4, 1 mM EDTA). After incubation on ice for 10 min, the lysate was centrifuged and the supernatant was collected.

Luminescence response of 1 towards different forms of DNA

The G-quadruplex DNA-forming sequences (HTS) was 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 HTS G-quadruplex DNA in Tris-HCl buffer (20 mM Tris, pH 7.0). Emission spectra were recorded in 500−730 nm range using an excitation wavelength of 360 nm.

Detection of T4 PNK activity

The random-coil oligonucleotide (HG_{55}, 50 μM) was incubated in Tris buffer (20 mM, pH 7.0). The solutions were heated to 95 °C for 10 min, cooled to room temperature at 0.1 °C/s, and further incubated at room temperature for 1 h to ensure formation of the hairpin substrate. The annealed product was stored at –20 °C before use. For assaying T4 PNK activity, 50 μL of 1× T4 Polynucleotide Kinase Reaction Buffer (70 mM Tris-HCl, 10 mM $MgCl_2$, 5 mM DTT, pH 7.6) with 10 U/mL λ exo, 0.1 mM ATP and indicated concentrations of T4 PNK were added to the solution containing the hairpin substrate (1 μM). The mixture was heated to 37 °C for 60 min to allow the T4 PNK catalyzed phosphorylation coupled with λ exo digestion of HG_{55} to take place. The mixture was cooled down and was subsequently diluted using Tris buffer (20 mM Tris, 50 mM KCl, pH 7.0) to a final volume of 500 μL. Finally, 0.75 μM of complex **1** was added to the mixture. Emission spectra were recorded in the 500−730 nm range using an excitation wavelength of 360 nm.

For T4 PNK inhibitor screening, the hairpin substrate HG_{55} was incubated with the indicated concentrations of T4 PNK inhibitor ADP and an equal amount of T4 PNK. The mixture was heated to 37 °C for 60 min and the mixture was cooled to room temperature. The mixture was then diluted with Tris buffer (20 mM Tris, 50 mM KCl, pH 7.0) with a final volume of 500 μL. 0.75 μM of complex **1** was subsequently added to the mixture. Emission spectra were recorded in the 500−730 nm range using an excitation wavelength of 360 nm.

For the detection of T4 PNK activity in cell extract, 50 μL of 1× T4 Polynucleotide Kinase Reaction Buffer (70 mM Tris-HCl, 10 mM $MgCl_2$, 5 mM DTT, pH 7.6) with 10 U/mL λ exo, 0.1 mM ATP and indicated concentrations of T4 PNK were added to the solution containing the hairpin substrate (1 μM) and cell extract. The mixture was heated to 37 °C for 60 min to allow the T4 PNK catalyzed phosphorylation coupled with λ exo digestion of HG_{55} to take place. The mixture was cooled down and was subsequently diluted using Tris buffer (20 mM Tris, 50 mM KCl, pH 7.0) to a final volume of 500 μL. Finally, 0.75 μM of complex **1** was added to the mixture. Emission spectra were recorded in the 500−730 nm range using an excitation wavelength of 360 nm.

Table S2. 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	278 (9.1×10^4), 238 (7.2×10^4)	580 (4.452)	0.079

Table S3. Photophysical properties of complex **1** in Tris buffer in the absence and presence of HTS G-quadruplex DNA.

Complex	Without HTS G-quadruplex	With HTS G-quadruplex
1 (Quantum yield Φ)	0.0177	0.0333

Fig. S1 Chemical structures of other iridium(III) complexes investigated in this study.

