Electronic Supporting Information

An oligonucleotide-based label-free luminescent switch-on probe for RNA detection utilizing a G-quadruplex-selective iridium(III) complex†

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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). All oligonucleotides were synthesized by Techdragon Inc. (Hong Kong, China)

DNA sequences used in this project:

	Sequence
ssDNA	5′-$C_2AGT_2CGTAGTA_2C_3$-3′
ds26	5′-$CA_2TCG_2ATCGA_2T_2CGATC_2GAT_2G$-3′
ds17	5′-$C_2AGT_2CGTAGTA_2C_3$-3′ 5′-$G_3T_2ACTACGA_2CTG_2$-3′
PS2.M	5′-$GTG_3TAG_3CG_3T_2G_2$-3′
Pu27	5′-$TG_4AG_3TG_4AG_3TG_4A_2G_2$-3′
Pu22	5′-$GAG_3TG_4AG_3TG_4A_2G$-3′
TBA	5′-$G_2T_2G_2TGTG_2T_2G_2$-3′
P1	5′-$C_2AGCTAG_3T_2G_3$-3′
P2	5′-$G_3ATG_3C_2G_3AG$-3′
Target RNA	5′-$CUC_3G_2UAGCUG_2$-3′
Single-base mismatched RNA strand	5′-$CUC\underline{A}CG_2UAGCUG_2$-3′
Two-base mismatched RNA strand	5′-$CUC\underline{A}CG_2UA\underline{A}CUG_2$-3′
Three-base mismatched RNA strand	5′-$C\underline{A_3}CG_2UAGCUG_2$-3′
Random coil RNA	5′-$GA_2CGCG_2UA_3UAG$-3′
Target DNA	5′-$CTC_3G_2TAGCTG_2$-3′
Single-base mismatched DNA strand	5′-$CTC\underline{A}CG_2TAGCTG_2$-3′
Two-base mismatched DNA strand	5′-$CTC\underline{A}CG_2TA\underline{A}CTG_2$-3′
Three-base mismatched DNA strand	5′-$C\underline{A_3}CG_2TAGCTG_2$-3′
Four-base mismatched DNA strand	5′-$C\underline{A_2}C_2G_2TA\underline{A_2}TG_2$-3′
Random coil DNA	5′-$GA_2CGCG_2TA_3TAG$-3′
F21T	5′-FAM-$(G_3[T_2AG_3]_3)$-TAMRA-3′
F10T	5′-FAM-TATAGCTA-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[1-3]: the precursor complex $[Ir_2(ptpy)_4Cl_2]$, $[Ir(ptpy)_2(dmph)]PF_6$. All complexes are characterized by ^{1}H-NMR, ^{13}C-NMR, high resolution mass spectrometry (HRMS) and elemental analysis.

$[Ir(ptpy)_2(dmph)]PF_6$ (**1**, where ptpy = 2-(p-tolyl)pyridine; dmph = 2,9-dimethyl-1,10-phenanthroline) A suspension of $[Ir_2(ptpy)_4Cl_2]$ (0.2 mmol) and 2,9-dimethyl-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: 62%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.74 (d, J = 8.3 Hz, 2H), 8.25 (s, 2H), 8.18 (d, J = 8.1 Hz, 2H), 7.91 (ddd, J = 8.2, 7.5, 1.5 Hz, 2H), 7.84 (d, J = 8.3 Hz, 2H), 7.78 (dd, J = 5.9, 0.7 Hz, 2H), 7.75 (d, J = 8.0 Hz, 2H), 6.98 (ddd, J = 7.3, 5.9, 1.4 Hz, 2H), 6.82 (dd, J = 8.0, 1.0 Hz, 2H), 6.08 (s, 2H), 2.25 (s, 6H), 2.04 (s, 6H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 168.81, 166.07 , 150.90 , 150.17 , 148.91 , 141.92 , 140.45 , 140.07 , 139.24 , 133.19 , 130.90 , 128.92 , 128.12 , 125.64 , 124.08 , 123.39 , 120.28 , 27.62 ,

21.82. MALDI-TOF-HRMS: Calcd. For $C_{38}H_{32}IrN_4$ $[M–PF_6]^+$: 737.2256 Found: 737.2275. Anal. ($C_{38}H_{32}IrN_4PF_6 \cdot H_2O$) C, H, N: calcd 50.72, 3.81, 6.23; found: 50.81, 3.60, 6.26.

