Electronic Supporting Information

A Label-Free Luminescent Switch-on Probe for Ochratoxin A Detection Using a G-quadruplex-Selective Iridium(III) Complex

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Experimental section

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 (acetone-d_6: ^{1}H δ 2.05, ^{13}C δ 29.8). 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).

Stock solution preparation. The Ir(III) complexes' stock solution were prepared in acetonitrile with a concentration of 1 mM. The OTA, OTB and warfarin were dissolved in methanol with an initial concentration of 250 μM, and were stored at –20 °C before use.

Photophysical measurement. Emission spectra, lifetime measurements and Luminescence quantum yields were determined according to a reference.[1]

FRET melting assay. The ability of **1** to stabilize G-quadruplex DNA was investigated using a fluorescence resonance energy transfer (FRET) melting assay and it was performed as previously described.[3]

Luminescence response of iridium(III) complex towards different forms of DNA

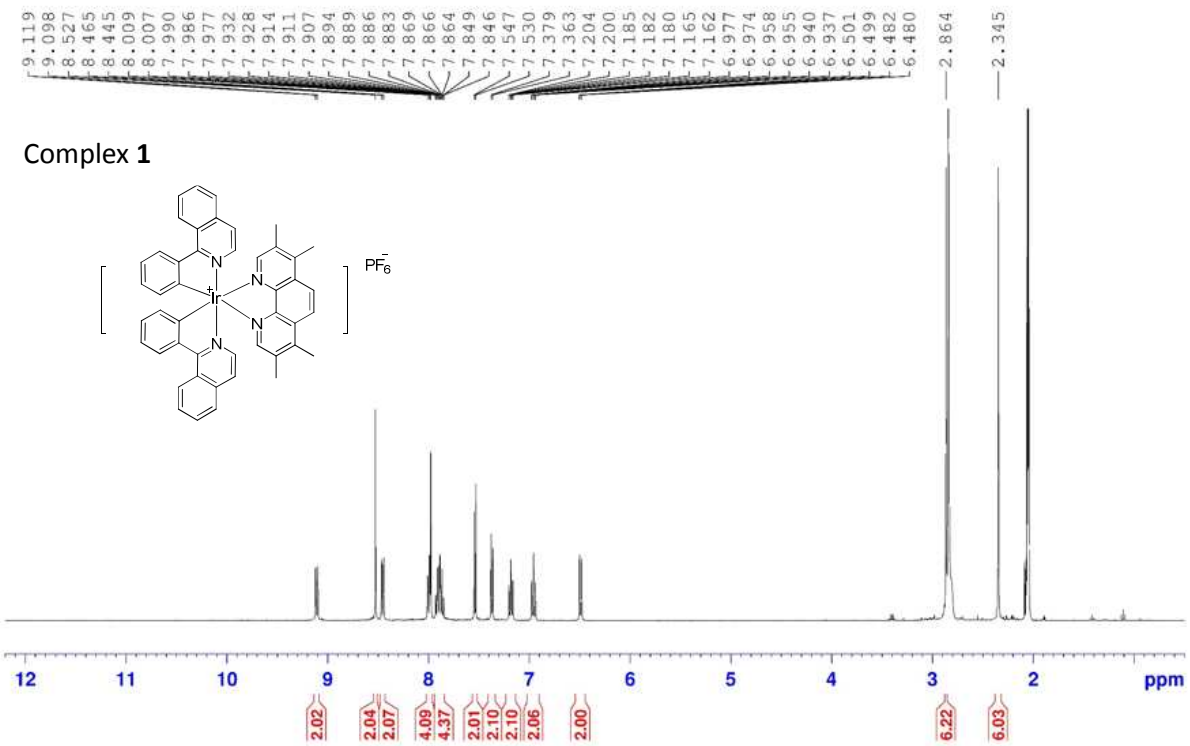
The G-quadruplex DNA-forming sequences (PS2.M) was annealed in Tris-HCl buffer (20 mM Tris, 100 mM KCl, pH 7.0) and were stored at –20 °C before use. Complexes **1**–**6** (0.5 μM) was added to 5 μM of ssDNA, dsDNA or PS2.M G-quadruplex DNA in Tris-HCl buffer (20 mM Tris, pH 7.0), then their emission intensity were tested.