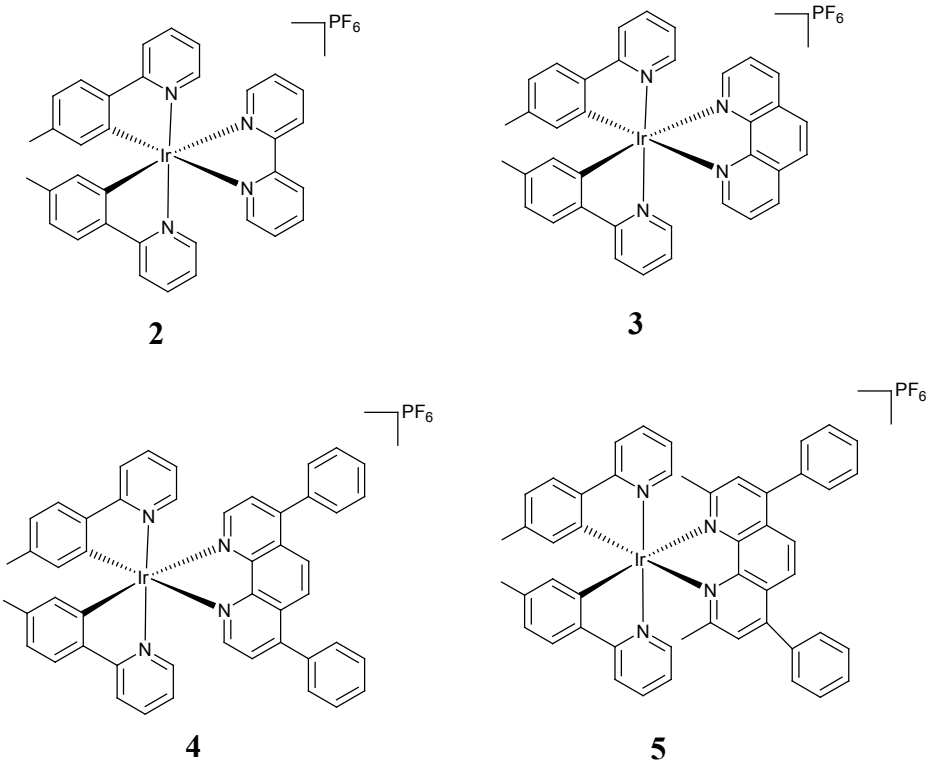

Fig. S2 Luminescence response of complexes **1–5** (1 μM) in 20 mM Tris buffer (pH 7.0) in the presence of 5 μM ctDNA; 5 μM ssDNA, and 5 μM HTS G-quadruplex, respectively. HTS G-quadruplex was pre-annealed in Tris buffer (20 mM, 100 mM KCl, pH 7.0). Ex = 360 nm and Em = 580 nm for **1**, Ex = 380 nm and Em = 600 nm for **2**, Ex = 360 nm and Em = 580 nm for **3**, Ex = 380 nm and Em = 600 nm for **4**, Ex = 320 nm and Em = 565 nm for **5**.

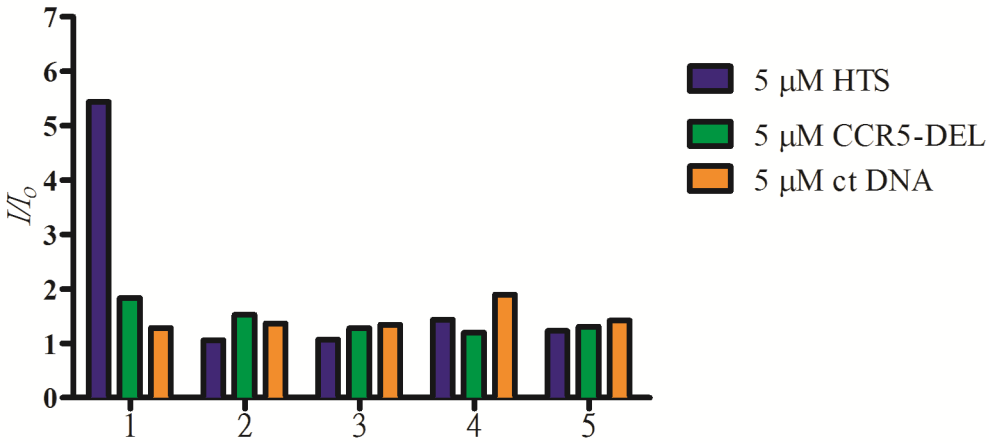

Fig. S3 UV/Vis absorption of complex **1** (5 μM) in acetonitrile solution at 298 K.