Luminescence response of 1 towards different forms of DNA

The G-quadruplex DNA-forming sequences (PS2.M, Pu22, 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). The luminescence intensity was monitored after excitation of the sample at 360 nm.

Emission measurement

A mixture of P1, P2 (10 μL, each 100 μM) and different concentration of RNA was mixed in hybridization buffer (90 μL, 50 mM Tris-HCl containing 20 mM KCl, pH 7.2). The mixture was annealed at 95 °C for 10 min, and was slowly cooled to 25°C. In the emission measurement, 100 μL of the DNA/RNA mixture stock solution was diluted with 400 μL of Tris-HCl buffer (50 mM, 20 mM KCl, pH 7.2) to obtain a DNA/RNA mixture solution in a cuvette, followed by the addition of iridium(III) complex **1** at a final concentration of 1.5 μM. The mixture was allowed to equilibrate at 25°C for 10 min. Emission spectra were recorded on a PTI QM-4 spectrofluorometer at 25°C. The luminescence intensity was monitored after excitation of the sample at 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 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 **1**. 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.

G4-FID assay

The FID assay was performed as previously described.[4] 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. $PD = 100 - [(FA/FA_0) \times 100]$ (FA_0 = fluorescence area of DNA-TO complex in the absence of **1**; FA = fluorescence area in the presence of **1**).

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)	547 (3.311)	0.662

[a] sh = shoulder peak

Table S2. Photophysical properties of complex **1** in Tris buffer in the absence and presence of G-quadruplex DNA.

Complex **1**	Tris buffer	5 µM of PS2.M	5 µM of Pu27
Quantum yield Φ	0.067	0.298	0.249

Fig. S1 UV/vis absorption of complex **1** in dichloromethane (DCM) at 298 K.

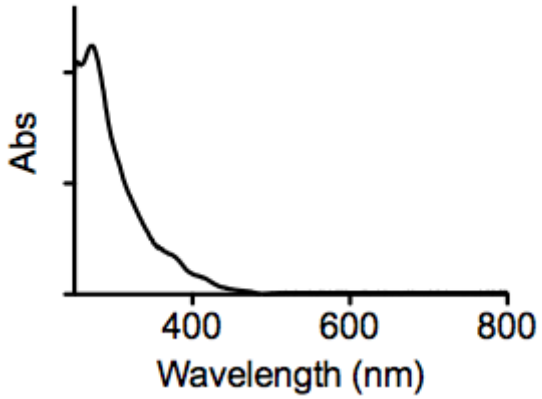
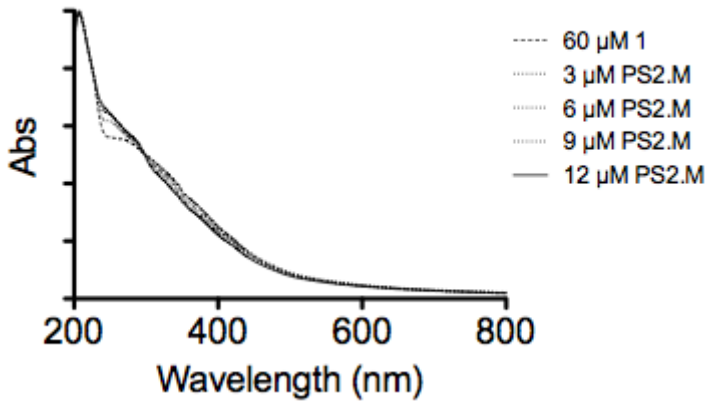


Fig. S2 UV/vis spectrophotometric titration of complex **1** with PS2.M.



References

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