Synthesis. The complex **1** was prepared according to (modified) literature methods, and is characterized by ^{1}H-NMR, ^{13}C-NMR, high resolution mass spectrometry (HRMS) and elemental analysis. The precursor iridium(III) complex dimer $[Ir_2(piq)_4Cl_2]$ was prepared as reported method.[2] Then, a suspension of $[Ir_2(piq)_4Cl_2]$ (0.2 mmol) and corresponding N^N ligand 2,3,8,9-tetramethyl-1,10-phenanthroline (tmphen), (0.44 mmol) in a mixture of DCM:methanol (1:1, 20 mL) was refluxed overnight under a nitrogen atmosphere. An aqueous solution of ammonium hexafluorophosphate (excess) was added in the gradual transparent reaction solution, and the mixture was reduced in volume by rotary evaoration until precipitation of the crude product appeared. The precipitate was then filtered and washed with several portions of water followed by diethyl ether. The product was recrystallized by acetonitrile:diethyl ether vapor diffusion to yield the titled compound.

Complex **1**. Yield: 56%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.11 (d, J = 8.4 Hz, 2H), 8.53 (s, 2H), 8.46 (d, J = 8.0 Hz, 2H), 8.01-7.98 (m, 4H), 7.93-7.86 (m, 4H), 7.54 (d, J = 6.8 Hz, 2H), 7.37 (d, J = 6.4 Hz, 2H), 7.20-7.16 (m, 2H), 6.98-6.94 (m, 2H), 6.50-6.48 (m, 2H), 2.86 (s, 6H), 2.35 (s, 6H); ^{13}C

NMR (100 MHz, Acetone-d_6) δ 169.8, 155.2, 152.3, 147.4, 146.7, 146.3, 141.8, 138.0, 136.3, 133.3, 132.8, 131.6, 131.4, 130.9, 130.0, 128.5, 127.7 127.1, 125.4, 123.0, 122.6, 18.1, 15.1; MALDI-TOF-HRMS: Calcd. for $C_{46}H_{36}IrN_4[M–PF_6]^+$:837.2569 Found: 838.2690. Anal.: ($C_{46}H_{36}IrN_4PF_6$+2H_2O) C, H, N: calcd.55.25, 3.83, 5.60; found 55.03, 3.77, 5.72.