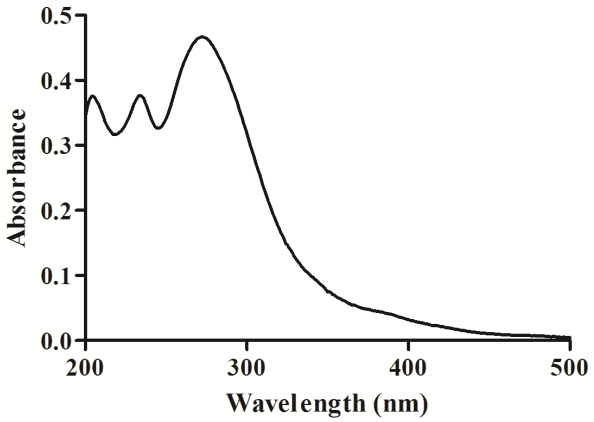

Fig. S4 Normalized excitation and emission spectra of complex **1** (20 μM) in acetonitrile solution at 298 K.

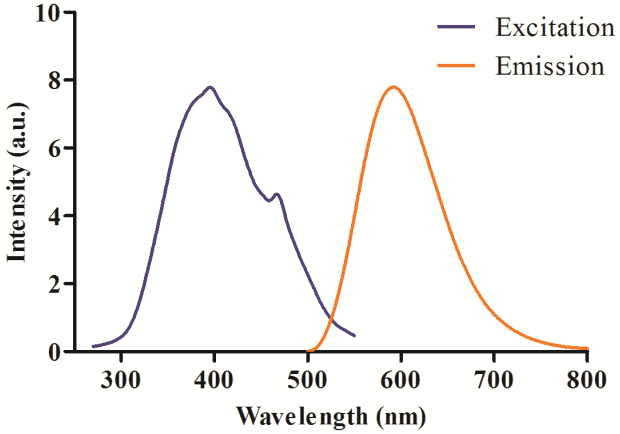

Fig. S5 Plot of $D/\Delta\varepsilon_{ap}$ *vs.* concentration of DNA for calculating the intrinsic binding constant (K) of complex **1** (20 μM). Absorbance was measured at 315 nm. Intrinsic binding constant of **1** to (a) HTS G-quadruplex DNA= 3.34×10^5 M^{-1}; (b) ds17 duplex DNA = 9.90×10^4 M^{-1}; (c) CCR5-DEL = 1.10×10^5 M^{-1}.

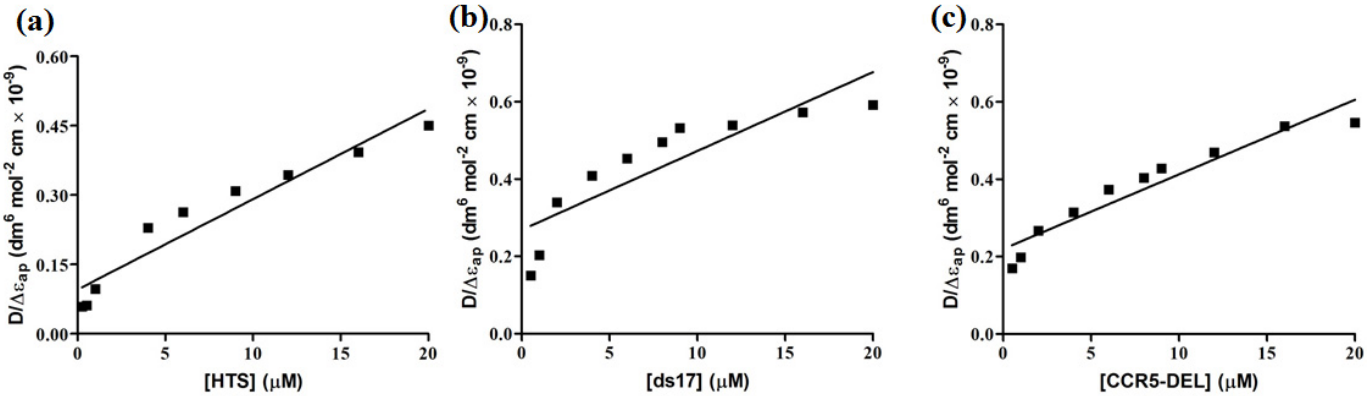

Fig. S6 G4-FID titration curves of DNA duplex ds17, ds26 and HTS G-quadruplex in the presence of increasing concentration of complex **1** in Tris-HCl buffer. DC_{50} value is determined by the half-maximal concentration of compound required to displace 50% TO from DNA.

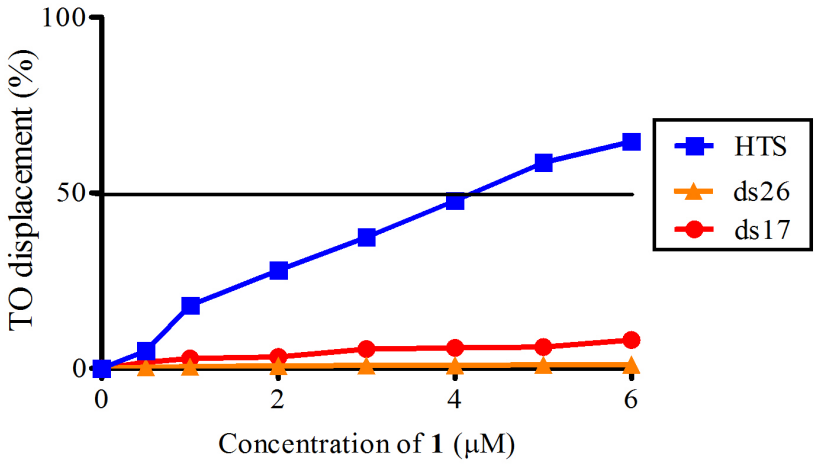

Fig. S7 (a) Luminescence response ratio of complex **1** and **6** (1 μM) in 20 mM Tris buffer (pH 7.0) in the presence of 5 μM HG_{55} hairpin DNA and 5 μM HTS G-quadruplex, respectively. HTS G-quadruplex was pre-annealed in Tris buffer (20 mM, 100 mM KCl, pH 7.0). (b) The structure of complex **6** is also displayed.

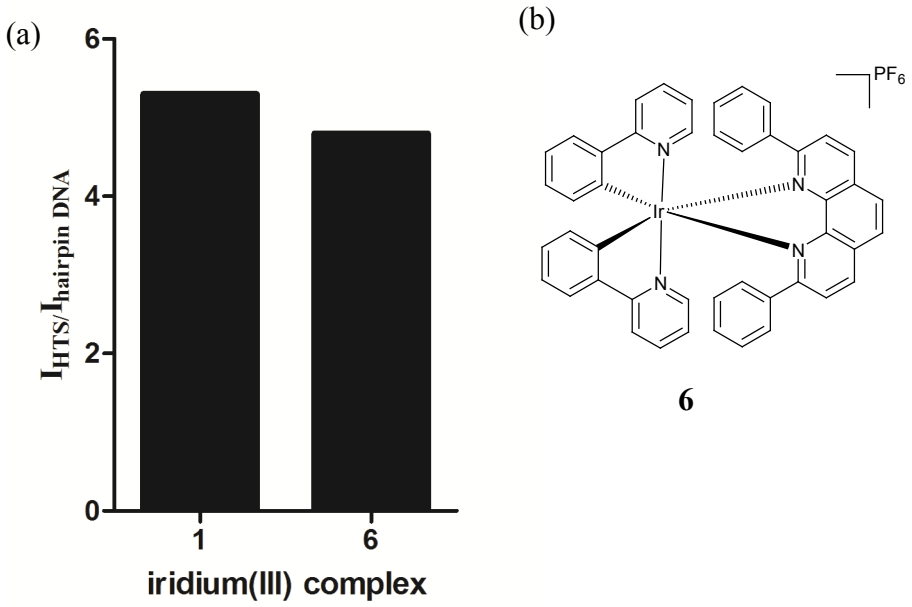

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Fig. S8 Luminescence response of the system with the complex ([complex **1**] = 1 μM) in the absence of HG_{55} with increasing concentrations of T4 PNK (0, and 10 U/mL).