Complex **1**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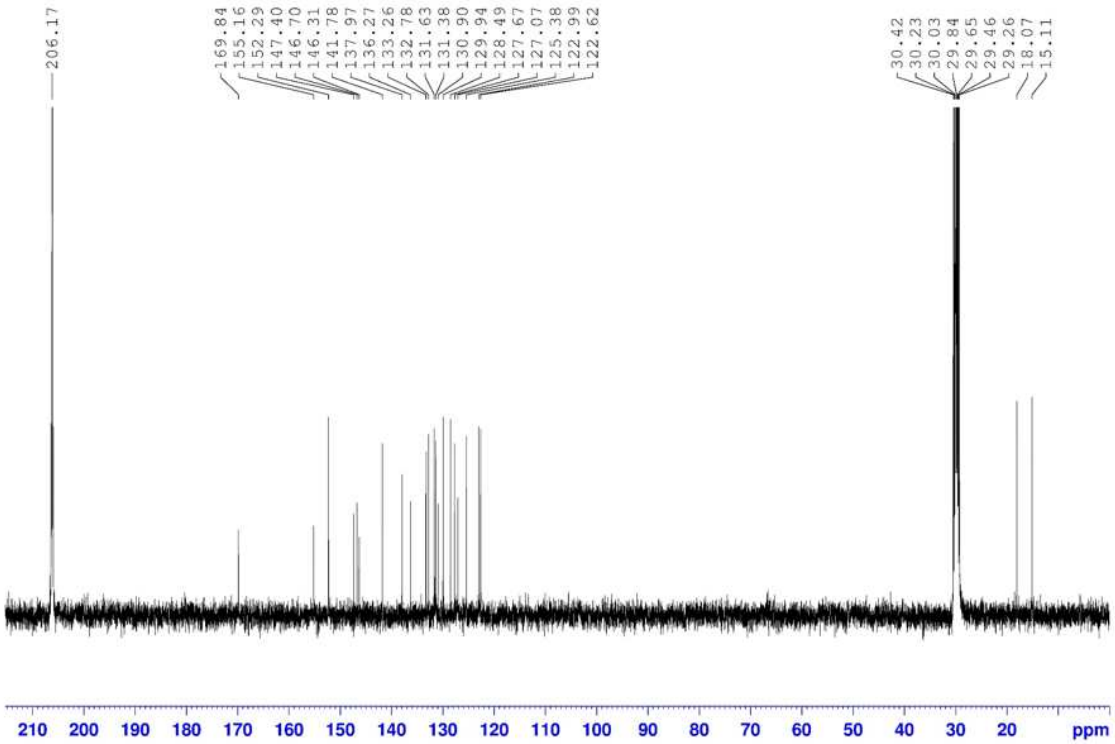
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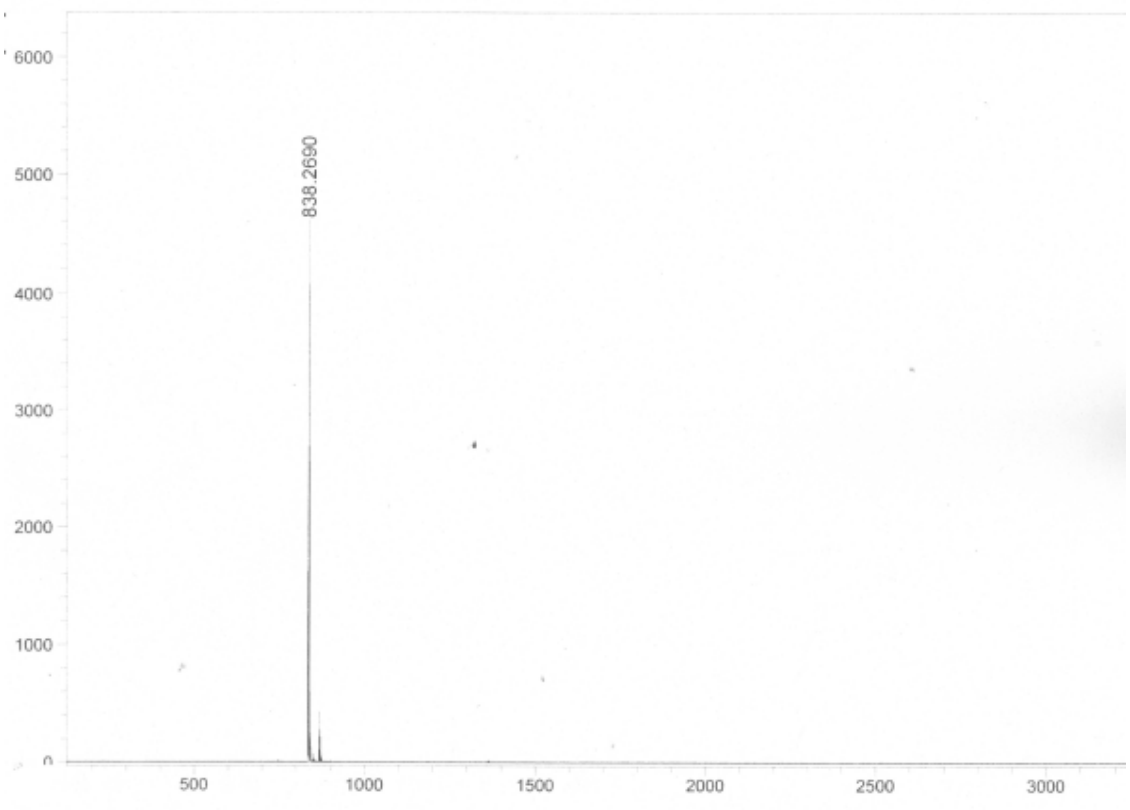


Complexes **2**–**6**. Reported.[3]

Table S1 Photophysical properties of iridium(III) complexes **1**–**6**.