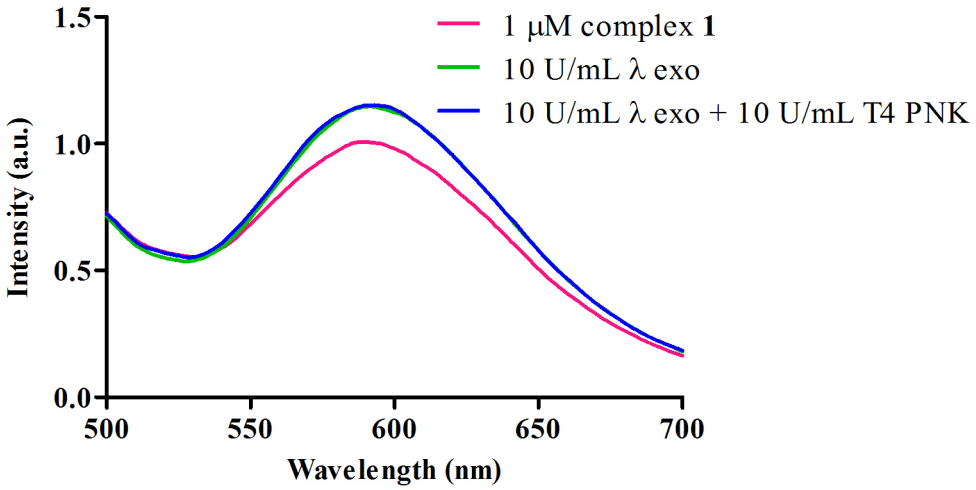

Fig. S9 Luminescence response of the mutant of hairpin substrate ([HG_{55m}] = 1 μM, [λ exo] = 10 U/mL, [ATP] = 0.1 mM, T4 PNK reaction time = 60 min) in the absence or presence of T4 PNK (2.5 U/mL).

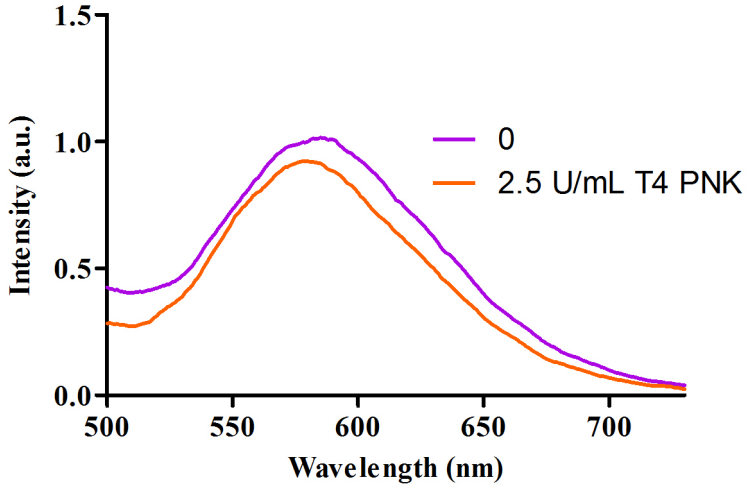

Fig. S10 Circular dichroism (CD) spectrum of 4 μM hairpin substrate HG_{55} in the absence (purple) or presence (blue) of 2.5 U/mL T4 PNK recorded in Tris buffer. The CD spectrum of HG_{55} treated by λ exo alone exhibits an intense positive peak at 270 nm and a strong negative peak at 240 nm, which is characteristic for duplex DNA. Upon incubation with both λ exo and T4 PNK, the spectrum changes to reveal a positive band at around 290 nm, and a weak negative peak at around 240 nm.

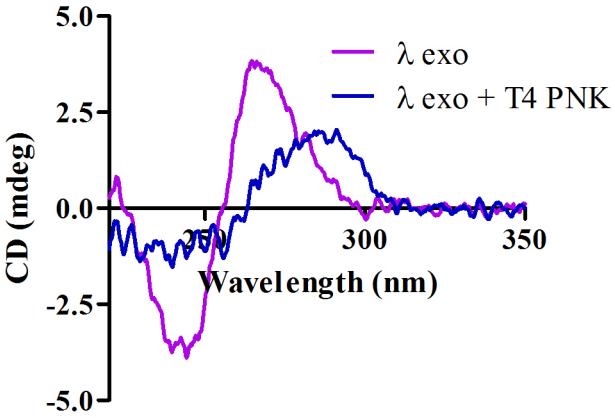

Fig. S11 Relative luminescence intensity at 580 nm of the system ([complex **1**] = 0.75 μM, [T4 PNK] = 2.5 U/mL, [λ exo] = 10 U/mL, [ATP] = 0.1 mM, T4 PNK reaction time = 60 min) at various concentrations of the hairpin substrate HG_{55} (0.25, 0.5, 1 and 2 μM) in aqueous buffered solution (20 mM Tris, 50 mM KCl, pH 7.0). It was observed that the luminescence response of the system was highest at 1 μM of HG_{55}. A higher concentration of the hairpin substrate would result in a higher background signal of the system, while a lower concentration of the hairpin substrate results in a lower luminescence enhancement after treatment with PNK.

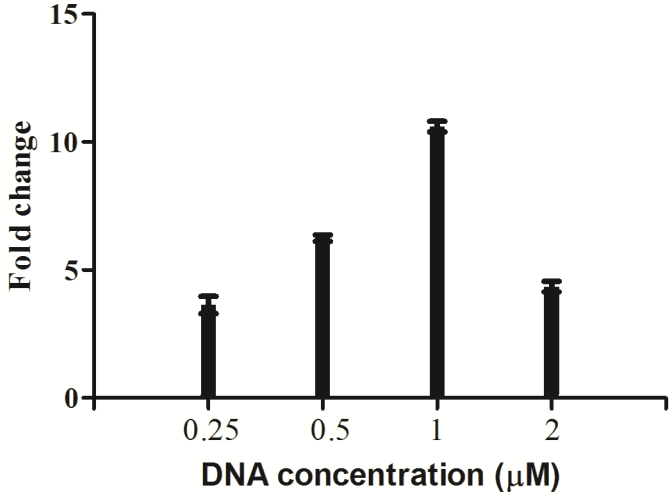

Fig. S12 Relative luminescence intensity at 580 nm of the system ([HG_{55}] = 1 μM, [T4 PNK] = 2.5 U/mL, [λ exo] = 10 U/mL, [ATP] = 0.1 mM, T4 PNK reaction time = 60 min) in the presence of different concentrations of complex **1** (0.5, 0.75, 1 and 1.5 μM) in aqueous buffered solution (20 mM Tris, 50 mM KCl, pH 7.0). 0.75 μM of complex **1** offered the highest luminescence fold-change response compared to 0.25, 1 or 1.5 μM of complex **1**. Both lower concentration and higher concentration of complex **1** gave a lower luminescence response.

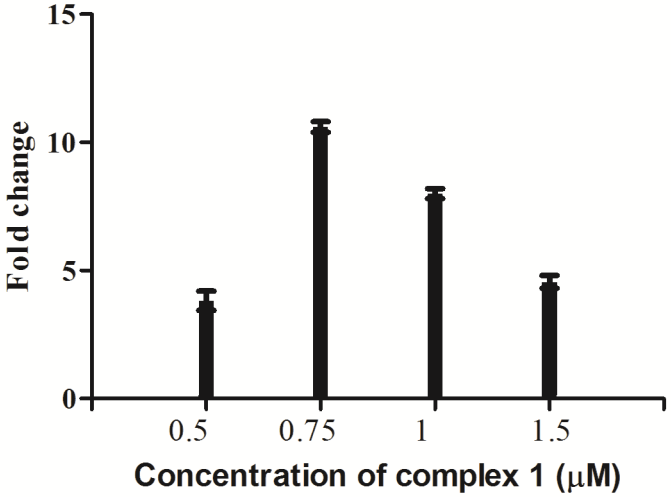

Fig. S13 Relative luminescence intensity at 580 nm of the system ([complex **1**] = 0.75 μM, [T4 PNK] = 2.5 U/mL, [λ exo] = 10 U/mL, [HG_{55}] = 1 μM, T4 PNK reaction time = 60 min) at various concentrations of the ATP (0.05, 0.1, 0.5, 1 and 2.5 mM) in aqueous buffered solution (20 mM Tris, 50 mM KCl, pH 7.0). The luminescence response of the system was highest at 0.1 mM of ATP. Higher concentrations of ATP resulted in a decreased fold change of the system, which may be partially due to the blockage of the PNK binding site for DNA at higher concentrations of ATP.[8]