Complex	Quantum yield	λ_{em}/ nm	Life time/ μs	UV/vis absorption λ_{abs} / nm (ε/ $dm^3mol^{-1}cm^{-1}$)
1	0.058	600	4.296	235 (1.57×10^5), 276 (1.17×10^5), 350 (4.18×10^4), 445 (1.48×10^4)
2	0.091	602	4.1	275(6.42×10^5)
3	0.063	598	4.1	272(4.5×10^5), 382(1.2×10^4), 438(9.6×10^3)
4	0.063	593	4.6	279(2.0×10^5), 382(1.56×10^4), 439(6×10^3)
5	0.049	602	4.6	278(2.65×10^5), 380(1.76×10^4), 439(8×10^3)
6	0.091	590	4.371	274 (7.98×10^4), 354 (1.13×10^4)

Table S2. DNA sequences used in this project:

DNA	Sequence
ON1	5′-GATCG_3TGTG_3TG_2CGTA$_3$$G_3$AGCATC$G_2$ACA-3′
ON2	5′- C_2ACA_3GATC-3′
PS2.M	5′-GTGGGTAGGGCGGGTTGG-3′
CCR5-DEL	5′-CTCAT$_4$$C_2$ATACAT$_2$$A_3$GATAGTCAT-3′
ds26	5′-CA_2TCG_2ATCGA_2T$_2$CGATC$_2$GAT$_2$G-3′
ds17	5′-C_2AGT$_2$CGTAGTA$_2$$C_3$-3′ 5′-$G_3T_2$ACTACGA$_2CTG_2$-3′
F21T	5′-FAM-(G_3[T$_2$AG_3]$_3$)-TAMRA-3′
F10T	5′-FAM-TATAGCTA-HEG-TATAGCTATAT-TAMRA-3′
ON1$_m$	5′-GATC*T*$_3$TGT*C*$_3$TG$_2$CGTA$_3$*C*$_3$AGCATCG_2ACA-3′[a]
ON2$_m$	5′- *G*$_2$AC*A*$_3$GATC-3′

a. The italic bases are mutant bases.

Figure S1.(a-f) Luminescence response of complexes **1**–**6** (0.5 μM) in 20 mM Tris buffer (pH 7.0) in the presence of 5 μM ssDNA (CCR5-DEL), 5 μM ds17 and 5 μM PS2.M G-quadruplex, respectively. PS2.M G-quadruplex was pre-annealed in Tris buffer (20 mM, 100 mM KCl, pH 7.0). (g) Diagrammatic bar array representation of the luminescence enhancement selectivity of complexes **1**–**6** (0.5 μM) in 20 mM Tris buffer (pH 7.0) in the presence of 5 μM ssDNA (CCR5-DEL), 5 μM ds17 and 5 μM PS2.M G-quadruplex, respectively. Error bars represent the standard deviations of the results from three independent experiments.