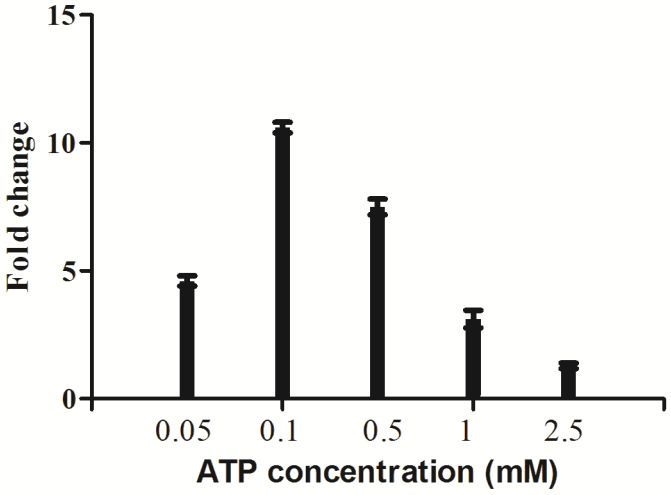

Fig. S14 Relative luminescence intensity at 580 nm of the system ([complex **1**] = 0.75 μM, [T4 PNK] = 2.5 U/mL, [HG_{55}] = 1 μM, [ATP] = 0.1 mM, T4 PNK reaction time = 60 min) at various concentrations of the λ exo (2, 5, 10, 15 and 20 U/mL) in aqueous buffered solution (20 mM Tris, 50 mM KCl, pH 7.0). 10 U/mL of λ exo offered the highest performance for PNK activity assay. Lower concentrations of λ exo may not have been able to fully digest the hairpin substrate, while higher concentrations of λ exo resulted in a higher background signal of the system, leading to a decreased luminescence fold-change response.

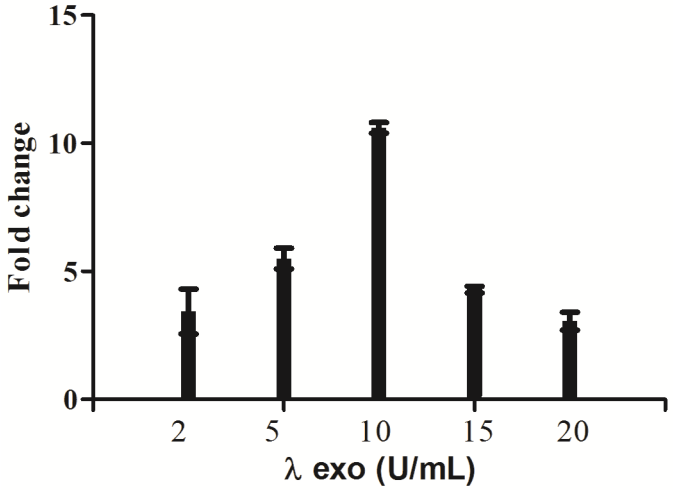

Fig. S15 Relative luminescence intensity at 580 nm of the system ([complex **1**] = 0.75 μM, [T4 PNK] = 2.5 U/mL, [λ exo] = 10 U/mL, [HG_{55}] = 1 μM, [ATP] = 0.1 mM) at different T4 PNK reaction time (20, 40, 60, and 80 min) in aqueous buffered solution (20 mM Tris, 50 mM KCl, pH 7.0). A time-course experiment revealed that the luminescent enhancement of the system reached saturation at approximately 60 min, after which no luminescence enhancement was observed.