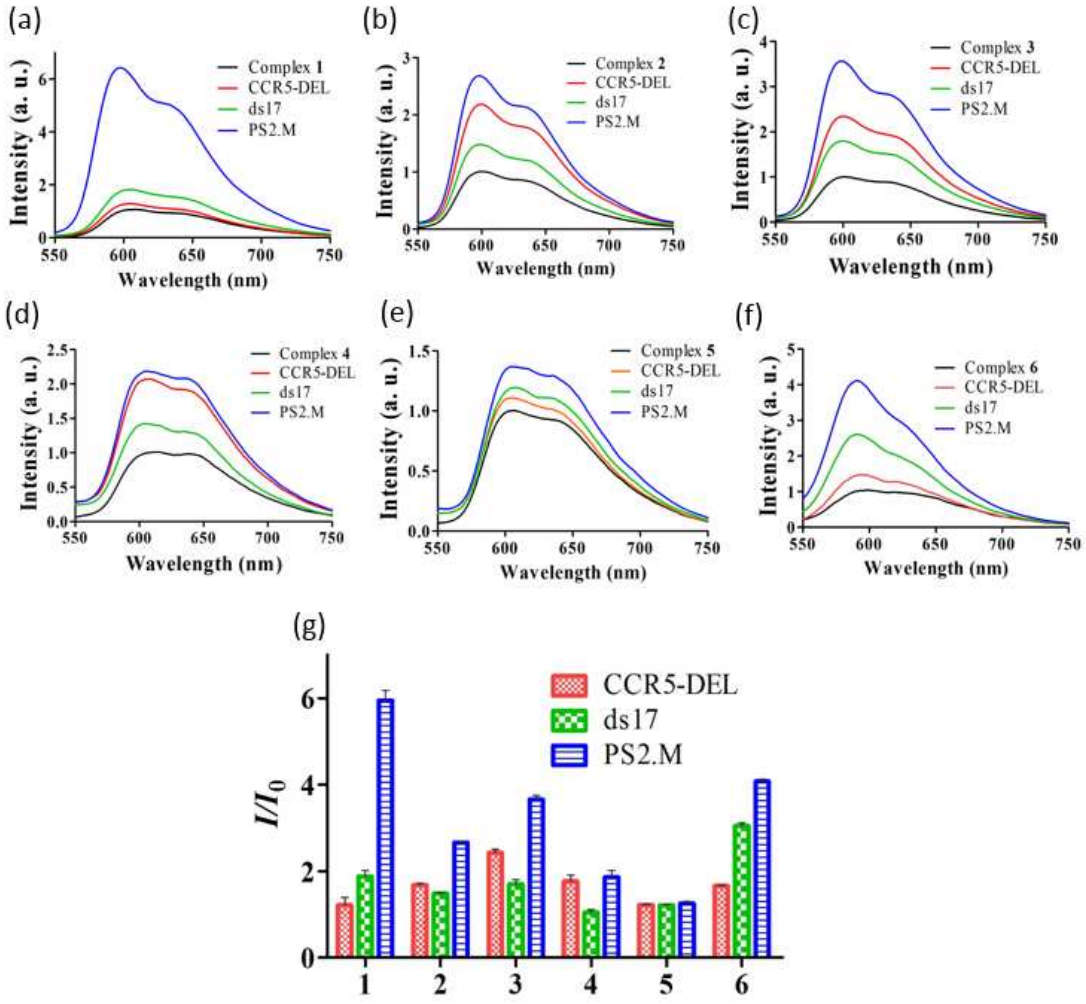


Figure S2. Plot of $D/\Delta\varepsilon_{ap}$ *vs.* concentration of DNA for estimating the intrinsic binding constant (K). The absorbance of **1** (20 μM) at 325 nm was used for calculation. Intrinsic binding constant of **1** to PS2.M G-quadruplex $K = 1.26 \times 10^5$ M^{-1}, ds26 duplex DNA $K = 0.4 \times 10^5$ M^{-1} and CCR5-DEL ssDNA $K = 0.21 \times 10^5$ M^{-1}.

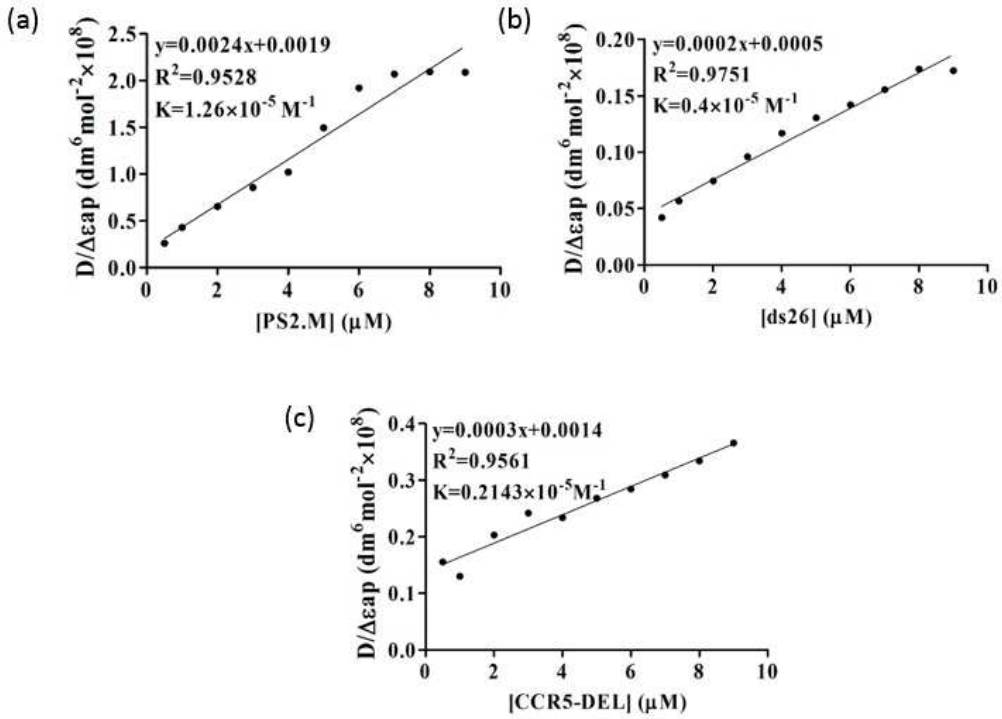


Figure S3. Luminescence response of the system with the complex alone ([complex **1**] = 0.5 μM) in the absence and presence of OTA (150 nM).