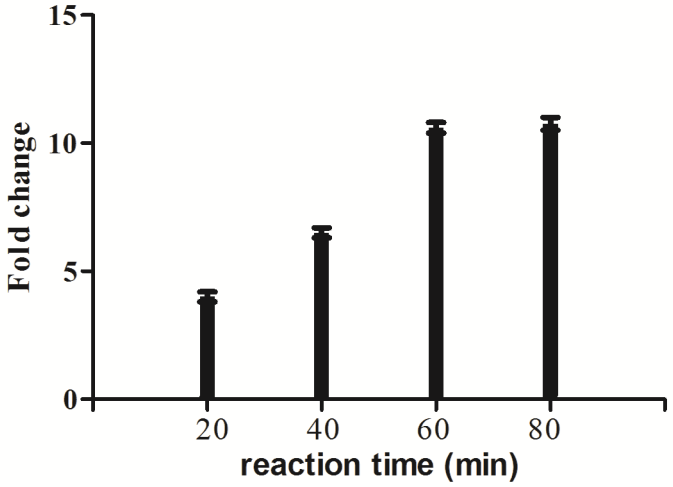

Fig. S16 Emission spectral traces of complex **1** (0.75 μM) and HG_{55} (1 μM) upon incubation with T4 PNK (0.05 U/mL) in Tris-HCl buffer (20 mM, 50 mM KCl, pH 7.0), showing a signal-to-noise ratio greater than 3 (3σ). Experimental conditions: [complex **1**] = 0.75 μM, [λ exo] = 10 U/mL, [HG_{55}] = 1 μM, [ATP] = 0.1 mM, and T4 PNK reaction time = 60 min.

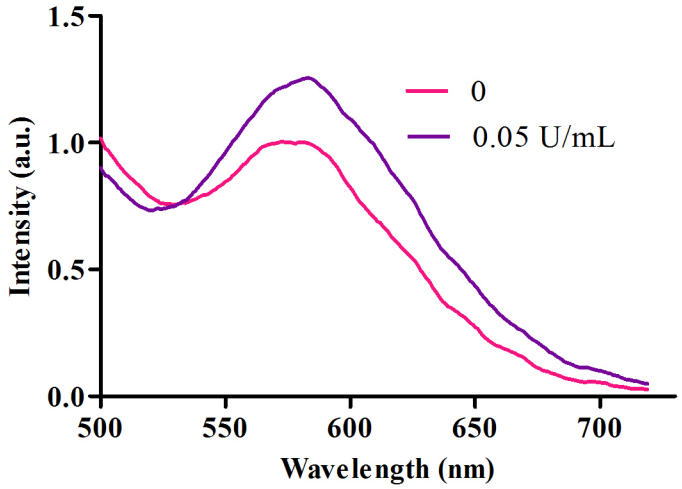

Fig. S17 Luminescence response of 0.75 μM of complex **1** in the presence or absence of 10 mM of T4 PNK inhibitor ADP.

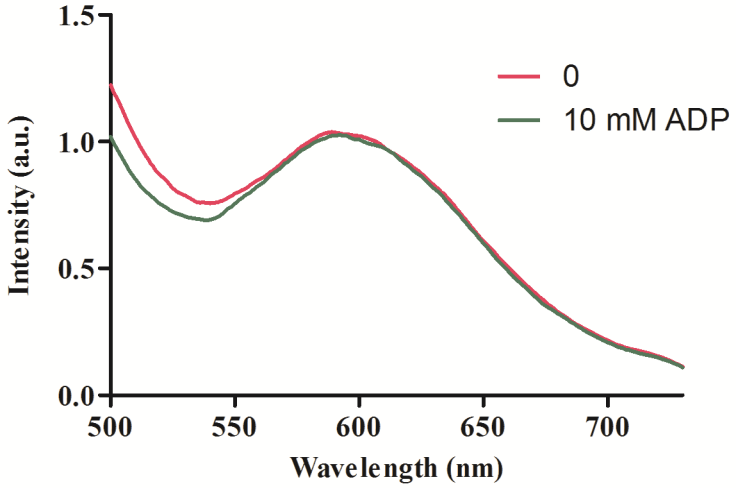

Table S4. Comparison of detection limit and detection range for PNK activity assays recently reported in the literature.

Method	*Detection limit*	*Range*	*Signal output*	*Ref.*
Molecular beacon DNA probe	0.002 U/mL	0.002 − 4 U/mL	fluorescent	6
Singly labeled DNA-hairpin probe	0.04 U/mL	0.04 − 10 U/mL	fluorescent	7
Nanochannel biosensor	0.01 U/mL	0.01 − 5 U/mL	fluorescent	9
Singly labeled DNA-hairpin probe with graphene oxide	0.001 U/mL	0.001 − 0.3 U/mL	fluorescent	8b
Perylene probe	0.003 U/mL	0.003 − 1.6 U/mL	fluorescent	10
Singly labeled DNA-duplex probe with graphene oxide	0.05 U/mL	0.05 − 10 U/mL	fluorescent	8a
Present study	0.05 U/mL	0.05 − 2.5 U/mL	luminescent	

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