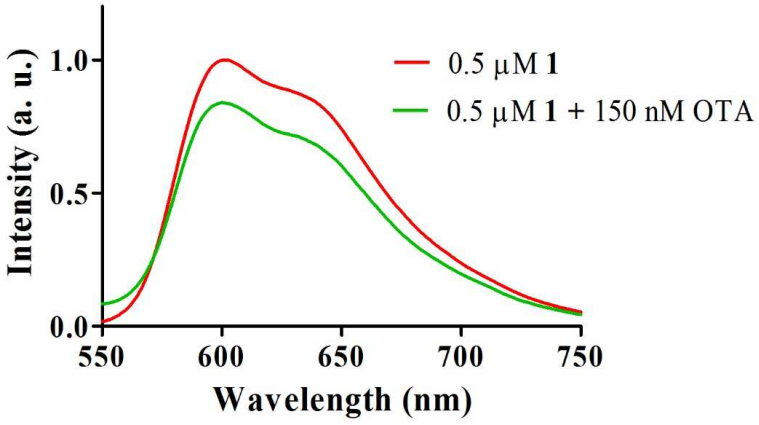


Figure S4. Relative luminescence response of complex **1** (0.5 μM) in the presence of OTA (60 nM) and duplex DNA (0.5 μM) or duplex DNA mutant (0.5 μM). Experimental conditions: 0.5 μM of complex **1,** duplex DNA or duplex DNA mutants (0.5 μM) in OTA binding buffer (10 mM Tris-HCl, 120 mM NaCl, 10 mM $MgCl_2$, 10 mM KCl, pH 8.4). Duplex DNA or duplex DNA mutants were treated with OTA at 25 °C for 10 min. Error bars represent the standard deviations of the results from three independent experiments.

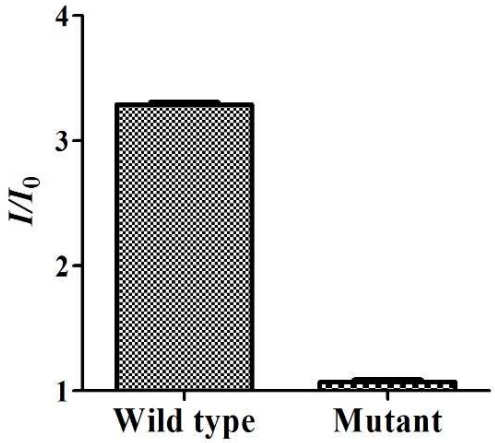


Figure S5. Circular dichroism (CD) spectrum of 2 μM duplex substrate in the absence (red) or presence (green) of 1 μM OTA recorded in OTA binding buffer (10 mM Tris-HCl, 120 mM NaCl, 10 mM $MgCl_2$, 10 mM KCl, pH 8.4).

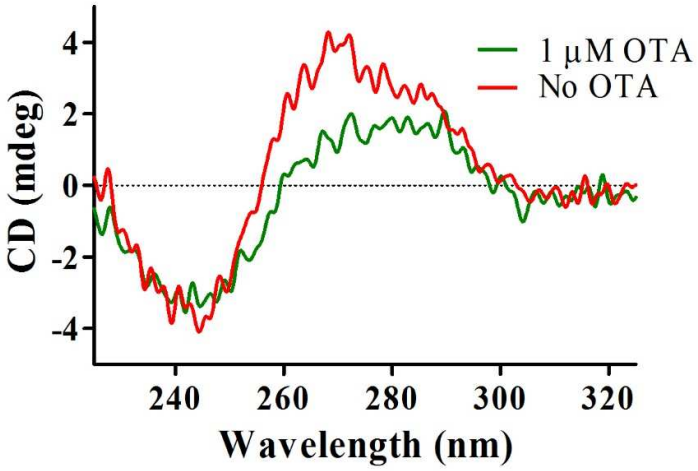


Figure S6. Relative luminescence response of the system in the absence or presence of OTA (20 nM) at various concentrations of duplex DNA (0.1, 0.25, 0.5, and 1 μM). Experimental conditions: complex **1** (0.5 μM) in OTA binding buffer (10 mM Tris-HCl, 120 mM NaCl, 10 mM $MgCl_2$, 10 mM KCl, pH 8.4). Duplex DNA (0.5 μM) were treated with OTA at 25 °C for 10 min. Error bars represent the standard deviations of the results from three independent experiments.

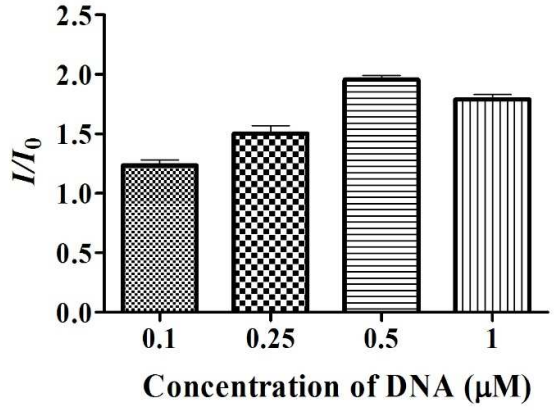


Figure S7. Relative luminescence response of the system in the absence or presence of OTA (20 nM) at various concentrations of complex **1** (0.25, 0.5, 1, and 2 μM). Experimental conditions: duplex DNA (0.5 μM) in OTA binding buffer (10 mM Tris-HCl, 120 mM NaCl, 10 mM $MgCl_2$, 10 mM KCl, pH 8.4). Duplex DNA (0.5 μM) were treated with OTA at 25 °C for 10 min. Error bars represent the standard deviations of the results from three independent experiments.

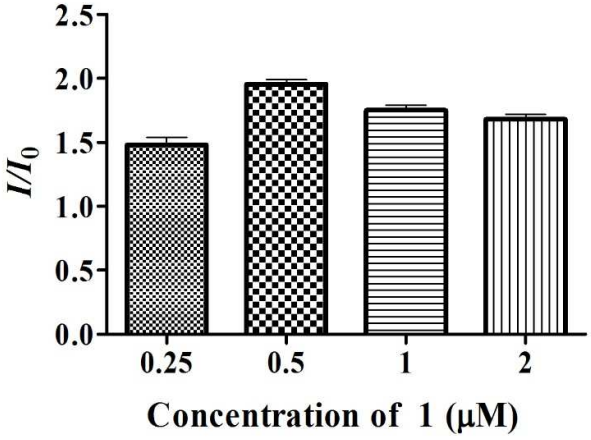
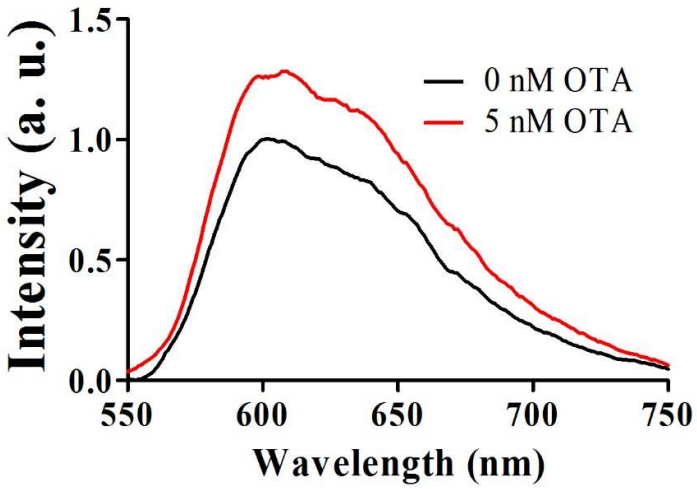


Figure S8. Emission spectral traces of complex **1** (0.5 μM) and duplex DNA (0.5 μM) in the presence of OTA (5 nM) in OTA binding buffer (10 mM Tris-HCl, 120 mM NaCl, 10 mM $MgCl_2$, 10 mM KCl, pH 8.4). Duplex DNA (0.5 μM) were treated by OTA at 25 °C for 10 min.



References

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